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Li-Yun Lin

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Characterization of the protective antibody response induced following vaccination or infection / Caractérisation de la réponse anticorps protectrice induite après vaccination ou après infection

THÈSE dirigée par :

Mme MOOG Christiane	Directrice de recherche, INSERM, Strasbourg

RAPPORTEURS :

M. VERRIER Bernard	Directeur de recherche, CNRS, Lyon
Mme GODOT Véronique	Professeure des Université en Immunologie, Université Paris-Est Créteil

AUTRES MEMBRES DU JURY :

M. PAILLART Jean-Christophe	Directeur de recherche, Université de Strasbourg
M. NEGRONI Matteo	Directeur de recherche, Université de Strasbourg
Mme RICHERT Laura	Professeure, Université de Bordeaux

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French Summary

Selon les données publiées par l'Organisation mondiale de la santé (OMS) en 2020, 37,7 millions de personnes vivent avec le virus de l'immunodéficience humaine (VIH) et 68% millions d'entre elles sont africaines. Outre le traitement, le développement d'un vaccin prophylactique efficace est crucial et urgent pour lutter contre la pandémie croissante du VIH. Différentes stratégies vaccinales ont été développées afin d'induire une réponse immunitaire spécifique efficace contre le VIH. Cependant, de nombreuses questions demeurent. 1) Les anticorps (Abs) inhibiteurs protecteurs peuvent-ils être induits par la vaccination ? 2) Cette réponse est-elle similaire partout dans le monde ou la variation ethnique ou géographique a-t-elle un impact sur la réponse immunitaire et sur la protection contre l'infection ? 3) Quel est le profil de la réponse Ab protectrice induite tôt après l'infection ?

Le VIH est un lentivirus infectant les cellules immunitaires humaines et présentant une diversité extrêmement élevée. En comparaison avec les isolats prédominants de sous-type B du VIH-1 d'Europe et d'Amérique, l'Afrique a élargi la diversité du VIH, les sous-types A et D étant majeurs en Afrique de l'Est ; C en Afrique australe ; A, G, CRF02_AG et CRF06_cpx en Afrique de l'Ouest ; et sous-type B et CRF02_AG en Afrique du Nord.

Pour lutter et éradiquer la pandémie de VIH, un vaccin protecteur efficace sera nécessaire. Pourtant, en raison de la grande diversité des sous-types de VIH, l'induction d'Abs avec des fonctions largement inhibitrices sera nécessaire. Les anticorps neutralisants (Nabs) sont des Abs capables de protéger les cellules contre les particules infectieuses, conduisant à l'inhibition de la réplication du VIH. Induire de tels Abs est considéré comme le Graal. Cependant, ce type d'Ab est difficile à induire en raison de la nécessité d'une longue maturation de la réponse humorale et essentiellement de la différentiation des lymphocytes B en types et sous-types d'immunoglobulines (Ig). En plus de ces NAbs difficiles à induire, des Ab capables d'inhiber le VIH par la fonction médiée par le domaine Fc des Abs ont été décrits. Dans ce cas, les Abs vont se lier aux récepteurs Fc (FcR) à la surface des cellules immunitaires effectrices afin d'activer ces fonctions inhibitrices médiées par les FcR. Il est intéressant de noter que le polymorphisme spécifique du FcyRIIA de génotype rs 10800309 s'est avéré significativement augmenté dans une cohorte de patients VIH qui contrôlent leur infection

(cohorte de contrôleurs VIH). Ce polymorphisme s'accompagne d'une expression accrue du récepteur FcγRII a la surface des cellules dendritiques, suggérant un renforcement de la réponse immunitaire grâce à la régulation positive du FcRII. Nous proposons donc que ce polymorphisme pourrait servir de marqueur prédictif à un meilleur contrôle du VIH par les Abs. Ainsi, aussi bien les types d'Ig ainsi que les polymorphismes des FcR à la surface des cellules effectrices pourraient contribuer à la protection du VIH par les Abs.

Les approches vaccinales actuelles proposent d'induire des Abs neutralisants ainsi que des Abs inhibiteurs médiés par les FcR. Par conséquent, les types et le sous-types d'Ig induites, ainsi que la quantité et la qualité des récepteurs Fc (FcR) exprimés sur les cellules immunitaires devront être pris en considération. Comme ces facteurs varient selon l'ethnicité, les futurs vaccins devront peut-être adapter leurs stratégies à l'origine ethnique génétique de la population ciblée ainsi qu'aux souches locales de VIH en circulation.
L'objectif de ma thèse est d'analyser la réponse immunitaire humorale, en particulier la neutralisation et l'inhibition médiée par les domaines Fc des Abs, dans le cadre de nouvelles stratégies vaccinales, et de décrypter l'implication de l'origine génétique dans les fonctions inhibitrices des Abs induits.

1) Développement de nouvelles stratégies vaccinales

En collaboration avec le consortium européen Horizon 2020 EHVA, de nouvelles stratégies vaccinales ont été développées. Mon objectif était de caractériser les réponses Ab induites afin de sélectionner les protocoles vaccinaux les plus prometteurs.

I) Trimères env modifiés :
   a. Masquage sélectif des épitopes immunogènes non fonctionnels
      1) Modèle Lapin *versus* 2) modèle Macaques
   1) Modèle lapin

Un trimère d'enveloppe stabilisée (env) BG505-SOSIP et CON ont été généré. Sur la base de ce trimère stabilisé, différentes nouvelles constructions de trimère env (ConC-GT ou ConC-GT-CL) ont été produites par notre partenaire H2020 (groupe de R. Wagner, Regensburg, Allemagne). Ces nouveaux trimères du VIH contiennent une délétion ou un masquage de la

glycosylation afin de rediriger la réponse immunitaire vers le domaine conservé de liaison du VIH au CD4.

Suite à l'immunisation de lapins avec ces envs modifiées, je détecte des Abs spécifiques du VIH. Ces Abs apparaissent plus précocement avec la construction ConC-GT. Cette immunisation est rattrapée avec la construction ConC-GT-CL dès la deuxième immunisation. Ces réponses Ab sont maintenue pour les 3ème et 4ème immunisations.

J'ai évalué l'activité neutralisante des Abs induits vis-àvis de diverses souches de VIH. Pour ce faire j'utilise un test de neutralisation TZM-bl conventionnel, standardisé et validé et je calcule l'IC50 (concentration inhibitrice 50). Pour ces conditions expérimentales, nous avons détecté une activité neutralisante contre les souches faciles à neutraliser (souche de VIH classifier Tier 1 comme MW956.26. Fait intéressant, une neutralisation homologue contre le virus ConC a également pu être détectée chez certains animaux. Par contre, nous ne détectons pas d'activité neutralisante vis-à-vis de virus primaires hétérologues

Ces résultats ont montré que nos nouvelles constructions env sont hautement immunogènes dans le modèle lapin.

2) Modèle Macaque

Dans le modèle de primates non Humains (NHP), je détecte des Abs spécifiques du VIH à la semaine 6 après les deuxièmes immunisations avec ConC-G. Pour l'env BG505 ancestral, une troisième immunisation est nécessaire pour la détection des Abs spécifiques du VIH. La réponse Ab induite est donc plus lente et le niveau d 'immunisation est plus faible que dans le modèle Lapin.

Ces résultats démontrent l'amélioration de l'immunogénicité de nos nouvelles constructions d'enveloppe modifiées. Cependant, par rapport à la réponse Ab détectée chez le lapin, les IC50 sont de 10 à 100 fois inférieures chez le NHP. De plus, cette réponse diminue après chaque immunisation.

Ainsi, les Abs spécifiques du VIH ainsi que l'activité neutralisante ont été plus fortement induits dans le modèle Lapin que dans le modèle NHP. Cette forte diminution de l'efficacité de l'immunisation dans le modèle NHP a été reportée dans des études antérieures.

II) Nouvelles plateformes vaccinales

a. Vaccination avec le vecteur VSV chez le lapin

Outre les différents candidats protéine/env, des plateformes de délivrance ont été développées pour potentialiser la réponse Ab. Ici, nous avons évalué la plate-forme VSV-GP exprimant les envs du VIH à leur surface (Figure 8). Cette plateforme a été testée dans le modèle lapin soit sous forme de deux immunisations VSV successives suivies de rappels protéiques, soit sous forme d'immunisation protéique VSV séquentielle

Des Abs anti-VIH élevés ont déjà été détectés après la 1ère immunisation (semaine 4) avec VSV-GP-sC23v4. Il n'y a pas de différence significative dans l'induction d'Abs entre les groupes (VSV/ VSV/ Env/ Env et VSV/ Env/ VSV/ Env). Par conséquent, la réponse immunitaire potentiellement induite contre le vecteur VSV n'a pas modifié l'induction de la réponse Ab spécifique du VIH contre les envs ConC et sC23.

Une activité neutralisante contre le virus Tier 1, MW965.26 a été détectée à 6 et 26 semaines après immunisation, dans les deux groupes et quelle que soit la stratégie d'immunisation, c'est-à-dire VSVenv/ VSVenv/ Env/ Env versus VSVenv/ env/ VSVenv/ env.

Ces résultats démontrent l'amélioration de la réponse Ab par la plateforme VSV utilisée seule ou en association avec les protéines env.

b. Vaccination avec le vecteur VSV chez le Macaque et libération prolongée de l'immunogènes (Pompe Osmotique)

Les plates-formes vectorielles VSV ont été testées plus avant dans le modèle NHP. Afin d'améliorer la réponse Ab induite dans les NHP, la protéine env ConCv5 KIKO a été administrée

en tant que rappel, soit en utilisant la voie d'administration intramusculaire (IM) classique, soit par libération lente et persistante à l'aide d'une pompe osmotique (OP). Ce processus de délivrance lent a récemment été décrit comme capable d'améliorer la réponse Ab induite.

Cette immunisation de la protéine par OP a permis d'induire une activité neutralisante contre le pseudovirus sCON KIKO à la semaine 28. Au contraire, l'immunisation classique en IM n'a pas permis d'induire des Abs neutralisants.

Ainsi, l'immunisation avec l'env sCON KIKO par voie OP augmente les Abs spécifiques du VIH et l'activité neutralisante contre le virus sCON KIKO autologue. Ces résultats démontrent les avantages de l'immunisation en OP par rapport à la voie IM classique. De nouvelles stratégies d'immunisation avancées, y compris l'administration lente d'immunogènes, devraient être entreprises pour améliorer l'immunogénicité des nouveaux candidats vaccins.

2) Interaction hôte-vaccin
    - Réponses immunitaires humorales et Impact génétique/ Ethnicité

Un projet soutenu par HIP (HVTN Initiative Project) a été développé en collaboration avec le College of Health and Allied Sciences (UDSM-MCHAS, Tanzanie) et le Medical Center de l'Université de Munich (LMU, Allemagne). Ce projet vise à comparer l'induction de la réponse Abs spécifique au VIH et leur fonction entre les individus d'origine caucasienne et africaine. Pour ce projet, l'essai clinique de phase II HVTN 204 a été choisi car le même protocole vaccinal a été mené aux États-Unis et en Afrique du Sud. Nous avons eu accès à des échantillons historiques (sérum et cellules) de 137 volontaires de vaccination à deux moments : la visite 2 (V2) collectée avant la première injection de vaccin et la visite 10 (V10) collectée après que les volontaires ont reçu trois immunisations et un rappel.

J'ai déterminé les Abs IgG et IgA totaux et spécifiques au VIH, la neutralisation, la fonction médiée par le Fc (cytotoxicité dépendante des Abs, ADCC) et le polymorphisme du FcR et j'ai comparé ces activités entre Africains et Caucasiens. Pour l'étude statistique, j'ai bénéficier de l'aide technique de E. L'Homme, de l'Unité de Soutien Méthodologique à la Recherche clinique et épidémiologique (USMR), Service d'Information Médicale (SIM) - CHU de Bordeaux.

Pour cette étude, nous avons observé :

- une quantité statistiquement significativement plus élevée d'immunoglobine totale G et A chez les Africains par rapport aux Caucasiens
- un génotype AA du polymorphisme rs 10800309 du FcyRIIA significativement plus élevé (10 %) chez les Caucasiens, par rapport aux Africains (2 %) ($p < 0,0001$). Des études antérieures de notre équipe avait montré une augmentation de ce polymorphisme AA dans la population de personnes « contrôleurs du VIH » et une augmentation de hausse l'expression de FcɣRII sur les cellules dendritiques associés à ce polymorphisme. Le génotype AA du polymorphisme rs 10800309 FcyRIIA pourrait donc participer à la protection de l'infection ou de la maladie.
-

Concernant la réponse à la vaccination (visite 10 – Visite 2) nous avons observé que :

- Les Caucasiens ont un rapport plus élevé entre les Abs spécifiques du VIH et les IgG totales par rapport aux Africains. Cette différence est due à un plus faible taux d'IgG totales chez les Caucasiens. Si nous comparons l'Abs spécifique au VIH à V10 avec le résultat pré-immun, les Africains ont un ratio plus élevé en raison du faible bruit de fond dans leur sérums pré-immuns.
- Les Caucasiens ont une activité neutralisante contre les virus MW965.26 à la visite 10 significativement plus élevée que celle détectée chez les Africains.

Ainsi, bien que la réponse Abs spécifique au VIH induite soit faible pour cet essai vaccinal, nous avons observé un rapport significativement plus élevé d'Abs spécifiques chez les Caucasiens par rapport aux Africains. De plus, la quantité totale d'Ig et les polymorphismes du FcR diffèrent significativement entre les Caucasiens et les Africains, suggérant fortement que la réponse immunitaire globale induite pourrait varier selon l'ethnicité.

3) Analyse de la réponse Ab précoce suite à une infection par le VIH

En plus de l'étude de la réponse Ab humorale induite dans les vaccins, j'ai participé à l'analyse de la réponse Ab précoce induite suite à une infection. Notre objectif final est d'améliorer la réponse immunitaire vaccinale sur la base de nos connaissances sur la réponse Ab suite à une

infection. Comme le vaccin doit induire une réponse immunitaire précoce et efficace, nous avons concentré notre étude sur la réponse induite lors d'une infection aiguë précoce.

Nous avons analysé la réponse Ab fonctionnelle induite précocement après infection dans les sérums prélevés dans la cohorte ANRS PRIMO à 2 temps : Jour 0 (J0) correspondant au jour d'inclusion dans la cohorte (jour de détection, moins de 3 mois après l'infection) et 6 ou 12 mois plus tard (M6/M12). S'agissant d'une cohorte historique, les patients sélectionnés n'étaient pas traités au moment du prélèvement.

Concernant l'activité neutralisante, nos résultats ont montré que certains patients étaient capables d'induire des Abs neutralisants à large spectre (bNabs) contre les virus transmis/fondateurs (T/F) peu de temps après l'infection. Ces résultats diffèrent de ce qui était précédemment publié pour les virus non-T/F, virus isolés plus tardivement au cours de l'infection. En effet, les études précédentes ne détectaient pas de bNab au début de l'infection. Il fallait attendre plus de deux ans pour la mise en place d'une réponse bNabs dirigé contre des isolats primaire (non T/F). La détection de bNabs contre les virus T/F chez les individus infectés après quelques mois ouvre de nouvelles perspectives pour le développement de tels Abs fonctionnels par vaccination.

Conclusion

Les différents résultats obtenus au cours de ma thèse sur la réponse Ab induite dans différentes stratégies vaccinales et suite à l'infection donnent des informations originales sur le type d'Ab à induire par la vaccination.

Ainsi, la réponse Ab spécifique du VIH induite par vaccination:

- est plusieurs fois inférieure chez les NHP et les humains par rapport à celle détectée chez les rongeurs. Ces résultats montrent les limites du modèle de petit animal et renforcent la nécessité de confirmer l'induction des Ab chez l'homme. Ces résultats suggèrent également qu'il faudrait améliorer l'induction des Abs par de nouvelles stratégies d'immunisation plus efficaces.

- dépend du contexte génétique et ethnique. Ce résultat indique la nécessité de caractériser la réponse immunitaire vaccinale dans les différents pays géographiques du monde afin de fournir des informations supplémentaires pour la conception future de vaccins.

Par ailleurs, la réponse Ab fonctionnelle détectée dans les prélèvements collectés au tout début de l'infection fournit de nouvelles données inspirantes pour le développement de nouveaux immunogènes pour les essais de vaccins futurs.

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Annex 2.Conformational Stabilization of Gp41-Mimetic Miniproteins Opens Up New Ways of Inhibiting HIV-1 Fusion..... 277

Abbreviation

Ab	Antibody
Ad	Adenovirus
ADCC	Antibody-dependent cellular cytotoxicity
ADCD	Antibody-dependent complement deposition
ADCP	Antibody-dependent cellular phagocytosis
ADCT	Antibody-dependent cellular trogocytosis
ADCVI	Antibody-dependent cell-mediated virus inhibition
AIDS	Acquired immunodeficiency syndrome
ALCAC	Canarypox
ART	Antiretroviral Therapy
ASC	Antibody secreting cells
AZT	Zidovudine
BCR	B-cell receptor
bnAb	Broadly neutralizing antibody
BP	Budding protein
CA	Capsid protein
cART	Combined antiretroviral therapy
CCR5	CC cytokine receptor 5
CD4	Cluster of differentiation 4
CD8	Cluster of differentiation 8
CDR3	Complementarity determining region 3
CH	Constant domain
CHR	C-terminal heptad repeat
CRF	Circulating recombinant form
CXCR4	CXC cytokine receptor 4
DAF	Decay-accelerating factor

DC	Dendritic cell
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
Env	Envelope
Fab	Fragment antigen binding (Fab) portion
Fc	Crystallizable fragment (Fc) portion
FcγR	Fcγ receptor
FDA	Food and Drug Administration
FP	Fusion peptide
FWPV	Fowlpox
gag	Group-specific antigen
GC	Germinal center
gp120	Viral surface glycoprotein 120
gp41	Viral surface glycoprotein 41
GPI	Glycosylphosphatidylinositol
HAART	Highly Active Antiretroviral Therapy
HCDR3	Heavy chain complementarity-determining region 3
HIV	Human Immunodeficiency virus
HIV-1	Human immunodeficiency virus type 1
HIV-2	Human immunodeficiency virus type 2
HLA	Human leukocyte antigen
HR	Heptad repeat
Ig	Immunoglobulin
IL	Interleukin
IM	Intramuscular
IN	Integrase
INF	Interferon

ITAM	Immunoreceptor tyrosine-based activation motif
ITIM	Immunoreceptor tyrosine-based inhibitory motif
iTreg	Induced Treg
LMQ	Neutral liposomes containing monophosphoryl lipid A (MPL) and Quillaja saponaria derived QS21 saponin
LPS	Lipopolysaccharide
LTR	Long Terminal Repeat
MA	Matrix protein
MAp	Matrix proteins
MCP	Membrane cofactor protein
MHC	Major histocompatibility complex
MoAb	Monoclonal antibody
MoNAb	Monoclonal neutralizing antibody
MPER	Membrane proximal external region
MPLA	Liposomes Containing Monophosphoryl Lipid A
mRNA	Messenger ribonucleic acid (mRNA)
MVA	Modified vaccinia virus Ankara
Nab	Neutralizing antibody
NC	Nucleocapsid protein
Nef	Negative regulatory factor
NHP	Non-human primat
NHR	N-terminal heptad repeat
NK	Natural killer cell
NKT	Natural killer T cells
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NPC	Nuclear pore complex
NRTI	Nucleoside/nucleotide reverse transcriptase inhibitor

nTreg	Natural Treg
OP	Osmotic pump
PC	Long-lived plasma cells compartments
PEP	Post-exposure prophylaxis
PI	Protease inhibitor
Pol	DNA polymerase
PR	Protease
PrEP	Pre-exposure prophylaxis
Rev	Regulator of expression of virion proteins
RNA	Ribonucleic acid (RNA)
RT	Reverse transcriptase
RT-PCR	Reverse transcription polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
sIgA	Secretory immunoglobulin A
SIV	Simian immunodeficiency virus
SIVcpz	Simian immunodeficiency virus in wild chimpanzees
SIVgor	Simian immunodeficiency virus in wild gorilla
SP1	Spacer peptide 1
SP2	Spacer peptide 2
T/F	Transmitted/founder
TasP	Treatment as prevention
Tat	HIV-trans-activator
Tfh	Helper follicular T lymphocytes
Tfr	Regulatory helper follicular T lymphocytes
TGF	Transforming growth factor
ThL	Helper T lymphocytes
TNF	Tumor necrosis factor

tRNA	Transfer ribonucleic acid
UNAIDS	Joint United Nations Program on HIV and AIDS
VH	Variable domain
Vif	Virion infectivity factor
Vpr	Viral protein R
Vpu	Viral protein U
VSV	Vesicular stomatitis virus
WHO	World Health Organization

1. Introduction

With the expansion of new technologies and the further understanding of virus pathologies, vaccines recently developed against pathogenic viruses saved millions of lives. For the most recent Sars-Cov-2 pandemic, which already killed more than 6 million people, Covid-19 vaccines show significant efficacy against hospitalization, severe disease and death rate.[1,2]

Despite this considerable progress in vaccine design, there are still some pathogens for which vaccines need to be developed.[3–5] Among them, vaccines against dengue or respiratory syncytial virus are under investigation. Remarkably, for human Immunodeficiency virus (HIV), vaccine have been examined since it's discovered in the 1980s.[6] For more than 30 years, researchers have dealt with developing a protective vaccine to fight against HIV pandemic.

1.1. Human Immunodeficiency Virus (HIV)

1.1.1. General information

HIV belongs to the genus Lentivirus (a group of retroviruses) from the Retroviridae family Retroviruses are enveloped viruses that replicate from a positive-sense single-stranded RNA diploid genome. Their name comes from the enzyme they possess, reverse transcriptase, which allows the reverse transcription of viral RNA into DNA.[7,8]

Retroviruses show common morphological, structural, and functional characteristics. They possess gag, pol and env genes which respectively lead to structural, enzymatic and envelope proteins.[7] HIV belongs to the Lentivirus genus: the name of the genus is from Latin (lenti = slow), and the viruses from this genus are characterized by needing a long incubation period before diseases break out due to their cytopathogenicity. [9]

The long incubation period of HIV viruses allowed their spreading in the early 80s. At that time, there were no screening tests available. The hosts did not notice that they were infected before the syndromes appeared after years of infection.

1.1.1.1. Discover and Origin

HIV infect human and AIDS over time.[10,11] In the middle of 1981, one report described Pneumocystis pneumonia in previously healthy homosexual men in Los Angeles. This document has been identified as the first official document reporting the acquired

immunodeficiency syndrome (AIDS) pandemic. [12] Sooner, Kaposi's sarcoma, candidiasis and tuberculosis within isolated clusters were found to be potential disease outcomes with long tern HIV-infection the United States. [13,14]

Shortly after the first formal report of AIDS was published, the disease was detected in the heterosexual population in Central and East Africa, suggesting the African HIV pandemic was older.[12,15,16] Scientists started searching for the origin of HIV viruses. They found viruses in the blood and tissue samples previously collected in 1959-1960, demonstrating that HIV was hidden in history. [17,18] Two types of HIV viruses were found to cause AIDS at the advanced stage of infection: human immunodeficiency virus type 1 (HIV-1) and human immunodeficiency virus type 2 (HIV-2). According to sequencing analysis, HIV-1 and HIV-2 were predicted to enter the human population around the 1920s and 1940s, respectively. Then they rapid grow exponentially around the 1960s to 1970s. [19–21]

1.1.1.2. Classification and phylogenetic evolution

HIV-1 is a zoonotic infection of staggering proportions and social impact. The groups M and N emerged from the chimpanzee simian immunodeficiency virus (SIV), and groups P originate from the SIV-infected gorillas.[21–24] HIV-2, which is less prevalent, comes from the inter-species transmission between SIV-infected mangabey and is divided into eight groups (A-H). [20,21,25]

Phylogenetic studies of different retroviruses have shown the tremendous genetic diversity of HIV-1, which constitutes one of the major obstacles to its eradication. [19,22,26] (Figure.1) As what is mentioned above, HIV-1 can be classified into four groups[22,24,27] :

- Group M (major) : This group is the oldest lineage and responsible for more than 97% cases in humans. [28] This group is divided into nine subtypes (A, B, C, D, F, G, H, J, K) and all of these subtypes are genetically distinct. What's more, there are sub-subgroups in subgroups A and F (A1, A2, A3, A4, F1 and F2).[26] Recombinant form appears (circulating recombinant form (CRF)) when a patient is co-infected with several virus subtypes. Some of these recombinant forms take the lead and are responsible for around 18% of infections worldwide. [21,29]

- Group N (non-M, non-O): It's a rare lineage, and the circulation of the variants of this group is only detected in Cameroon.[24]

- Group O (Outlier): group O is rare and mainly spreading in West Central Africa. [27,30,31]

- Group P: As the most recent discovered group, group P is closely related to the gorilla simian immunodeficiency virus (SIVgor) and shows no evidence of recombination with other HIV-1 lineages.[32]

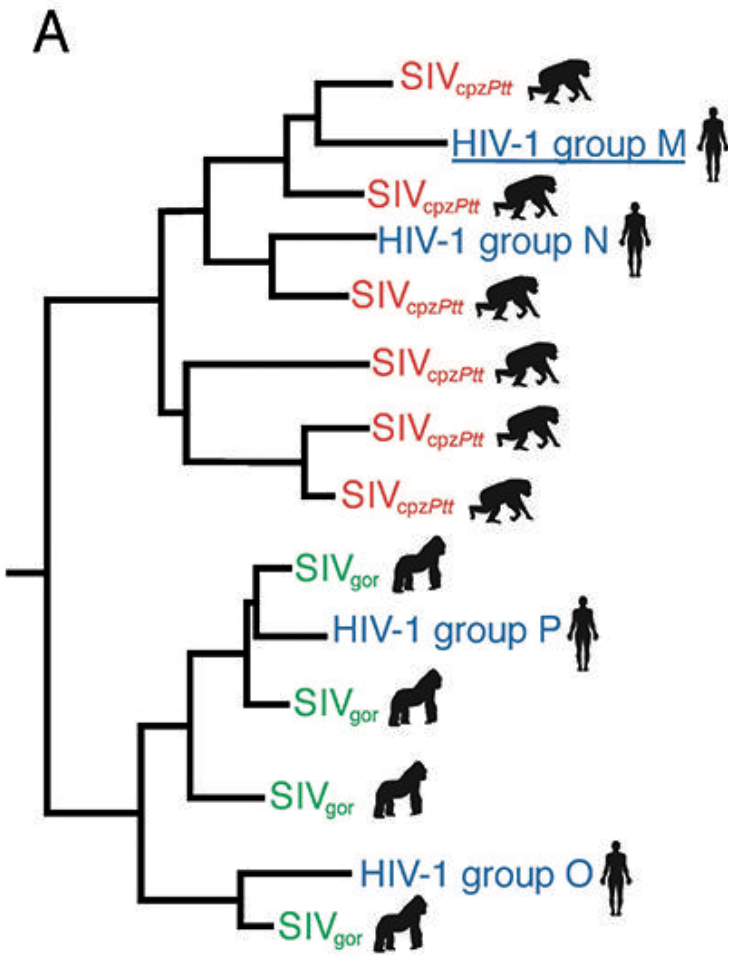

Figure 1. Phylogenetic relationships between the HIV-1, SIVcpz, and SIVgor lineages. Phylogenetic tree of HIV-1 and related simian immunodeficiency viruses (SIV) sequences reveals the multiple virus cross-species transmission events that originated HIV-1 groups. [33]

The diversity of HIV-1 gives it the advantage of spreading widely and makes it more difficult to be detected. It also points out the importance of monitoring humans for primate lentiviruses and studying the viral and host factors that govern cross-species infection and onward transmission.[21,31] I will mainly focus on HIV-1, further shortened as HIV for simplicity.

1.1.1.3. Epidemiological data

The distribution of HIV-1 group M subtypes varies according to geographical location. Subtype A is distributed mainly in Eastern Europe and Central Asia, while subtype B is the pre-dominant subtype in western Europe and America. Subtype C is mainly distributed in East Asia. Africa shows the highest HIV-1 diversity with subtypes A and D in eastern Africa, C in southern Africa, A, G, CRF02_AG, and CRF06_cpx in western Africa, and B and CRF02_AG in northern Africa (figure 2). [34,35]

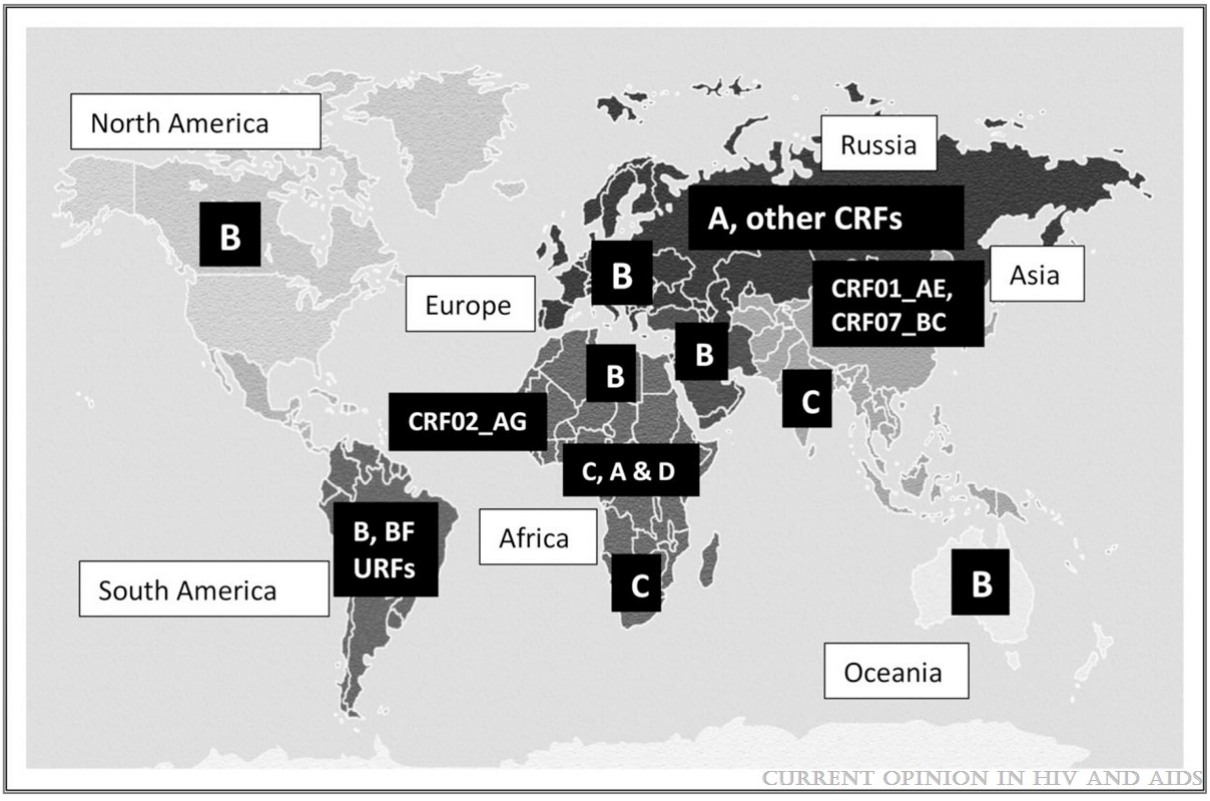

Figure 2. Global distribution of major HIV subtypes. Map showing the localization of the major HIV subtypes and circulating recombinant forms (Bbosa et al. 2019).[35]

According to data from the World Health Organization (WHO) and the Joint United Nations Program on HIV and AIDS (UNAIDS), 38.4 million people were living with HIV globally in 2021,

and two third of them are living in African regions.[36] (Figure 3A). During 2021, 1.5 million people were newly infected with HIV (Figure 3B) and 650,000 AIDS-related deaths were counted (Figure 3C).

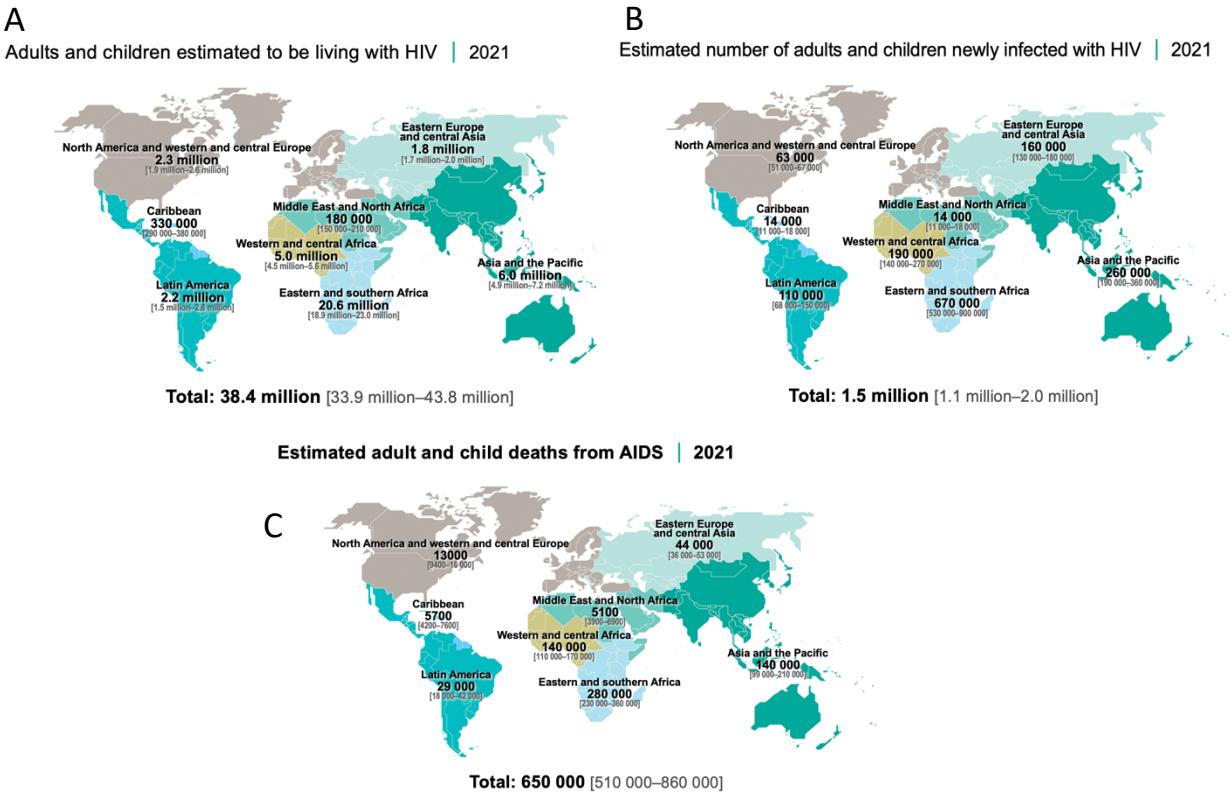

Figure 3. 2021 World HIV situation (UNAIDS, 2021).[37]

Estimated number of adults and children

- *A) living with HIV in 2021.*
- *B) newly infected with HIV in 2021*
- *C) deceased due to HIV infection*

Each region is presented by different color.

Compared with previous years' data, the number of new infection and death are decreasing. However, the limitation of medical resources in Africa makes the HIV pandemic harsher in these regions of the world. Moreover, the diversity of HIV-1 and the number of infection are highest in Africa (figure 3).

1.1.2. Structure and replication of HIV-1

1.1.2.1. HIV-1 genome and structure

HIV-1 comprises two strands of noncovalently linked, unspliced and positive-sense single strain-RNA.[38] The nine genes of the HIV-1 genome can be classified into three parts: viral structural proteins (gag, pol, env), essential regulatory elements (tat, rev) and auxiliary regulatory proteins (nef, vpr, vif, vpu). All these genes are surrounded by non-coding LTR (Long Terminal Repeat) sequences, which are essential for the replication of the virus.[38–41] (figure 4).

- Viral structural proteins:
    - Gag (group-specific antigen): It codes for CA (capsid protein, P24), MA (matrix protein, p17), NC (nucleocapsid protein, p7), p6 and two small peptides SP1 and SP2 (Spacer peptide 1 and 2).
    - Pol: encodes three enzymes: integrase (IN), reverse transcriptase (RT) and protease (PR).
    - Env (envelope): the env encodes the envelope proteins, gp160. After gp160 being cleaved by host protein and post-translational processing produces, it can turn to surface protein (gp120) and transmembrane (gp41) glycoproteins.
- Essential regulatory elements:
    - Tat (HIV-trans-activator): it is in charge of regulating the reverse transcription of viral genome RNA, ensuring efficient synthesis of viral mRNAs and regulating the release of virions from infected cells.
    - Rev (regulator of expression of virion proteins): Rev is important for the synthesis of major viral proteins.
- Auxiliary regulatory proteins
    - Nef (negative regulatory factor): N-terminal myristoylated membrane-associated phosphoprotein. Nef, historically called negative factor, favor

infection by rerouting a variety of cell surface proteins to disrupt host immunity and promote the viral replication cycle.

- vpr (lentivirus protein R): It's a virion-associated, nucleocytoplasmic shuttling regulatory protein.
- vif: A conservative phosphoprotein that can assist viral infection.
- vpu: A class I oligomeric integral membrane phosphoprotein that is involved in CD4 degradation and in the release of virions from infected cells. Recently, it is proposed as inhibitor of antibody-dependent cellular cytotoxicity (ADCC).[42,43]

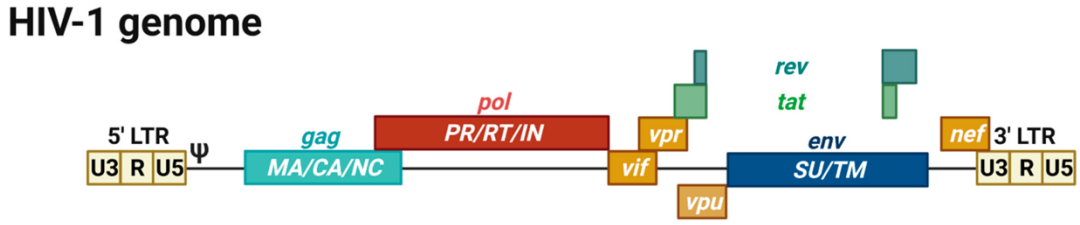

Figure 4. HIV-1 genome. [41]

1.1.2.2. HIV-1 envelope proteins and virus particle

The env gene encodes the glycosylated polyprotein gp160 (obtained by translating mono-spliced viral messenger RNA).[44] It is synthesized at the level of the endoplasmic reticulum. Then it reaches the Golgi apparatus, cleaved by a cellular protease to produce surface (gp120) and transmembrane (gp41) glycoproteins. [41,45,46]

1.1.2.2.1. Gp120

Gp120 is a glycoprotein that is exposed on the surface of the HIV envelope, and its molecular weight, 120kDa, names it[46]. It has five conserved (C1 to C5) and five highly variables (V1 to V5) regions that can be targeted by antibodies. [47,48] Gp120 is anchored to the viral membrane and variable sequences form a structure in loops exposed and linked together by disulfide bridges. The conserved regions form discontinuous structures interacting with the ectodomain of

gp41.[49] These two proteins, both combined in trimers, form the envelope spike that can interact with the receptor CD4 and mediate the attachment and viral entry into the host cell.[50–52] (figure 5).

Binding to CD4 induces conformational changes, causing displacement of the V1/V2 loops and resulting the exposure of third variable (V3) loop of the gp120, in order to bind the CCR5 or CXCR4 chemokine co-receptors. [53,54]

The variable regions of gp120 are highly exposed, immunogenic and targeted by neutralizing antibodies.[48] However, as they are highly variable, virus rapidly escape the humoral response. Moreover, the most conserved epitopes (or antigenic determinants) as the CD4 binding site are masked in pockets and, therefore, not easily accessible for the immune response. A few infected patients are able to develop a humoral immune response targeting these conserved masked epitopes. The strategies used to develop these neutralizing antibodies will be described in chapter 3.

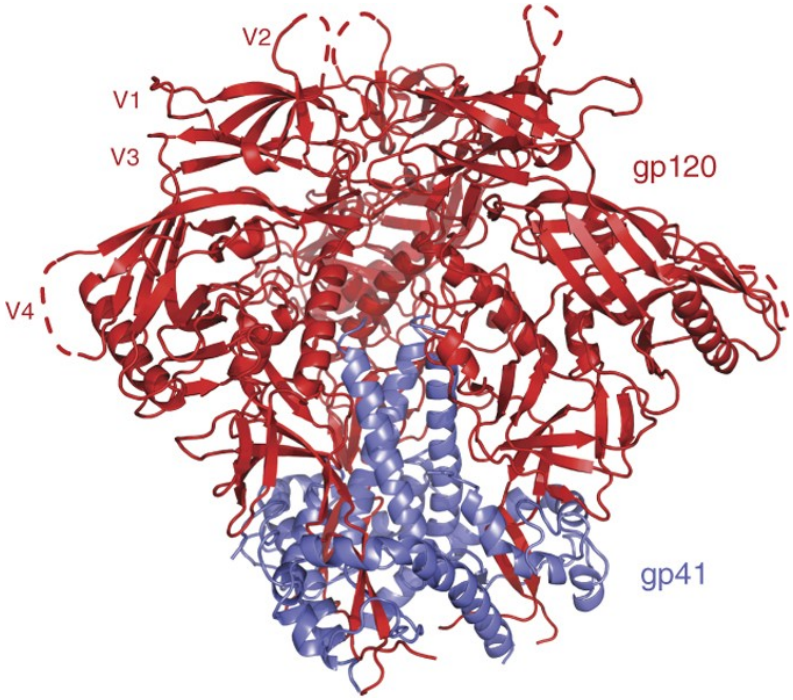

Figure 5. Structure of the HIV-1 Env trimer.[55]

1.1.2.2.2. Gp41

Gp41 is a highly glycosylated transmembrane glycoprotein organized into three major domains: the extracellular, transmembrane, and cytoplasmic domains.[56] The N-terminus of gp41 is a fusion peptide necessary for membrane fusion. It is followed by two helical heptad repeat (HR) regions : N-terminal heptad repeat (NHR) and C-terminal heptad repeat (CHR).[57–59] The CHR is followed sequentially by a membrane proximal external region (MPER). This region has been proposed to be a very promising target for drug and immunogen development. [60–62]

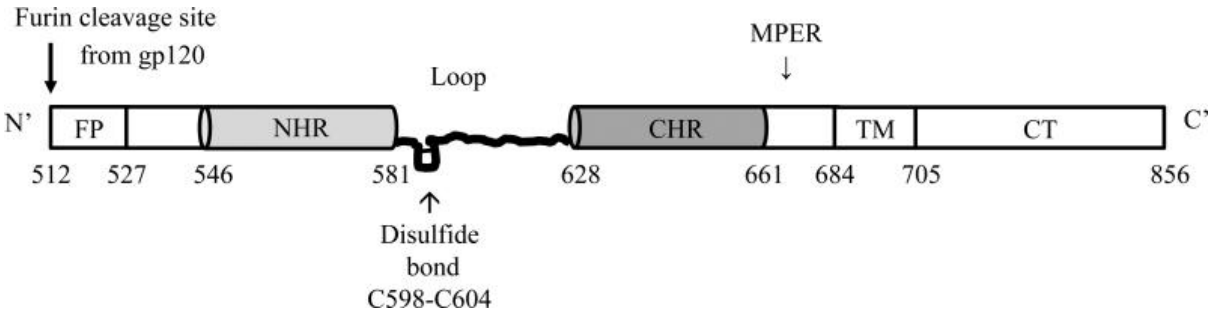

Figure 6. The primary structure of gp41.[62]

1.1.2.2.3. Virus particle

The mature HIV-1 virus particle is spherical and 100-120 nm in diameter. It comes from the assembly and maturation of the polyproteins Pr55Gag, Pr160Gag-Pol and Envgp160. (Figure 7) Its envelope is a lipid bilayer originating from the host cell's plasma membrane. The particle contains the viral Env glycoproteins in the form of polyproteins exposed on the surface of the viral particle (gp120) or anchored in the membrane (gp41). These envelope proteins have specific so-called “fusogenic” properties, allowing the virus to fuse to the target cell's plasma membrane.[7]

The inner side of the envelope is lined by matrix proteins (MAp17), which are closely associated with the lipid envelope by their myristoylated N-terminal end. The capsid proteins (CAp24) form the retroviral core. Assembly of the p24 protein into hexamers and pentamers forms a cone-shaped capsid. This capsid contains viral RNA in dimeric form and is associated with nucleocapsid proteins (NCp7), allowing protection against nucleases.[7,41]

It also contains the viral enzymes (PR, IN and RT), the auxiliary proteins Vpr, Vif and Nef, and the cell transfer RNA (tRNA), serving as a primer for reverse transcription. The capsid also contains cellular proteins and encapsidated cellular nucleic acids.[41,63,64]

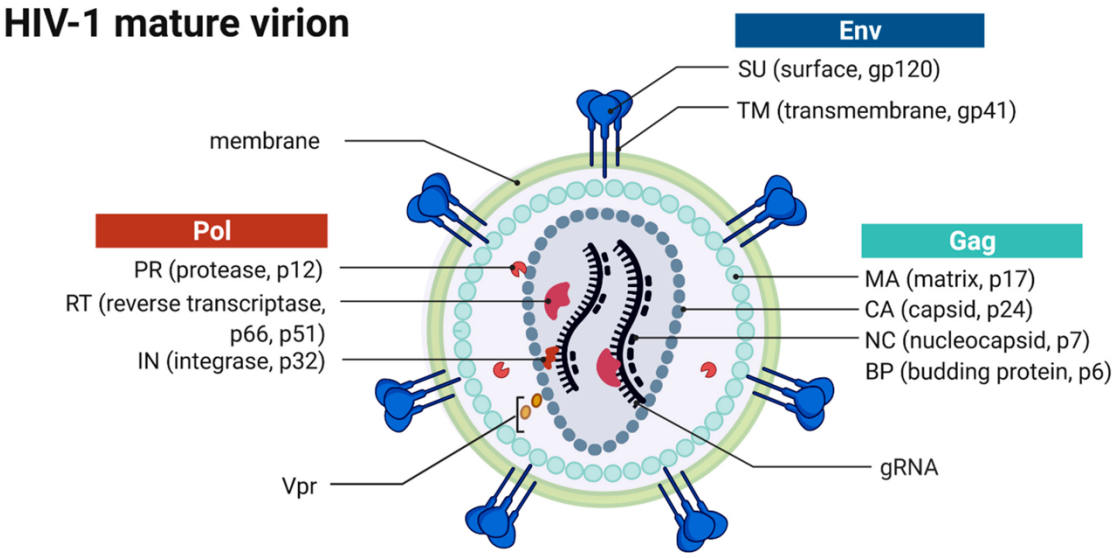

Figure 7. HIV-1 virion structure. [41]

1.1.2.3. HIV-1 life cycle

HIV spread mostly thought mucosa contact to a naïve host, by transmission of a single variant, called the Transmitted/Founder (T/F) virus selected from the multiple quasi-species present in the chronically infected donor .[67–69] The precise characteristics of the selected T/F virus is not well defined yet. The virus infects a target cell and spread to other target cells in the average of 1.2 days.[65,66]

The viral replication cycle (Figure 8) is divided into an early and a late phase. The early phase (Figure 8 on the left part) includes the steps of interactions of surface proteins of the viral particle with cell receptors, reverse transcription of viral RNA into proviral DNA and integration of proviral DNA into the genome of the host cell. [53,70,71] The late phase (Figure 8 on the right part) concerns the transcription and expression of the genome, as well as the budding and maturation of new viral particles.[72]

The first steps in the viral replication cycle are recognition, fusion, and entry of the virus into target cells. This interaction changes gp120's conformation and binds it to a second receptor

next to CD4: the co-receptor, CXCR4 and CCR5. Besides fusion, HIV-1 can enter cells by a the cell-to-cell transmission pathway via Env-induced, actin-dependent viral synapse.[73]

The cone shaped viral capsids move along the microtubules of the cell towards the nuclear pore complex (NPC), then enter the nuclear.[74,75] On contrary to what was previously described, this reverse transcription step occurs in the nucleus.[75,76] As the capsid enters the host cell nucleus, uncoating and reverse transcription are completed.[74,77] The viral genome in its RNA form is associated with reverse transcriptase (RT) and other proteins (viral and cellular) within a reverse transcription complex. Viral RNA is back-transcribed into double-stranded DNA.[78,79] The reverse transcriptase can cause errors frequently, which leads to the origin of the genetic variability of HIV and contributes to resistance to antiretroviral treatments.[80]

Integrase is the viral protein responsible and sufficient to catalyze the integration reaction. It cleaves a few nucleotides at both 3' ends of the DNA and integrates the linear viral DNA into the host cell genome. The integrated viral DNA is called provirus.[81,82] The provirus can remain silent through different mechanisms for several years, creating the latent phase.[83]

During productive infection, proviral DNA is transcribed into messenger RNA (mRNA). The viral proteins Tat and Nef hijack the cellular transcriptional machinery to synthesize mRNA from the integrated proviral DNA.[79,84]

Viral mRNAs are translated into precursors in different cellular compartments. Gag, Gag-Pol, Vif, Vpr, Nef, Tat and Rev mRNAs are translated into cytosolic polysomes, then mediated by the interaction of Rev-bound viral RNAs with the nuclear transport receptor, serving as a binding molecule to the nuclear pore complex (NPC).[85]

The new viral particles are assembled from two copies of viral genomic RNA, viral proteins (Gag, Gag-Pol, Env, Vif, Vpr and Nef) and cellular cofactors. Viral particles assemble and escape from the cell by budding.[72,85]

The final stage of the cycle is virion maturation. The maturation is carried out by the viral protease cleaving the Gag and Gag-Pol precursors sequentially into structural and non-structural proteins; then, the viral particles achieve their final structure to become infectious.[85,86]

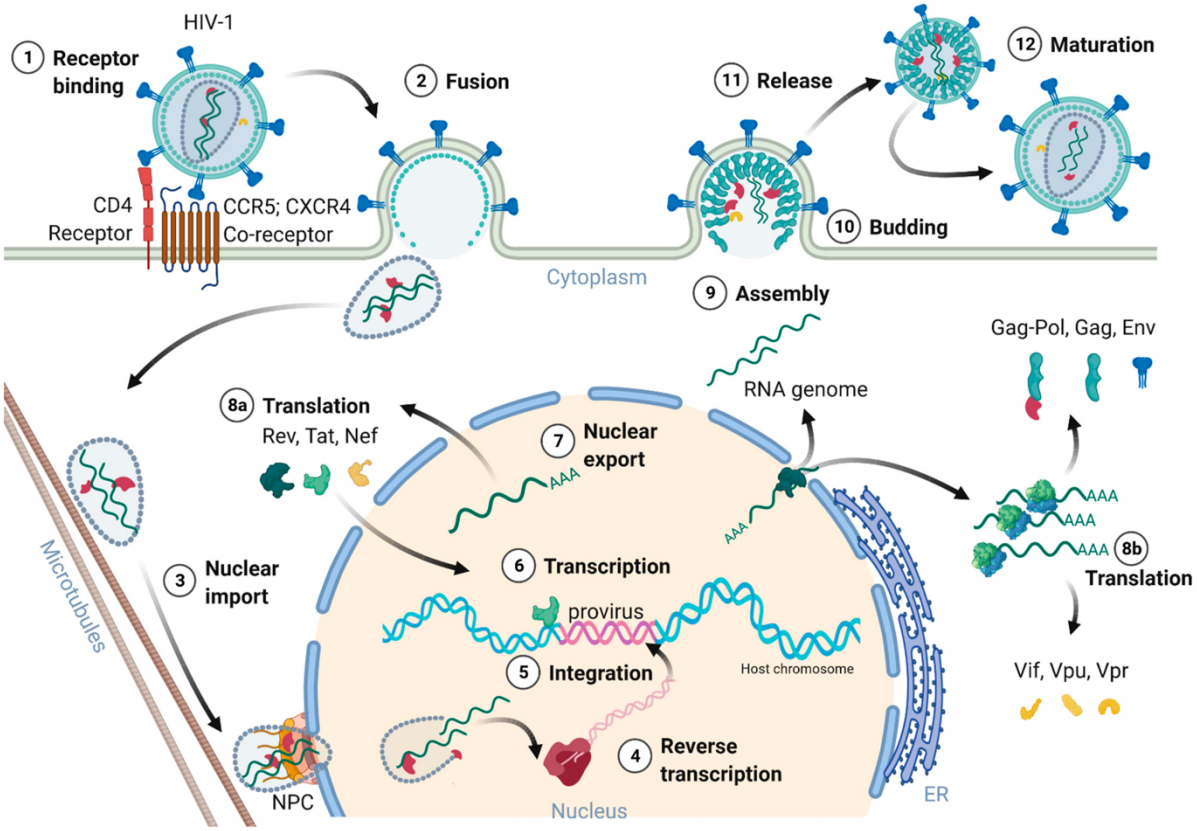

Figure 8. The Early and late phases of productive HIV infection. The early phase comprises attachment, viral fusion, reverse transcription, uncoating and proviral integration (left). The late phase includes viral replication, assembly, budding and HIV-1 maturation (right). [41]

1.1.2.4. HIV-1 target cells and reservoir

Different cell types present through the body express both the CD4 receptor and a co-receptor but not all are targeted by HIV[7]. The main HIV-1 target cells are CD4+ T lymphocytes (CD4+, CCR5+, CXCR4+). Monocytes/macrophages (CD4+, CCR5+), dendritic cells (CD4+, CCR5+, CXCR4+), microglial cells (CD4+, CCR5+) and astrocytes (CCR5+, CXCR4+) can also be infected by HIV-1.[87–89] NK cell (CD4+, CCR5+, CXCR4+) were described to replicate HIV by one team but this replication could never be confirmed.[90]

CD4 T cells are the principal HIV targets. When activated, their cellular machinery will be deviated to allow HIV replication. A small set of these cells which have integrated the virus into their genome establish latent infection and become HIV-1 cell reservoirs. These latent reservoirs will allows HIV to persist despite immune responses and antiretroviral therapy.[91,92]

The anatomical compartments identified as HIV-1 reservoirs are mainly the lymphoid tissue associated with the digestive tract and the lymph nodes. (Figure 9) The other compartments are blood, the central neuron system, bone marrow, the genital tract, etc.[91–93]

The diversity of cell types and their multiple anatomical locations, ranging from the genital tract to the central nervous system, create many sanctuaries that can harbor HIV-1.[91,94]

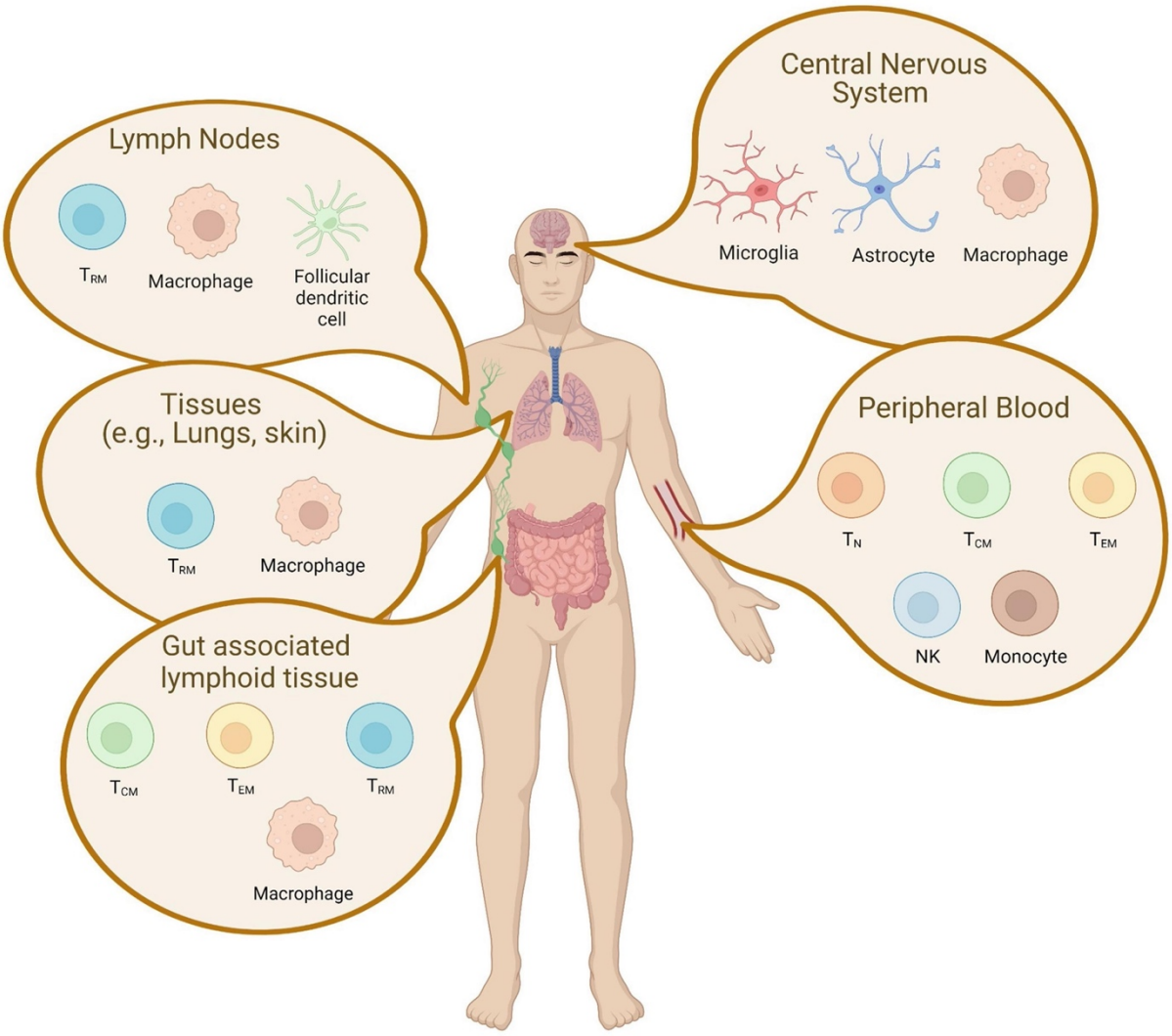

Figure 9. Latent cellular reservoir cells in tissue. A schematic representation of latently infected cells in tissues is depicted. The reservoirs are established in unique cell types and are localized across different tissues. CD4+ memory T cell subsets are found in the peripheral blood, the lymphoid tissue, gut-associated lymphoid tissue, and the central nervous system.[92]

1.1.3. Biological and clinical characteristics of HIV-1

1.1.3.1. Modes of transmission

HIV-1 is present in most of the body's biological fluids (blood, semen, seminal fluid, vaginal secretions, and breast milk) in different concentrations. Therefore, virus transmission occurs through these biological fluids by unprotected sex, contaminated blood transfusion, hypodermic needles, or mother to child transmission during pregnancy, delivery or breastfeeding.[95]

There are three main models of HIV-1 transmission:

- Unprotected sex (major): The virus enters through vaginal, oral, or anal mucous membranes. The risk of infection increases sharply if these mucous membranes have lesions and/or if there is the presence of other sexually transmitted diseases. Using condoms remains the best way to limit infection by this type of transmission.

- Contaminated blood and sharing needles: This route of transmission of HIV-1 was demonstrated soon after discovering the virus. Nowadays, this mode of transmission persists mainly among drug addicts (intravenous drug injection) or medical personnel who are injured by contaminated objects.

- Mother to child (vertical transmission): It occurs mainly during childbirth but also during pregnancy or breastfeeding. Disproportionately affects low- and middle-income countries, in particular the countries of Southern Africa.[37,96]

HIV-1 infection typically results from the transmission of a single viral variant, the transmitted/founder (T/F) virus.[97]The T/F virus selected is transmitted to a new host.[98] Virus evolve by numerous mutations and immune selections to form quasispecies. The virus directly isolated form the patient is called primary isolate[99]

1.1.3.2. Pathophysiology

HIV-1 infection leading to AIDS develops in three phases (Figure 10):

- Primary infection (acute viremia phase): Couple days to weeks post-exposure, HIV-1 replicate actively, the number of helper CD4+ T cells gradually decreases, and the virus spreads through the body. It takes adaptive immune systems six to eight weeks to develop an immune defense leading to reduced viral load. The decreased viral load is concomitant with a partial recovery of the number of CD4+ T lymphocytes. At this stage, patients might develop opportunistic infection as influenza or mononucleosis-like illness as fever, lymphadenopathy, pharyngitis, rash, myalgia etc. [100,101] These symptoms are nonspecific and often not recognized as signs of HIV infection. (Figure 11)

- Asymptomatic phase (latency phase): Patients have no symptoms at this stage, which can last more than ten years. During this period, HIV-1 virus load is relatively maintained, although numerous variants are generated and the total number CD4+ T lymphocytes decreases. Besides, the virus can remain latent in reservoir cells (memory CD4+ T lymphocytes or macrophages).[102–104]

- The symptomatic phase (AIDS): In this phase, the host immune system is no more able to contain HIV viral load due to the progressive decline of CD4 T lymphocytes (CD4+ T cell count below 200 cells per µL). A sharp increase of viral load pushes the immune system to collapse. The symptoms in this phase are mostly opportunistic infections caused by bacteria, viruses, fungi and parasites.[105] These infections like Pneumocystis pneumonia and Kaposi's sarcoma do not usually develop in individuals with healthy immune systems.[13,106] It might also lead to variety of neuropsychiatric sequelae.[107] The occurrence of opportunistic infections associated with the loss of immune system (AIDS) leads to death.[108]

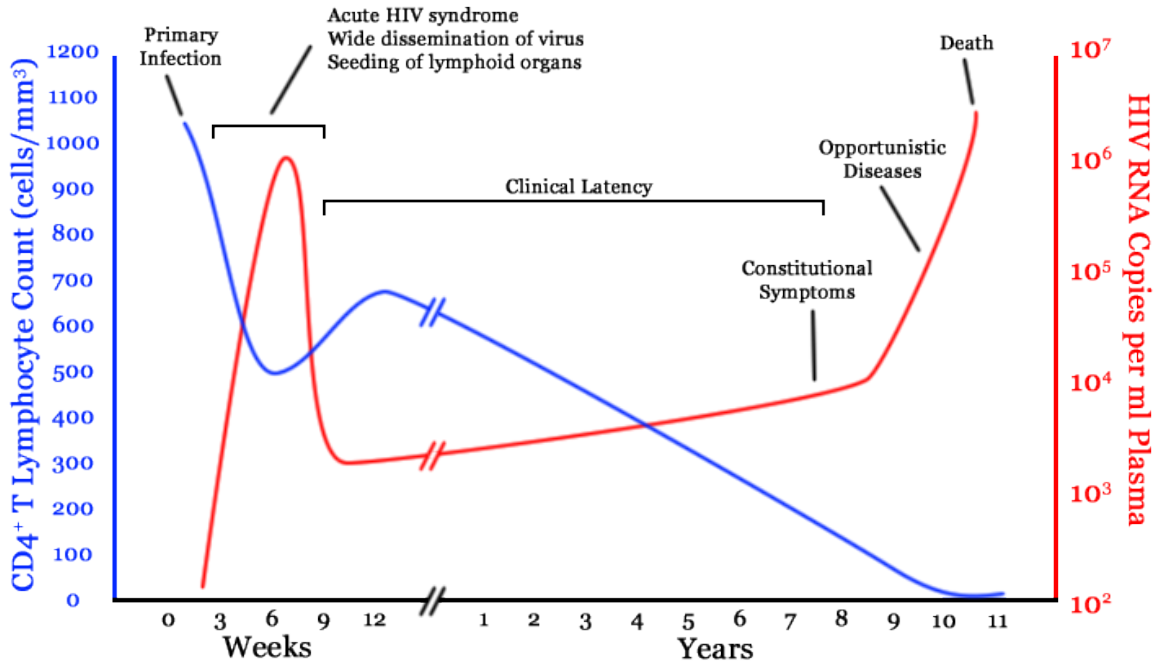

Figure 10. Evolution of the natural HIV-1 infection in the absence of therapy. The number of CD4+ T lymphocytes decreases (blue curve), unlike the viral load (RNA copies/mL of plasma) which increases (red curve) over time. [109]

1.2. HIV infected patient and the immune responses

1.2.1. Immune response to HIV-1 infection

HIV- infected individuals exhibit solid adaptive cellular responses (activity of CD4+ and CD8+ T cells) and humoral immune responses (production and activity of antibodies).

1.2.1.1. Adaptive cellular response

1.2.1.1.1. $CD4^+$ T cells

CD4+ T lymphocytes (helper T lymphocytes) are the main targets of HIV-1. They express the CD4 on their surface, which enables them to recognize the molecules of the major histocompatibility complex (MHC) or human leukocyte antigen (HLA) class II, present on the surface of cells presenting the antigens.[110–112]

These cells participate in the fight against infection through their central function of stimulating B lymphocytes and CD8+ T lymphocytes, and through their direct antiviral activity thanks to the production of cytokines (interferon (IFN)-γ and β- chemokines).[110]

Helper T lymphocytes (ThL) can be divided into LTh effectors and regulatory LThs.[113,114] (Figure 12) The ThL (Th1, supporting the cellular response; Th2, involved in the humoral response; Th17, Th9, Th22 and helper follicular T lymphocytes (Tfh)) participates in immune responses, and the latter (natural Treg (nTreg), induced Treg (iTreg) and regulatory Tfh (Tfr)) can control the immune responses.[112,114,115]

Notably, the Tfhs have emerged as the key cell type required for the formation of Germinal Centers (GCs) and the generation of long-lived serological memory. [112,116,117] They interact with B cells in secondary lymphoid organs, the organs where antigens are presented for the initiation of the adaptive immune response. This interaction stimulates B lymphocytes differentiation into plasmocytes producing high-affinity antibodies. Pathogen specific Abs are induced to constitute a practical and durable barrier against infection.[112]

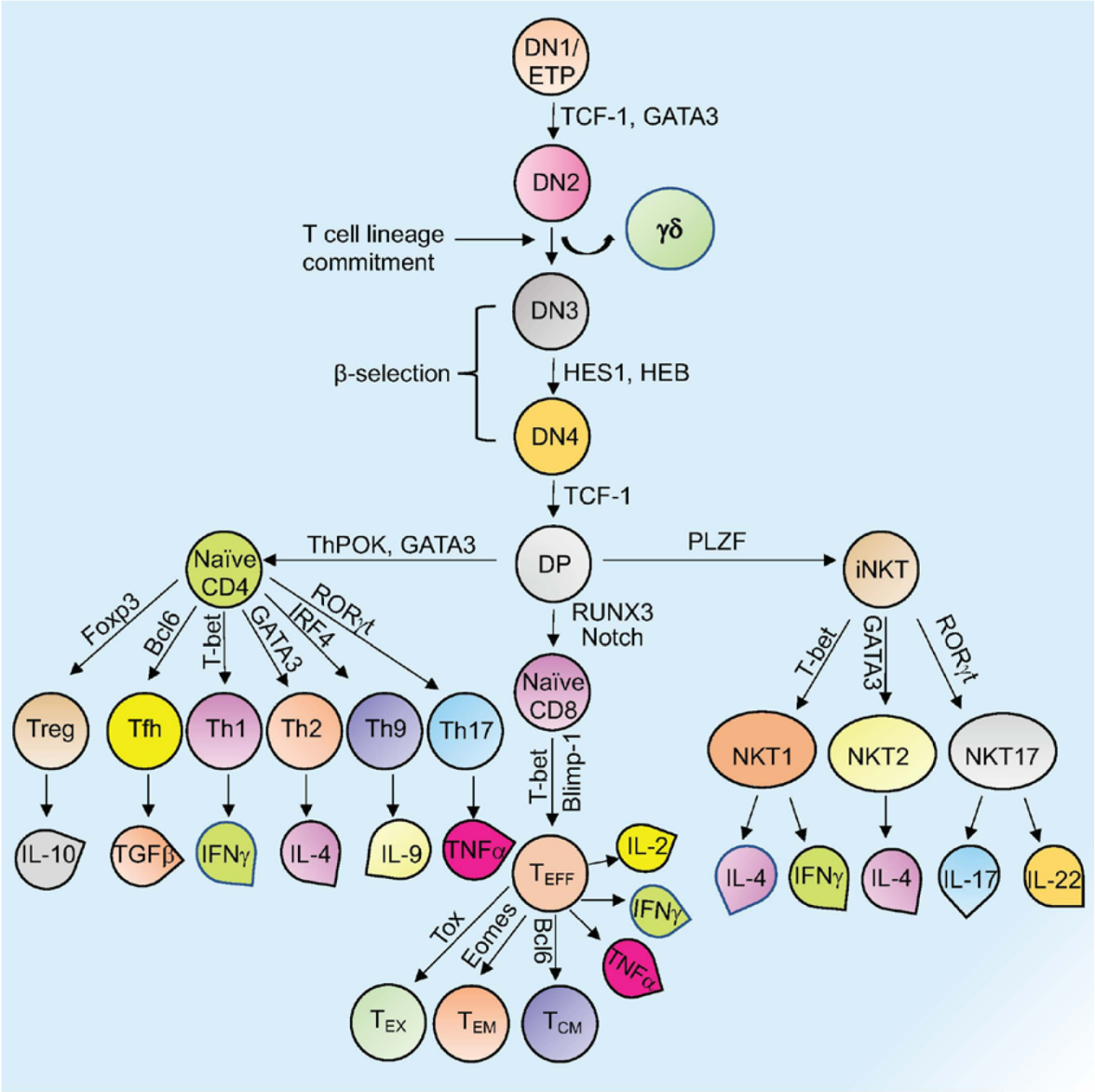

Figure 11. Overview of T cell development and differentiation. Following a series of DN (1–4) stages, DP cells develop into naïve CD4+, naïve CD8+, or natural killer T cells (NKT). Several transcription factors regulate this process. Different T cells secrete various cytokines to exert their activity. Signature transcription factors and cytokines designated to different cell types are shown. (Tex= exhausted T cells; TEM = T effector memory T cells; TCM= T central memory T cells; TEFF = effector T cells; Th= T helper T cells; Tfh= T follicular helper T cells; Treg= regulatory T cells).[113]

1.2.1.1.2. CD8⁺ T cells

CD8+ T lymphocytes (cytotoxic T lymphocytes) are a critical subpopulation of MHC class I-restricted T cells and are adaptive immunity mediators. The expression of CD8 on the cell

surface allows these cells to recognize class I MHC molecules present on all nucleated cells in the body.[111]

Acute viremia is associated with the activation of CD8+ T cells, while the number of CD4+ T lymphocytes decreases sharply. In the acute phase, CD8+ T cell responses can efficiently suppress viral replication via directly destroying HIV-infected cells or secreting cytokines (IFN-γ, tumor necrosis factor (TNF)-α, IL-2).[111,118,119]

1.2.1.2. Humoral response

The specific humoral response is characterized by the production of HIV-1 specific antibodies following the activation of B cells and their differentiation into antibody-secreting plasma cells.[120]

1.2.1.2.1. B cells

B lymphocytes can be divided into five populations:[121,122]

1)Immature/transitional B cells: Precursor to naïve mature B cells.

2)Naïve mature B cells: Precursor to GC, memory and antibody-secreting cells.

3)Germinal center (GC) B cells: After receiving T cells help in secondary lymphoid tissue follicles, activated B cells can become germinal center (GC) B cells. These cells can proliferate and class-switch the B-cell receptor (BCR) constant region from IgM/IgD to IgG, IgA, or IgE.[123]

4)Memory B cells. These long-lived quiescent cells can quickly be mobilized during a second encounter with the antigen for which they have been selected. They are essential for maintaining long-term humoral immunity.[124]

5)Antibody-secreting cells:

a) Plasma cells: Differentiating in the bone marrow and have a long lifespan.

b) Plasmablasts: Short-lived plasma cells that secrete large amounts of antibodies into secondary lymphoid organs and the blood.[125]

HIV infection lead to imbalanced B-cell perturbations, that may impact on HIV-specific Ab production..[124,126,127]

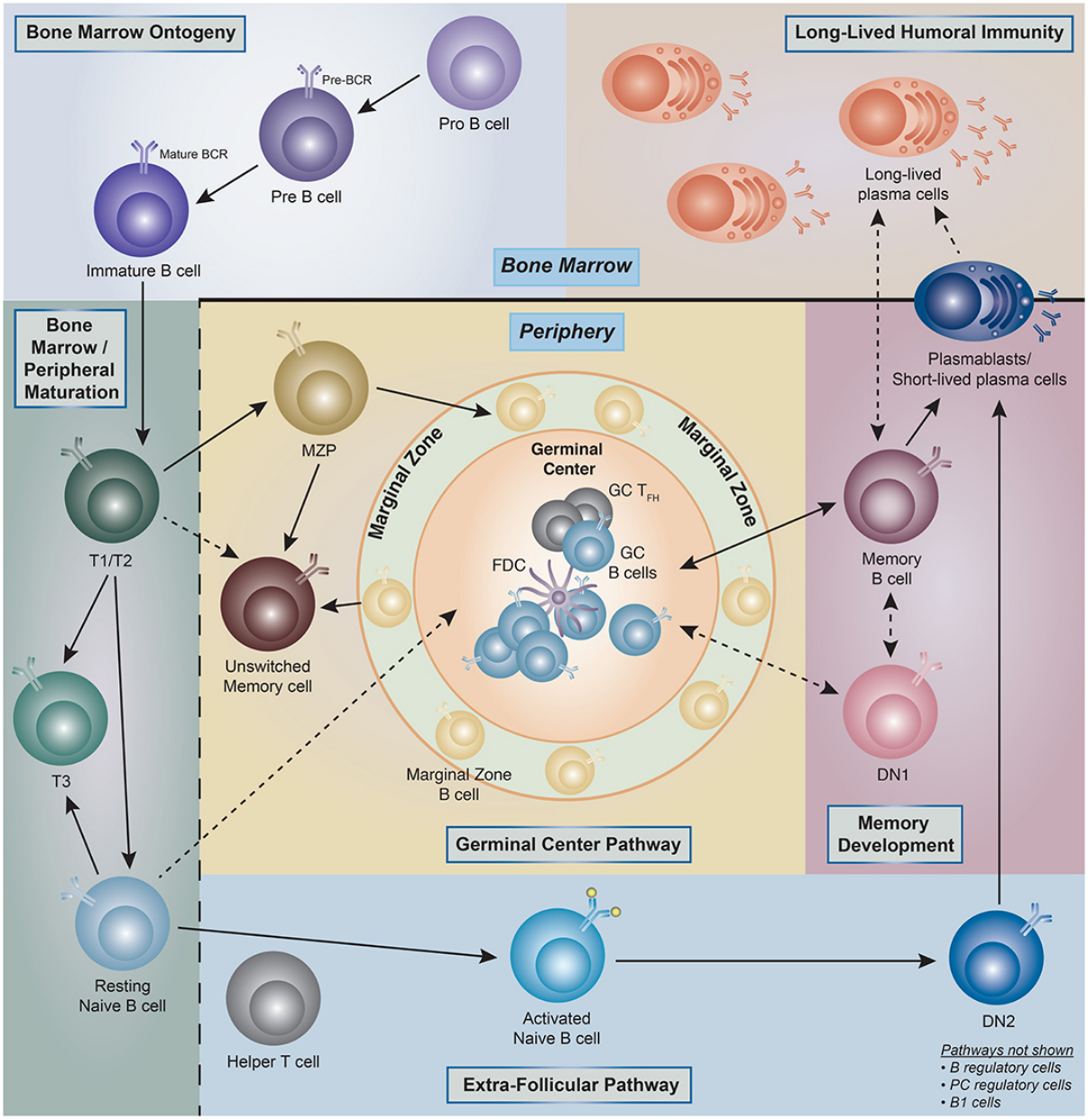

Figure 12. Human B cell ontogeny. Illustration of the current understanding of B cell ontogeny in human B cells from late bone marrow (pro- and pre-B cells) through peripheral activation pathways, and into antibody secreting cells (ASC)/long-lived plasma cells compartments (PC).[128]

1.2.1.2.2. Kinetics of appearance of HIV-1 specific antibodies in vivo

The initial humoral responses directed against HIV-1 can be detected approximately one week after infection. These antibodies are first directed against the fusion glycoprotein gp41, then against the envelope glycoprotein gp120 (around four weeks post-infection). [129,130]

The first autologous neutralizing activities, the antibody binds to the viral particle and makes it no longer infectious, appear several months post-infection. These so-called autologous Abs recognize the strains transmitted before, during the first events of the infection: the transmitted/founder (T/F) viruses.[129,131–133] This lag of neutralizing antibodies production makes them rarely target the contemporaneous viruses.[134] Virus have time to mutate. As a result, the new viral particles produced are able to escape autologous antibodies produced. [131,134]

Two to four years post-infection, neutralizing antibodies directed against different viral strains (so called cross-neutralizing Abs) can be detected However, only 10 to 30% of HIV-1 infected individuals develop this type of Abs.[135,136] An even smaller subset (1-10%) termed elite neutralizers produce broadly neutralizing Abs (bnAbs) which efficiently neutralize the majority of circulating primary isolates. [135,137,138] These bnAbs result from a long immune response maturation. They need repeating contacts with the antigen, and are characterized by numerous somatic mutations and a long heavy chain HCDR3 (heavy chain complementarity-determining region 3).[136,139,140] The long maturation required for acquiring broad-spectrum NAbs is an obstacle for the development of a preventive vaccine which seeks to induce this type of response.[140]

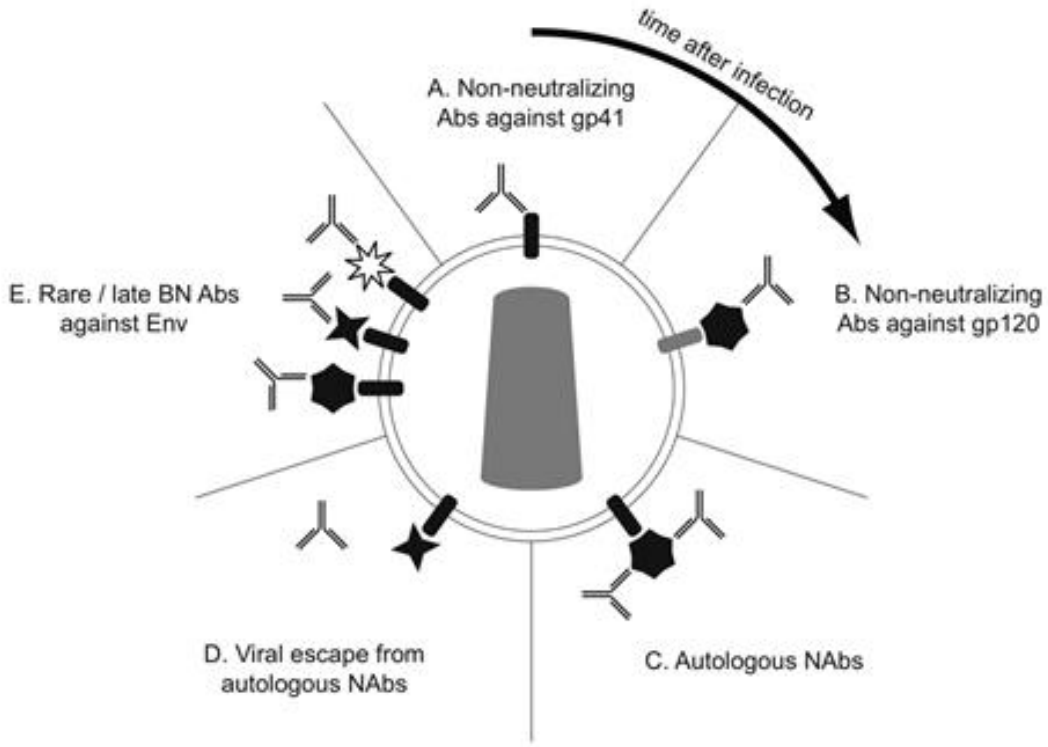

Figure 13. The antibody response to human immunodeficiency virus type 1 (HIV-1) occurs in a clockwise direction starting at the top A*, Initial antibody response to HIV-1 is nonneutralizing and directed at gp41.* B*, Nonneutralizing antibodies directed against gp120 arise soon thereafter.* C*, after a delay of weeks to months, autologous neutralizing antibodies (NAbs) arise that apply selection pressure on the virus.* D*, Viral mutation results in neutralization escape by HIV-1, represented here by a change in the shape of gp120.* E*, In some patients, antibodies that neutralize a wide range of HIV-1 isolates arise, represented here by a variety of shapes of gp120. Mixing of envelope shapes on a single virus particle is shown for illustrative purposes only. bnAbs, broadly neutralizing antibodies.*[141]

1.2.1.3. Immunoglobulins and IgG subtypes of HIV-1 control

The humoral immune response is an antibody-mediated immune response that induces immunoglobulins (Ig) specifically directed to antigens: the so-called antibodies (Ab). The structure of Ig can be divided into four polypeptide chains that are identical in pairs: two heavy

chains (H for heavy) and two light chains (L for light). Each H chain consists of an N-terminal variable domain (VH), three constant domains (CH1, CH2 and CH3), and an additional region: the hinge region, located between the CH1 and CH2 domains. Similarly, light chains consist of an N-terminal variable domain (V) and a constant domain (C).[142,143] (Figure 14) The fragment antigen binding (Fab) portion of the antibody determines the antigen-binding specificity, and the crystallizable fragment (Fc) portion mediates complement component binding and a myriad of Fc receptor-mediated activities of natural killer (NK) cells, monocyte/macrophages and dendritic cells.[144]

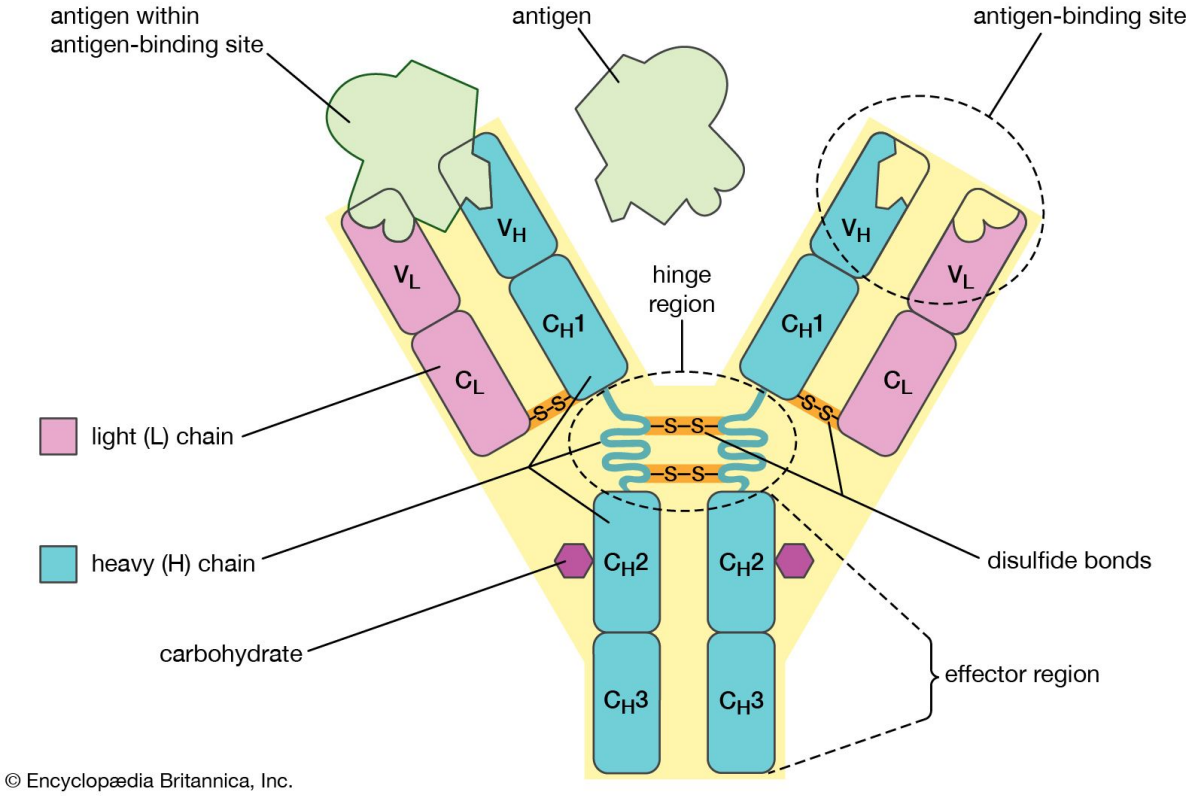

Figure 14. The four-chain structure of an antibody, or immunoglobulin, molecule. The basic unit is composed of two identical light (L) chains and two identical heavy (H) chains, which are held together by disulfide bonds to form a flexible Y shape. Each chain is composed of a variable (V) region and a constant (C) region. [145]

Humans have five types (isotypes) of Igs: IgM, IgD, IgG, IgA, and IgE determine by different heavy chains. During infection, Ig H chain class switching occurs rapidly after activation of mature naïve B cells, resulting in a switch from expressing IgM and IgD to expression of IgG,

IgE, or IgA. This switch improves the ability of antibodies to remove the pathogen. Class switching occurs by a deletional recombination between two different switch regions, each of which is associated with a heavy chain constant (CH) region gene; the variable region of the heavy chain stays the same. After HIV infection, antibodies of IgM type directed to gp41 are first induce followed by class switching to IgG and IgA antibodies (Figure 15).[129,146]

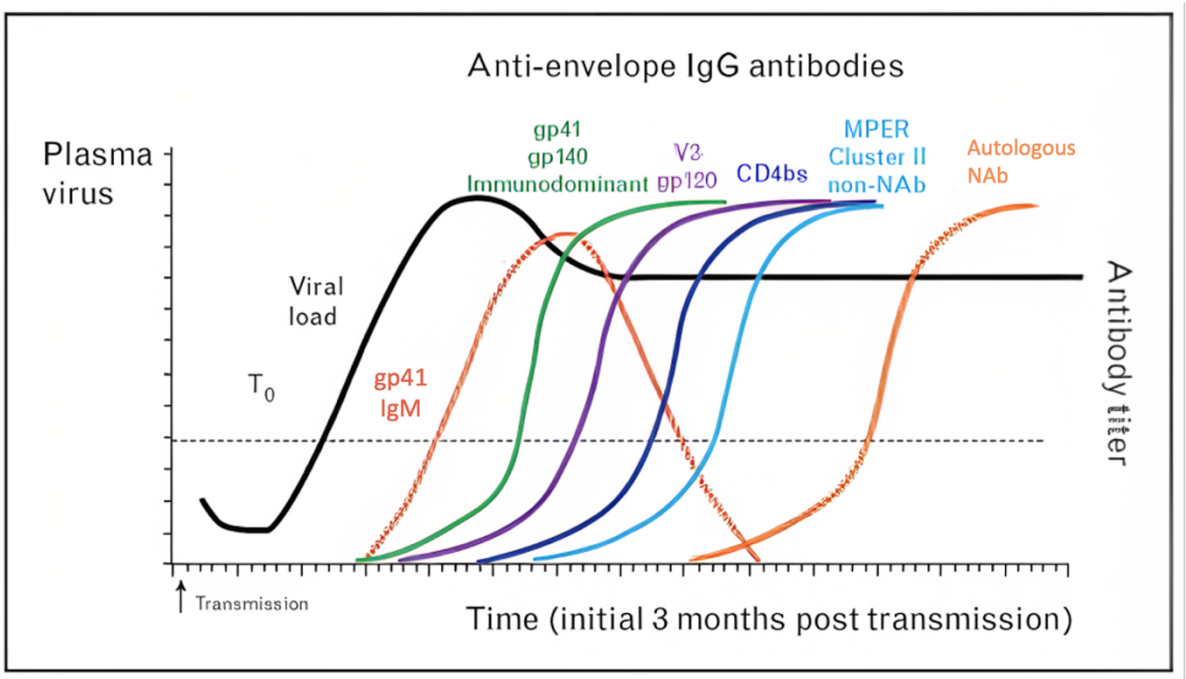

Figure 15. Sequentially elicited IgG antibodies to HIV-1 envelope epitopes. After HIV-1 transmission, antibody isotypes and specificities to the HIV-1 envelope are elicited sequentially. The first HIV-1-specific antibodies detected in the plasma are anti-gp41 IgM (red line). Anti-gp41 IgM undergoes class switching to IgG and IgA, making gp41 the first protein also recognized by IgG and IgA antibodies. Gp41 (green), gp120 (purple), CD4bs (dark blue), MPER (non-neutralizing) (light blue), autologous neutralizing antibodies (orange line (Modified from Tomaras, G.D. and Haynes, B.F., 2009).[146]

1.2.1.4. IgM

IgM is the first antibody appearing in response to the initial exposure to antigens.[129,147] It's a pentamer composed of five four-chain units, giving it a total of 10 antigen-binding sites and participates in tissue homeostasis by regulating inflammatory processes and autoimmune

diseases (Figure 16).[148,149] The anti-HIV-1specific gM is the first antibody class to emerge after HIV infection. Several human IgM-derived monoclonal Abs (MoAbs) obtained from healthy donors showed high non-specific binding avidity to gp120 antigens.[150,151] This non-HIV-1 specific IgM present in HIV-1 non-infected individuals has been proposed to prevent mucosal transmission, suggesting it may be able to stop HIV from crossing epithelial cell membranes. [152,153]

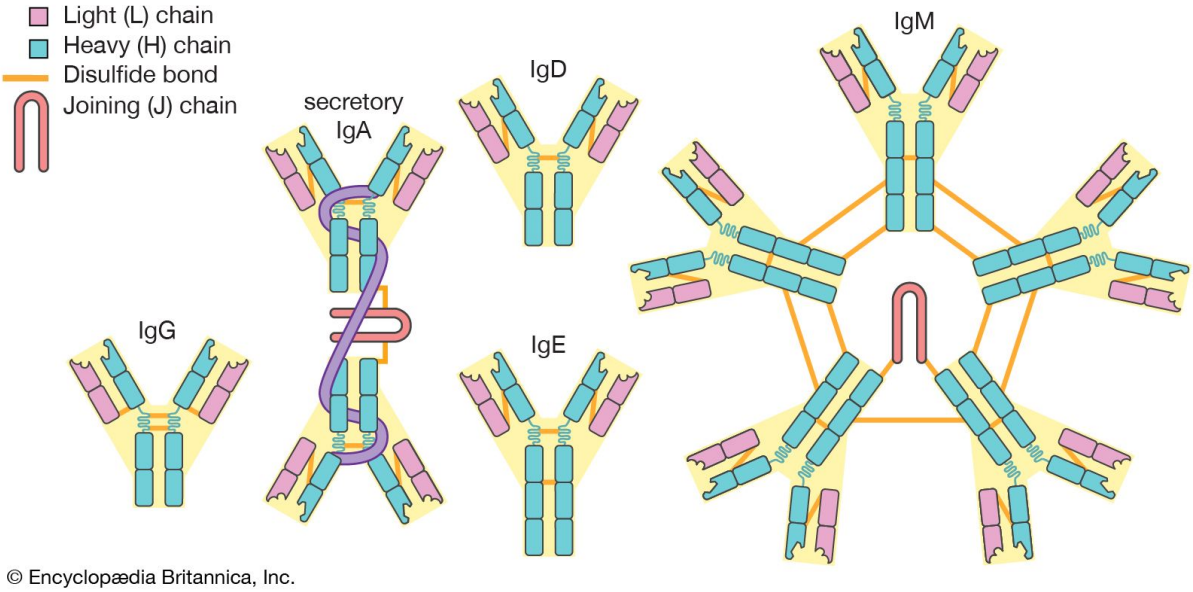

Figure 16. The five main classes of antibodies (immunoglobulins): IgG, IgA, IgD, IgE, and IgM.[145]

1.2.1.5. IgA

IgA antibodies are secreted in the respiratory or the intestinal tract and act as the main mediators of mucosal immunity.[154] They are monomeric in the serum, but appear as a dimer termed secretory IgA (sIgA) at mucosal surfaces. The secretory IgA is associated with a J-chain and another polypeptide chain called the secretory component. IgA antibodies are divided into two subclasses that differ in the size of their hinge region. IgA1 has a longer hinge region which increases its sensitivity to bacterial proteases.[155] Therefore, this subclass dominates the serum IgA, while IgA2 is predominantly found in mucosal secretions.

HIV is mainly transmitted through mucosal surfaces. Kaul et al. found that the HIV-specific IgA is present in the genital tract of most HIV-1-resistant Kenyan sex workers which suggest mucosal HIV-1-specific IgA responses might play a role in HIV-1 resistance. [156] Mucosal HIV-1-

specific IgAs have been shown to inhibit the transcytosis of HIV-1 migration through epithelial cells.[157,158] Moreover, IgAs found in cervicovaginal fluids can inhibit HIV-1 via Fc receptor-mediated functions, such as ADCC.[159–161] Besides, secretory IgA directed to V1/V2 loop of HIV demonstrated cross-neutralizing capacity against various HIV-1 clades (A, B, and C). [162] Lastly, IgA isolated from sera of HIV infected individuals are able to neutralize HIV efficiently.[163]
On contrary, some studies found that IgA response might be a risk factors. Indeed, HIV-specific plasma IgA were associated with increased risk of HIV acquisition in RV144 vaccine trial. In vitro, vaccine-induced IgA decreased the ADCC activity of Env-specific vaccine-induced IgG.[164–166]

From passive and active immunization studies in macaques, we learn that mucosal IgA can prevent SHIV acquisition. However, whether anti-HIV IgA responses is beneficial or harmful for the host is still questioned.[167–169]

1.2.1.6. IgG

Among five types of antibodies, IgG is the major Ig isotype (75%) and accounts for 10-20% of all plasma proteins. IgG can be classified into four subtypes: IgG1, IgG2, IgG3 and IgG4 in order of decreasing abundance.[170,171]

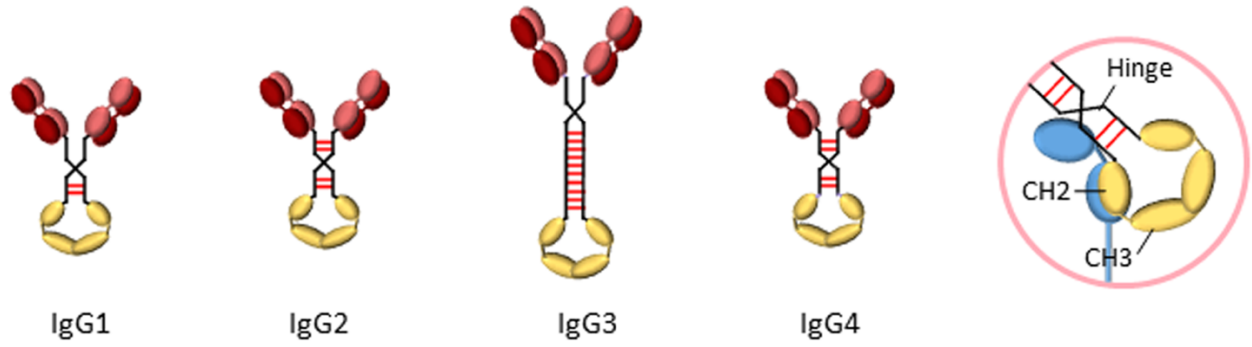

Figure 17. Structure of IgG subclasses, indicating how the different heavy and light chains are linked, the length of the hinge, and the number of disulfide bridges connecting the two heavy chains.[172]

The four IgG subtypes are highly conserved and differ in the structure of their constant domain, particularly in the hinge region and the CH2 domain (Figure 17).[171] It broadens IgG Fc

domain-mediated functions and structural differences depending on the IgG subtype. The interaction between naïve B cells with follicular dendritic cells and follicular helper T cells, that happens in the germinal centers of lymphoid follicles, induces IgG isotype diversification [112,173] By recombining immunoglobulin heavy chain genes, Abs switch to IgG2 and IgG4 downstream of IgG3 and IgG1.[174] The IgG subtypes have various affinities for the different Fcγ receptors (FcγR) present on the surface of immune cells (Figure 18). In addition, the affinity varied according to the FcγR polymorphism. This difference of affinity will directly impact on Abs Fc-mediated functions. Numerous studies pointed out the interest of the different IgG subtypes in controlling HIV-1 infection. This will be discussed in the following paragraphs.

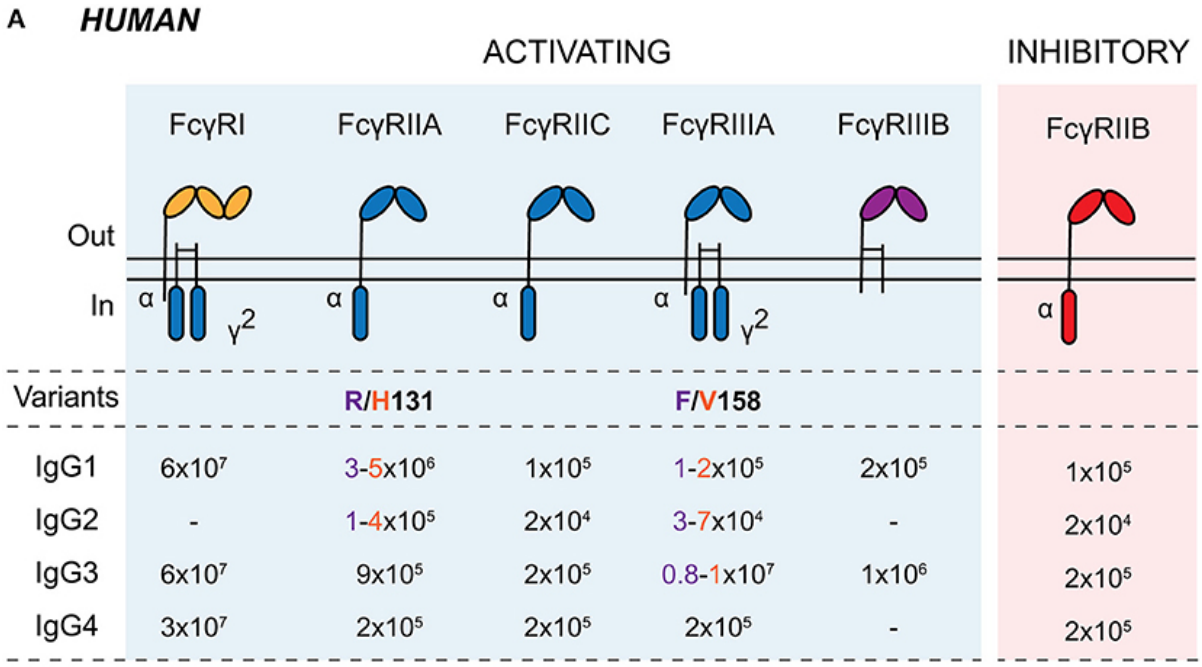

Figure 18. Human and murine Fcγ receptors. Schematic of human classical Fcγ receptors (discussed in 2-3-2-1) embedded in the plasma membrane IgG affinity-altering variants are highlighted beneath the respective human FcγR, with the low- and high-binding variants and associated IgG affinities colored in purple and orange, respectively, in the table. Binding affinities are indicated as KA (M^{-1}).[175]

1.2.1.6.1. IgG1

Richardson et al.'s study showed that there is a link between the diversity of IgG subtypes and the development of broad-spectrum neutralizing antibodies, also correlated with the polyfunctionality of the Fc domains of the antibodies.[176]

IgG1 is the main Ig subtype in the blood. Anti-HIV antibodies of IgG1 subtype are the efficiently induced rapidly, during acute infection and persist in chronic stage. They are mainly directed against Env, Gag, and Pol proteins. [177–179] According to the individuals' HLA typing and clinical parameters, the HIV-1 specific responses, the proportions of the different IgG subtypes, are variable. [180–182] Most monoclonal NAbs (MoNAbs) developed so far against HIV are of the IgG1 subtype (2F5, IgG1 b12, 2G12, etc.). Only a few Abs of other IgG subclasses were directly isolated from blood. In vitro modification of IgG subclasses demonstrated that, indeed, the Fc domain of Ab participate in HIV inhibition.[183]

IgG1, together with igG3,has strong affinities for six classic FcγRs.[175] HIV-1-specific IgG1 can bind to FcR and mediate antibody-dependent cellular cytotoxicity (ADCC) of HIV-1-infected cells. [175,184]

1.2.1.6.2. IgG2

Anti-Env IgG2 can be detected at various stages throughout HIV-1 infection but the concentration is lower than IgG1.[185–187] The low anti-gp41 IgG2 levels could be correlated with clinical manifestations.[188]

In addition to neutralization and weak Fc-mediated inhibition, IgG2 could also regulate the immune response. Studies also found a correlation between the level of HIV-1-specific (Env or Gag) IgG2 antibodies and virus control.[146,189] Classically, IgG2 antibodies are induced by bacteria and recognize phospholipids and carbohydrates. The role of IgG2 HIV specific Abs remain elusive.

1.2.1.6.3. IgG3

IgG3 are described to be a sign of B cell maturation. IgG3 has been proposed to have increased in-vitro neutralizing ability compared with IgG1. IgG3 have also a strong affinity to FcγR which might lead to potent inhibitory Fc-mediated Ab functions. They are therefore be considered as good prognostic in HIV disease.It.[171,190,191] Indeed, several studies found a decreased anti-HIV IgG3 during disease progression.[184,186] In HIV-1 infected individuals, IgG3 is associated with enhancing the neutralization potency and Fc-mediate function of HIV-1 V2 specific BNAbs.[192] As for vaccination, the studies from the RV144 phase III and HVTN702 phase IIb/III vaccine trial demonstrated that vaccine-induced anti-V1V2 Abs of IgG3 subtype correlated with a reduced risk of infection.[193–195] How exactly these anti-V1V2 IgG3 may contribute to the decreased risk need further investigations.[196]

1.2.1.6.4. IgG4

IgG4, the less predominant IgG subclass, is typically induce following chronic antigenic stimulation. During HIV-1 infection, HIV-1-specific IgG4 was found more readily during the chronic phase.[188,197] Till now, no study has identify a specific role of IgG4 subtype in HIV infection.

1.2.1.7. Mechanisms of HIV-1 inhibition by antibodies

1.2.1.7.1. Neutralization

Neutralization has been defined as the loss of infectivity which ensues when antibody molecule(s) bind to a virus particle without other components, such as complement molecules or antibody Fc domain (Fc) receptors.[198] Neutralization requires the interaction between the paratope located at Fab domain of the antibody and the epitope present on the virus. This interaction prevents virus entry into target cell. The NAb can therefore protect cells from pathogens or infectious particles by inhibiting various steps leading to infection via the binding of their Fab domain to the infectious agent.[198,199]

1.2.1.7.1.1. Neutralizing antibodies directed against the HIV-1 envelope.

HIV-specific NAbs recognize epitopes on the surface glycoproteins of the virus. Several MAbs directed against defined epitopes of the virus envelope have been described for their abilities to neutralize a broad spectrum of primary isolates of HIV-1. These specific virus targets are highlighted in figure 19. Noteworthy, HIV-1 isolates display different sensitivity to neutralization. They were therefore classified from easy to difficult to neutralized by Tier 1 to Tier 4.[200,201] This classification has important implications for the monitoring and interpretation of vaccine-elicited neutralizing antibody responses.[200] Most of the primary isolates are classified as Tier 2, with a few exceptions for the other Tiers. bNAbs need therefore to neutralize a broad range of Tier 2 stains to inhibit the majority of the circulating HIV strains BNAbs directed to HIV-1 epitopes (including VRC01, 3BNC117, 10-1074, VRC01-LS, VRC07-523LS, PGT121 and N6LS) have been used in clinic as potential therapeutic agents. The clinical trial performed so far have shown that Ab treatment is safe and well tolerated.[208–212]

However, administration of anti-HIV-1 bNAbs to humans shown modest and transient suppression of viremia, lowering viraemia and maintaining viral suppression of Ab-sensitive viruses for a short period in the absence of ART.[202–207] Rapidly, Ab escape variants are generated, strongly indicating that therapies using multiple bNAbs in combination will be necessary for virus eradication.

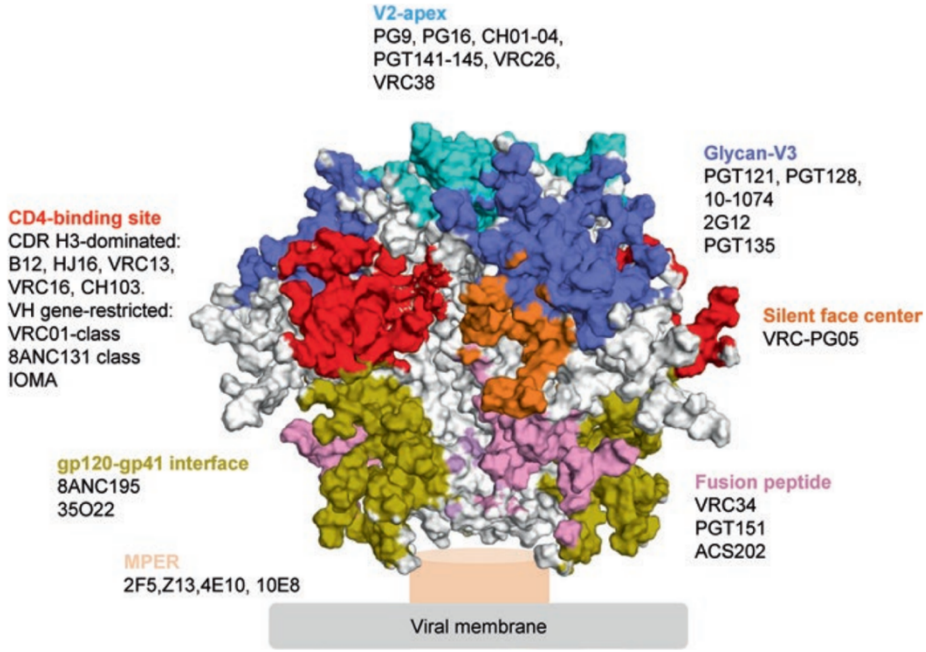

Figure 19. Major sites of vulnerability to neutralizing antibodies on the pre-fusion closed HIV-1 Env trimer. The target sites for CD4-binding site antibodies, the V1V2 apex antibodies, the glycan V3 antibodies, the fusion peptide-targeting antibodies, the gp120-gp41 interface antibodies, the MPER antibodies and silent face.[212]

1.2.1.7.1.2. CD4-binding site antibodies

The CD4-binding site on HIV-1 gp120 is a conserved and conformational epitope responsible for CD4 receptor binding. It mediates the initial step of virus-host interaction is functionally conserved for efficient association with the CD4 receptor. This epitope is located in a pocket, therefor highly masked for the immune response. However, various Abs directed to this epitope have been identify. These Abs with potent and broadly neutralizing activity demonstrate specific characteristics, as the recognition via the heavy chain for b12. [213,214] Or a highly mature phenotype with long CDR3 (complementarity determining region 3) domain, or high mutation from germline for VRC01, VRC07, NIH45-46, 3BNC117, VRC-PG04, N49-P7, etc.[213,215,216]

1.2.1.7.1.3. V1V2 apex antibodies

The V1V2 site is another major site of vulnerability to NAbs. BNAbs PG9, PG16, CH01-04, PGT141-145, and PGDM1400 are among them. These antibodies target the top of the Env trimer. They also have a long heavy chain (long CDR3 domain), which allows them to reach the 71 epitopes through the glycan shield.[211,212]

1.2.1.7.1.4. Glycan V3 antibodies

The V3-glycan epitopes are considered as the "supersite of vulnerability" and depend on the N332 glycan. The Abs that target this region are commonly induced following HIV infection, but only a few of them (include PGT121–123, PGT135, 10-1074, 2G12, etc.) are able to display broad neutralizing activity.

1.2.1.7.1.5. Fusion peptide-targeting antibodies

The fusion peptide (FP) is thought to be sequestered. However, bNabs VRC34 and PGT151 directed to this region display neutralizing activity. This demonstrates FP to be a neutralizing epitope that could be target for vaccine design.[217–219]

1.2.1.7.1.6. MPER region antibodies

The gp41 MPER is a highly conserved and critical component of the viral entry process. It thus represents an exciting site to target for the inhibition of early fusion stages.[220,221] BNAbs directed against the MPER region of gp41, such as 4E10, 2F5, 10E8, etc., recognize hydrophobic residues, specific peptides, and sequences located before the transmembrane domain. [210,222,223]

1.2.1.7.1.7. gp120/gp41 interface antibodies

Recently, additional bNabs, Abs 35O22, 8ANC195 and PGT151-158, have been isolated form infected individuals. They recognize a new viral epitopes on the pre-fusion closed HIV-1 Env at the interface of gp120 and gp41.[224,225]

1.2.1.7.1.8. Silent face of the antibodies

One of the most glycosylated regions on the HIV-1 Env trimer is located on the outer domain of gp120.[53] This highly glycosylate sites allow an efficient escape from immune response. Nonetheless, after several years of infection, Abs recognizing these glycosylated sites are induced. The most original one is Ab 2G12 with a dimer structure to allow the binding to two related carbohydrates.[226]

As a trimer, the region in the center is not accessible by the immune response and therefore call the "silent face".[48] However, following gp120 dissociation, this silent face becomes immunologically active, although the Abs induced recognize gp120 monomer and have therefore no neutralizing activity. Recently, anAb recognizing the center of the “silent face” on the gp120 subunit, VRC-RG505, was found to display neutralizing activity suggesting that this part of the env structure can be recognized by the immune response.[227]

1.2.1.7.2. Mechanisms related to Fcγ receptors

The family of Fc receptors for IgG (FcγRs) is broadly expressed by cells of haematopoietic origin and consists of one inhibitory and several activating receptors. They show distinct affinities for the different IgG subtypes (IgG1 to 4).[228–230]

1.2.1.7.2.1. Fc receptor structure and immunology

There are nine FcγR human express: FcγRI (CD64), FcγRIIa (CD32a), FcγRIIb (CD32b), FcγRIIc (CD32c), FcγRIIIa (CD16a), FcγRIIIb (CD16b, specific for neutrophils), FcnR (neonatal), FcRL5 (CD307) and TRIM21 (tripartite motif-containing protein 21) (Figure 20).[231] These receptors expressed on the surface of immune cells bind to the different IgG subtypes (IgG1 to 4) with distinct affinities.

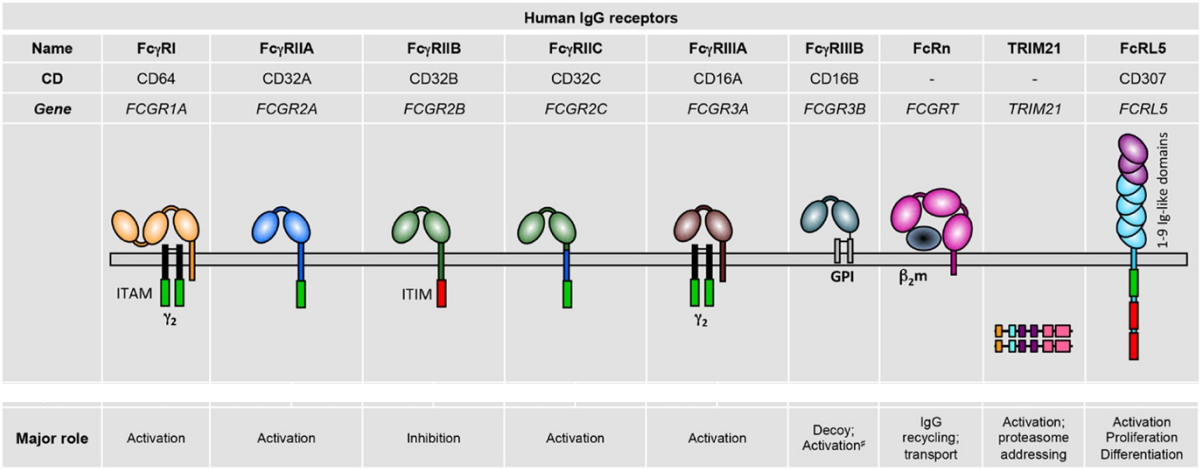

Figure 20. Human IgG receptor family and its major role. ITAM, immunoreceptor tyrosine-based activation motif; γ2, dimer of FcRγ subunits; ITIM, immunoreceptor tyrosine-based inhibitory motif; GPI, glycosyl-phosphatidylinositol; β2m, β2-microglobulin.(modified from Gillis et al. 2014) [231]

The six classic FcγR (FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, and FcγRIIIb)trigger different intracellular signals (Figure 21).[172,231,232] FcγRIIb is transmitting an inhibitory signal via an ITIM (immunoreceptor tyrosine-based inhibitory motif) and FcγRIIIb is anchored by GPI (glycosylphosphatidylinositol). The other four receptors transmit activating signals via an ITAM (immunoreceptor tyrosine-based activation motif).[171,233] The expression of activating FcγR can be stimulated by pro-inflammatory factors like lipopolysaccharide (LPS), Th1 type cytokines (IFN-γ) or certain components of the complement system (C5a). Th2-type cytokines (interleukin (IL)-4, IL-10, transforming growth factor (TGF)-β), on the contrary, can induce dysregulation of the expression of activator-type FcγR in favor of increasing the expression of inhibitory FcγR.[230,231] The role of these FcR were highly investigated in HIV-1 research.

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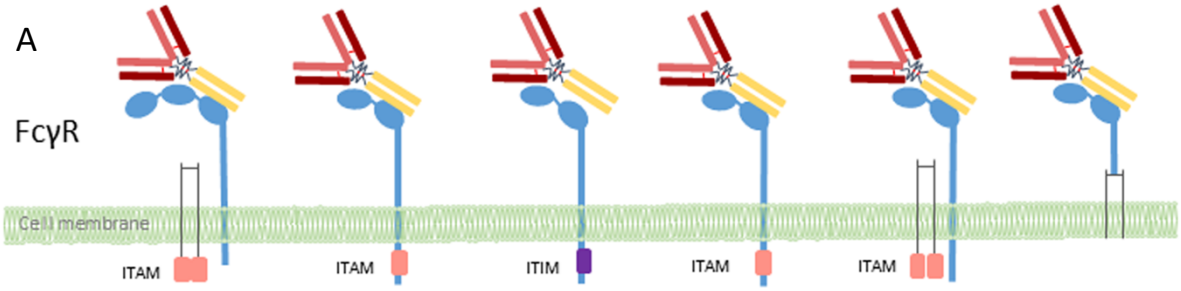

FcγR Main Functions	CDC Phagocytosis Opsonization	Antigen presentation, ADCC, ADCP			ADCC/ ADCP CDC degranulation	
Distribution	Mo, Mϕ, DC, Neu, Eos	Mo, Mϕ, DC, Neu, PLT	Mo, Mϕ, DC, MC, Neu, Bas, BC	NK	Mo, Mϕ, DC, NK	MC, Neu, Eos

B

FcγR	I	IIa		IIb	IIIa		IIIb	
Polymorphism		H131	R131		V158	F158	NA1	NA2
IgG1	+++	+++	+++	+	+++	++	+++	++
IgG2	-	++	+	-	+	-	-	-
IgG3	++++	++++	++++	++	++++	++++	++++	+++
IgG4	++	++	++	+	++	-	-	-

Figure 21. Six classic Fc gamma receptors and the binding affinities to IgG subclasses. (A) Six classic Fc gamma receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, FcγRIIIb), their main function, polymorphisms, and distribution on immune cells. (B) FcγR binding affinities of IgG subclasses. CDC complement dependent cytotoxicity, ADCC antibody-dependent cellular cytotoxicity, ADCP antibody-dependent cellular phagocytosis, Mo Monocyte, Mϕ Macrophage, DC Dendritic cell, MC Mast cell, Neu Neutrophil, Bas Basophil, Eos Eosinophil, NK Natural killer cell, BC B cell, PLT Platelet.((modified from Lin et al, 2022). [172]

Different FcγR induces different function (Figure 21). The interaction between Abs and FcγR plays a key role in the immune response against HIV-1, via the activities of ADCP (antibody-dependent cellular phagocytosis), ADCC (antibody-dependent cell-mediated cytotoxicity) or ADCVI (antibody-dependent cell-mediated virus inhibition).[234]

1.2.1.7.2.2. Antibody-dependent cellular phagocytosis (ADCP)

After the antibody's Fab domain recognizes the viral particle, the Fc domain of the antibody engages with the FcRs expressed by innate immune cells (monocytes, macrophages, neutrophils, dendritic cells (DCs), and mast cells). These interaction lead to phagocytosis of the opsonized viral particle.[183,228,235,236]

Moreover, ADCP, the phagocytosis of infected cells, can be induced by Nabs or specific non-NAbs. Several studies indicate that the detection of ADCP in vitro leads to efficient inhibition of HIV infected cells.[183,228,235] It also associates with the protection and reduced viremia in the macaque model. [237–240] ADCP was correlated with decreased risk of infection in HVTN505 vaccine trials.[194]

1.2.1.7.2.3. Antibody-dependent cell-mediated cytotoxicity (ADCC)

ADCC is a potent Fc-mediated effector function involved in clearing malignant or infected cells. It's the most considered Fc-mediated function in the field of HIV-1. ADCC is observed with neutralizing as well as non-neutralizing IgG, mainly from IgG1 and IgG3 subclasses.[241] The antibody's Fab domain bonds to the infected cell (expressing the HIV-1 envelope proteins on the surface), and its Fc domain recognizes the FcRIII of the NK cells. The cytotoxic granules (granzymes and perforin), chemokines, nitric oxide or reactive oxygen species released from the NK cells destroy the infected cells.[234,242,243] ADCC seem to play a critical role in protection against HIV-1 acquisition.[166] Also, the ADCC activity of IgG in breast milk correlated inversely with infant infection risk.[244] Moreover, analysis of Ab responses in the RV144 trial reveals that ADCC correlate with lower infection risk. [195,245] Anti-HIV-1 Abs with ADCC activity can be a key to reducing HIV latent reservoirs and some HIV-1 bNAbs have been proposed to diminish latent reservoirs by Fc-mediated mechanisms such as ADCC.[246–251]

1.2.1.7.2.4. Antibody-dependent cell-mediated virus inhibition (ADCVI)

Antibody-dependent cell-mediated virus inhibition (ADCVI) is focuses on the overall effect of Abs on virus replication in the presence of effector cells in vitro.[252,253] It detects ADCC and other Fc-related inhibitory functions. Even though ADCVI and ADCC activities likely overlap, the non-cytolytic mechanisms, such as the FcγR-triggered production of β-chemokines, can also participate in the virus inhibition measured in ADCVI assays.[229,252] The ADCVI is detected as early as the first week after symptom onset or the first month after exposure, much earlier than what was reported for the NAb response.[244,252] ADCVI antibody activity is not associated with the risk of HIV-1 superinfection; however, higher serum ADCVI activity against a clinical R5 strain of HIV-1 correlated inversely with the infection rate.[254,255]

1.2.1.7.2.5. Antibody-dependent complement deposition (ADCD)

The complement system comprises several proteins and is a crucial component of the innate immune response. It can also link the innate and adaptive response by activation following the recognition of a pathogen (alternate pathway and lectin pathway) or following the detection of an antibody attached to the surface of a pathogen or infected cell.[256] There are three complement pathways: classical, lectin pathway and alternate pathway; all converge towards the formation of the membrane attack complex, which allows the lysis of the pathogens or the infected cells. [257]

Cellular proteins, such as CD46 (MCP, membrane cofactor protein) or CD55 (DAF, decay-accelerating factor), are involved in complement regulation during HIV-1 budding. They are incorporated into the viral particle's envelope, allowing the virus to escape the complement system.[258,259] Although the classical complement pathway plays a role in HIV-1 lysis during infection, some studies show this activity is weak compared to other viruses and might play a detrimental role by promoting the infection of cells attracted by chemotaxis (dendritic cells, macrophages or T lymphocytes).[257,259,260] Besides these, some studies suggested that ADCD might have a strong association with neutralization breadth, which might be associated with the binding of C3 to complement-receptor-2 on follicular dendritic cells. [176,261,262] It may

improve antigen presentation in the germinal centers leading to higher affinity maturation and improved antibody breadth.[261,262]

1.2.1.7.2.6. Antibody-dependent cellular trogocytosis (ADCT)

Trogocytosis is a biological process during which fragments of the plasma membrane are transferred between two immune cells within an immunological synapse. There are two types of trogocytosis (Figure 22).[263,264]

1. Adhesion molecule-mediated trogocytosis: MHC molecules are damaged on antigen-presenting cells to CD8+ T lymphocytes.
2. Antibody-dependent cellular trogocytosis (ADCT): Involving the binding of an antibody to an antigen on the target cell's surface and the concomitant binding of the Fc portion of the antibody to an Fc receptor on the effector cell.

It has been proposed that patients with high ADCT activities develop broad-spectrum neutralizing Abs later on.[176] In 2018, Richardson et al. developed a test measuring anti-HIV ADCT, and proposed that this new antiviral Fc effector function mediated by HIV-specific Abs could be harnessed for vaccination and cure strategies.[265]

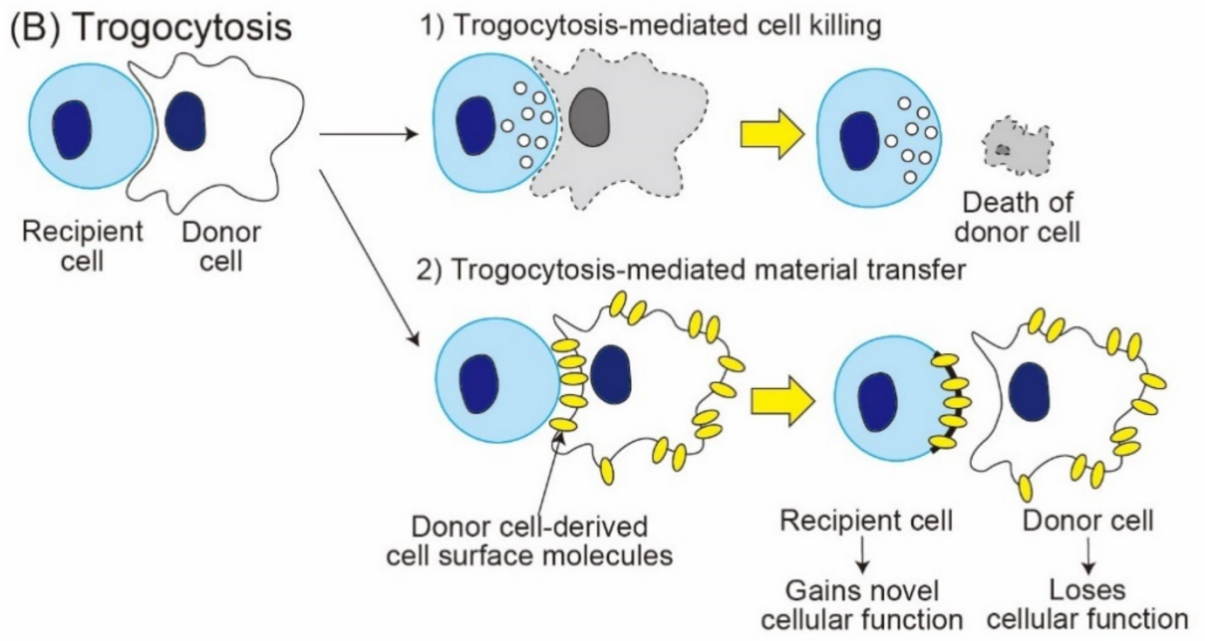

Figure 22. Characteristics of two types of trogocytosis. In the process of trogocytosis, recipient cells nibble the cell body of donor cells. Trogocytosis results in either (1) the death of target cells (trogocytosis-mediated cell death) or (2) the transfer of cell surface molecules, together with membrane patches, from donor cells to recipient cells (trogocytosis-mediated material transfer).[263]

1.2.1.8. Study of the Ethnicity impacts on immune responses and Fcγ receptor polymorphism

Research focusing the impacts of ethnicity/ genetic on Fcγ receptor polymorphism and humoral immune responses are currently under investigation. The studies analyzing the impact of ethnicity on HIV infection and vaccination will be discussed in the following paragraphs.

1.2.1.8.1. Immunoglobulins

Several studies have shown that serum Ig concentrations vary according to ethnicity, sex, and age. Total IgG and IgA levels increase with age and reach the adult concentration at around ten years of age.[266,267] The levels of serum IgG were found to be significantly reduced with age, and the level of IgA was found to be maintained.[268]

The IgA, IgG1, IgG2 and total IgA were found to be higher in people with African origin when compared to Caucasian populations living in the same country.[269–272] A similar result of higher total IgG levels in Africans than in Caucasians and Hispanics was found HIV-infected individuals.[272–275] Other studies extend the analysis to Asians, Amazonians, or Melanesians, and consistently found higher total IgG in these populations compared to Caucasians. [271,276–281]

Notably, all these studies comparing Ab profiles according to ethnicity were performed with individuals living in the same country.

For sure, geographical location plays a role in immunoglobulin concentration.[280,282] E.g.: Mexico City residents' low IgG and IgM levels, especially IgM, might be a compensatory mechanism for the increase in blood viscosity caused by high-altitude erythrocytosis.[282] Among same ethnicity, there are still some difference between tribes and genders, which might be related to environment or diet.[283–285] New studies need therefore to integrate other factors as geographic origin in order to better evaluate the difference of immune response in people living in different countries and continents. This may drastically influence the vaccine immune response.

1.2.1.8.2. Fcγ receptor polymorphism

As discussed in chapter 2-3, FcR mediates Fc-Ab-functiona that are important for HIV protection. The FcR genes significantly differ by single-nucleotide polymorphisms (SNPs) frequencies among ethnic groups[286–290]. These differences might modify FcR binding efficacy and impact on FcR mediated function**.**[286–290] Indeed, an association was found between FcR polymorphisms and HIV-1 protection or disease outcome.[291–293]

However, FcGR association differed according to vaccine modalities, targeted HIV-1 subtypes, study populations, mode of HIV-1 transmission (Figure 23). A general association of FcR with vaccine outcome could not be evidenced so far. The FCGR2C rs114940536, rs138747765, and rs78603008 polymorphisms vas associated with a decreased risk for HIV acquisition in the RV144 trial.[294] In VAX004, enhanced HIV-1 acquisition occurred in vaccinees homozygous for

the FcγRIIIa-176V allele. Vaccinees with minor *FCGR2A* and *FCGR2B* alleles enhanced ADCVI and ADCP *i*n VAX004 and HVTN505 and were associated with reduced risk of HIV-1 acquisition.[255,295] On contrary, the vaccinated ones carrying the FCGR2C-TATA or the FCGR3B-AGA haplotype had significantly higher incidences of HIV acquisition in HVTN505 trial.[296] Moreover, an FCGR2A SNP (rs2165088) and two FCGR2B SNPs (rs6666965 and rs666561) influenced the anti-gp140 ADCP which associated with HIV risk.[296]

All these results indicate that, for future vaccine or therapy designs, ethnicity (related to Ig concentration and FCGR polymorphisms) need to be taken into account in addition of other factors as gender, and age (Further discussed in section 4 of the result).[297,298]

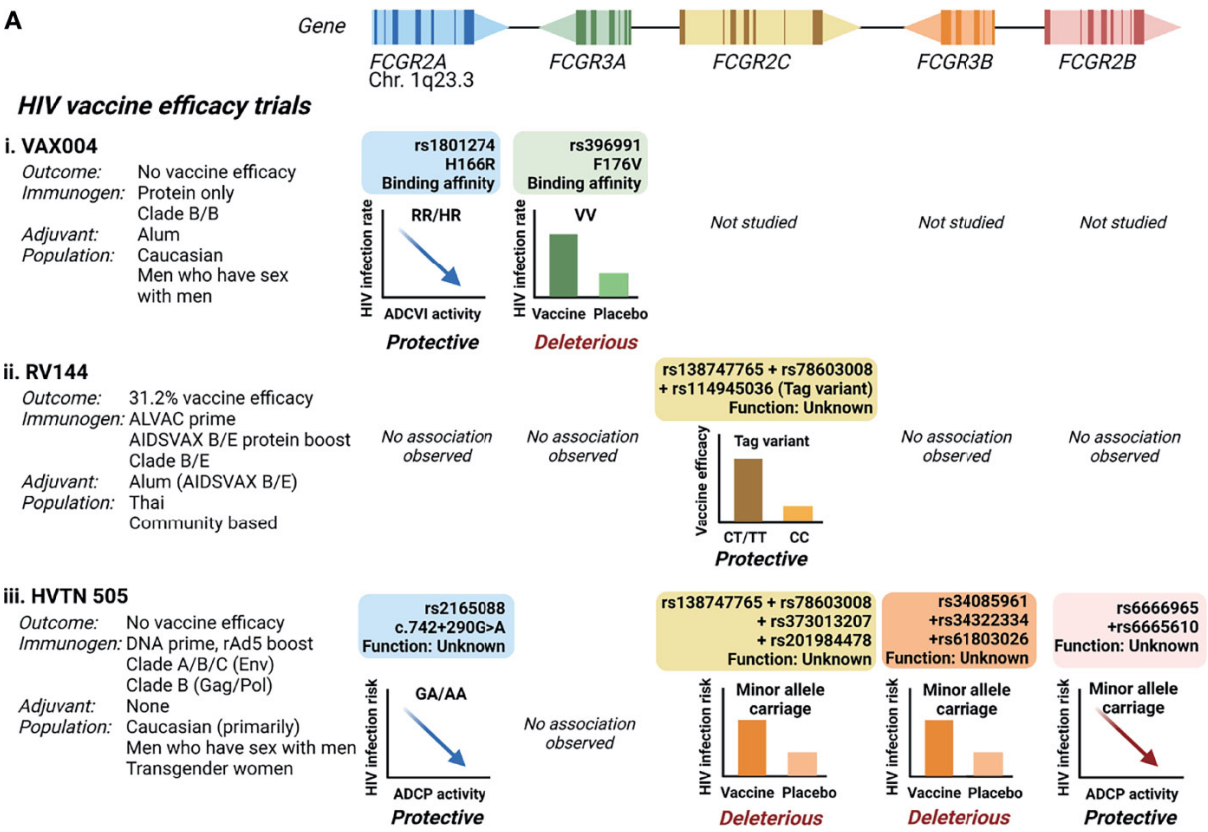

Figure 23. FCGR variant associations with HIV-1 vaccine efficacy trial outcomes. Three HIV-1 vaccine efficacy trials investigated the association between FCGR *variants and HIV-1 acquisition risk: VAX004, RV144 and HVTN505. The trials differed concerning vaccine modalities, target HIV-1 subtypes, study populations, mode of HIV-1 transmission, and host ethnicities.*[297]

1.3. Chapter 3. Prevention, Treatment and Vaccine development

1.3.1. The 90-90-90 strategy to end the HIV Pandemic

With the aim of ending the AIDS epidemic by 2020, an ambitious target was set by UNAIDS in 2014: 90% of people living with HIV should be screened, 90% of those these should be on long-term antiretroviral treatment and 90% of those treated should have an undetectable viral load (Figure 25). [299] This target would allow to triple the number of people with an undetectable HIV load. As HIV infected individual with undetected virus load are potentially non-infectious, epidemic modelling predicts, that AIDS epidemic would end by 2030 if this goal is achieved. [299]

Onit 14 countries, sadly not including France, have already achieved the 90-90-90 targets.[300,301] However, in most of the countries, the goal will only be achievable by addressing gaps in the HIV testing and treatment cascade. The global target could be accomplished with combined efforts to improve testing and treatment from all key players.[302–304]

Figure 24. The 90-90-90 strategy. From the left to the right are: 90% of people living with HIV should know their HIV status, 90% of those these should receive antiretroviral treatment, and 90% of those treated should have viral suppression. [305]

1.3.2. Prevention and treatment of HIV-1 infection

To achieve the 90-90-90 strategy to end the HIV pandemic, people with unknown HIV status should accept screening tests and get access to treatment. HIV prevention methods are also essential to reduce newly infected patients.

1.3.2.1. Prevention

In order to avoid being contaminated or infecting someone, several prevention strategies are now available. In addition to the use of condoms, regular screening for HIV but also for other sexually transmitted infections will allow to limit transmission. Preventive medicines have been developed, such as pre-exposure prophylaxis (RrEP) and post-exposure prophylaxis (PEP).[306]

1.3.2.2. Screening

There are three types of HIV tests: [307]

- Antibody test (most used): They detect antibodies produced by the immune system in response to viral infection. They cannot be used immediately after risk exposure since several weeks are necessary for the body to produce a quantity of antibodies high enough to be detectable. These tests allow a diagnosis in a few minutes by sampling blood or saliva. This method is generally used in self-tests which provide a reliable result on the serological status of an individual. In the event of a positive result, these tests must be confirmed by tests coupling the detection of antibodies and viral antigens.
- Antigen/Antibody test: It is also called combined ELISA (enzyme-linked immunosorbent assay) tests. This type of test allows the coupled detection of antibodies directed against HIV-1 and the p24 antigen (corresponding to the capsid domain of the Gag protein or the mature capsid protein). A negative result of this test carried out six weeks after the supposed exposure can consider the absence of HIV infection.
- Nucleic acid detection test: This test directly measures the quantity of virus in the blood. The quantitative RT-PCR (reverse transcription polymerase chain reaction) technique allows viral RNA to be quantified. This test is quite expensive and cannot be used for routine screening. However, it can detect people with primary infection.

1.3.2.3. Antiretroviral therapy

Antiretroviral therapy has significantly altered the prognosis of HIV infection. In 1987 the Food and Drug Administration (FDA) approved zidovudine (AZT), a nucleoside reverse transcriptase inhibitor, to use on HIV-infected patients.[308,309] Years later, combining medicines for HIV treatment was demonstrated to be highly effective, currently known as Highly Active Antiretroviral Therapy (HAART) or cART (combined Antiretroviral Therapy).[310] HAART typically consists of three medications of at least two different classes.[311,312] The medicines used for HAART are classified into six classes from their mode of action, including co-receptor antagonists, fusion inhibitors, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors and protease inhibitors (PIs) (Figure 24).[311–313] Current antiretroviral therapies are highly efficacious in maintaining undetectable viral loads for an extended period. A prolonged virological success allows restoration of immune functions, a reduction of inflammation and thus, a virtual normalization of life expectancy.[311,313]

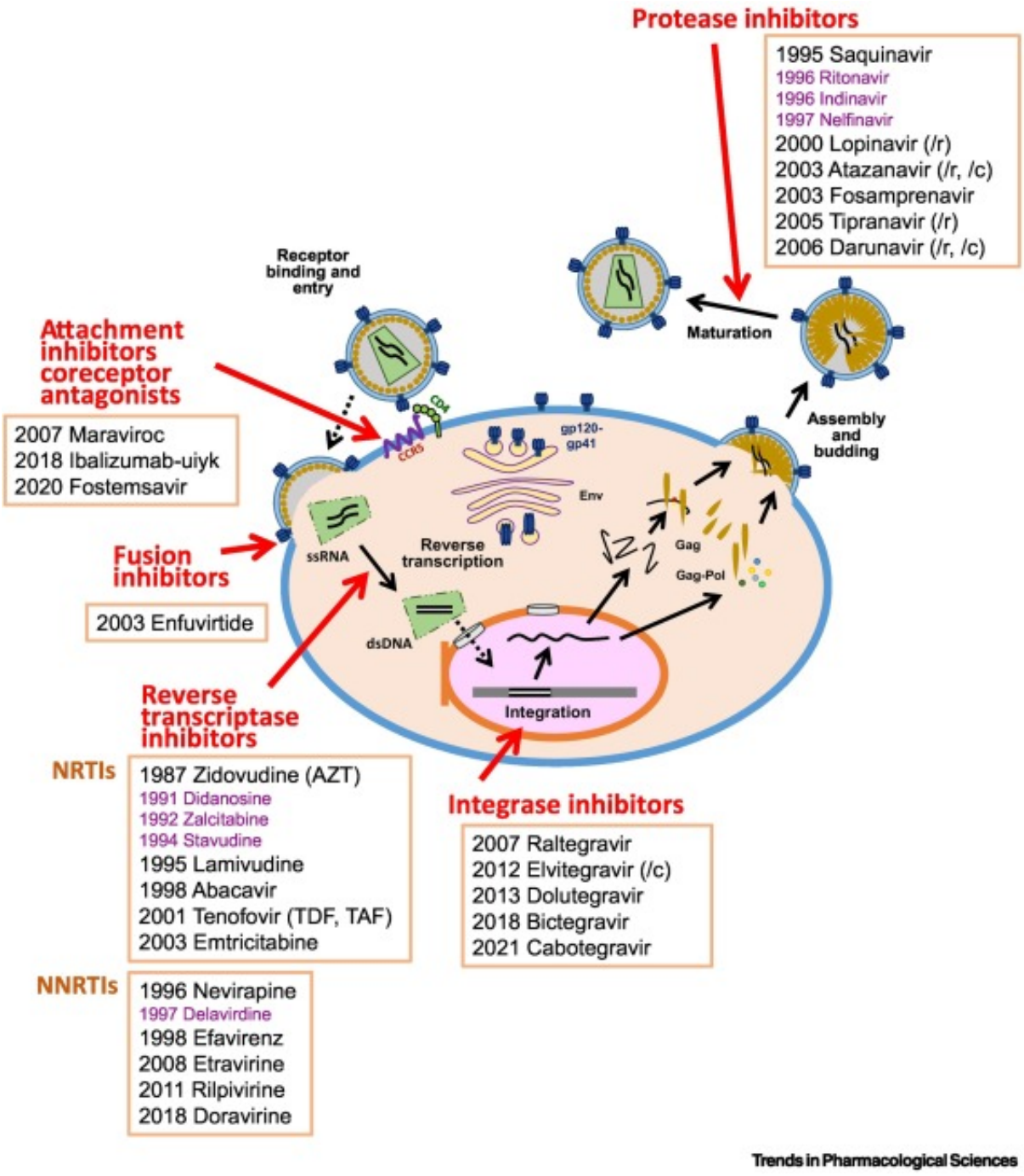

Figure 25. The mechanism of antiretroviral therapy and medicine. The mode of antiretroviral therapy actions includes co-receptor antagonists, fusion inhibitors, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), integrase inhibitors and protease inhibitors (PIs). [313]

The goal of cART includes decreasing viral replication, preventing the early onset of diseases with an inflammatory component, preventing the emergence of resistance to antiretrovirals,

improve or preserve the patient's quality of life and prevent the transmission of the virus (treatment as prevention or TasP).[314] However, cART is not curative since the viral reservoir cannot be completely eliminated and inflammation persist.

1.3.2.4. Prophylaxis therapies

Besides cART, there are two categories of HIV prophylaxis therapy which depend on the time therapy is started: Pre-exposure (PrEP) and post-exposure (PEP) prophylaxis.[315]

- PrEP therapy: It uses antiretroviral drugs in high-risk, HIV-negative patients to prevent future HIV infections. This therapy consists of a single daily pill containing two nucleoside reverse transcriptase inhibitors: tenofovir disoproxil fumarate and emtricitabine. When taken as prescribed, PrEP has been found to be over 90% effective in preventing HIV infection.[315–317]
- PEP therapy: It is a combination of three drugs, including tenofovir, emtricitabine like PrEP and raltegravir, an integrase inhibitor that prevents provirus insertion into the host genome.[318–320] This therapy must start within 72 hours following potential exposure and it might reduce the risk of infection by 80%.[315,318]

Until now, no universe therapy can cure HIV. Thus, effective HIV vaccines are urgently needed to fight against the HIV pandemic.

1.3.3. HIV Vaccine trials

The AIDS pandemic continues to challenge us with unique scientific and public health issues. The development of a preventive vaccine against HIV represents one of the principal axes in the fight against this virus.

1.3.3.1. Animal models

Animal models offer apparent advantages in studying HIV/AIDS. The animal models are pre-request, allowing for more invasive investigations of the disease and preclinical testing of drugs and vaccines efficacy. Two types of HIV animal models have been developed: small animal and non-human primate (NHP) models.[321–323]

- Small animal model: This model includes mice, rats, and rabbits. Among these small animal models, only the humanized mice, which are genetically immunocompromised and have been engrafted with human tissues to reconstitute the human immune system allows experiment challenge. This mouse model is now wildly used in HIV research.[322]
- The Non-human primate (NHP) models: Vaccine studies are hampered by the lack of animal models infected by HIV-1. In particular, studying the efficacy of Nabs depends on the ability to challenge the animals with viruses encoding the HIV-1 ENV gene.[324] Although NHP are not infected by HIV, the innate and adaptive immune responses that NHPs elicit against the related simian virus (SIV) are, for the most part, very similar to human responses.[325] Compared to African monkeys, which are the natural host of SIV, the non-natural monkey host suits for HIV/AIDS research. In addition, chimeric simian-human immunodeficiency viruses (SHIVs), SIV viruses contain the env from HIV, were developed to overcome the limitations due to SIV/HIV differences.[326,327] There are three old world monkey species that are routinely used as animal models for HIV/AIDS studies: the rhesus macaque (*Macaca mulatta*), the pig-tailed macaque (***Macaca nemestrina***) and the cynomolgus macaque (***Macaca fascicularis***) (Figure 26).[321,325] Besides them, the marmosets (***Callilthrix jacchus***) from new world monkey species and Ape species also play a vital role in HIV research to help us better understand the post-infection and post- vaccination immune response.[321,325,327]

Notheworthy, animal models are different to human. Especially, their humoral system is different. For example, Ig isotypes differ between species. Mice only have one IgA isotype, hominoid primates (with the exception of orangutan) have two IGHA, and rabbits have 15.[328–330] Therefore, the animals are good models to screen for new vaccine concepts but they give incomplete results on the safety and the immunogenicity that will be effectively observed in humans[321]

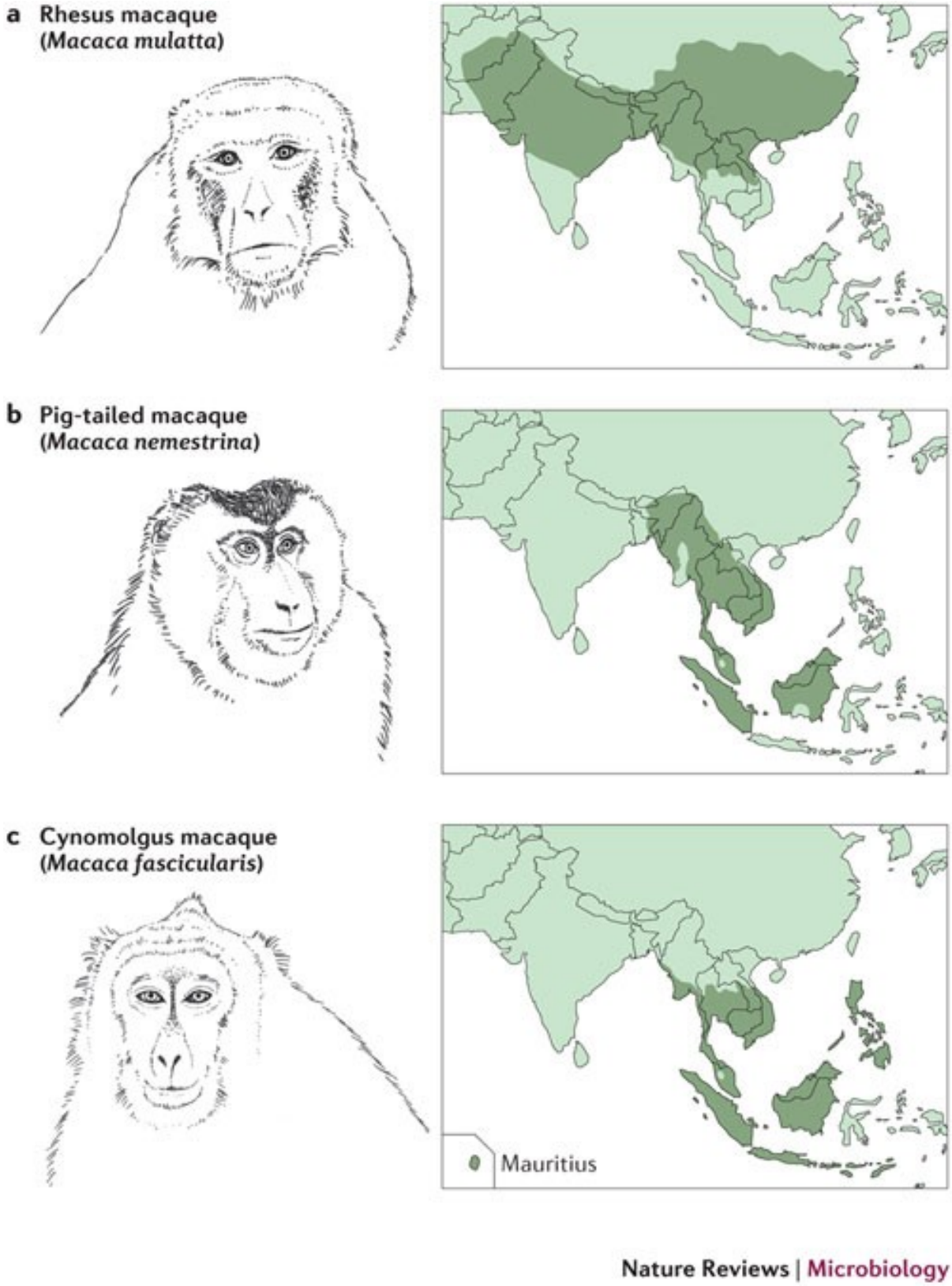

*Figure 26. The geographical range of the common monkeys used in NHP models. A) Rhesus macaque (**Macaca mulatta**), exsist in western India and Pakistan across China. B) Pig-tailed macaque (**Macaca nemestrina**) is native to Southeast Asia, Malaysia and Indonesia. C)*

*Cynomolgus macaque (**Macaca fascicularis**) is native to regions of Indochina, Malaysia, Indonesia and the Philippines.*[321]

1.3.3.2. Immunogen

Several HIV vaccine designs are available and perform in different vaccine trials (Figure 27).

1.3.3.2.1. Envelop proteins

The structure of recombinantly produced Env is thought to be critical to HIV-1 vaccine design because it can display bNAb epitopes in a conformation-dependent manner that may stimulate the bNAb unmutated common ancestors.[55,331,332] The first two phase III trials using the env protein as vaccine is VAX003 and VAC004. Sadly, there is no protectivity shown in these two trails.[333–336] Nowadays, there are several strategies to improve and stabilize the native-env-like trimers.[337–342] In animal models, the new modified trimers were found to induce tier 2 neutralizing Abs and deserved testing in human clinical trials.[343–345] Additional modified trimers need to be developed. Especially, envs focusing on conserved neutralizing epitopes need to be constructed in order to induce Abs with broad activity controling virus immune escape.[346]

1.3.3.2.2. Vectors expressing envs

Besides improving the proteins used in HIV vaccine, the safety profile, immunogenicity, and variety of available candidates make the nonreplicating viral vectors attractive in HIV vaccine development.[347] They are mainly four types of nonreplicating vectors used in HIV vaccines:

- Poxvirus vectors: The canarypox (ALVAC), modified vaccinia virus Ankara (MVA), Copenhagen-derived NYVAC, and fowlpox (FWPV) belong to this category. Studies show this type of vector can increased HIV-specific T-cell responses and expanded pre-existing T cell responses. [348–350] In prophylactic clinical trials, the attenuated poxvirus vectors ALVAC, MVA, NYVAC and fowlpox have proven to be suitable activators of specific immune responses (Review in ref [351]).[351] MVA and NYVAC are given higher immune response parameters than the other vectors.[351,352]
- Adenoviruses: This category includes Adenovirus serotype 5 (Ad5), adenovirus serotype 26 (Ad26), adenovirus serotype 35 (Ad35) etc.[353–355] Ad5 has been well-

studied and used in Step study (HVTN505), it shown no efficacy but elicited IFN-g ELISPOT responses and polyfunctional T cells.[356,357] Ad26 is used in the *Ad26.COV2.S* vaccine (Johnson & Johnson) to fight against the Sars-Cov-2 pandemic.[358,359] In HIV vaccine studies, Ad26 utilizes different cellular receptors, exhibits different in vivo tropism, interacts with dendritic cells and improves preclinical protective efficacy compared with Ad5.[360–364] Adenovirus 35 (Ad35) offers similar advantages to Ad26. It can elicit more robust cellular immune responses to HIV proteins.[365]

- Rhabdoviruses: Rhabdovirus vector as vesicular stomatitis virus (VSV) are suitable HIV vaccines vectors. They has become the focus of intense research due to their low seroprevalence and little pre-existing immunity in humans.[366–370] Moreover, they are able to replicate at high titers without safety issues. They are therefore good immunogens that may elicit a potent anti-HIV Env humoral response and may provide adequate or optimal protection.
- Alphaviruses: The AVX101 alphavirus-based HIV vaccine was studied in two consecutive phase I clinical trials (HVTN 040 and 059) and passed the safety test. However, the cellular and humoral immune responses it induced were limited.[371]

1.3.3.2.3. DNA and RNA vaccine

DNA-based vaccines have excellent immunogenicity in animals and are easy to manufacture, which makes excellent scalability, and storage.[372] Their poor immunogenicity in human can be improved with intradermal delivery or administration in conjunction with molecular adjuvants.[373–376] The phase I HVTN087 trial using HIV-1 DNA vaccine showed increased CD8+ T-cell responses but decreased CD4+ responses compared to vector-based immunization. [375,377]

RNA based vaccines provides us a fast responses to Covid-19 pandemics. [378,379] This type of vaccines can induce high anti-HIV protective Abs and CD4+ cell responses in animal models.[380,381] In a recent study, mRNA vaccine shows the ability of inducing tier 2 NAbs and decreasing the risk of SHIV infection in monkeys.[382] Until now, there is no HIV mRNA vaccine entering human clinical trial.

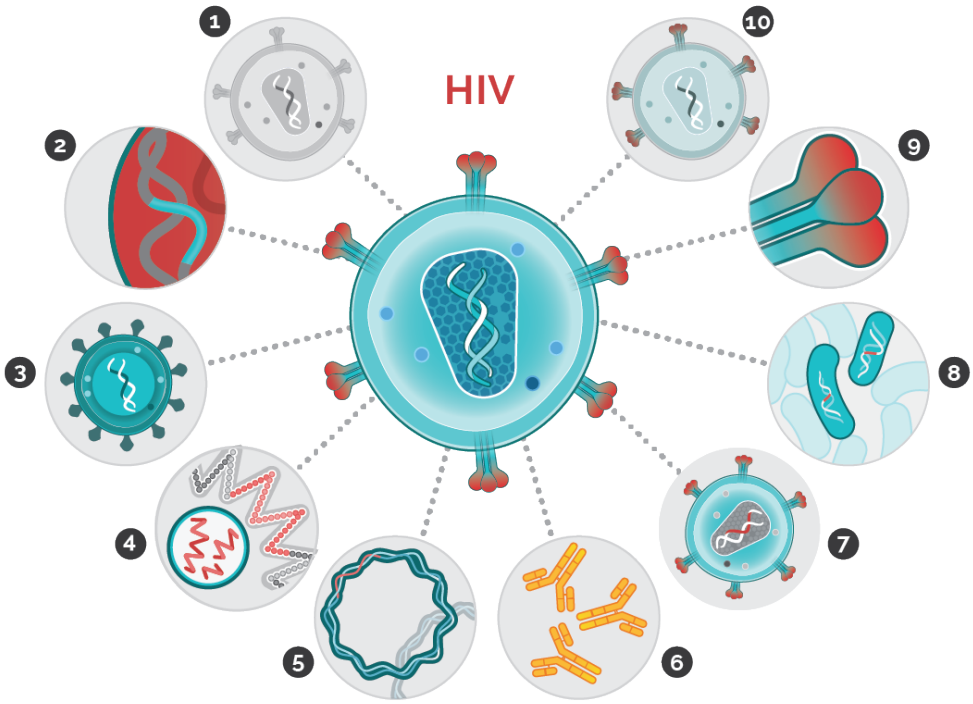

Figure 27. Current HIV vaccine Designs. 1) Whole-inactivated / inactivated HIV; 2) synthetic peptide / laboratory-made piece of protein; 3) recombinant viral vector / another virus carries pieces of HIV; 4) mRNA / mRNA carries pieces of HIV; 5) DNA / DNA carries pieces of HIV; 6) broadly neutralizing antibodies / binds to and neutralizes HIV; 7) virus-like particles / same shape as HIV, insides changed; 8) recombinant bacterial vector / bacteria used to carry pieces of HIV; 9) recombinant sub-unit / HIV protein made in a lab; 10) live-attenuated / weakened HIV (HVTN, Lisa Donohue). [383]

1.3.3.3. Adjuvants

Adjuvants are critical to enhance the immunogen response. They will increase humoral response's magnitude and persistence of the immunogen used for vaccination. Still, the characteristics of the immunogens may sometimes be altered by adjuvants. Protective response to vaccination has been exceptionally challenging for HIV vaccination. Several adjuvants have been applied on HIV vaccine (Figure 28)[384,385], but the immune response still need to be improved. [385,386] Tailoring adjuvant choices may help to improve the immune response and consequently HIV vaccines protection.

Adjuvant	Description	Proposed Mechanism
placebo	alum only no antigen	Negative control
alum	Alhydrogel® Used in VAX003, VAX004, RV144	Most used, broad safety profile across both antigens and populations Immunostimulatory effect includes both antigen retention and immune cell signaling
MPL	Monophosphoryl lipid A*	MPL potentiates the immune system as a toll like receptor 4 (TLR4) agonist
Liposomal MPL	MPL and antigen in oil-in-water emulsion adsorbed to Alhydrogel®	Oil-in-water emulsions enhances recruitment of macrophages to injection site
MF59	oil-in-water emulsion comprised of squalene and surfactants, Tween80 and Span85 Used in HVTN702 and HVTN 100	In context of influenza vaccines, Ag specific antibody titers increase and it is useful in antigen sparing.
MF59 + MTP-PE	Muramyl tripeptide-phosphatidyl ethanolamine (MTP-PE)	MTP-PE is an immunopotentiator acting as a NOD2 ligand
SAF/2	Syntex adjuvant formulation, oil-in-water emulsion comprised of squalene and surfactants, pluronic L121 and Tween 80	In contrast to MF59, the chemical properties of L121 bind antigen proteins to emulsion droplet surface
SAF/2 MDP	Muramyl dipeptide (MDP)	MDP is another immunopotentiator and NOD2 ligand, smaller in size than MTP-PE

Figure 28. The adjuvants uses in HIV vaccine research (Modified from Xu et al. 2022).[385]

1.3.3.4. Immunize strategies

Several immunization strategies were tested in previous HIV vaccine clinical trials.[387,388] They have been developed based on difference immune approaches according to vectors, adjuvants and ethnicities (Table 1). Four vaccine concepts have been evaluated in efficacy trials: protein subunit vaccine, recombinant adenovirus vector, vector prime followed by a protein subunit boost, and DNA prime followed by recombinant adenovirus vector boost. The first vaccine trials aiming to develop immunogens capable of inducing broad-spectrum NAbs from envelope glycoproteins (recombinant gp120 and gp160 proteins) failed. Thereafter, the vector prime followed by a protein subunit boost in the RV144 vaccine trial showed 31% efficacy.[245] The other two concepts, recombinant adenovirus vector or DNA prime followed by recombinant adenovirus vector boost did not show efficacy.

Several vaccine strategies has already been tested. The difficulties for these vaccine strategies is to generate the appropriate immunogen at the right place and at the right time to orchestrate a potent and durable response.[389,390] New vaccine platforms, such as nanoparticles, hydrogels, osmotic pump and microneedles has be engineered to spatially and temporally control the interactions of vaccine components with immune cells.[391–396] New immunization strategies are still desperately needed to improve efficacy, by inducing more

sustain functional antibody responses, T cell responses and/or long-lasting immune memory response.

1.3.3.5. Current vaccine trails

Despite the difficulties encountered during the development of vaccine trials, the search for new immunogens, vectors, adjuvants, and immune strategies capable of inducing effective humoral and cellular responses continues. However, so far, no human HIV vaccine trial has been shown to be effective in inducing broad-spectrum NAbs.[260,333,397–400] Some research teams focused on developing immunogens capable of inducing an effective cytotoxic cellular response, particularly cytotoxic CD8+ T lymphocytes.[401,402]

Sadly, there is no available vaccine to fight the HIV pandemic yet, but the precious experience from HIV research assists the Sars-Cov-2 vaccine development and saves millions of lives.

Vaccine Trial	Year	Location	Target population	Vaccine	Ab function		Fc Receptor			Vaccine Efficacy	Ref
					Neutralization	Fc mediate	FcR	Polymorphism	Association with risk		
VaxSyn	1987	Canada	72 adults	Recombinant envelope glycoprotein subunit (rgp160) of HIV	Low Tier 1	NFD	-	-	-	No	403,404
HIVAC-1e	1988	USA	35 male adults	Recombinant vaccinia virus designed to express HIV gp160	N/F	NFD	-	-	-	No	405
Vax004	1998-2002	North America	5,417 MSM and 300 women	AIDSVAX B/B gp120 with alum	Tier 1	ADCC ADCP	FCGR2A	rs1801274	↓	No	255,295,406–408
							FCGR3A	rs397991	↑		
Vax003	1999-2003	Thailand	2,545 mem and women IDUs	AIDSVAX B/E gp120 with alum	Tier 1	ADCC	-	-	-	No	333,406,408,409
STEP/HVTN 502	2004-2007	North America, Caribbean South America and Australia	3,000 MSM and heterosexual men and women	MRKAd5 HIV-1 gag/pol/nef trivalent vaccine	Low Tier 1	NFD	-	-	-	No	356,410–412

Phambili/HVTN503	2003-2007	South Africa	801 adults	rAd5 (gag/ pol/ nef)	Low Tier 1	NFD	-	-	-	No	413–415
RV144	2003-2009	Thailand	16,402 community-risk men and women	ALVAC-HIV (vCP1521) and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	FCGR2C	rs114945036 rs138747765 rs78603008	↓	31.2%	166,294,409,413,416–420
HVTN204	2005-2008	USA and South Africa	480 HIV-negative adults	Three vaccines with DNA encoding HIV-1 subtype A gp120, subtype B gp140ΔCFI, subtype C Gag. Follow by a booster rAd5 expressing the same env gene (subtype A, B, C) and a subtype B Gag-Pol fusion protein.	Low Tier 1	-	-	-	-	No	397,421
HVTN505	2009-2013	USA	2,504 men or transgender women who have sex with men	Three vaccinations with DNA encoding HIV clade B gag, pol and nef as well as env from HIV clades A, B and C followed by an Ad5 vector-	Low Tier 1	ADCC ADCP	FCGR2A	rs2165088	↓	No	194,296,397
							FCGR2C	rs138747765 rs78603008 rs373013207 rs201984478	↑		
							FcGR3B	rs34085961 rs34322334	↑		

				based vaccine encoding clade B gag and pol as well as env from clades A, B and C				rs61803026			
							FCGR2B	rs6666965 rs6665610	↓		
HVTN305	2012-2017	Thailand	162 women and men	ALVAC-HIV and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	-	-	-	No	416,422,423
HVTN306	2013-2020	Thailand	360 men and women aged 20-40 years old	ALVAC-HIV and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	-	-	-	No	424,425
HVTN097	2012-2013	South Africa	100 black Africans (men and women) aged 18-40 years old	ALVAC-HIV (vCP1521) and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	-	-	-	No	426,427
HVTN100	2015-2018	South Africa	252 men and women	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59	Low Tier 1	ADCC ADCP	-	-	-	No	428–430
HVTN702	2016-2020	South Africa	5,400 men and women	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59	Low Tier 1	-	-	-	-	No	431–434

HVTN 703	2016-2020	Sub-Saharan Africa	1900 women	VRC01 broadly neutralizing monoclonal antibody	Low Tier 1	-	-	-	-	No	435–437
HVTN 704	2016-2020	Brazil Peru Switzerland, United States	2701 men and transgender persons	VRC01 broadly neutralizing monoclonal antibody	Low Tier 1	-	-	-	-	No	435–437
HVTN705/ Imbokodo	2017-2021	Sub-Saharan Africa	2,637 women ages 18 to 35 years	Ad26.Mos4.HIV, adjuvanted clade C and Mosaic gp140 HIV bivalent vaccine	-	-	-	-	-	Comparing with RV144, unable to improve the efficacy on Sub-Saharan Africa women	419,438–440
HVTN706/ Mosaico	2017-2023	America and Europe	3,800 HIV-negative men and transgender people aged 18 to 60 years who have sex with men and/or transgender people	Ad26.Mos4.HIV, adjuvanted clade C and Mosaic gp140 HIV bivalent vaccine	-	-	-	-	-	No	438,441,442

PrepVacc	2018-2024	Mozambique South Africa, Tanzania, and Uganda	1668 adults	DNA/AIDSVAX and DNA/CN54gp140 + MVA/CN54gp140) with PrEP	-	-	-	-	-	Pending	438,443

Table 1. Illustration of completed and documented or on-going major phase 1b to phase 3 HIV trials that analyzed the Ab and/or Fc Receptor functions. NFD: no Fc-mediated function detected, –: no related publications found

2. Objective

The current vaccine approaches expect to induce neutralizing as well as Fc-mediated inhibitory Abs. Therefore, the type and subtype of the immunoglobulins (Ig) induced, and the quantity and quality of the Fc-receptors (FcRs) expressed on immune cells will need to be taken into consideration. As these factors varied according to ethnicity, future vaccines may need to adapt their strategies to the genetic ethnicity background of the targeted population in addition to the choice of immunogen according to the local HIV circulating strains.

The aim of my thesis is to select new vaccine strategies with improved Ab responses. We have therefore characterize the humoral immune response, especially the neutralization and Fc-mediated inhibition by Abs induced:

1. early after HIV-1 infection (PRIMO Cohort)
2. by New vaccine strategies (Horizon 2020 and Labex VRI)
3. in association with genetic ethnic background (HVTN initiative program)

My thesis work is presented in the form of two published manuscripts, one submitted manuscript and one manuscript in preparation, which will soon be submitted for publication.

In the appendix are presented published works to which I have contributed within the laboratory.

3. Material and Methods

3.1. Material and methods

3.1.1. Cohorts

3.1.1.1. PRIMO

The primo Cohort is a cohort of patient sampled early after the detection of HIV infection. Sera from 24 patients from the ANRS PRIMO cohort were obtained at 2 time points: Day 0 (D0) corresponding to the day of inclusion in the cohort (day of detection, less than 3 months after infection) and 6 or 12 months later (M6/M12). As this is a historical cohort, early treatment at HIV detection was not yet recommended. The patients selected were therefore not treated at the time of sample collection. IgG were purified by protein G columns (Sepharose™ 4 Fast Flow, Amersham Biosciences) according to manufacture instructions.

All subjects provided their written informed consent to give their blood sample for researchpurposes. The PRIMO cohort was funded and sponsored by ANRS and approved by the Ile de France III Ethics Committee, July 2, 1996, with amendment N°15 approved June 08. The study was conducted according to the principles expressed in the Helsinki Declaration.

3.1.2. Vaccine trails

3.1.2.1. EHVA studies on animal model

Different vaccine trails were developed under the Horizon 2020 European HIV Vaccine Alliance (EHVA) consortium. The aim of this consortium was to discover and evaluate novel prophylactic and therapeutic vaccine candidates. We were involved in the characterization of the antibody response induced following immunization with novel vaccine immunogens, adjuvants and delivery routes.

3.1.2.2. HVTN204

A grand from HVTN Initiatives Program (HIP) was obtained as a Pilot study for systematic comparison of HIV vaccine-induced immune responses between Caucasian and

African/African American populations. The proposed study aims to systematically compare HIV vaccine-induced immune responses in African vs Caucasian vaccinees in relation to ethnicity, host genetic and geographic background. For this study HVTN204 vaccine trial was chosen as this vaccine was preform using with exactly the same protocol in South Africa and US. We receive serum and cryopreserved cells form the HVTN repository to perform this comparison.

3.1.2.3. VRI06

As participants of the Labex Vaccine Research Institute (VRI) sponsored for more than 10 years in France, we were actively involved in the characterization of the Ab response induced by the phase I clinical trial VRI06. This project aims to increase antobody response via using the envelopes targeting CD40 on the surface of DC cells.

3.1.3. ELISA (enzyme-linked immunosorbent assay)

3.1.3.1. Total and HIV- specific Ab detection

MAXISORP 96-well plates (Sigma) were coated overnight with a sheep anti-human IgG/ IgA (1 µg/mL in carbonate buffer, Binding Site) overnight at 4 degree for the detection of total IgG and IgA. Plates coated with gp160 MN LAI (A hybrid oligomeric gp160 Env with gp120 derived from HIV-1 MN and gp41 derived from HIV-1 LAI, subtype B), gp160 MN LAI did (Recombinant Env with gp120 from HIV-1 92TH023 linked to gp41 from LAI, with a deletion in the immunodominant region, subtype CRF01-AE), gp70 V1V2 (a murine leukemia virus gp70 scaffold containing HIV-1 gp120 variable regions 1 and 2 from HIV-1 isolates 92TH023, subtype AE) and gp140 ConS (corresponding to the consensus envelope antigen, subtype B) (0.5µg/mL in carbonate buffer for HIV-specific IgGs and 2 µg/mL in carbonate buffer for HIV-specific IgAs) were used to detect the anti-gp 160 IgGs, anti-gp70 V1V2 IgGs, anti-gp140 IgGs and anti-gp160 IgAs.

Plates were washed and saturated with PBS (Gibco) contain 10% milk (1h, at 37 °C) and with the diluted sera (2 h, at 37 °C). Then plates were washed and a secondary goat anti-human IgG-HRP (HorseRadish Peroxidase) or anti-human IgA-HRP added (1 h at 37 °C, 0.2 µg/mL in

PBS, Southern Biotech). After all the incubation, 100 µL of TMB (3,3',5,5'-tetramethylbenzidine) (KPL) substrate is added before a kinetic reading. After 30 minutes of incubation in the dark and at room temperature, the reaction is stopped with sulfuric acid (25 µL of 1 M H_2SO_4 per well) and the endpoint optical density (OD) is read at 450 nm (reference 650 nm).

The total IgG and IgA concentrations are calculated using a range of concentrations established with a reference serum.

3.1.4. Cellular culture

3.1.4.1. Cell lines

3.1.4.1.1. TZM-bl

TZM-bl cell is a HeLa-derived indicator, adherent cell line. These cells express CD4 and CCR5 as well as the luciferase reporter gene under the control of the LTR regions of HIV-1[444]

The maintenance of these cells is done every three to four days. They are cultured in 75 cm^2 culture dishes in DMEM medium (Dulbecco/Vogt modified Eagle's minimal essential medium) 1 g/L of glucose (Gibco) supplemented with 10% FBS (fetal Bovine serum) (Dutscher) and supplemented with penicillin (50 IU/mL) and streptomycin (50 ng/mL) (Gibco). After removing the depleted culture medium by pouring it into a waste bottle, the cells are washed with 5 mL of cold PBS. Then 2.5 mL of room temperature trypsin-EDTA (tetraacetic ethylenediamine) (Gibco) is added to cover the cell layer for 1 minute at room temperature. Eliminating part of the trypsin then put the culture dish in the incubator at 37°C, 5% CO_2 for 3 minutes to enhance the action of the trypsin. The cells are later resuspended in 7 mL of fresh 10% FBS DMEM medium. 1 mL of cell suspension is taken up in a 75 cm^2 culture dish with 12 mL of 10% FBS DMEM medium. The cells are finally incubated at 37°C, 5% CO_2.

3.1.4.1.2. CEM.NKR.CCR5

CEM.NKR.CCR5 are suspension cells from a cloned human T lymphoblastoid cell line (T-LCL). They are resistant to lysis by NK cells and express the HIV-1 coreceptor CCR5. [445]

The maintenance of these cells is done every three to four days. They are cultured in 75 cm^2 culture dishes in RPMI-1640 medium (Roswell Park Memorial Institute medium) (Gibco) supplemented with 10% FBS (Dutscher) and supplemented with penicillin (50 IU/mL) and streptomycin (50ng/mL) (Gibco). 1.5 mL of cell suspension are diluted in 13 mL of 10% FBS RPMI medium. The cells are finally incubated at 37°C, 5% CO_2.

3.1.4.1.3. HEK293T

HEK293T are adherent cells. It is a human cell line, derived from the HEK 293 cell line (human embryonic kidney 293 cells), which expresses a mutant version of the large T antigen of SV40.[446] The maintenance of these cells is done every three days on the same principle as the TZM-bl. They are cultured in DMEM 4.5 g/L glucose medium (Gibco) supplemented with 10% FBS (Dutscher) and supplemented with penicillin (50 IU/mL) and streptomycin (50 ng/mL) (Gibco).

3.1.4.2. Primary cells

Peripheral blood mononuclear cells (PBMC) are isolated from leucocyte concentrates (buffy coat) of anonymous donors who are HIV-negative and hepatitis C virus (HCV)-negative. All the buffy coat are from the French Blood Establishment (EFS).

To isolate the PBMC, the Ficoll® gradient sedimentation technique is used (Eurobio).The leukocyte concentrate is diluted 1/4 in PBS Ca2+/Mg2+ (containing 0.68 mM CaCl2 and 0.05 mM MgCl2) (Gibco), supplemented with citrate at 1/100 (9 mM tri-sodium citrate dihydrate and 1.55 mM citric acid monohydrate). Then, 25 mL of this cell dilution are gently placed on 20 mL of separation medium (Ficoll®) with a density of 1.078. The tubes are then centrifuged for 25 minutes at 1200 g and the cells at the interface are removed and then washed three times with PBS. The cells thus obtained are taken up in 50 mL of PBS and counted using a KOVA® Glasstic® Slide (Hycor) counting cell after 1/10 dilution in trypan blue.

3.1.5. Virus Preparation

Pseudoviruses used in Neutralization assay were produced by cotransfecting 293T cells with HIV-1 env expression plasmid and the env-deficient HIV-1 backbone plasmid (pSG3ΔEnv).

3.1.6. Neutralization Tests

Pseudoviruses used in Neutralization assay were produced by co-transfecting 293T cells with HIV-1 env expression plasmid and the env-deficient HIV-1 backbone plasmid (pSG3ΔEnv).

The neutralizing ability is determined by a luciferase test and the values obtained in RLU (relative light unit or relative light unit) are expressed as a percentage of the control. The samples are diluted in 10% FBS DMEM. Serial dilution of sera beginning at 1 : 20 dilution and 25 µL of these dilutions are deposited in a 96-well plate and incubated for 1 hour at 37° C with 25 µL of diluted virus. 25 µL of TZM-bl at 4.105 cells/mL diluted in DMEM 10% FBS+DEAE (diethylaminoethyl) Dextran (Sigma) at 37.5 µg/mL are then added. After 36 hours, the cells are incubated for 10 minutes at room temperature and in the dark with Bright-Glo™ (firefly luciferase assay system) (Promega) diluted 1/2 in sterile water. The reaction is then stopped by adding 25 µL of sterile water and the luminescence is detected by passing the plates through a luminometer (PerkinElmer).

The inhibitory reciprocal dilution 50% (IRD50) were defined as the sample's dilution that can cause 50% reduction of relative luminescence units. IRD50> 60 will be considered as positive.

3.1.7. Cell lysis activity test (Antibody dependent cellular cytotoxicity: ADCC)

The ADCC assay was performed using purified PBMCs as effector cells and the CEM.NKR.CCR5 cell line as target cells. four HIV-1 clones expressing the viral envelope and the Renilla luciferase reporter gene were used for infection.

Serial fold diluted samples (beginning at 1/50 for sera) were added to the 96 well flat bottom plates. 4 day-post infection, HIV-1 infected CEM.NKR.CCR5 cells were incubated with PBMCs (30:1, effector: target ratio) in the medium contains RPMI-1640, 10% FBS, and 50 IU interleukin-2 (R&D Systems) for 5 hours. Renilla-Glo luciferse assay substrate substrate (Promega) was added to determine the luminescence intensity generated by infected target

cells. To define lysis of target cells by Abs, the percentage of infected target cell in presence of Abs was normalized to control wells without Abs using the following formula:

$$\%\ \text{of lysis} = \left(\frac{\text{RLU of infected target cells with effectors-RLU of infected target cells with effectors and samples}}{\text{RLU of infected target cells with effectors}} \right) * 100$$

The AUC was calculated as the integrated background-subtracted net activity truncated above zero over a range of dilutions using the trapezoidal method.

For the HIV-1 that were not modified to express the luciferase, we developed another ADCC assay. The CEMNKR cells infected or non-infected were stained with arboxyfluorescein Diacetate Succinimidyl Ester (CFSE) in day 4 before incubating with PBMC. Serial fold diluted samples (beginning at 1/50 for sera) were added to the 96 wel half-areal flat bottom plates togeteher with cell mixture: 4 day-post infection, HIV-1 infected CEM.NKR.CCR5 cells were incubated with PBMCs (30:1, effector: target ratio) in the medium contains RPMI-1640, 10% FBS, and 50 IU interleukin-2 (R&D Systems) for 5 hours. P24 stained (APC) was performed and infected cells were measured by flow cytometry.

3.1.8. FcR

Genomic DNA was extracted from 3× 10^6 PBMCs with the Qiagen QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following manufacturer's instructions. The rs1801274 (H131R) in FCGR2A (FcɣRIIa) and rs10800309 in FCGR2A (FcɣRIIa) were genotyped with custom Taqman assays (Thermo Fisher Scientific, Waltham, MA, USA).[294]

3.1.9. Flow cytometry

To characterize the target cell population, cells are marked by fluorescent antibodies specific for the various chosen determinants. The acquisition of the results is carried out on an Attune™ NxT Acoustic Focusing Cytometer (ThermoFisher), calibrated by Attune™ performance tracking beads (performance test, ThermoFisher) to ensure the quality and consistency of the measurements between the different experiments.

3.1.10. Statistical analyzes

Comparisons between groups are analyzed using a two-tailed Mann-Whitney test. P values less than 0.05 are considered statistically significant (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). Part of the statistical analyzes are performed with Prism 10 software (GraphPad, San Diego, CA).

4. Result

4.1. Analysis of the early Ab response following HIV infection

As vaccine need to induce an early effective immune response, we first focused our study on the response induced during early acute infection.

This study aimed to improve our knowledge of early potential Ab response in order to induce such Abs by vaccination.

We analyzed the functional Ab response induce early after infection in sera collected from the ANRS PRIMO cohort at 2 time-points: Day 0 (D0) corresponding to the day of inclusion in the cohort (day of detection, less than 3 months after infection) and 6 or 12 months later (M6/M12). As it is an historical cohort, the patients selected were not treated at the time of sample collection.

In collaboration with Julie Lucas, student in our group, we detected broadly neutralizing Abs (bNabs) in some sera collected early after infection. This activity differ from what was previously publish. Indeed, previous studies detected bNabs against non-T/F viruses only after one to two years of infection. Noteworthy, in our study, we analyzed neutralizing activity directed against Transmitted/Founder (T/F) viruses and not against the Tier 2 primary viruses classically used for neutralization assessment. Results from this study have been publish in AIDS (see publication below). For this study, I participated in neutralizing activity detection, Ab detection, IgG purification from serum samples to confirm that the activity was related to the Ab, not other particles.

The early detection of bNabs against T/F viruses in infected individuals open new perspective for the development of such functional Abs by vaccination.

4.1.1. Publication 1

OPEN

CONCISE COMMUNICATION

Identification of early-induced broadly neutralizing activities against transmitted founder HIV strains

Julie Lucas[a], Li-Yun Lin[a], Nicodème Paul[a], Géraldine Laumond[a], Jéromine Klingler[a], Sylvie Schmidt[a], Julia Frappier[a], Asma Essat[b], Laurence Meyer[b,c], Alicia Castro Gordon[d], Cécile Goujard[b,d], Seiamak Bahram[a,e], Christiane Moog[a,f], for ANRS CO6 Primo Cohort

Objectives: Broadly neutralizing antibodies have been proposed as key actors for HIV vaccine development. However, they display features of highly matured antibodies, hampering their induction by vaccination. As protective broadly neutralizing antibodies should be induced rapidly after vaccination and should neutralize the early-transmitted founder (T/F) viruses, we searched whether such antibodies may be induced following HIV infection.

Design: Sera were collected during acute infection (Day 0) and at viral set point (Month 6/12) and the neutralizing activity against T/F strains was investigated. Neutralizing activity in sera collected from chronic progressor was analyzed in parallel.

Methods: We compared neutralizing activity against T/F strains with neutralizing activity against non-T/F strains using the conventional TZM-bL neutralizing assay.

Results: We found neutralizing antibodies (nAbs) preferentially directed against T/F viruses in sera collected shortly after infection. This humoral response evolved by shifting to nAbs directed against non-T/F strains.

Conclusion: Although features associated with nAbs directed against T/F viruses need further investigations, these early-induced nAbs may display lesser maturation characteristics; therefore, this might increase their interest for future vaccine designs.

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Keywords: acute infection, HIV, neutralizing antibodies, transmitted/founders, vaccine

[a]Laboratoire d'ImmunoRhumatologie Moléculaire, Institut national de la santé et de la recherche médicale (INSERM) UMR_S 1109, Institut thématique interdisciplinaire (ITI) de Médecine de Précision de Strasbourg, Transplantex NG, Faculté de Médecine, Fédération Hospitalo-Universitaire OMICARE, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, [b]Centre de Recherche en Epidémiologie et Santé des Populations (CESP), INSERM U1018, Université Paris-Saclay, Le Kremlin-Bicêtre, France, [c]AP-HP, Service d'Epidémiologie et de Santé publique, Hôpital Bicêtre, Le Kremlin-Bicêtre, France, [d]AP-HP, Service de Médecine interne et d'Immunologie clinique , Hôpital Bicêtre, Le Kremlin-Bicêtre, France, [e]Laboratoire d'Immunologie, Plateau Technique de Biologie, Pôle de Biologie, Nouvel Hôpital Civil, Strasbourg, and [f]Vaccine Research Institute (VRI), Paris, France.

Correspondence to Christiane Moog, U1109, Institute of Virology, 3 rue Koeberlé, 67000 Strasbourg, France.
Tel: +33 368853742; e-mail: c.moog@unistra.fr
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Introduction

Treatment with anti-HIV broadly neutralizing antibodies (bnAbs) has shown that the predominant mechanism of antiviral activity of these antibodies is through neutralization of virus entry, their Fc-mediated functions contributing only to 21% of plasma-virus decay slopes [1]. BnAbs are therefore considered to be the Grail for the development of an effective HIV vaccine. Kinetics of neutralizing antibody (nAb) induction have been largely studied. Following HIV infection, autologous nAbs are first detected [2], followed about 1 year by heterologous nAbs [3,4]. Neutralization breadth against Tier 2 strains is only detected in a subset of individuals during the chronic phase and following numerous rounds of viral replication and neutralization escape [4]. This delay of bnAbs response led to the postulate that the presence of viral quasispecies is needed to drive the humoral response for the selection of B cells directed to conserved epitopes [5]. Vaccine efforts have therefore focused on targeting conserved epitopes under evolutionary constraints thereby limiting the virus's adaptive space. However, so far, these approaches have failed *in vivo*. Indeed, antibodies displaying the characteristics of bnAbs have all the same features with high levels of somatic hypermutation, insertions and/or deletions (indels) and, often, unusually long CDRH3 (heavy chain complementarity-determining regions) [4,6]. These specific Ab characteristics have strong disadvantages in the setting of vaccine strategies, as they require long-lasting maturation and the path to bnAbs induction will therefore be difficult.

Another vaccine approach would be to focus on transmitted founder (T/F) viruses that have established the actual infection, given the hypothesis that transmissible phenotypes are limited or antigenically conserved [7]. This is indeed the case during sexual HIV transmission as only a limited number of HIV variants are transmitted. The unique traits of these T/F variants and their evolutionary trajectories with antibody response remain an unresolved question [8].

Different studies have been performed to identify common features for T/F viruses. Shorter V1V2 regions [9], lower glycosylation sites [3] and less efficient binding to $CD4^+$ [10] have been identified. These characteristics may increase infectivity and affinity to mucosal receptors or reduce sensitivity to neutralization [9,11]. However, these special features could not be confirmed by others describing increased neutralizing sensitivity [12] and less infectivity for viruses isolated early after infection [11], further suggesting that the pattern of Env evolution observed may be specific to the host and infecting strains [11]. The explanation of the viral bottleneck selecting T/F strains remains elusive. However, vaccine should induce antibodies able to neutralize these selected transmitted variants.

As only a limited number of HIV variants are acquired during transmission, we hypothesized that less potent nAbs, with limited breadth, but nonetheless able to neutralize T/F strains may be sufficient for protection. Noteworthy, previous studies have analyzed the neutralization of T/F vs. chronic viruses but did not specifically focus on serum samples from very early infection. We searched for such antibodies in sera collected from infected individuals grouped according to the time following infection. Remarkably, we detected nAbs against T/F in some samples collected during early acute infection. Neutralization was waived over time in favor of nAbs against non-T/F viruses. These results demonstrate that nAbs against T/F strains can be induced rapidly the following infection. As they were induced rapidly, they may not display the highly maturated and difficult-to-induce profile of the nAbs detected after several years of infection. They may therefore be of high interest in future vaccine designs.

Methods

Patient cohorts

Sera from 24 patients from the ANRS PRIMO cohort were obtained at two time points: Day 0 (D0) corresponding to the day of inclusion in the cohort (day of detection, <3 months after infection) and 6 or 12 months later (M6/M12). As this is a historical cohort, the patients selected were not treated at the time of sample collection. We used a historical non-treated, HIV-infected chronic progressors cohort ($n = 16$) of individuals infected with a median of 8 years. These chronic progressors had normal CD4T cell levels (median of 696/mm^3 at the time of blood sampling) but high viral load and went on therapy shortly after sample collection because of disease progression. IgG were purified by protein G columns (Sepharose 4 Fast Flow, Amersham Biosciences, Amersham, UK) according to manufacturer instructions.

All patients provided their written informed consent to give their blood sample for research purposes. The PRIMO cohort was funded and sponsored by ANRS and approved by the Ile de France III Ethics Committee, 2 July 1996, with amendment No. 15 approved 8 June. The study was conducted according to the principles expressed in the Helsinki Declaration.

TZM-bl neutralization assays

The conventional TZM-bl neutralization assay was used [13]. Serial dilution of sera (beginning at 1 : 20 dilution) was tested for its ability to neutralize various HIV-1 strains (Supplemental Table S1, http://links.lww.com/QAD/C626), and the inhibitory reciprocal serum dilution 50% (IRD50), the inhibitory reciprocal serum dilution 70% (IRD70) or the inhibitory antibody concentration 50%

(IC50) were calculated [13]. The capacity of individuals' sera to neutralize murine leukemia virus (MuLV) was assessed as a control. Two sera (M6/12) with IRD more than 50 for MuLV pseudovirus were excluded from the study. Samples with values at least 50 IRD50 and samples with values of less than 1 μg/ml IC50 were recorded as positive for neutralizing activity.

HIV-specific Ig detection

HIV-specific antibodies directed against gp41S30, gp160 HIV MN LAI (kind gift from R. El Habib, Sanofi Pasteur) or trimer folded JRFL NFL TD (kind gift from R. Wyatt, Scripps Research, San Diego, CA, USA) were detected by ELISA as previously described [14].

Statistical analysis

The statistical significance (*P* values) was evaluated using a two-tailed paired (T/F versus non-T/F) Wilcoxon test of the median neutralizing activity of sera using the Prism software (GraphPad Software Inc., San Diego, California, USA).

Results

Distinct patterns of neutralizing antibodies against T/F variants compared with non-T/F strains over time

We searched for nAb in sera collected early after infection (D0), 6–12 months after infection (M6/12) or in sera from chronic progressors. In opposition to what has been previously described, antibodies able to neutralize some HIV strains were already detected at D0 of infection (Fig. 1a and Supplementary S2, http://links.lww.com/QAD/C625). In sera from patients 350 115, 440 138, 680 115, 750 518 and 751 206, IRD50 titers with values of at least 50 against T/F viruses were detected. At that time point, the median IRD50 was significantly higher for T/F viruses compared with non- T/F viruses ($P = 0.016$). 426c, CH077, CH058 and RHPA- T/F viruses were the best neutralized (Fig. 1d). Moreover, we analyzed the neutralizing activity after IgG purification. We found a similar neutralizing profile (Supplemental Fig. S1, http://links.lww.com/QAD/C625) indicating that the neutralization detected was not due to non-specific factors present in the sera. These results show that nAbs against some T/F strains can be induced very rapidly during acute infection.

At M6/12, Samples with IRD50 of at least 50 increased with the highest neutralizing activity in patients 330 209 and 940 106. At that time point neutralization was similar for T/F and non-T/F strains ($P = 0.14$) (Fig. 1b) and was recovered in the IgG fraction (Supplement Fig. S1, http://links.lww.com/QAD/C625).

In chronic progressors, on contrary, we detected significantly more neutralizing activities against non-T/F strains compared with T/F viruses ($P > 0.0005$), suggesting that the neutralizing activity is redirected against non-T/F viruses after several years of infection (Fig. 1c).

Indeed, by following the neutralizing profile over time, neutralizing activity against T/F viruses did not increases at M6/12 and in chronic progressors compared with a gradually increased neutralization for non-T/F Tier 2 and Tier 1 strains (Fig. 1d). The general profile of neutralizing responses depicted by the radar plot showed that the mean neutralizing activity against T/F viruses was similar for the three time points (Fig. 2a). Therefore, T/F strains may display special features supporting early nAb development by the humoral immune response.

Detection of cross-neutralizing activity against T/F viruses at D0

For the vaccine design, induction of cross-neutralization against different strains is essential for a large coverage of HIV protection. We hence analyzed the capacity of the sera to cross neutralize several strains (Fig. 2b). Significantly, 17% of sera collected at D0 already neutralized four to five of the seven T/F viruses analyzed with an IRD value of at least 50, whereas none of them cross-neutralized four to five non-T/F Tier 2 strains. Cross-neutralization of non-T/F viruses appeared later with 29 and 56% of sera neutralizing more than four non-T/F s Tier 2 strains at M6/12 and for chronic progressors, respectively. This cross-neutralization only slightly evolved for T/F viruses from 17 to 29% and 31% cross-neutralization of more than four T/F viruses at M6/12 and for chronic progressors, respectively. Similar profile with neutralization detected against T/F viruses at D0 was observed by using an IRD70 read out (Supplemental Fig. S2, http://links.lww.com/QAD/C625). Again, this point to a different neutralizing activity against T/F viruses that switch over time to nAbs against non-T/F strains. Whether this cross-neutralizing activity refers to bNAbs or to polyclonal responses with induction of various nAbs needs further investigation. Moreover, to identify specific env-binding features associated with this cross-neutralizing activity, we analyzed antibody-binding capacity to various envelop proteins (Supplemental Fig. S3, http://links.lww.com/QAD/C625). Binding to gp41 protein, gp160 monomer or JRFL-folded trimer did not allow to discriminate cross-neutralizing activity (colored dots) from the other samples (black dots) as the color dots were distributed all over the panel. More precise immunomapping should be performed to identify epitopes involved in cross-neutralize activity in sera collected at D0.

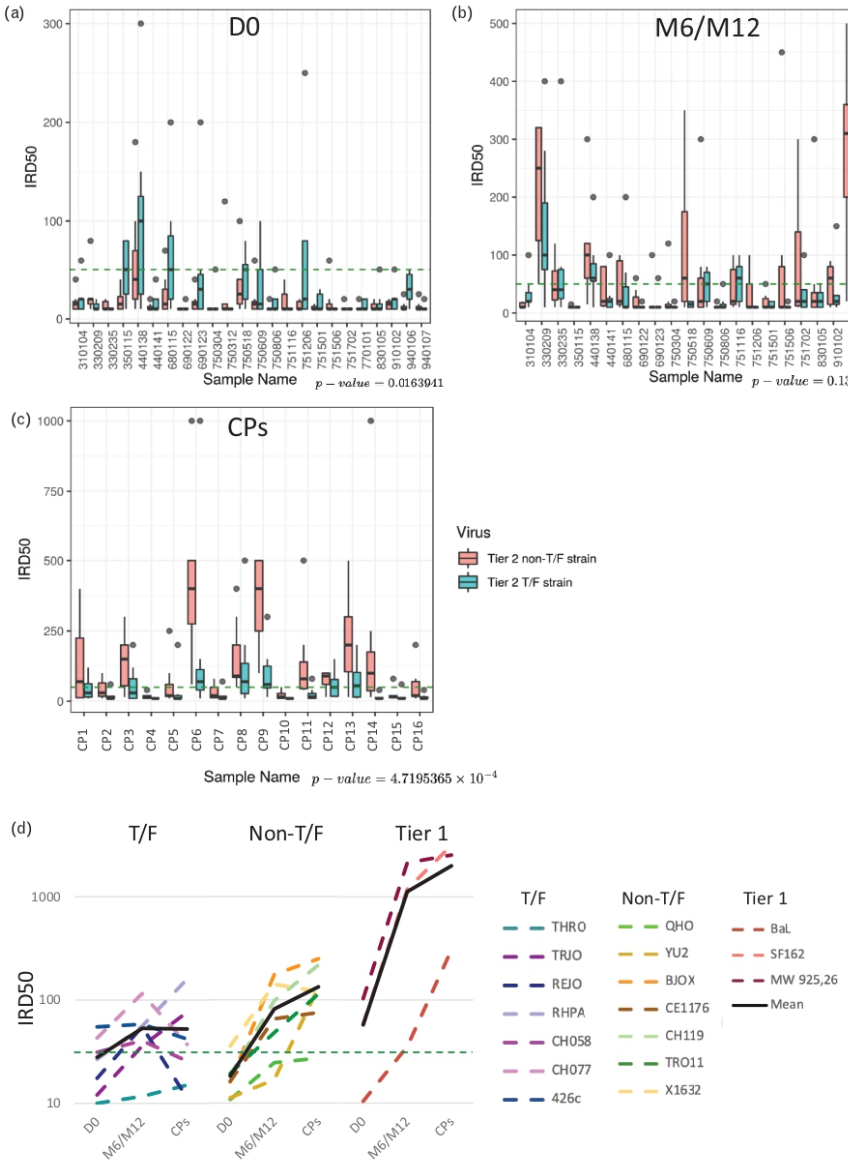

Fig. 1. Distinct pattern of neutralizing activity against transmitted founder (T/F) variants over time in HIV-1-infected patients. Neutralization for each serum collected at PRIMO D0 (a), month M6/12 (M6/12) (b) and chronic progressors (c) against T/F (red) and non- T/F (green) strains. Box plots indicate median inhibitory reciprocal dilution 50% (IRD50) values. Mean neutralizing activity for each virus: T/F, non- T/F and Tier 1 strains at different time points (d). Seven T/F s Tier 2 viruses (CH058, CH077, RHPA, THRO4156.18, REJO4541.67, 426c and TRJO4551.58), seven non- T/F Tier 2 viruses (QH0692.42, YU2, X1632_S2_B10, TRO.11, CH119.10, BJOX2000.03.2 and CE1176_A3) and three Tier-1 reference strains (SF162.LS, MW 925.26 and BaL) were used. Two-tailed paired (T/F versus non- T/F) Wilcoxon test of the median neutralizing activity was performed for each serum.

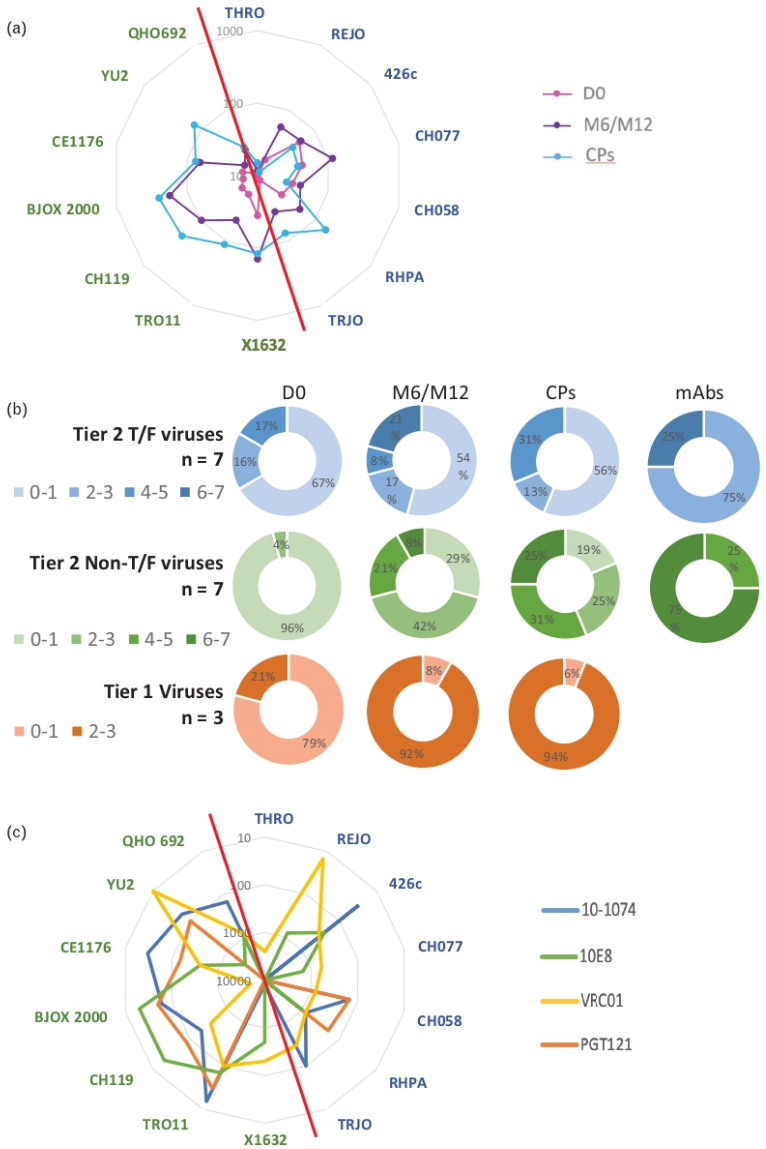

Fig. 2. Detection of cross-neutralizing activity against transmitted founder (T/F) viruses at D0. Radar chart against T/F viruses (in blue) and non- T/F (in green) for mean inhibitory reciprocal dilution 50% (IRD50) neutralizing activities of sera collected at three time points (a) or inhibitory concentration 50 (IC50) of four bmNAbs: 10–1074 and PGT121 targeting V3 glycan-dependent epitopes, 10E8 targeting the remarkably conserved gp41 membrane-proximal external region and VRC01 targeting the conformational $CD4^{+}$-binding site. (c). Pie charts representing the percentage of viruses neutralized with an IRD50 value of at least 50 for sera collected at D0, M6/M12 and chronic progressors or IC50 less than 1 µg/ml for bmNAbs 10–1074, PGT121, 10E8 and VRC01 for Tier 2 T/F (blue), non- T/F (green) and Tier 1 (orange) strains (b).

Effects of neutralizing antibodies against T/F strains on viral load

We further searched for an association between nAbs detected at D0 and viral load evolution. We did not detect any correlation between nAbs responses directed against T/F or non- T/F strains and their corresponding viral load (Supplemental Table S2, http://links.lww.com/QAD/C626 and Fig. S4, http://links.lww.com/QAD/C625). We also analyzed whether nAbs detected at D0 may associate with lower viral load at D0 or at set point at M12. Although patients displaying cross-neutralizing activity at D0 had often low post-acute viral load, no significant association could be detected between nAbs and viral load at the set point (Supplemental Fig. S4, http://links.lww.com/QAD/C625). This shows that the unexpected cross-neutralizing response detected at D0 did not influence post-acute viral load evolution at the set point.

Neutralizing sensitivity of T/F strains

Next, we determined if differences of cross-neutralizing activity between the T/F and non- T/F could be attributed to differences of virus-neutralizing sensitivity. We therefore analyzed the neutralizing capacity of four well-known monoclonal bnAbs (mbnAbs) against our T/F and non- T/F viruses (Fig. 2c). We found that these mbnAbs poorly neutralized (Fig. 2c) and cross-neutralized (Fig. 2b and Supplemental Table S3, http://links.lww.com/QAD/C626) T/F viruses (with only 25% neutralization against four or five T/F), whereas 100% of them neutralized more than four non-T/F strains tested (Fig. 2b). This low neutralization sensitivity corroborated the resistant phenotype described for transmitted founder viruses. Of note T/F viruses were significantly less inhibited by bnAbs PGT121 and 10E8 (Fig. 2c and Supplemental Table S3, http://links.lww.com/QAD/C626) further suggesting that V3 glycan-dependent epitopes and the conserved MPER may be less exposed on these viruses.

Discussion

In this study, we assessed the neutralization sensitivity against T/F strains and compared them with other Tier 2 variants. We found a modest cross-neutralizing activity against T/F strains in sera collected during acute infection (D0). This unexpected neutralizing activity barely increased over time. On the contrary, nAb response shifted to the development of neutralization against non-T/F Tier 2 strains suggesting that the early humoral immune response developed against T/F viruses was discarded in favor of other non- T/F strains. Moreover, the highly potent monoclonal bnAbs that display more than 60% coverage at IC50 less than 1 μg/ml using a large panel of selected Tier 2 isolates [4], were found to poorly neutralize the T/F viruses. This further indicates that these T/F viruses display a neutralization-resistant phenotype. Although the number of T/F strain analyzed was relatively small, this unexpected cross-neutralizing activity detected at D0 against our 'neutralization resistant' T/F viruses deserve further investigation.

Whether this unexpected cross-neutralizing activity has potential, protective effects need to be further assessed on larger cohorts of acute patient and against multiple types of T/F and non- T/F viruses. Moreover, it is unknown whether vaccine targeting more specifically T/F viruses with unambiguous identification of transmissibility signatures will give promising protection. Our data clearly demonstrate that nAbs against T/F viruses can be induced during acute infection. As these nAbs were induced very early after infection, they may display specific features (epitope recognized, less constrained maturation phenotypes) distinct from the currently identified mbnAbs. nAbs directed against T/F strains therefore open new perspectives for the development of such antibodies in future vaccine designs.

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Author contribution: Conceptualization: C.M.; Methodology: J.L., L.-Y.L., G.L., J.K., S.S., J.F., C.G. and C. M.; Software: J.L. and N.P.; Validation: C.M.; Formal analysis: J.L. and C.M.; Investigation: J.L., C.M., C.G. and L.M.; Resources: C.G., A.E., L.M. and A.C.-G.; Data curation: J.L. and C.M.; Writing – original draft: J. L. and C.M.; Writing – review and editing: J.L., C.M., S. B., L.-Y.L. and C.G.; Visualization: C.M.; Supervision: C.M. and S.B.; Project administration: C.M.; Funding acquisition: C.M. and S.B.

Conflicts of interest

The authors declare no conflict of interests. This work was not presented before.

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Supplementary Figures:

Figure S1

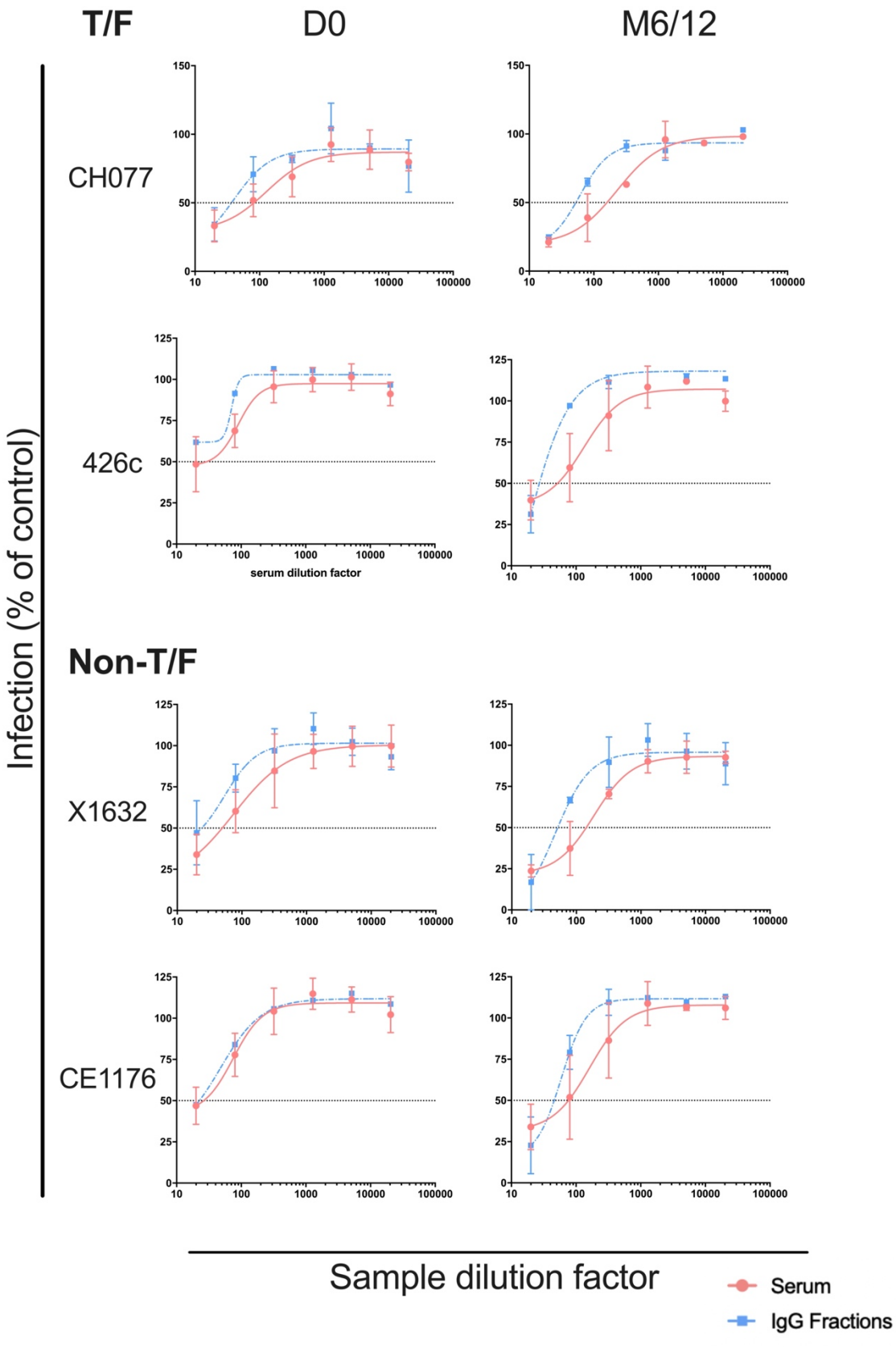
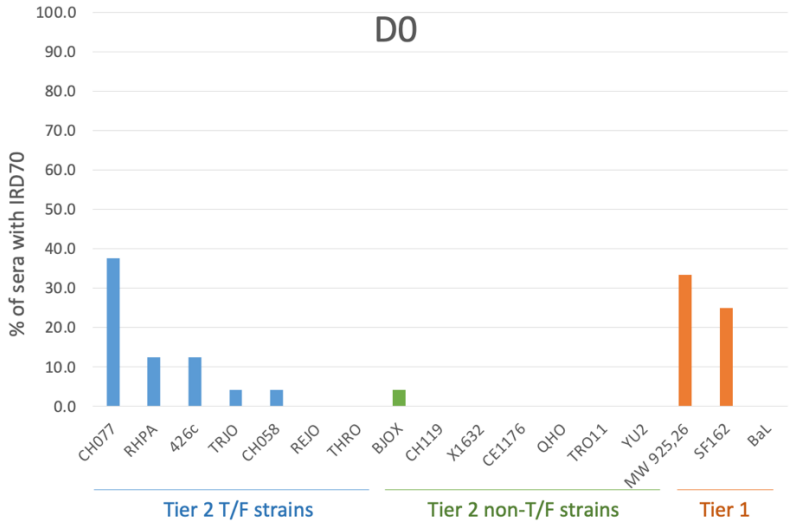
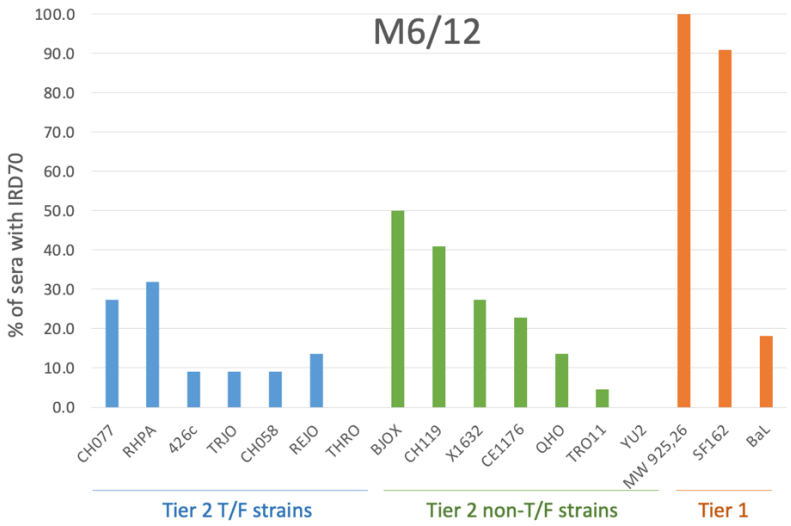


Figure S2


D0
% of sera with IRD70
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CH077
RHPA
426c
TRJO
CH058
REJO
THRO
BJOX
CH119
X1632
CE1176
QHO
TRO11
YU2
MW 925,26
SF162
BaL
Tier 2 T/F strains
Tier 2 non-T/F strains
Tier 1


M6/12
% of sera with IRD70
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CH077
RHPA
426c
TRJO
CH058
REJO
THRO
BJOX
CH119
X1632
CE1176
QHO
TRO11
YU2
MW 925,26
SF162
BaL
Tier 2 T/F strains
Tier 2 non-T/F strains
Tier 1

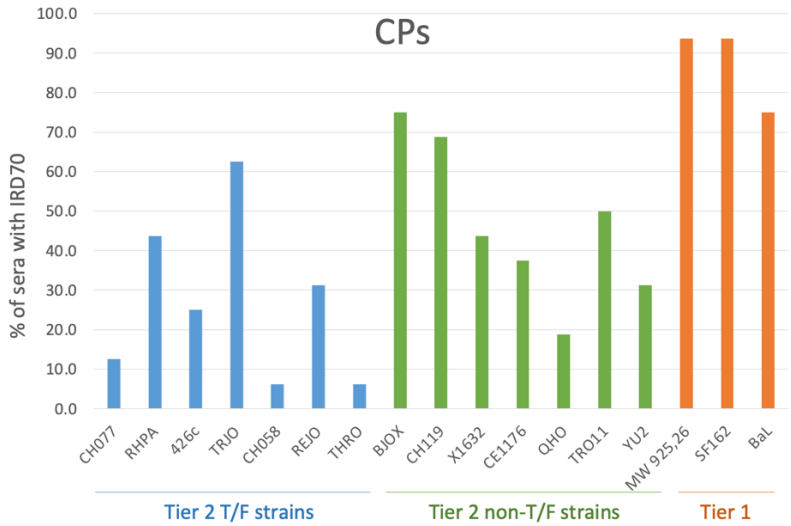

CPs
% of sera with IRD70
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
CH077
RHPA
426c
TRJO
CH058
REJO
THRO
BJOX
CH119
X1632
CE1176
QHO
TRO11
YU2
MW 925,26
SF162
BaL
Tier 2 T/F strains
Tier 2 non-T/F strains
Tier 1

Figure S3

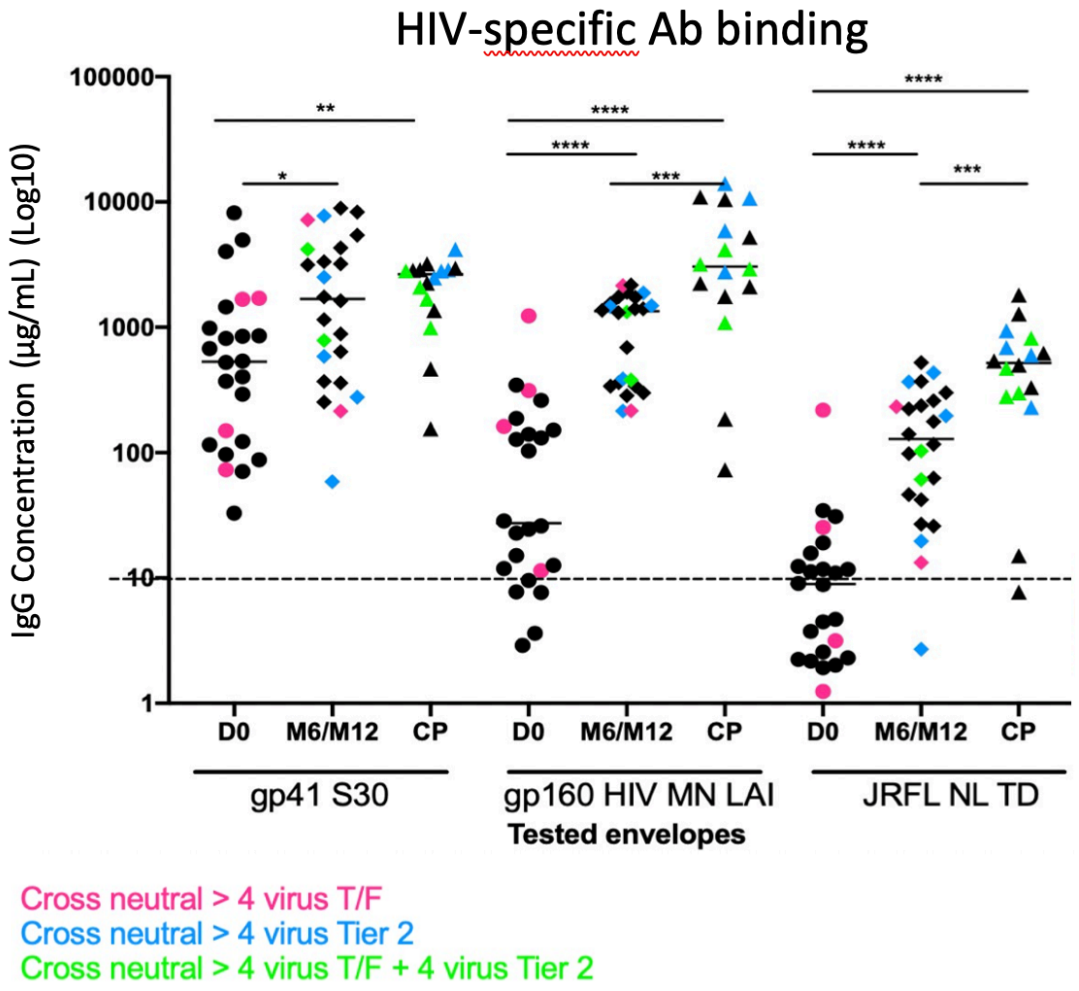
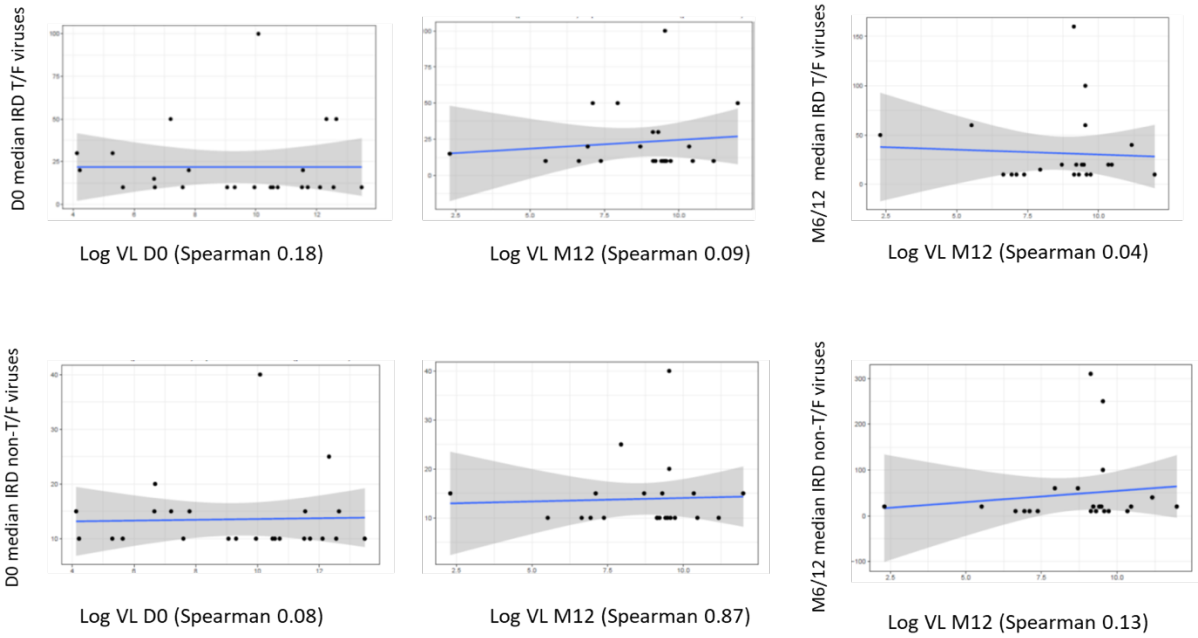

HIV-specific Ab binding
IgG Concentration (µg/mL) (Log10)
100000
10000
1000
100
10
1
**
*
****
****
***
****
****
***
D0
M6/M12
CP
D0
M6/M12
CP
D0
M6/M12
CP
gp41 S30
gp160 HIV MN LAI
JRFL NL TD
Tested envelopes
Cross neutral > 4 virus T/F
Cross neutral > 4 virus Tier 2
Cross neutral > 4 virus T/F + 4 virus Tier 2

Figure S4


D0 median IRD T/F viruses
Log VL D0 (Spearman 0.18)
Log VL M12 (Spearman 0.09)
M6/12 median IRD T/F viruses
Log VL M12 (Spearman 0.04)
D0 median IRD non-T/F viruses
Log VL D0 (Spearman 0.08)
Log VL M12 (Spearman 0.87)
M6/12 median IRD non-T/F viruses
Log VL M12 (Spearman 0.13)

Virus name	Abbreviations	Clade	T/F strains	Neutralization level (Tier)	
Tier 1 strains	BaL	BaL	B	No	1
	SF162/BB	SF162	B	No	1
	MW 925,26	MW 925,26	C	No	1
Tier 2 non-T/F strains	QHO692	QHO	B	No	2
	YU2	YU2	B	No	2
	BJOX2000.03.2	BJOX	CRF07	No	2
	CE1176_A3	CE1176	C	No	2
	CH119.10	CH119	CRF07	No	2
	TRO11.IMC.LucR	TRO11	B	No	2
	X1632_S2_B10	X1632	G	No	2
Tier 2 T/F strains	THRO4156.18	THRO	B	Yes	2
	TRJO4551.58	TRJO	B	Yes	2
	REJO4541.18	REJO	B	Yes	2
	p.RHPA.c/2635	RHPA	B	Yes	2
	pCH058.c/2960	CH058	B	Yes	2
	pCH077.t/2627	CH077	B	Yes	2
	426c	426c	C	Yes	2

Supplementary Tables:

Table S1

Virus strains analyzed for neutralizing activities. T/F are Infection Molecular Clones corresponding to the first virus acquired following transmission. Non-T/F strains were mainly isolated from patients during their acute phase.

Table S2: Neutralizing titer (IRD50, blue colors) for sera against Tier 1, Tier 2 non-T/F and T/F strains; Total and HIV-specific Abs (µg/mL, green/yellow colors)

		Tier 2 T/F strains							Tier 2 non-T/F strains							Tier 1			Specific IgG concentration			
	Sérums	426c	CH077	CH058	RHPA	REJO	TRJO	THRO	X1632	TRO11	CH119	CE1176	BJOX	YU2	QHO	MW 925,26	SF162	BaL	IgG total	gp41 S30	gp160 HIV MN LAI	JRFL NFL TD
PRIMO D0	310104	60	20	20	20	10	10	10	40	15	20	10	10	15	10	100	50	10	7725	402,75	151,5	10,95
	330209	10	20	10	10	20	10	10	80	15	20	20	20	15	10	200	80	10	18615	122,75	140	11,2
	330235	10	10	10	10	10	10	10	20	10	15	10	10	10	20	85	100	10	23925	1455	28,5	8,85
	350115	80	80	80	50	20	30	10	25	15	40	20	10	10	10	40	10	10	18710	72,95	11,45	1,24
	440138	150	300	100	40	100	10	10	180	100	40	30	40	10	10	330	500	20	45635	1672	1235	218
	440141	10	20	20	40	10	10	10	20	10	10	10	15	10	10	20	10	10	24910	676,55	24,45	11,75
	680115	200	100	70	50	25	15	10	40	70	10	10	10	15	20	100	100	10	13815	149,75	161,5	3,155
	690122	10	20	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	15050	70,8	26,05	11,7
	690123	200	50	30	40	10	10	10	40	20	10	10	15	15	10	100	10	10	11225	96,4	131	12,35
	750304	50	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	17690	32,945	12,6	1,925
	750312	10	10	10	10	10	10	10	120	10	10	15	15	10	10	20	10	10	36660	87,55	15,1	2,015
	750518	50	80	60	50	30	10	10	40	20	25	100	40	10	10	40	10	10	52140	1705	312	25,3
	750609	10	100	60	40	15	10	10	60	10	15	20	15	10	10	80	10	10	17290	115,35	11,9	3,74
	750806	10	50	20	20	10	10	10	10	10	10	10	20	10	10	10	10	10	12015	292,4	127,5	9,05
	751116	10	10	10	10	10	10	10	30	10	40	10	20	10	10	18	10	10	34825	525,35	3,615	2,17
	751206	250	20	80	80	10	10	10	10	20	15	10	10	20	10	120	10	10	51865	983,05	9,555	2,3
	751501	10	20	30	30	10	10	10	15	10	15	10	10	10	10	20	10	10	68350	817,15	103,2	15,75
	751506	10	10	10	10	10	10	10	10	10	60	10	20	10	10	10	80	10	69550	4974,5	187,4	19,05
	751702	10	10	10	10	10	10	10	20	10	10	10	10	10	10	18	10	10	86575	371,1	2,895	4,47
	770101	10	20	20	10	20	10	10	20	10	10	10	10	10	10	60	10	10	50100	538,15	7,74	2,235
	830105	50	20	10	10	10	10	10	20	20	10	10	10	10	10	18	200	10	118225	8170,5	345,7	30,9
	910102	50	10	20	20	20	10	10	10	15	20	15	20	10	10	22	10	10	135450	847,45	22,79	4,685
	940106	50	20	40	50	20	30	10	15	25	10	10	10	10	10	320	10	10	43840	853,85	7,645	2,57
	940107	10	20	10	10	10	10	10	10	10	15	10	25	10	10	600	35	10	47450	4020,5	261,65	34,5
PRIMO M6/12	310104	50	100	20	20	20	20	10	10	15	10	20	20	10	10	1000	2000	50	23575	1628	1545	141
	330209	80	400	100	100	280	70	10	320	250	320	150	320	100	50	1200	1500	150	42440	586	1460	197
	330235	80	400	70	20	30	40	10	100	25	40	45	120	10	20	300	1000	50	26685	1749	1405	117
	350115	10	10	10	10	10	10	10	10	10	10	15	10	10	10	5000	500	10	14260	370	299	98
	440138	60	100	70	50	200	60	10	100	300	120	100	120	15	20	1300	2000	75	44075	2514	1880	434
	440141	10	100	20	20	10	30	10	80	10	80	15	80	10	20	3000	3000	60	35310	3205	1735	301
	680115	10	200	10	20	10	70	10	100	10	80	20	100	10	20	1000	3000	200	35900	887	1755	523
	690122	10	10	10	10	20	10	10	40	10	10	15	60	10	10	800	500	15	27285	1151	1900	237
	690123	10	10	10	10	60	10	10	10	10	10	10	100	10	10	500	250	10	20055	360	1365	177
	750304	10	10	10	10	20	10	10	120	10	10	10	20	10	10	6000	30	10	37620	254	340	26
	750518	10	20	20	20	15	10	10	250	20	100	60	350	20	10	800	100	10	48015	786	1325	103
	750609	50	80	60	80	30	10	10	300	10	20	40	80	10	10	3000	30	10	35690	214	216	13
	750806	50	10	10	20	10	10	10	20	10	10	10	10	10	10	2000	130	10	20550	638	340	42
	751116	80	100	80	60	10	30	10	90	20	60	10	100	10	20	320	700	10	49965	7209	2144	233
	751206	10	10	10	10	10	10	10	10	10	100	10	90	10	10	13000	500	10	71650	5427	362	46
	751501	10	20	20	20	10	10	10	20	10	50	10	30	10	10	280	80	10	65750	3143	285	27
	751506	10	20	10	10	10	10	10	100	10	60	10	450	10	10	1000	2000	10	33460	4297	1310	259
	751702	20	100	40	40	10	10	10	200	10	80	15	300	10	20	1000	3000	10	52910	3336	691	63
	830105	50	50	20	20	10	10	10	10	20	20	10	300	10	50	60	2000	10	94425	8300	1403	224
	910102	150	20	30	30	20	10	10	90	20	85	75	60	10	10	1800	120	10	163500	4181	380	61
	940106	>320	400	160	160	220	100	10	500	280	400	320	310	20	120	1000	5000	10	44390	7736	1487	367
	940107	10	100	20	400	60	10	10	20	20	150	120	140	10	10	6000	800	10	51065	8925	2175	370
Progressors	SKU/CH	10	10	10	40	10	20	10	20	80	60	20	200	10	15	4500	2500	400	25902	2880	10450	1810
	NOE/OL	10	15	15	80	10	40	10	40	50	80	40	200	500	80	5000	5000	700	32094	1679	2922	470
	LEV/JE	200	70	15	500	10	70	40	80	100	300	90	400	90	50	4000	5000	500	32333	2862	14050	940
	MOL/SE	10	10	10	10	10	10	10	10	40	15	15	50	10	10	10	15	10	35736	155	73	8
	RIO/RO	10	10	10	15	10	40	10	100	15	1000	100	250	60	15	800	2500	120	22352	993	1090	300
	SEK/LA	10	10	10	15	10	60	10	15	20	20	15	80	10	10	1300	200	100	40565	2839	2240	620
	FOU/NI	150	60	75	1000	20	70	10	500	300	500	250	1000	400	60	2500	1000	300	81250	4183	5910	690
	CHA/FE	10	10	10	10	10	10	10	10	20	20	15	40	10	10	500	150	10	25446	465	185	15
	ONF/AL	100	15	55	150	15	50	20	80	90	100	40	100	100	15	2500	5000	180	53284	2482	2770	230
	PAP/PH	10	55	55	150	10	200	20	500	300	200	150	300	60	15	4500	5000	720	41605	3210	10970	1280
	ENN/MO	10	25	10	15	10	200	10	20	20	100	20	250	15	10	1500	2000	160	30599	2249	5220	540
	BAL/DO	10	20	10	15	10	60	10	30	20	100	40	90	15	10	350	15000	80	43033	2957	1763	330
	AZI/CO	50	75	30	120	10	20	10	10	400	250	70	200	15	10	1300	1500	40	50203	2823	3181	280
	LES/CH	10	20	10	15	10	70	10	20	15	80	20	80	10	10	1000	1000	120	20422	1363	2105	500
	BON/OL	10	40	30	120	10	200	10	90	150	150	20	250	300	15	5500	3000	700	33325	2088	4150	820
	MAK/MA	60	150	55	300	15	100	40	400	200	500	300	500	500	100	5500	1500	750	52691	2816	10710	600

IRD 50

<50
50-150
150-400
> 400

Concentration (µg/mL)

<50
50-300
300-2000
>2000

Concentration (µg/mL)

<10 000
10 000-40 000
40 000-60 000
>60 000

Table S3

	Tier2 non-T/F strains								
mNAbs	X1632	TRO11	CH119	BJOX	QHO	YU2	CE1176	median	% coverage
10-1074	8,000	15	200	60	150	60	30	60	86
PGT121	10,000	30	80	50	10,000	100	150	100	71
10E8	500	70	20	20	1,000	3,000	400	400	86
VRC01	200	100	350	5,000	800	10	400	350	86

	Tier2 T/F strains								
mNAbs	426c	pCH058	PCH077	pRHPA	pREJO	pTRJO	pTHRO	median	% coverage
10-1074	30	150	10,000	800	10,000	100	10,000	800	43
PGT121	10,000	150	10,000	200	10,000	10,000	10,000	10,000	29
10E8	250	10,000	1,500	1,000	800	10,000	10,000	1,500	43
VRC01	350	800	600	800	15	300	2,500	600	86

* *

IC50 (µg/mL)	
	>1 000
	100-1 000
	10-100

4.1.2. Discussion

The results from this paper demonstrated that Abs induced in the early phase of HIV infection already displayed neutralization against T/F viruses. Later on, a shift of neutralizing activity against tier 2 T/F viruses to tier 2 non-T/F viruses was observed.

To further characterize the Ab response induced early after infection, I investigated the Fc-mediated inhibitory activity of these samples against different HIV strains. We performed ADCC experiments against three non-T/F viruses and one T/F virus. The three tested non-T/F viruses were CE1176 (Clade C), CH119 (Clade CRF07) and X1632 (clade G). As described in the material and method, ADCC was evaluated by the detection of the RLU (associated with HIV inefectd cells. Results were expressed as the peak area under the curve (PAUC). We used an HIV-positive patient sample (Bon/OL) as positive control.

For ADCC assay, we made several technical modifications to improve the experiment. For detecting the ADCC ability against CE1176, we performed several experiments (Figure 29). As we detected variations of our internal control from one experiment to another, we performed larger experiments allowing the testing of all the samples at ones. Moreover, we used half area plates to increase the effector cells/infected cells interactions. After improving the experiment method, the ADCC ability against virus CH119 (Figure 30) and virus X1632 (Figure 31) were tested.

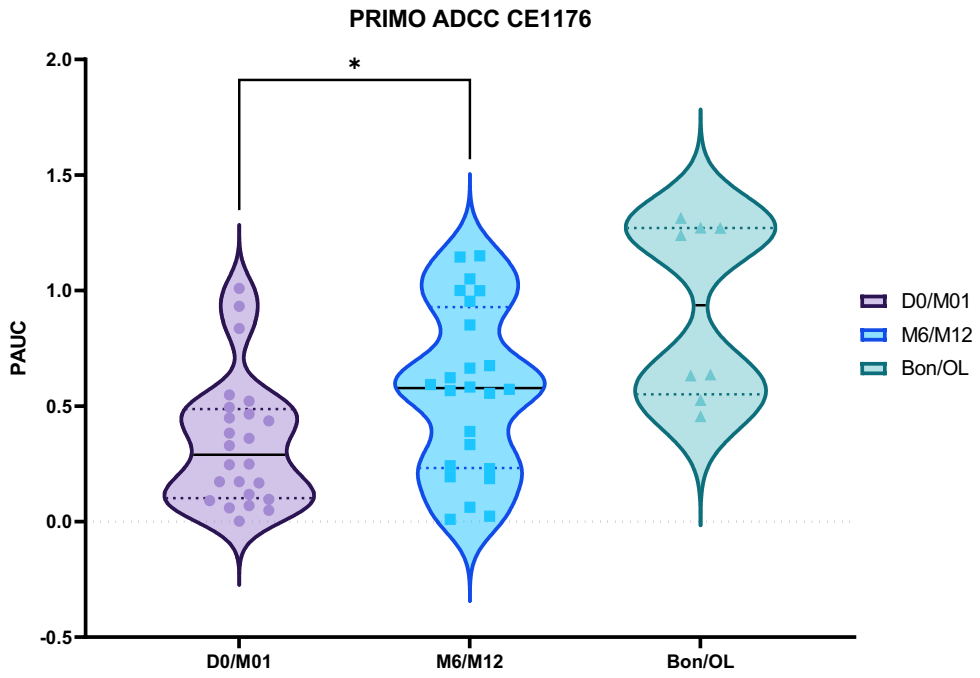


Figure 29. The ADCC ability against clade C non-T/F virus CE1176. P= 0.0198

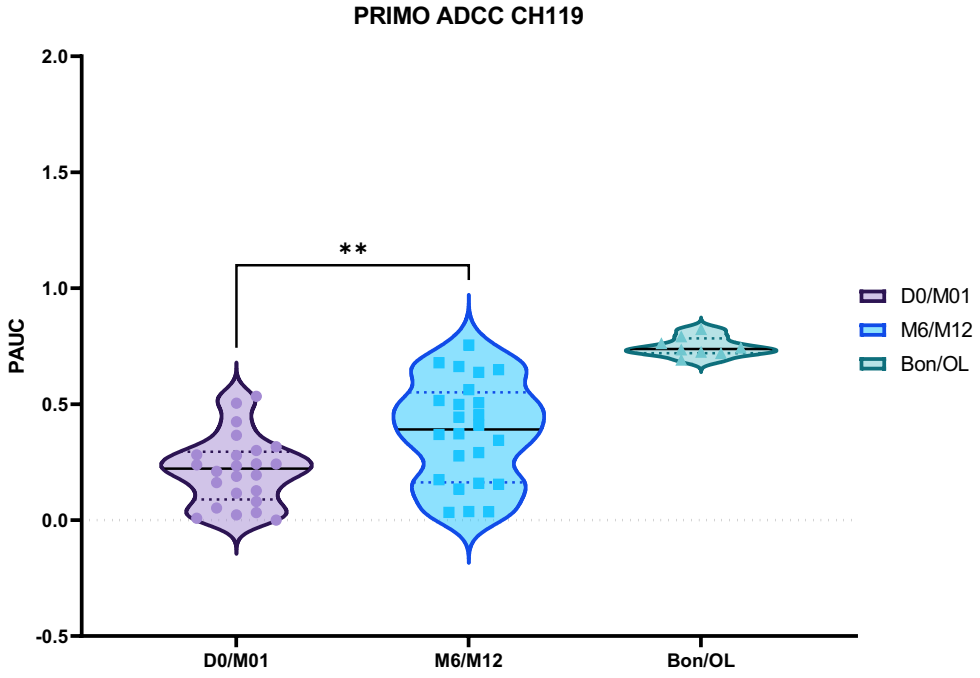


Figure 30. The ADCC ability against clade CRF07 non-T/F virus CH119. P= 0.0036

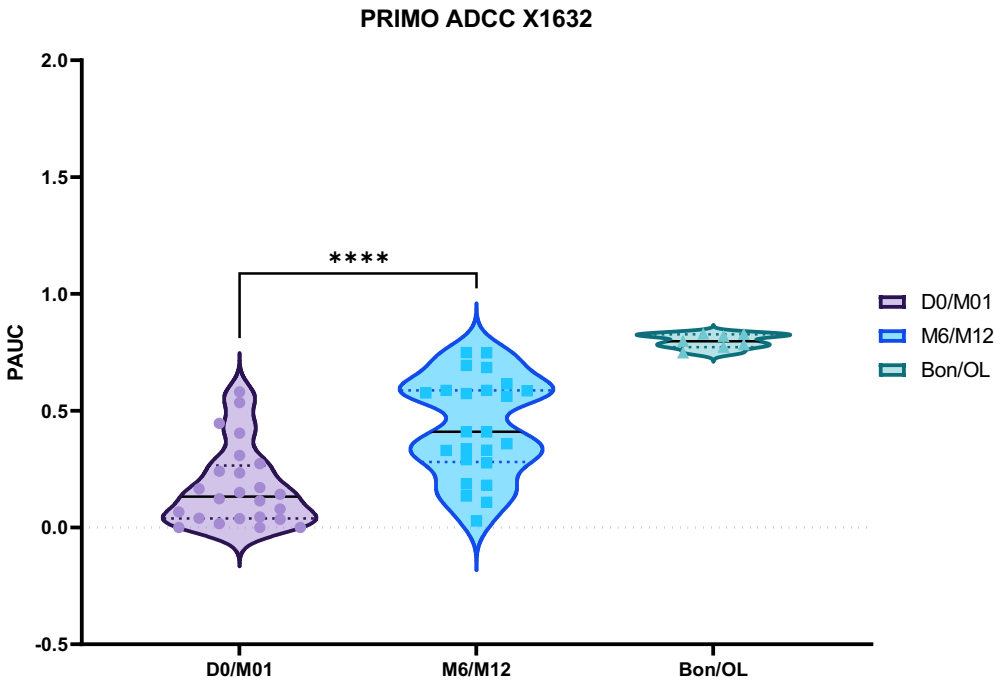


Figure 31. The ADCC ability against clade G non-T/F virus X1632. P < 0.0001

ADCC results for these three non-T/F viruses showed that some ADCC was detected at early time point and increased in M6/M12 samples.

As our T/F viruses do not express Renilla Luc in their genome, pCH058 T/F virus infected cells were revealed by flow cytometry (Figure 32). Under these conditions, we could not detect ADCC ability against pCH058 in PRIMO samples and in our control infected sample. Whether this difference of ADCC sensitivity is due to the virus used (TF versus non-T/F strain) or to the protocol used (readout of infected cells by luciferase versus p24 staining) will need further investigations.

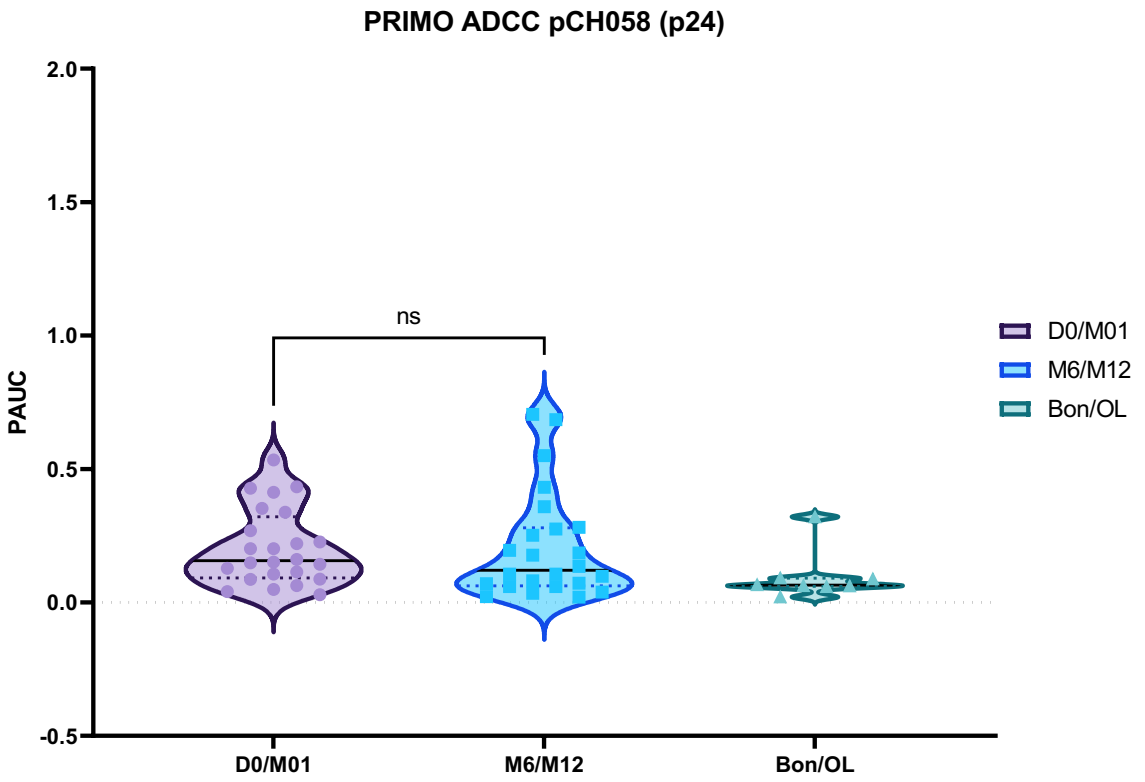


Figure 32. The ADCC ability against clade B T/F virus pCH058. P = ns

Mielke et al. suggest that HIV-1 could be sensitive to neutralization and resistant to ADCC or vice versa but were rarely sensitive or resistant to both responses.[447] In our results, neutralizing as well as ADCC abilities against CH119 and X1632 were high in M6/M12 samples. For CE1176 virus, the samples had high ADCC ability but low neutralization. We detected some neutralizing abilities against pCH058 at early time points, but no ADCC against this virus. Additional viruses should therefore be teste for neutralizing and ADCC activities of PRIMO samples in order to complete their functional characterization.

In conclusion, these results inform on the functional activity induced after infection. We found that the neutralizing ability against T/F viruses can be detected in PRIMO samples at the early stage of HIV-1 infection. In addition, early Fc-mediated functions might be able to provide productive help. This early functional Ab response is encouraging as it gives new hits for the development of functional Abs that should be induced with new vaccine strategies.

4.2. Development of new vaccine strategies

New vaccine strategies have been developed in collaboration with the Horizon 2020 European consortium European HIV Vaccine Alliance (EHVA). I aimed to characterize the Ab responses (binding and neutralizing activity) induced by several new immunogen constructs, new vectors and new immunization routes on animal models (rabbit or NHP). These studies will allow to select the more promising vaccine candidates to be tested in humans.

In these studies, I determined the HIV specific Ab response induced by newly generated immunogen on rabbit and NHPs, by VSV vectors expressing HIV envs on rabbits, and a slow delivery immunization route on NHP model. The HIV-specific antibodies were detected by "direct ELISA", and the neutralizing antibodies were detected using the conventional "TZM-bl neutralizing assay".

4.2.1. Defining the immunogenicity of new env immunogens

4.2.1.1. Generation of new envelope immunogens

Recently, a stabilized envelope (env) trimer BG505-SOSIP was generated. Based on this stabilized trimer, different new env trimer constructs were produced by our H2020 partner (group of R. Wagner, Regensburg Germany). These new HIV trimers contain glycosylation deletion or masking in order to redirect the immune response to the conserved CD4 binding domain of HIV (Figure 33).

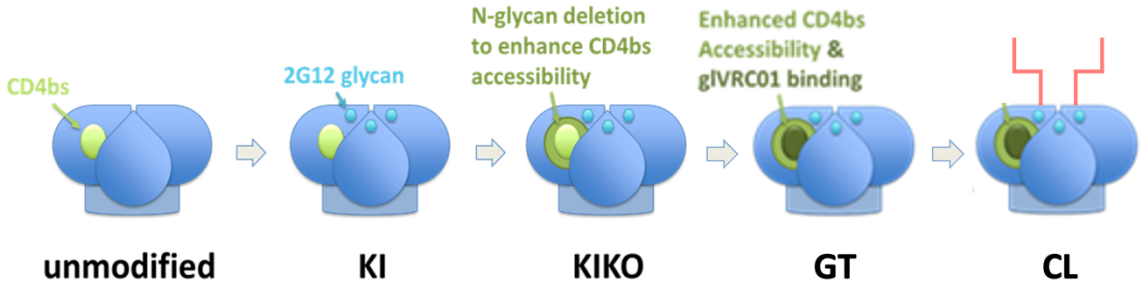


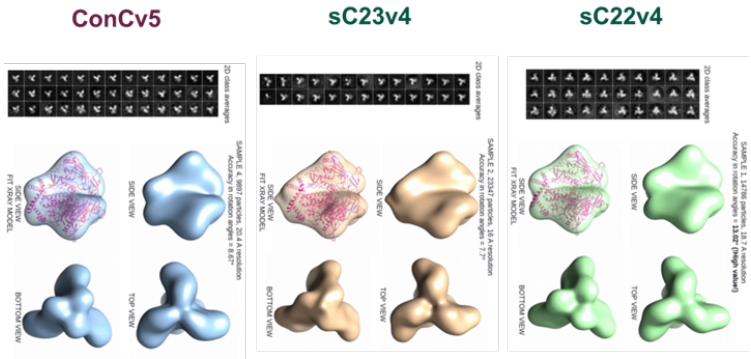
Figure 33. Chemical modifications of the env trimers

Three envs (sC22, sC23 and ConC) have been selected based on their stability and well-folding as env trimers (Figure 34).

Figure 34. Structure of the env trimers

4.2.1.2. The immunogenicity of these new constructs were tested in animal models.

4.2.1.2.1. Characterization of the Ab response induced following rabbit immunization



This assay aimed to analyze the immunogenicity of new env ConC constructs carrying various modifications of the env glycosylation sites (GT, KIKO, CL). Prime/boost strategies was conducted (Table 2) on rabbit immunized with 40 µg modified protein mix with a new adjuvant LMQ (neutral liposomes containing monophosphoryl lipid A (MPL) and Quillaja saponaria derived QS21 saponin). This adjuvant can not be currently used in human as it is not yet approved by the FDA.[448]

Group	D0 bleed	W0-D0 IM	D14 bleed	W4-D28 IM	D42 bleed	W12-D84 IM	D98 bleed	W20-D140 IM	D154 final bleed
1		ConCv5-GT		ConCv5-GT		ConCv5-GT		ConCv5-GT	
2		ConCv5-GT		ConCv5-GT		ConCv5-KiKo		ConCv5-Ki	
3		ConCv5-GT-CL		ConCv5-GT-CL		ConCv5-KiKo-CL		ConCv5-Ki-CL	

Table 2. Prime/boost protocol using various modified ConCv5 envs for rabbit immunization

First, the induction of HIV-specific antibodies was analyzed by ELISA. We identified the presence of Abs able to bind two distinct env, either the classical env trimer BG505, or the homologous ConC env trimer in sera from rabbits following immunization. This binding was measured at different sera concentration in order to determine the half-maximal effective concentration (EC50) of specific Abs induced over time (Figure 35)

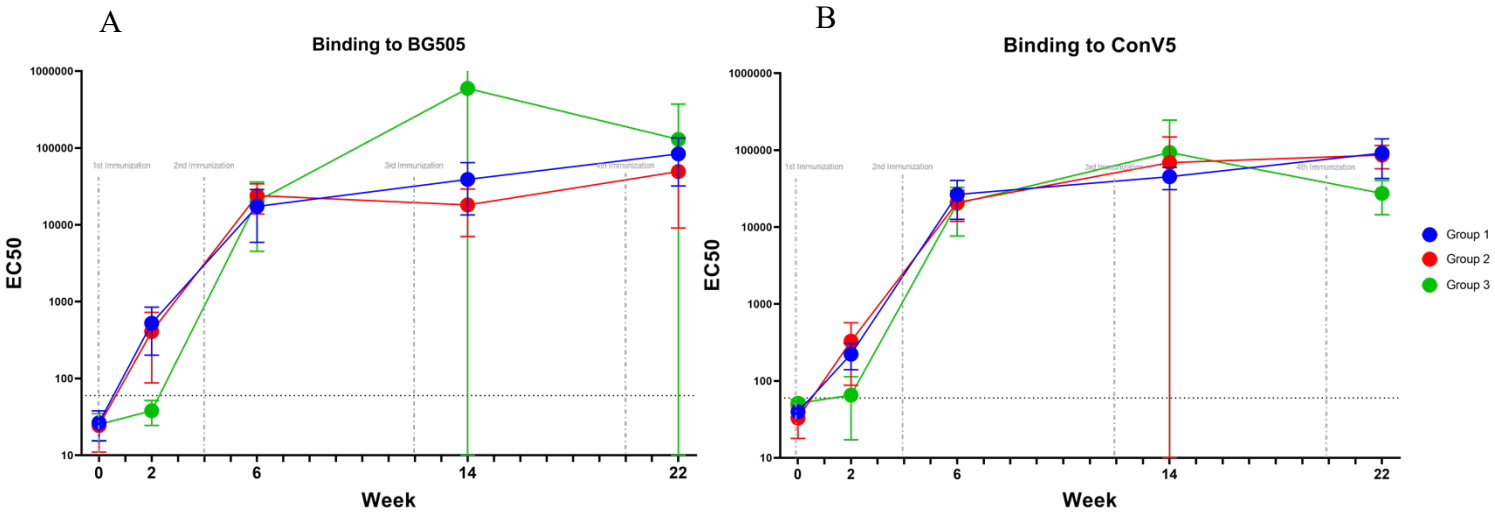


Figure 35. IC50 of HIV specific antibody binding to A) BG505 env and to B) ConC env. High level of HIV specific antibody was detected following the first two immunizations. HIV-specific-Ab concentrations are maintained at the 3rd and 4th immunization.

HIV-specific Abs were already detected following the first immunization (Week 2) with ConC-GT (group A and B) compared to ConC-GT-CL (group C). After the second immunization, the group C immunized with ConC-GT-CL induced specific Abs similar to group 1 and 2. This Ab response is maintained over time at the 3rd (W14) and 4th (W22) immunization.

The neutralizing ability was assessed against various HIV strains using the conventional TZM-bl assay and the IC50 (inhibitory concentration 50) was calculated. We detected neutralizing activity against the easy-to-neutralize Tier 1 strains MW956.26 in all three groups but not against the Tier 1 strain SF162 (Figure 36). Interestingly, homologous neutralization against virus ConC could also be detected for some animals (Figure 37).

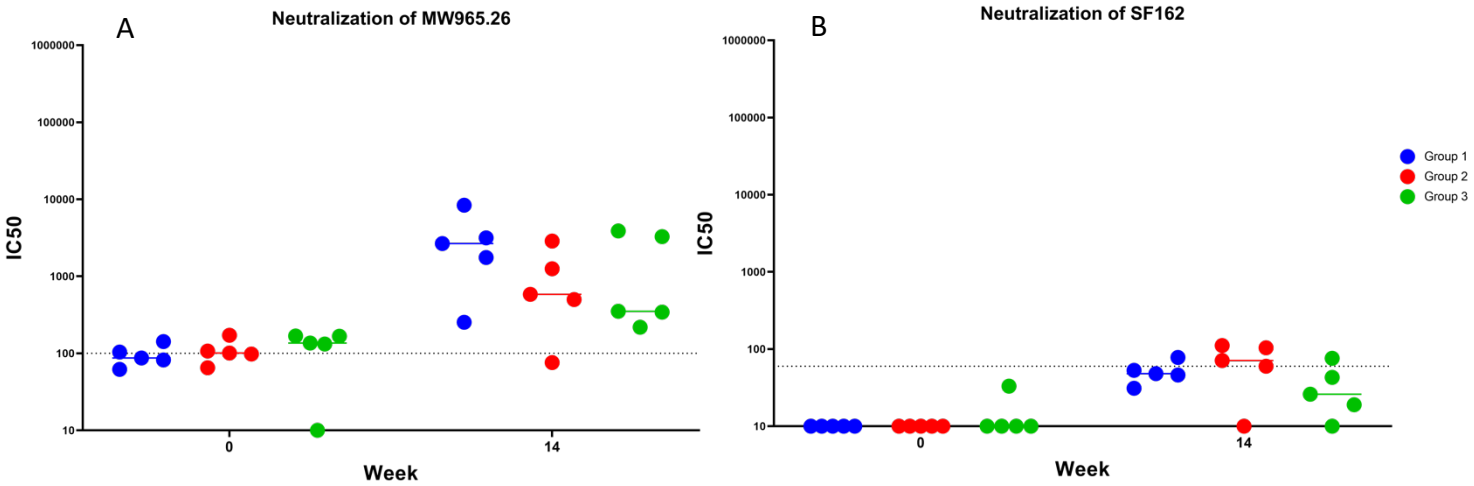


Figure 36. Neutralizing activity against two tier 1 viruses : A) MW965.26 and B) SF162.

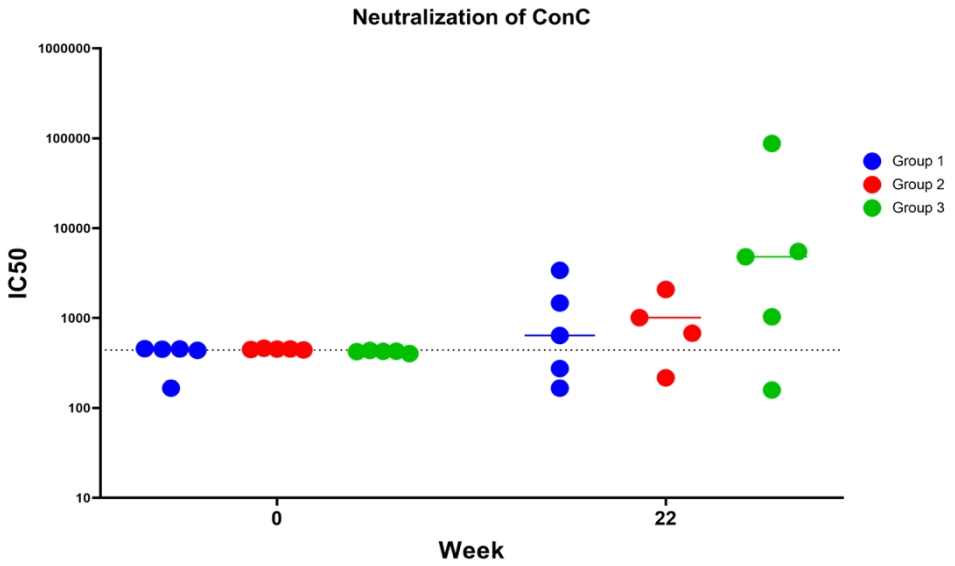


Figure 37. Neutralizing activity against the homologous strains ConC.

These results showed that our new env constructs are highly immunogenic in rabbits.

4.2.1.2.2. Characterization of the Ab response induced following immunization of non-human-primates

As the various env constructs ConC-GT and ConCv5- KIKO demonstrated efficient inductions of Ab responses in rabbits, these constructs were further analyzed in the non-human-primates (NHP) model. NHP model is a highly relevant as macaques are genetically close to human and can be challenges with viruses (SIV or SHIV) similar to HIV. Immunization protocol comparing the ancestral env BG505 with the new constructs ConC-GT and ConCv5- KIKO were performed as described in Table 3. For this immunization, MPLA (Liposomes Containing Monophosphoryl Lipid A) was used as FDA approved adjuvant.[449–451]

Gr	Size	Route	Protein Dose	Wk0	Wk4	Wk12	W24	W36	Wk 36 / 48
1A	6	IM	100 µg	BG505	BG505	BG505	BG505	-	
2B	6	IM	100 µg	ConCv5-GT	ConCv5-GT	BG505	BG505	BG505	Memory responses
3C	6	IM	100 µg	ConCv5-GT	ConCv5-GT	ConCv5-KIKO	ConCv5-KIKO	BG505	

Table 3. Immunization protocol using several env trimers conducted in NHP.

First, the induction of HIV-specific Abs able to bind two distinct env, either the classical env trimer BG505 or the homologous ConC env was measured by ELISA. The binding capacity of the induced Abs were analyzed in sera at different dilutions in order to define the EC50 of HIV-specific Abs induced over time (Figure 38)

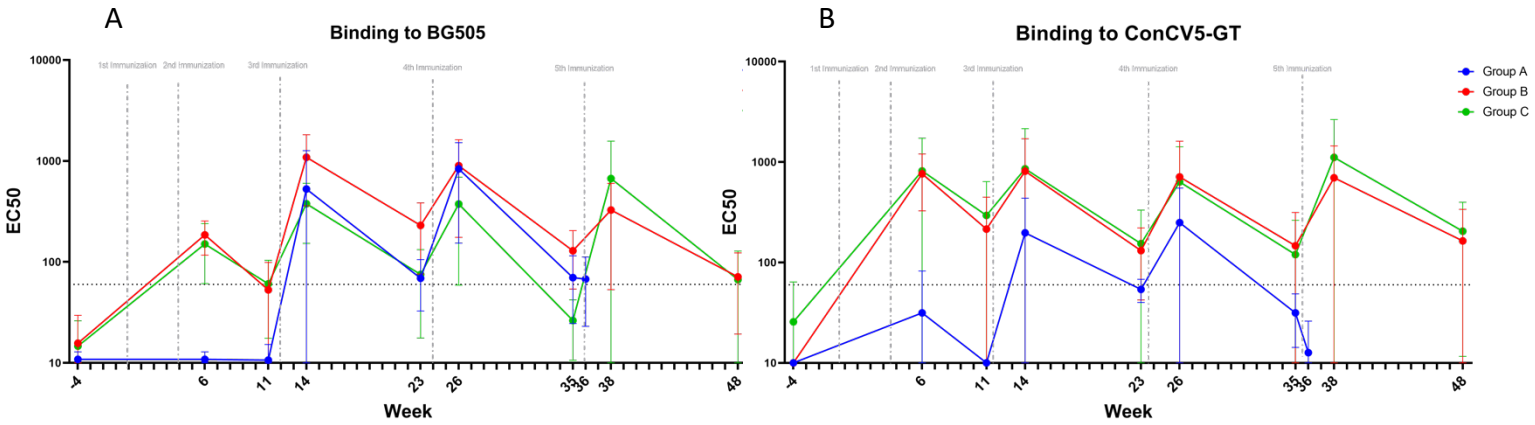


Figure 38. EC50 of HIV specific antibody binding to BG505 env A) or to ConCV5-GT env B) induced over time following prime boost-immunization strategy in NHP.

In the NHP model, HIV-specific antibodies were detected at week 6 after the second immunizations with ConC-G (group B and C). A third immunization with the ancestral env BG505 was necessary for HIV-specific Ab detection.

These results demonstrated the improvement of immunogenicity of our new constructs. However, compared to the Ab response detected in rabbit, the IC50 was 10 to 100 folds lower in NHP. Moreover, this response decreased after each immunization.

The neutralizing activity was assessed against the easy-to-neutralized Tier 1 MW965.26 in pre-immune sera and in sera collected at Week 26 (Figure 39).

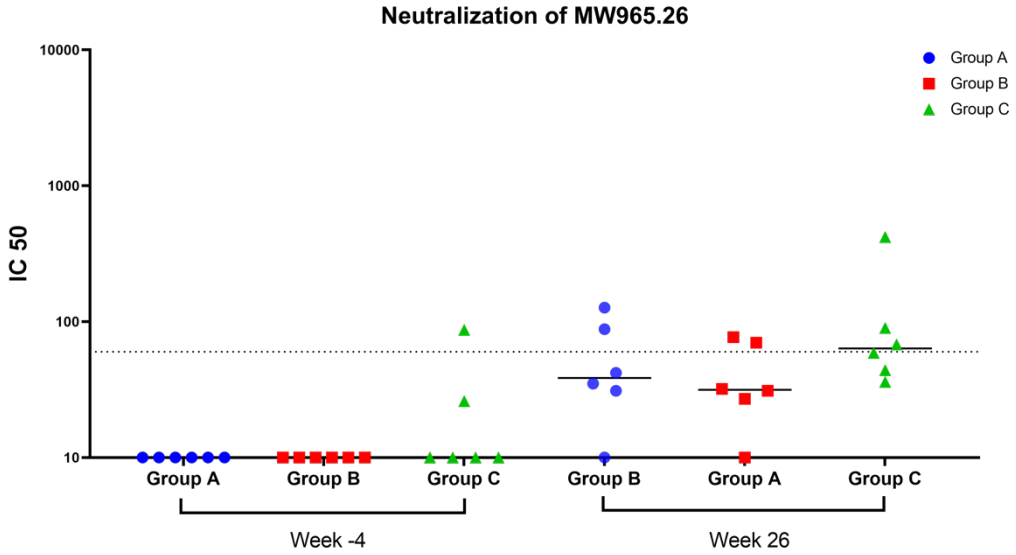


Figure 39. IC50 Neutralizing activity against virus MW965.26 in sera collected at Week 26 for the three immunization groups.

The neutralizing activity detected against this easy-to-neutralized Tier 1 virus was borderline. Compared to rabbit immunization, HIV specific Abs as well as neutralizing activity were strongly reduced in NHP. This strong decrease of immunization efficacy observed in NHP compared to small rodents was observed in previous studies. The decrease of immunogenicity may also be explained by the use of a different adjuvant, MPLA, which may be less potent as the newly developed adjuvant MLQ.

4.2.2. Improvement of immunization delivery

4.2.2.1. Immunization with the VSV (vesicular stomatitis virus) vector platforms in the rabbit model

Besides the different protein/env candidates, delivery platforms have been developed to potentiate the Ab response. Here we evaluated the VSV-GP platform expressing the HIV envs at their surface (Figure 40). This platform was tested in the rabbit model either as two successive VSV immunization following by protein boosts or as sequential VSV protein immunization (Table 4). Immunization was performed with 40 µg protein (with MPLA, ratio 2:1) and 2E08 $TCID_{50}$ VSVenv..

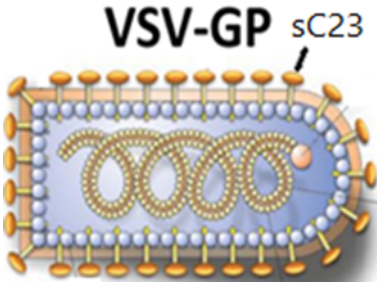


Figure 40. VSV expressing HIV env at its surface.

Group #	Week 0	Week 4	Week 12	Week 20
A	VSV-GP-sC23v4	VSV-GP-sC23v4	sC23v4	sC23v4
B	VSV-GP-sC23v4	sC23v4	VSV-GP-sC23v4	sC23v4

Table 4. Sequential VSV-GP immunization in the rabbit model.

High HIV-specific Ab were already detected following the 1st immunization (Week 4) with VSV-GP-sC23v4. There is no significant difference in the Ab induction between group A and B (VSV/ VSV/ Env/ Env and VSV/ Env/ VSV/ Env). Therefore, the potential immune response induced against the vector did not modify the induction of HIV-specific Ab response against ConC and sC23 envs (Figure 41).

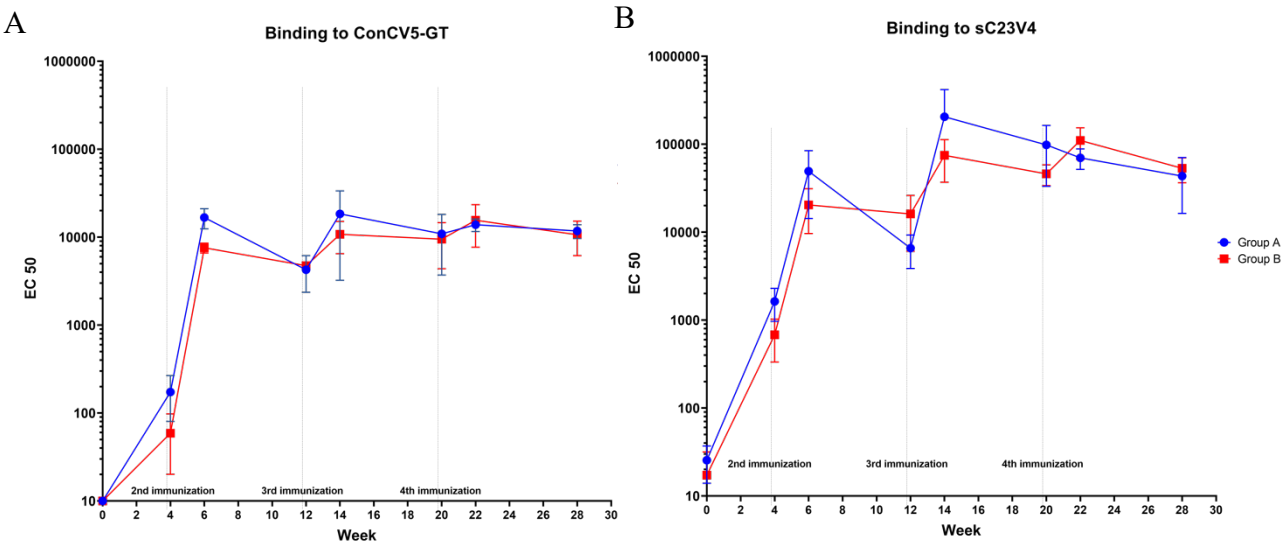


Figure 41. Ec50 of HIV-specific Abs against ConCV5-GT env A) and against sC23V4 env B). HIV specific antibody was already high following first two immunization.

Neutralizing activity against tier 1 virus MW965.26 was detected after W6 in the two groups irrespective of the immunization strategy, i.e., VSVenv/ VSVenv/ Env/ Env versus VSVenv/ env/ VSVenv/ env (Figure 42).

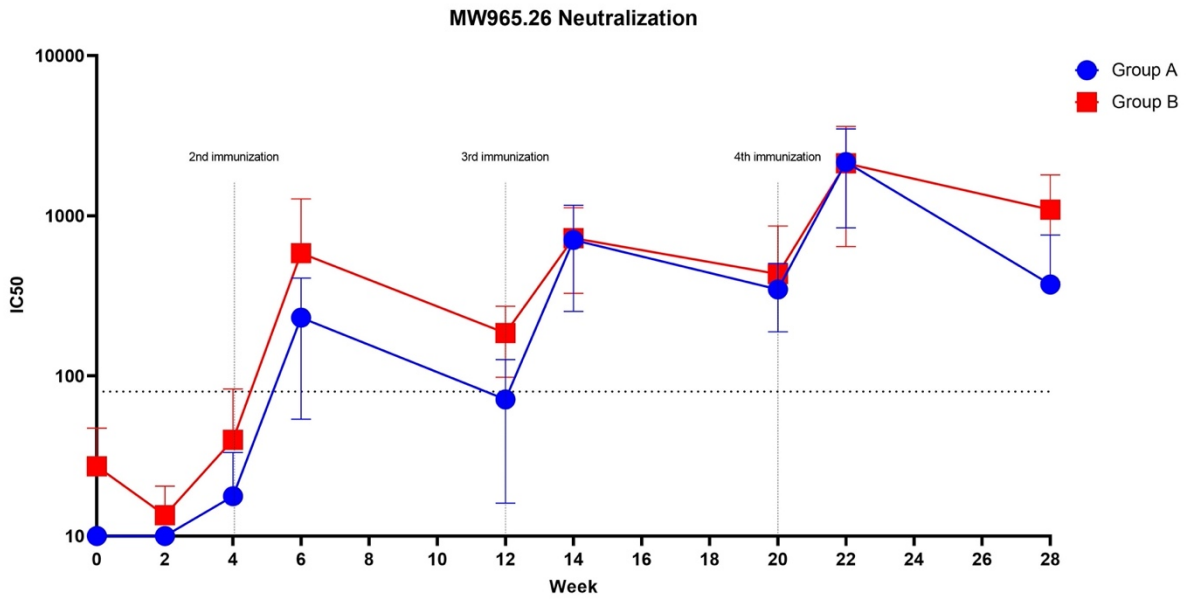


Figure 42. IC50 Neutralizing activity against tier 1 virus MW965.26 following different VSV Env or Env immunization strategies (Table 4).

The neutralizing activities against tier 2 viruses sC23-R6 and sC23 KIKO were also tested (Figure 43, 44). There was no neutralizing activity detected against sC23-R6 at all the time points but the neutralizing activity against sC23 KIKO was detected at W6 and follow up in the two groups.

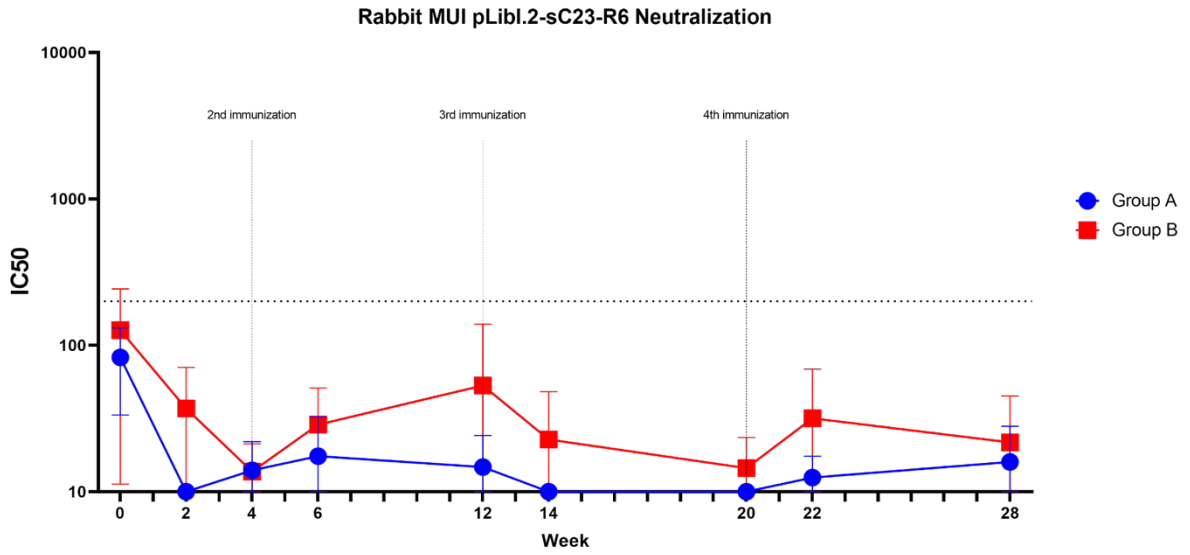


Figure 43. Neutralizing activity against tier 2 sC23-R6 following different VSV Env or Env immunization strategies (Table 4)

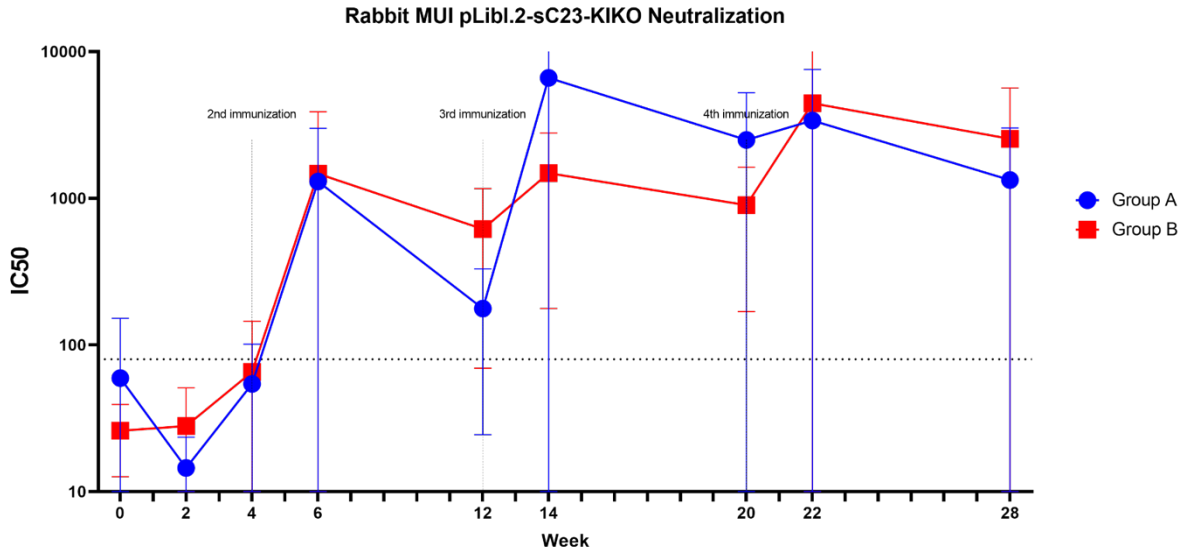


Figure 44. Neutralizing activity against tier 2 sC23-KIKO following different VSV Env or Env immunization strategies (Table 4).

These results demonstrate the improvement of the Ab response by the VSV platform used alone or in combination with env proteins. Moreover, the switch VSP protein boost did not improved immunogenicity compared to the VSV first and protein boost strategy (manuscript in preparation with our colleagues under EHVA consortium).

4.2.3. Testing of different immunization routes with the NHP model

The VSV vector platforms and protein boost immunization were further tested in the NHP mode using different immunization routes. In order to improve the Ab response, the env protein ConCv5 KIKO (at 100 µg) was further given by slow persistent release using an osmotic pump (OP) (Table 5). This slow delivery process has recently been described to improve Ab response.

Group	Size	Week 0	Week 4	Size	Week 12	Week 24
1a	10	VSV (IM)	VSV (IM)	5	Protein (IM)	Protein (IM)
1b				5	Protein (OP)	Protein (OP)
2a	10	VSV (IM)	VSV (IM)	5	VSV (IM)+ Protein (IM)	VSV (IM)+Protein (IM)
2b				5	VSV (IM)+ Protein (OP)	VSV (IM)+ Protein (OP)

Table 5. Immunization protocol comparing IM versus OP delivery in NHP model

The induction of HIV-specific Ab response binding to the autologous env ConC-V5 KIKO was analyzed over time (Figure 45). The two VSV vector prime (VSV-GP vector expressing the ZM96gp140 env, $2X10^8$ TCID50) gave similar HIV specific Ab response (EC50 around 800) as two env ConC-GT immunization (Figure 46), suggesting the VSV platform did not increased NHP immunization on its own. But it demonstrated the relevance of the use of the VSV platform as a prime. In addition, the slow delivery by OP significantly increased (P=0.0007) HIV-specific Ab binding to the autologous ConC-V5 KIKO env response (group G1b and G2b) compared to the classical intramuscular (IM) delivery route (group G1a and G2a). OP immunization also increases HIV specific Abs against BG505 SOSIP env (not shown).

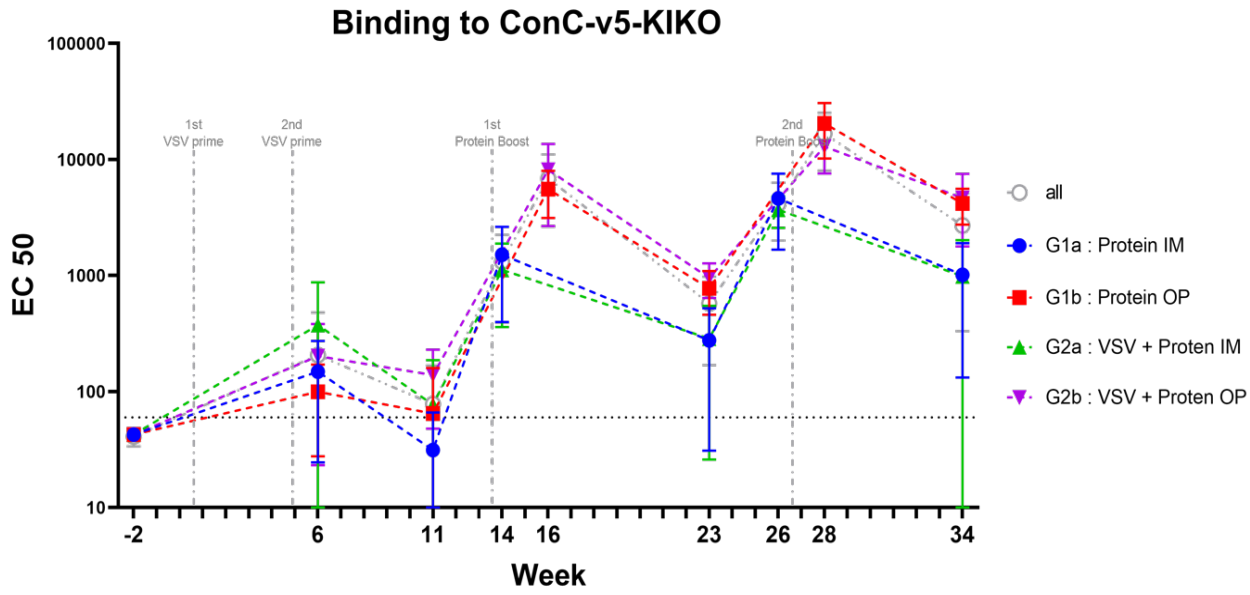


Figure 45. EC50 HIV specific Ab response directed against ConC-V5 KIKO env.

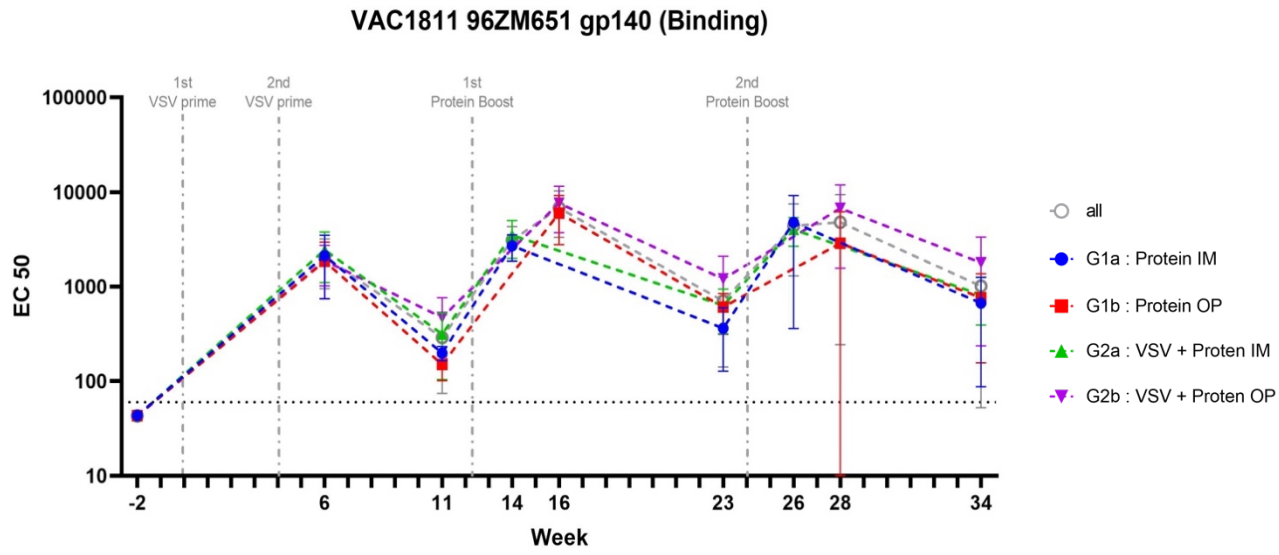


Figure 46. EC50 HIV specific Ab response directed against ZM96gp140 env.

Neutralizing activity induced against Tier 1 viruses MW965.26 (Figure 47) and against the homologous sCON KIKO virus (Figure 48) was analyzed over time following immunization. We detected high neutralizing activities with IC50 > 1000 after the first protein boost. This activity increased for OP delivery.

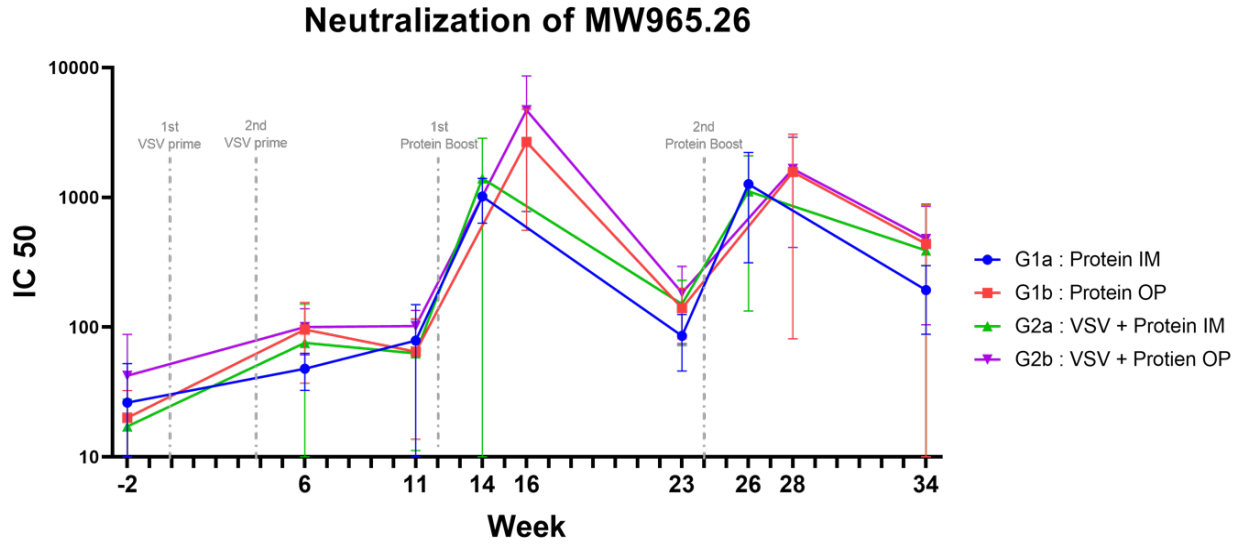


Figure 47. IC50 Neutralizing activity against Tier 1 viruses MW965.26.

Neutralizing activity against sCON KIKO pseudovirus was detected following VSV+ protein OP immunization at W16 (purple curve) and following OP immunization route at W28 (red curve) (Figure 47). On the contrary, neutralizing activity against the homologous sCON KIKO virus could not be detected following the classical IM immunization route (blue and green curves). These results demonstrate the benefits of OP immunization compared to classical IM route.

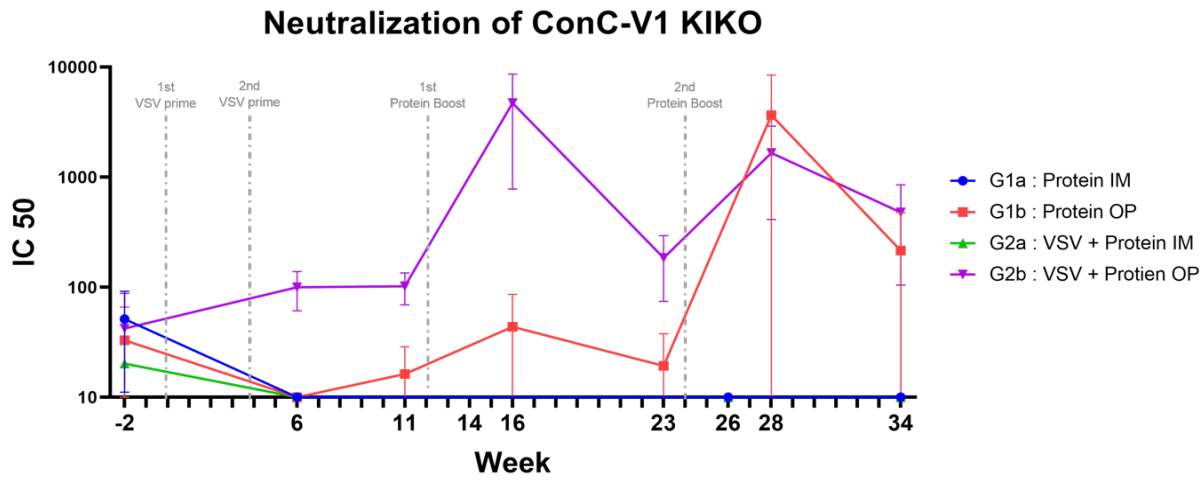


Figure 48. Neutralizing activity against sCON KIKO.

Immunization with sCON KIKO by OP route increases HIV-specific Abs and neutralizing activity against the autologous sCON KIKO virus. New advanced immunization strategies, including slow delivery of immunogens, should be undertaken to improve immunogenicity of vaccine candidates.

To sum up, generating new immunogen, VSV vector and OP improved the immunogenicity and neutralizing antibodies. However, the immune responses detected in NHP are lower as what we observed in rabbit as previously observed. The humoral responses induced with this new strategies need to be further assessed in Human in order to confirm the immunogenicity of the new designed immunogens.

4.3. New immunogen: Env targeting CD40 on DC

Human trials are necessary to evaluate whether the vaccine candidates are capable of inducing an efficient immune response potentially able to protect from HIV infection. Previous experiment on animal models showed that a new immunogen, env targeting CD40 at the surface of DC, demonstrated high immunogenicity.[452] This vaccine strategy was shown to form germinal center and stimulate functional CD4+ T cell and B cell maturation.[452–455] This immunogen was further selected for a phase I HIV vaccine trial in Human called VRI06. I participated in detecting the functional antibody responses, neutralization and ADCC.

VRI06 is a phase I, multicenter, double-blind, placebo-controlled, dose-escalation trial of CD40-HIVRI.Env (anti-CD40 monoclonal antibody fused with Env GP140 clade C ZM-96 of HIV) adjuvanted vaccine, combined or not with the HIV-1 vaccine DNA-HIV-PT123 in healthy volunteers (NCT04842682) (Figure 49). [456]

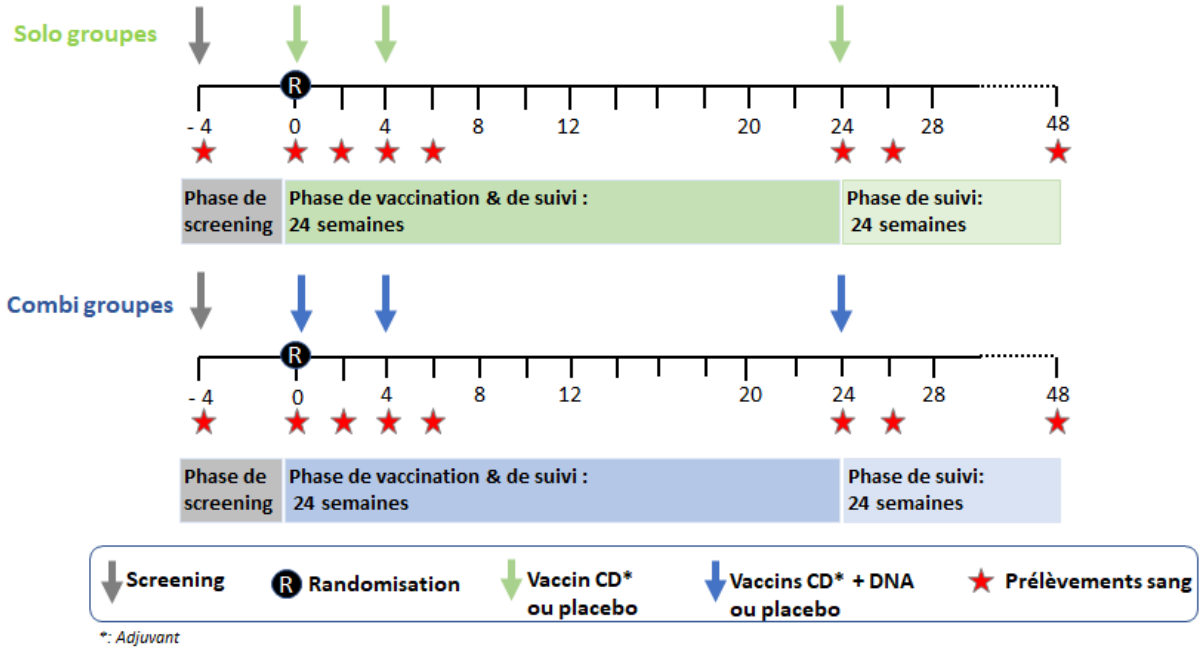


Figure 49. Protocol design of the VRI06 trial.

It aims to evaluate different doses of CD40.HIVRI.Env (adjuvanted with Hiltonol) alone and in co-administration with DNA-HIV-PT123. There are six groups in this trail: Solo 0.3, Solo 1 and Solo 3 (with 0.3, 1 and 3 mg/ml doses of CD40.HIVRI.Env), and Combi 0.3, Combi 1 and Combi 3 (CD40.HIVRI.Env combined with DNA-HIV-PT123) (Figure 50). Notably, since it's an on-goin trial, not all the samples are yet tested.

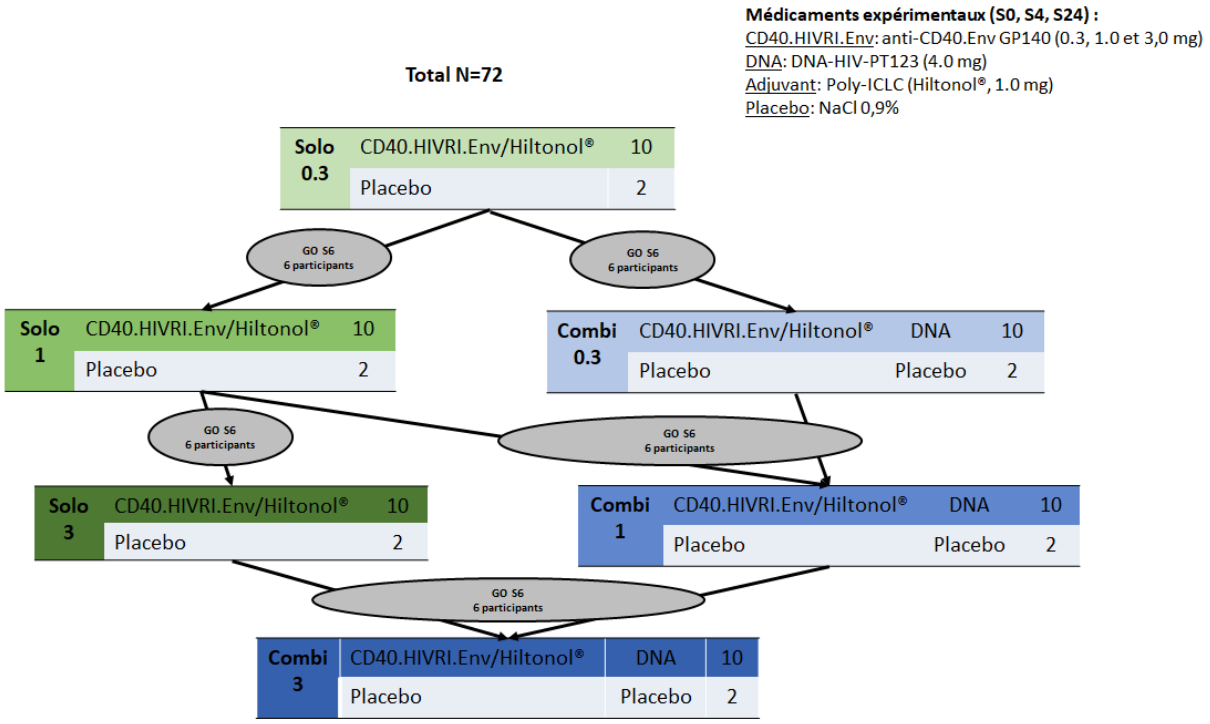


Figure 50. The six groups of this trail. These six groups include: Solo 0.3, Solo 1, Solo 3, Combi 0.3, Combi 1, and Combi 3.

This immunization strategy induced high and sustained HIV specific Ab response (not shown). I performed the neutralization abilities against Homologous 96ZM651 virus and two tier 1 viruses, SF162 and MW965.26 in Solo 0.3, Solo 1, Solo 3, Combi 0.3 and Combi 1 samples.

There is no neutralizing activity against homologous 96ZM651 detected in all the samples (not shown).

Neutralizing activity against Mw965.26 viruses were detected in the W6 and W 26 sample of the three solo groups (Figure 51). Interestingly, the neutralizing activity increased as the envelope concentration of vaccine got higher. There is significant difference between the neutralizing activities at W26, 2 weeks after 4th immunization, between Solo 0.3 and Solo 3 but not in other time points. This suggest that the higher immunogen treatment allow higher neutralizing Ab maturation in Solo 3 compared to the groups with lower immunogen treatments.

The neutralizing activity decreased at W48 and there is no significant difference between Solo 0.3 and Solo 1 at this time point (Figure 50). The immunization group Solo 3 at W48 is not yet available for Ab testing.

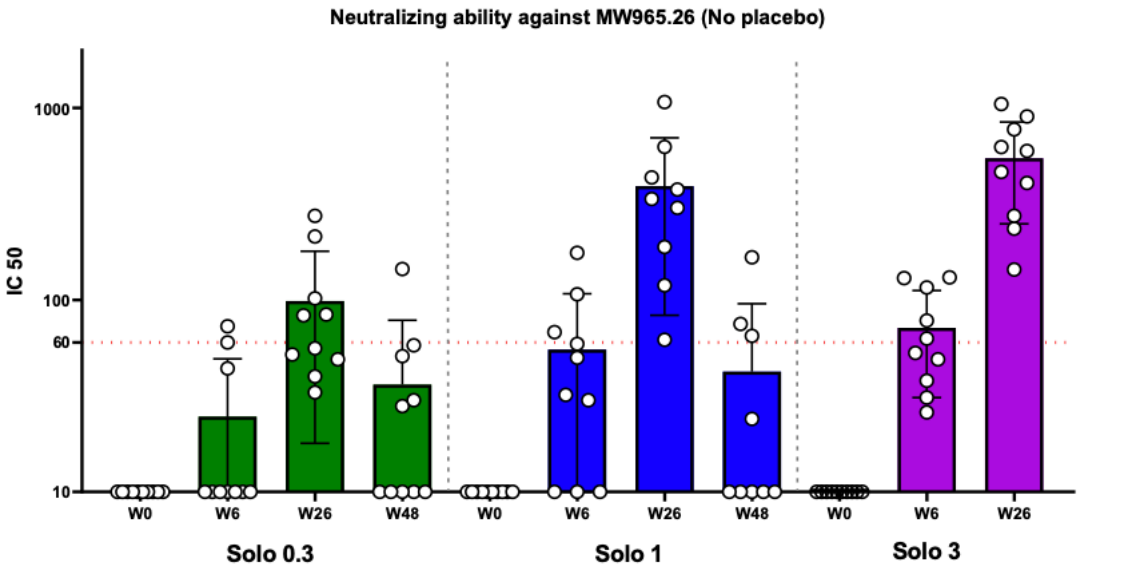


Figure 51. The neutralizing ability againsts MW965.26 pseudovirus. Three time points of three groups' result are displayed on the figure. There is significant difference between the neutralzing activities at W26 between Solo 0.3 and Solo 3, P = 0.0002.

The ADCC abilities against clade C HIV-1 CE1176 and HIV BJOX of Solo 0.3 W0, W6 and W26 samples were tested (Figure 52). I could not detect ADCC against these two viruses induced following vaccination (compared to pre-immune samples) using the ADCC protocol as described in material and method. Bon/OL, a serum sample form an HIV infected individual was used as positive control. ADCC was not detected against HIV-1 BJXO virus with Bon/Ol suggesting this virus is less susceptible to induce ADCC as compared to HIV-1 CE1176.

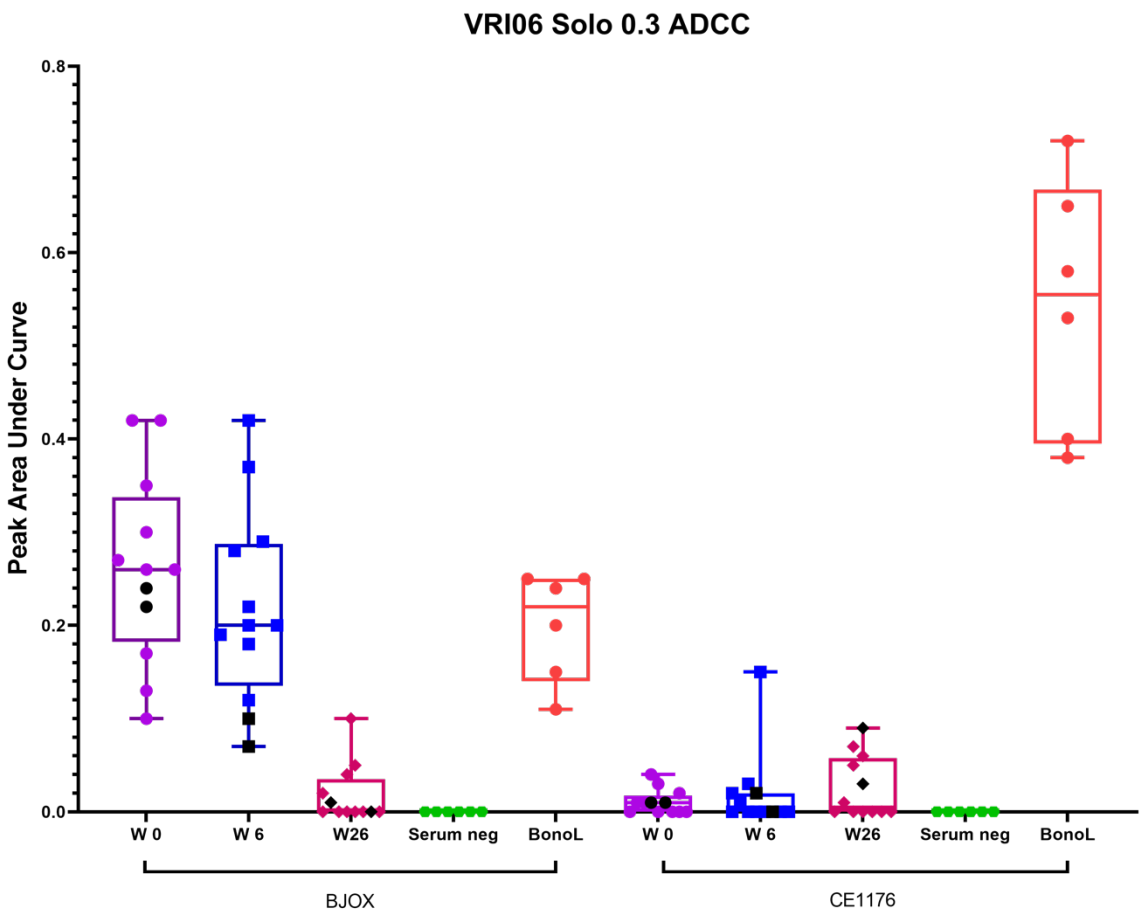


Figure 52. The ADCC abilities against CE1176 and BJOX viruses. There is no ADCC ability detected in the Solo 0.3 samples at W6 and 26 after immunization.

Besides the Solo groups, the neutralizing abilities of two Combi group, combi 0.3 and combi 1, were tested at two time points. I detected neutralizing activities against MW965.26 (Figure 53) but not against SF 162 and the homologous 96ZM651 strain (not shown). More time points' samples will be analyzed as they get available in order to evaluate the impact of the DNA boost on functional Ab response induced with combined immunization.

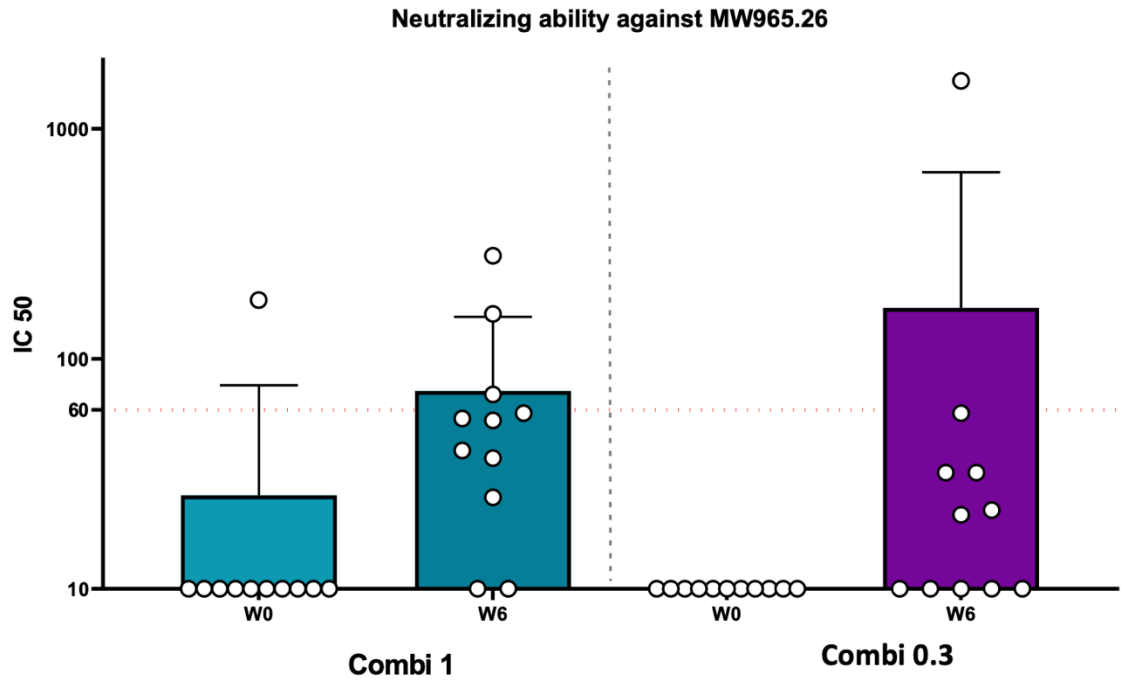


Figure 53. IC50 neutralizing activity against MW965.26 virus of Combi 0.3 and Combi 1. There is no significant difference between two groups at W6.

In conclusion, this new VRI06 immunization strategy aiming to target DC is highly promising as we could detect neutralizing Abs after only three immunizations with low doses of immunogens. The Fc-mediated functions and, immune memories will to be investigate for Solo 1 and Solo 3 in order to characterize more deeply the Ab response induced with this new vaccine strategy.

4.4. Fc-mediate function, FcR polymorphism and current pandemics

The interplay between IgG subclasses, multiple FcRs and polymorphisms thereof contribute to the complexity of the Fc-mediated response.[457,458] Multifaceted antibody functions and demographic attributes of patients must be considered for a desperately needed effective vaccine against HIV. Along with the HIV pandemic, the Sars-Cov-2 (Covid 19) pandemic caused urgent medicine and vaccine needs. Comparing to HIV, there are efficient Covid-19 vaccines to fight the pandemic, but the role Fc-mediated functions play still need further analysis and investigation. In this part, we use two review articles to discuss the current known Fc-mediated functions of two pandemics deeply.

4.4.1. Review 1: FcR polymorphism and the diversity of Ab Responses to HIV infection and vaccination *(Lin et al. Gene and Immunity)*

Besides neutralizing and Fc-mediated activity, additional factors such as Ab type, concentration and kinetics of induction, and Fc-receptor expression and binding capacity also influence the protective effect conferred by Abs. As these immune responses varied according to additional factors as ethnicity, age and sex, these additional factors should also be considered for the development of an effective immune response.

4.4.2. Review 1

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REVIEW ARTICLE OPEN

Check for updates

Fc receptors and the diversity of antibody responses to HIV infection and vaccination

Li-Yun Lin[1], Raphael Carapito[1,2], Bin Su[3] and Christiane Moog[1,4]✉

The development of an effective vaccine against HIV is desperately needed. The successive failures of HIV vaccine efficacy trials in recent decades have shown the difficulty of inducing an appropriate protective immune response to fight HIV. Different correlates of antibody parameters associated with a decreased risk of HIV-1 acquisition have been identified. However, these parameters are difficult to reproduce and improve, possibly because they have an intricate and combined action. Here, we describe the numerous antibody (Ab) functions associated with HIV-1 protection and report the interrelated parameters regulating their complex functions. Indeed, besides neutralizing and Fc-mediated activity, additional factors such as Ab type, concentration and kinetics of induction, and Fc-receptor expression and binding capacity also influence the protective effect conferred by Abs. As these parameters were described to be associated with ethnicity, age and sex, these additional factors must be considered for the development of an effective immune response. Therefore, future vaccine designs need to consider these multifaceted Ab functions together with the demographic attributes of the patient populations.

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INTRODUCTION

According to World Health Organization (WHO) data from 2020, 37.7 million people are living with HIV-1/AIDS and 68% of them are Africans [1]. In contrast to western Europe and America, where subtype B is predominant, subtype A is largely distributed in Eastern Europe and Central Asia and subtype C in East Asia. Africa shows the highest HIV-1 diversity with subtypes A and D in eastern Africa, C in southern Africa, A, G, CRF02_AG, and CRF06_cpx in western Africa, and B and CRF02_AG in northern Africa [2–4]. To fight against and end the HIV-1 pandemic, an efficient protective vaccine is needed. However, due to the high diversity of HIV-1 subtypes, vaccines need to induce antibodies (Abs) with broad inhibitory activity, i.e., antibodies able to inhibit numerous HIV-1 variants. This requirement is considered as one of the main limitations for the development of an efficient HIV vaccine [5, 6].

Over more than three decades, several HIV-1 vaccine trials have been conducted all over the world [7]. However, in HIV-1 vaccine history, only the RV144 phase III trial performed in Thailand showed a statistically significant decreased risk for HIV-1 acquisition at 42 months (31.2%) [8]. Interestingly, analysis of immune correlates for risk showed that Abs binding to the V1V2 region of gp120 correlated with a decreased risk for infection [9]. The IgG1 and IgG3 subclasses mediating antibody-dependent cell-mediated cytotoxicity (ADCC) seem to play a predominant role in protection against HIV-1 acquisition [10]. Moreover, the concentration of plasma envelope (Env)-specific IgA Abs was found to be directly correlated with a higher risk for HIV acquisition [10, 11]. These correlates of risk highlight the predominant role of isotypes and Fc-mediated functions in addition to the previously known protective role of neutralizing antibodies (NAbs). Knowledge of these new factors opens windows of opportunities for innovations in inducing a broad inhibitory humoral immune response to fight HIV and introduces new parameters to be considered, such as Fc domain/Fc receptor (FcR) interactions [12–17].

ANTIBODIES AND THE PLEIOTROPIC FUNCTION OF THE HUMORAL RESPONSE

Induction of HIV-specific Abs of various isotypes

The B cells of the immune system produce Abs that are classified into five major immunoglobulin (Ig) classes or isotypes: IgM, IgG, IgA, IgD, and IgE [18]. IgG is further divided into four subclasses (Fig. 1A) that are diversely distributed according to ethnicity, sex and age, with IgG1, IgG2, IgG3, and IgG4 representing 60–72%, 20–31%, 5–10%, and <4% of total IgG, respectively [19]. IgG subclass prevalence has been reported to change over time following the course of disease and symptoms [20]. Following HIV-1 infection, the adaptive immune response predominantly induces IgG1, IgG3 and IgA [21]. In the RV144 vaccine trial, high levels of HIV-1-specific IgG3 and low Env-specific IgA correlated with a decreased risk of HIV-1 infection [10]. The various Ab isotypes and subclasses bind differently to Fc receptors at the surface of immune cells, including dendritic cells and mainly macrophages

[1]Laboratoire d'ImmunoRhumatologie Moléculaire, Institut national de la santé et de la recherche médicale (INSERM) UMR_S 1109, Institut thématique interdisciplinaire (ITI) de Médecine de Précision de Strasbourg, Transplantex NG, Faculté de Médecine, Fédération Hospitalo-Universitaire OMICARE, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France. [2]Laboratoire d'Immunologie, Plateau Technique de Biologie, Pôle de Biologie, Nouvel Hôpital Civil, Strasbourg, France. [3]Beijing Key Laboratory for HIV/AIDS Research, Sino-French Joint Laboratory for Research on Humoral Immune Response to HIV Infection, Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing, China. [4]Vaccine Research Institute (VRI), Créteil, France. ✉email: c.moog@unistra.fr

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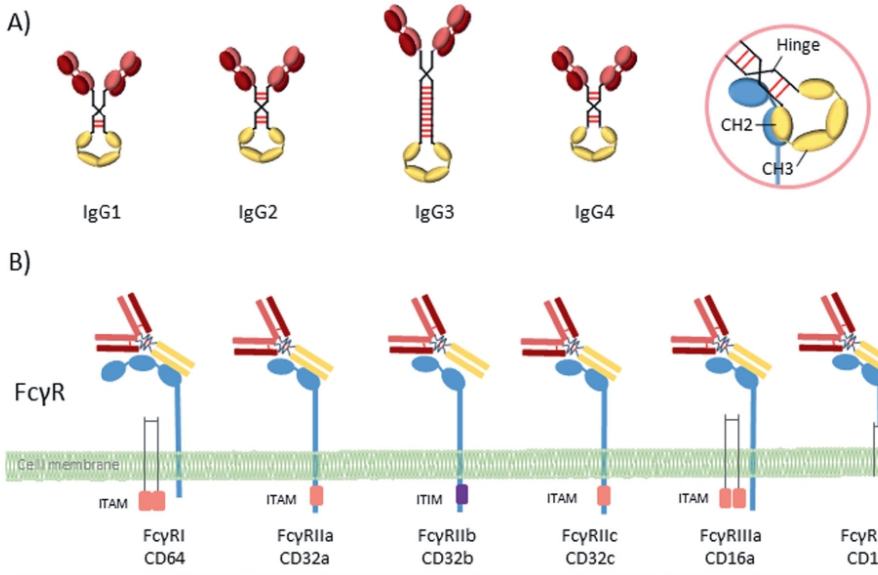

FcγR Main Functions	CDC Phagocytosis Opsonization	Antigen presentation, ADCC, ADCP			ADCC/ ADCP CDC degranulation	
Distribution	Mo, Mϕ, DC, Neu, Eos	Mo, Mϕ, DC, Neu, PLT	Mo, Mϕ, DC, MC, Neu, Bas, BC	NK	Mo, Mϕ, DC, NK	MC, Neu, Eos
SNP associated with modification of risk in HIV vaccine studies	-	rs1801274 rs2165088 rs10800309	rs6666965 rs6665610	rs114945036 rs138747765 rs78603008 rs373013207 rs201984478	rs396991	rs34085961 rs34322334 rs61803026

C)

FcγR	I	IIa		IIb	IIIa		IIIb	
Polymorphism		H131	R131		V158	F158	NA1	NA2
IgG1	+++	+++	+++	+	+++	++	+++	++
IgG2	-	++	+	-	+	-	-	-
IgG3	++++	++++	++++	++	++++	++++	++++	+++
IgG4	++	++	++	+	++	-	-	-

Fig. 1 Antibodies and FcR-mediated functions. A IgG subclasses. **B** Fc gamma receptors (FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa, FcγRIIIb), their main function, polymorphisms, and distribution on immune cells. **C** FcγR binding affinities of IgG subclasses. CDC complement dependent cytotoxicity, ADCC antibody-dependent cellular cytotoxicity, ADCP antibody-dependent cellular phagocytosis, Mo Monocyte, Mϕ Macrophage, DC Dendritic cell, MC Mast cell, Neu Neutrophil, Bas Basophil, Eos Eosinophil, NK Natural killer cell, BC B cell, PLT Platelet.

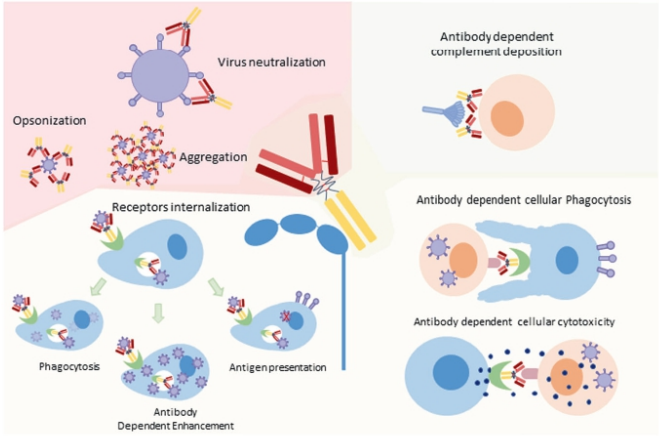

Fig. 2 HIV antibody functions. The functions are dependent on different Ab domains: The Fab domain is involved in virus neutralization, opsonization and aggregation; the Fc domain of Ab induces the activation of the complement system; dual binding of Ab via Fab and Fc domains leads to Fc-mediated antibody function: antibody-dependent cellular phagocytosis and antibody-dependent cellular cytotoxicity; FcR internalization may lead to phagocytosis, antigen presentation or antibody-dependent enhancement.

(Fig. 1B). As these cells are the best-in-class antigen-presenting cells, different Ab isotypes and subclasses directly affect Ab binding to antigen-presenting cells, modulating immune cell activation and consequently the quality of the humoral immune response that is induced [22]. Comprehensively interrogating the extensive biological Ig diversity in patients may provide critical insights that can guide the development of effective Ab-based vaccines and therapies.

Two main antibody functions observed in HIV-infected patients and in vaccine trials: neutralization and Fc-mediated functions

NAbs protect cells from pathogens or infectious particles by inhibiting any effect leading to infection via the binding of their Fab domain to the infectious agent (Fig. 1B) [23, 24]. Studies of the passive injection of broadly NAbs in nonhuman primate (NHP) models demonstrate their high potential for conferring protection against HIV acquisition [23, 25]. Considering these data, immunogens aiming to induce the production of these NAbs were developed [23, 26]. Many vaccines have been designed to induce Abs targeting the envelope glycoproteins of the virus, mainly gp120 or gp160 [26–28]. However, these vaccines failed to induce broadly NAbs. Indeed, the production of broadly NAbs is extremely difficult to induce due to the need for an extensive maturation process [29, 30].

The success of the RV144 vaccine trial supported the development of new vaccine designs for the induction of Abs with additional functions, mainly Fc-mediated Ab functions [31, 32]. It has been proposed that several Fc-mediated mechanisms, including ADCC, antibody-dependent cellular phagocytosis (ADCP), antibody-dependent complement deposition (ADCD), aggregation and immune activation, participate in HIV inhibition (Figs. 1B, 2) [14, 33–37]. In addition, viruses can be directly opsonized by phagocytosis *via* Ab and FcR binding. The virus is then destroyed, and digested peptides can be retrieved by antigen-presenting cells for T cell activation (Fig. 2) [17, 34, 38, 39]. If the virus escapes this lysis process, opsonized virus entry may also lead to increased infection by a process called antibody-dependent enhancement (ADE) [40]. This ADE function should of course be avoided [41–43]. All these different Fc-mediated mechanisms involve the binding of the Fc domain of the Ab to the Fc receptor present on immune cells. The Fc-mediated functions of Abs are therefore also directly interconnected with FcR expression at the surface of immune cells [44, 45].

MODULATING FCR EXPRESSION AT THE SURFACE OF IMMUNE CELLS

FcRs are cell surface glycoproteins that bind to the Fc domain of Abs. This binding varies according to the isotype and subclass of the Ab but also according to the type of FcR (Fig. 1B, C) [44–46]. These FcRs are differentially expressed on most immune cells, including natural killer (NK) cells, monocytes, macrophages, eosinophils, dendritic cells, B cells and even some T cells [17, 46]. There are three family classes of FcRs (I, II, and III), each of which comprises a different number of proteins: FcγRI, FcγRIIa, FcγRIIb, FcγRIIc, FcγRIIIa and FcγRIIIb (Fig. 1B) [18]. All human FcγRs except FcγRIIB signal through an immunoreceptor tyrosine-based activating motif (ITAM), whereas FcγRIIB delivers inhibitory signals through an immunoreceptor tyrosine-based inhibitory motif (ITIM) [4, 46]. The diversity of human FcγRII and III is further increased by single nucleotide polymorphisms (SNPs) in their extracellular domains, the most studied of which are H131R in FcγR gene FCGR2A, 126C>T in FCGR2C, F158V in FCGR3A, and NA1/2 in FCGR3B (Fig. 1C). FcγRIIC has an unusual structure and is generated by an unequal crossover between FcγRIIA and FcγRIIB. FCGR2C signals through the ITAM similarly to FCGR2A. FcγRIIC (126C>T), rs114945036 presumably lead to an open reading frame with an atypical FcR protein sequence.

Importantly, the different FcR polymorphisms of the host need to be considered when analyzing FcR-mediated functions of Abs. FcγR SNPs will impact both on the the binding to the complementary Fc portion of the Abs and on the expression or activation state of the cells [46] (Fig. 1B). Increasing evidence suggests that FcγR SNPs impair receptor expression on DCs, which in turn influences the risk for HIV infection and vaccine efficacy [15, 16, 47]. Interestingly, a combination of polymorphisms may also influence FcR expression, such as the combination of rs1801274 and rs10800309 in the FcγRII coding gene FCGR2A, which affects the expression level of FcR on immature dendritic cells [48]. FcγRIIIA polymorphism appears to modify NK cell activation and, as a consequence, ADCC activity [49].

Table 1. HIV vaccine trials.

Vaccine trial	Year	Location	Target population	Vaccine	Ab function		Fc receptor			Vaccine efficacy	Ref.
					Neutralization	Fc mediated	FcR	Polymorphism	Association with risk of infection		
VaxSyn	1987	Canada	72 adults	Recombinant envelope glycoprotein subunit (rgp160) of HIV	Low Tier 1	*NFD*	–	–	–	No	[66, 67]
HIVAC-1e	1988	USA	35 male adults	Recombinant vaccinia virus designed to express HIV gp160	N/F	ADE	–	–	–	No	[68, 69]
Vax004	1998–2002	North America	5417 MSM and 300 women	AIDSVAX B/B gp120 with alum	Tier 1	ADCC ADCP	FCGR2A FCGR3A	rs1801274 rs397991	↓ ↑	No	[37, 50, 52, 77, 84]
Vax003	1999–2003	Thailand	2545 mem and women IDUs	AIDSVAX B/E gp120 with alum	Tier 1	ADCC	–	–	–	No	[77, 83, 84]
STEP/ HVTN502	2004–2007	North America, Caribbean South America, and Australia	3000 MSM and heterosexual men and women	MRKAd5 HIV-1 gag/pol/nef trivalent vaccine	Low Tier 1	*NFD*	–	–	–	1.4 increased risk of infection	[70, 71, 74, 75]
Phambili/ HVTN503	2003–2007	South Africa	801 adults	rAd5 (gag/pol/ nef)	Low Tier 1	*NFD*	–	–	–	1.7 increased risk of infection	[72, 73]
RV144	2003–2009	Thailand	16,402 community-risk men and women	ALVAC-HIV (vCP1521) and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	FCGR2C	rs1149455036 rs138747765 rs78603008	↓	31.2% decreased risk of infection	[8, 10, 51, 54, 82, 83, 85, 86]
HVTN505	2009–2013	USA	2504 men or transgender women who have sex with men	Three vaccinations with DNA encoding HIV clade B gag, pol and nef as well as env from HIV clades A, B and C followed by an Ad5 vector-based vaccine encoding clade B gag and pol as well as env from clades A, B and C	Low Tier 1	ADCC ADCP	FCGR2A FCGR2C FcGR3B FCGR2B	rs2165088 rs138747765 rs78603008 rs373013207 rs201984478 rs34085961 rs34322334 rs61803026 rs6666965 rs6665610	↓ ↑ ↑ ↓	No	[54, 81, 87]
HVTN305	2012–2017	Thailand	162 women and men	ALVAC-HIV and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	–	–	–	No	[86, 88]
HVTN306	2013–2020	Thailand	360 men and women aged 20–40 years old	ALVAC-HIV and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	–	–	–	No	[89, 90]
HVTN097	2012–2013	South Africa	100 black Africans (men and women) aged 18–40 years old	ALVAC-HIV (vCP1521) and AIDSVAX B/E vaccine	Low Tier 1	ADCC ADCP	–	–	–	No	[91]
HVTN100	2015–2018	South Africa	252 men and women	ALVAC-HIV (vCP2438) and bivalent subtype C gp120/MF59	Low Tier 1	ADCC ADCP	–	–	–	No	[92–94]

Table 1. continued

Vaccine trial	Year	Location	Target population	Vaccine	Ab function		Fc receptor			Vaccine efficacy	Ref.
					Neutralization	Fc mediated	FcR	Polymorphism	Association with risk of infection		
HVTN705/ Imbokodo	2017–2021	Sub-Saharan Africa	2637 women ages 18 to 35 years	Ad26.Mos4.HIV, adjuvanted clade C and Mosaic gp140 HIV bivalent vaccine	–	–	–	–	–	Comparing with RV144, unable to improve the efficacy on Sub-Saharan Africa women	[31, 76, 78–80]

Illustration of completed and documented or on-going major phase 1b to phase 3 HIV trials that analyzed the Ab and/or Fc Receptor functions.
NFD no Fc-mediated function detected, – no related publications found.

Specific polymorphisms at the FCGR2A (encoding Arg or His at position 131) and FCGR3A (encoding Phe or Val at position 158) gene loci have been associated with an HIV vaccine benefit [50]. The rs396991 SNP leads to an increased binding capacity of Abs for FcγRIIIA, which is the main receptor involved in ADCC, suggesting that the vaccine efficacy may be related to an increased efficacy of this function. More recently, Li et al. described that a tag SNP (rs114945036) in FCGR2C (126C>T, presumably leading to a stop codon or an open reading frame) was significantly associated with protection against infection with a subtype AE HIV-1 strain in the RV144 vaccine clinical trial [51]. The direct effect of this SNP is not well documented. Authors propose that it may lead to an alternative splicing, bypassing the FCGR2C-Stop codon to encode a product with an atypical FcR protein sequence, thereby modifying FcR expression or accessibility on cells [51].

Overall, the interplay between IgG subclasses, multiple FcRs and polymorphisms thereof contribute to the complexity of the Fc-mediated response [15, 46]. As a consequence, numerous studies have analyzed the association between FcR genes or their polymorphisms and the evolution of HIV disease or vaccine protection (Table 1) [50–55].

EFFECT OF ETHNICITY, SEX, AND AGE ON FC-MEDIATED AB RESPONSE TO HIV

Several studies have shown that serum Ig concentrations vary according to ethnicity, sex, and age. Total IgG and IgA levels increase with age and reach the adult concentration at ~10 years of age. Thereafter, the levels of serum IgG were found to be significantly reduced with age, and the level of IgA was found to be maintained. Total IgG and IgA concentrations are higher in Black populations than in White populations [19, 56, 57]. A similar result of higher total IgG levels in HIV-infected Africans than in Caucasians and Hispanics was also found [57–60]. Notably, all these studies comparing Ab profiles according to ethnicity were performed in individuals living in the same country. The difference in Ab responses in Africans living in Africa and Caucasians living in Europe or the USA needs to be investigated to integrate the effect of geographic origin in these studies.

In addition, age-related differences in clonal expansion with decreased IgA levels and skew toward IgG2 were observed after influenza vaccination [61, 62].

These results illustrate the importance of Ab classes in vaccine studies. This difference in Ab isotypes and concentrations according to ethnicity, age and sex may directly impact FcR functions and influence the efficacy of Ab induction in HIV-vaccinated individuals.

The demonstration of the role of Fc-mediated function also brings into question the importance of FcR features. The frequencies of SNPs of FcR genes differ significantly between ethnic groups [63–65]. These differences may strongly modify the association found between FcR polymorphisms and HIV-1 protection or disease outcome. In Kawasaki disease for example, the association with the FCGR2C-ORF haplotype becomes evident only when Asians, in whom FCGR2C-ORF is a nearly absent haplotype, are excluded from the cohort [64].

Overall, analyzing Fc-mediated Ab functions without considering ethnicity, sex, and age is hazardous. These factors need to be considered for genotype/phenotype association studies, as well as for the analysis of FcR involvement in HIV vaccine trials.

FCR AND AB FUNCTIONS IN VACCINE TRIALS

During the past three decades, several HIV-1 vaccine trials have been performed all over the world. The first vaccine trial tested the recombinant envelope glycoprotein subunit (rgp160) in 72 adults. This vaccine showed induction of NAbs but not Fc-mediated Ab responses [66, 67]. The second HIV-1 trial (HIVAC-1e) used

recombinant vaccinia virus that expressed HIV-1 gp160, and its administration resulted in no induction of neutralizing Ab or Fc-mediated Ab responses, even though ADE was detected [68, 69]. Whether this lack of detectable Ab function was due to technical issues needs to be further assessed. Thereafter the following vaccine trials using envelop antigens succeeded in inducing both neutralizing and Fc-medicated Ab responses (Table 1). Of note, the $CD4^+$ T cell-driven HIV immunogens used in the HVTN502 and HVTN503 vaccine trials did not contain envelop antigens, and led to an increased risk of infection [70–75]. FcR variants and their potential association with a decreased risk for infection were further investigated in three vaccine trials: Vax004, HVTN505 and RV144 (Fig. 1B). Although the Vax004 and HVTN505 vaccine strategies did not show efficacy, distinct FCGR polymorphisms have been associated with either an increased or decreased risk for HIV-1 acquisition (Table 1). For the RV144 vaccine trial conducted in Thailand, an association between the FCGR2C rs114940536, rs138747765, rs78603008 polymorphisms and a decreased risk for HIV acquisition was shown [51]. While focusing on fighting the HIV-1 pandemic in Africa, a similar strategy to that used in the RV144 trial was initiated in the South African area [76–79]. This trial, called HVTN702, did not reach the efficacy requirement of RV144 and was therefore stopped prematurely [80]. This failure could be explained by the fact that Black South Africans do not possess the FCGR2C haplotype that was associated with increased vaccine efficacy in the RV144 trial [63]. Collectively, the differences in FCGR2C polymorphisms in South Africa versus Thailand highlight the need for further mechanistic investigations to define the functional relevance of FcR polymorphisms in HIV-1 protection, especially in the context of vaccination. Interestingly, HVTN505 conducted in the USA showed different FcγR SNPs associated with a different hazard ratio of HIV-1 acquisition from that of RV144. In the HVTN505 trial, patients receiving the vaccine had significantly higher incidences of HIV acquisition than those receiving placebo among participants carrying the FCGR2C-TATA haplotype or the FCGR3B-AGA haplotype. Moreover, an FCGR2A SNP (rs2165088) and two FCGR2B SNPs (rs6666965 and rs666561) influenced the correlation of anti-gp140 antibody-dependent cellular phagocytosis with HIV risk [81]. Of note, the HVTN505 and RV144 trials differed in a number of points, i.e., canarypox prime/protein boost in a general low-risk Thai population in RV144 versus DNA prime/rAd5 boost in a high-risk U.S. population of men who have sex with men in HVTN505.

These results indicate that the functional impact of a given FcγR polymorphism on the risk for HIV-1 acquisition is highly context specific, depending on the specific vaccine regimen but also on other factors, such as demographics, virus quasi-species, and genetic background [53, 81, 82].

DISCUSSION OF FUTURE ASPECTS

RV144 was the sole HIV-1 vaccine trial that showed a limited but statistically significant decreased infection risk [8, 10, 82]. As this protection was not associated with neutralization but with specific Ab types and Fc-mediated function, increased efforts were made to obtain a more in-depth characterization of the induced HIV-specific Ab response [10, 54, 82]. Indeed, in addition to HIV-specific Ab response and neutralizing activity, the specificity of the recognized epitope and Fc-mediated functions were investigated (Table 1). In addition, the FcR polymorphisms associated with infection outcome were explored [50–52, 54, 55, 81, 82]. However, taken individually, none of these factors could be associated with protection. For example, attempts to associate FcR genotypes with HIV outcome resulted in variable, sometime contradictory, results (Table 1). These results largely suggest that multiple Ab factors, including Ab class and subclass, structures, Fc domain interactions with Fc receptors, FcR locus copy number and FcR polymorphisms, may impact vaccine efficacy with synergistic or sometimes antagonistic effects [83]. Moreover, as Ab concentrations and FcR polymorphism frequencies vary according to ethnicities, analysis of correlates of infection risk need to take these additional parameters into consideration [63–65]. These results shed light on the complexity of the humoral response that may be correlated with a decreased risk of HIV-1 acquisition. Future vaccine strategies need to address humoral Ab induction as a whole challenging the different characteristics of the Abs and FcRs required to obtain the most promising combination of humoral responses associated with protection.

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AUTHOR CONTRIBUTIONS

CM and LYL contributed to the writing. RC and BS corrected, amended and edited the paper. All authors provided feedback on the report.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Correspondence and requests for materials should be addressed to Christiane Moog.

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4.4.3. Review 2 : Are induction of Fc mediated immune responses important for HIV and SARS-Cov-2 vaccine protection ? (Submitted)

The major current pandemics involve two viruses, HIV and SARS-Cov-2. These pandemics already cause millions of deaths. Neutralizing antibodies were found to be critical for vaccine protection. However, it has to be recognized that additional Fc-mediate functional Abs provided unexpected help to counteract virus mutations gained to escape from Nabs. In this review, we discussed the current knowledge of Fc-mediated functions involved in HIV and Sars-Cov-2 inhibition and the limitation of the current experimental methods evaluating these Fc-mediated function.

4.4.4. Review 2

Are induction of Fc-mediated immune responses important for HIV and SARS-CoV-2 vaccine protection?

Li-Yun Lin [1], Bin Su [2] and Christiane Moog [1,3]*

[1] Laboratoire d'ImmunoRhumatologie Moléculaire, Institut national de la santé et de la recherche médicale (INSERM) UMR_S 1109, Institut thématique interdisciplinaire (ITI) de Médecine de Précision de Strasbourg, Transplantex NG, Faculté de Médecine, Fédération Hospitalo-Universitaire OMICARE, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France.
[2] Beijing Key Laboratory for HIV/AIDS Research, Sino-French Joint Laboratory for Research on Humoral Immune Response to HIV Infection, Clinical and Research Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China
[3] Vaccine Research Institute (VRI), Créteil, France
* Correspondence: c.moog@unistra.fr

Abstract: The development of effective vaccine is crucial to fight against new virus pandemics. For HIV and SARS-CoV-2, induction of neutralizing antibodies (NAb) were found to be key for vaccine protection. However, it has to be recognized that additional Fc-mediate functional Abs may provide unexpected help to counteract virus mutations gained to escape form NAbs. These additional humoral functions are complex and will need further consideration to understand the mechanisms underlying this protection in order to induce such Abs by vaccination. This review provides a comprehensive update on the current knowledge of Fc-mediated functions involvement in protection.

Keywords: HIV; SARS-CoV-2; Vaccine; Neutralizing antibody; Fc-mediated immune response

1) Introduction

History of vaccine inducing protective Abs

For the previously developed and approved vaccines, detection of specific Abs able to bind to the pathogen were used as surrogates for vaccine efficacy. This was mainly performed by detecting Abs able to bind to pathogen-specific epitopes by ELISA binding assay. The induction of such pathogen-specific antibodies was generally associated with vaccine protection. It rapidly become clear that these Abs were not associated with human immunodeficiency viruses (HIV) vaccine efficacy.

Discovered in 1983, HIV continues to be a major global public health issue, having claimed 40.1 million lives so far [1]. According to the data release from WHO, there

were an estimated 38.4 million people living with HIV at the end of 2021 [2]. To fight against HIV pandemic, an efficient vaccine is urgently needed. However, after more than 40 years of research, we are still deeply investigating new vaccine strategies leading to protection. Indeed, HIV wickedly hijack the immune response towards non-functional decoyed Abs. The HIV-specific Abs inducted by traditional vaccine strategies were found to be completely inefficient for protection [3–5].

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a coronavirus that causes the respiratory illness, COVID-19 (coronavirus disease 2019) is responsible for an on-going pandemic already leading to more than 6.4 million deaths [6]. As HIV, fighting against SARS-CoV-2 rely on development of an efficient vaccine. For SARS-CoV-2, vaccine was developed extremely quickly thanks to the new mRNA vaccine platform [7]. These vaccines efficiently protect from ancestral vaccine driven strain, but the protection drastically decline with the emergence of new variant of concerns (VOCs) displaying numerous mutations [8]. However, despite becoming infected, vaccinated people remain protected from server disease [9].

2) Role of neutralizing Abs in vaccine protection

For the development of an effective HIV vaccine, prototype immunogens able to target highly conserved masked epitopes on the HIV envelop trimeric spike need to be conceive. Abs directed to these specific epitopes were found to display functional neutralizing activities. Neutralizing antibodies (NAbs) were considered as the best-in-class functional Abs with the capacity to inhibit infectivity of pathogen [10,11]. This inhibitory mechanism did not require other immune components. Noteworthy, sterilizing protection against chimeric SIV/HIV (SHIV) acquisition could be repeatedly achieved by passive transfer of NAbs in the non-human primate model challenged experimentally [12, 13] These results of protection in the macaque model completely re-boosted HIV vaccine research programs. Unfortunately, NAbs were found to be very difficult to induce by vaccination. Indeed, virus mutations escape the immune response and only a limited number of Abs directed to specific epitopes were able to inhibit a broad range of HIV strains [14]. These broadly NAbs were only induced in a few HIV infected individuals, also called ELITE neutralizer, after several years of infection [14,15]. They need very high B cell maturation in order to target neutralizing conserved epitopes that are often hidden by the virus. Such very complex maturation cannot be obtained by classical vaccine strategies.

Concerning SARS-CoV-2 vaccination, mRNA platforms against this virus have been rapidly developed. This vaccine induced high and efficient SARS-CoV-2 specific

NAbs against the homologous vaccine strain [15]. NAbs have been consistently associated with decreased risk of SARS-CoV-2 acquisition [17].

However, vaccine protection is not unambiguously associated with neutralizing activity [18–21]. SARS-CoV-2 specific Abs rapidly decline and re-boosting is necessary to re-activate the SARS-CoV-2 specific and protective NAb response [22]. Moreover, the new VOCs progressively escape from these neutralizing responses response leading to infection with the new variants [23–25]. Yet, despite this drastic decrease in neutralizing potency, vaccinated individuals that became infected were still protected from sever disease suggesting the participation of additional Ab inhibitory functions [18, 26].

2) Role of Fc-mediated Ab inhibitory function in vaccine protection

In the HIV field, by searching for additional Ab functions, it became obvious that Fc domain of the Ab play an additional inhibitory role [27]. Later on, Fc-mediated functions were found to be associated with decreased risk of HIV acquisition in the phase III RV144 vaccine trial. This trial involving more than 16 000 volunteers with low risk of HIV infection showed a 31.2% decreased risk of HIV acquisition without induction of neutralizing Abs [28, 29]. The decreased risk was correlated with non-NAbs directed against V1/V2 and ADCC [29 - 33]. Interestingly, non-neutralizing anti-V2 monoclonal Abs elicited in HIV-1-infected patients showed strong cross-reactive ADCC activity using different primary subtype B and C isolates as well as subtype B Transmitted/Founder viruses *in vitro* [30, 34]. Moreover, monoclonal Abs against V1/V2 similar to those induced by RV144 vaccine trial demonstrated strong ADCC activity in vitro against primary infected cells [34].

Following these findings, numerous studies were conducted to research for correlates of protection distinct to neutralization. A new vaccine trial, HVTN 702, was conducted in South Africa using a quite related regiment as for RV144. Despite a lack of efficacy and distinct immunogenicity profile, HVTN 702 showed a similar correlation of IgG V1V2 induction with decreased risk of HIV-1 acquisition in a subset of volunteers [35]. Data obtained during HIV infection support the role of non-NAbs in HIV protection. First, in mother to child HIV-1 transmission, pre-existing functional Abs in mothers were found to correlate with lower morbidity, reducing the HIV-1 acquisition and improving the survival rate of infants [36 - 38]. Next, ADCC were found to correlate with a slowdown of disease progression [39, 40]. In the non-human primate (NHP) model, infusion of Abs with ADCC activities reduces viremia [41 – 45]. These different results run alongside with a role of Fc-mediated Ab function in HIV replication. However, whether induction of such Abs is key for fighting against HIV

pandemic will need further investigations. Moreover, we need to identify the mechanisms associated with this protection in order to improve the forthcoming vaccine designs [11, 46–49].

For SARS-CoV-2 infection, the rapid decline of NAbs following vaccination and the escape neutralizing response by VOCs clearly revealed additional functional Ab activities [50]. Abs displaying ADCC were also found to be associated with protection [51-53] [Table 1]. Abs displaying ADCC/ADCP activities were associated with lower systemic inflammation following SARS-CoV-2 infection [19, 51, 54]. Moreover, hybrid immunity of vaccinated individuals that previously experienced COVID-19 offers a qualitatively improved antibody response able to better leverage Fc effector and ADCC potential [54, 55]

Fc-mediated Ab induced	Correlation with	HIV	SARS-CoV-2
Pre-exist (Mother to Kid)	• Reduced mortality	Yes [36, 37]	NA[1]
Following infection	• Slowed down disease progression • reduction in viremia	Yes [38 - 40, 56, 57]	
	• lower systemic inflammation		Yes [19, 20, 54]
By vaccination	• Related to prevention (vaccine efficacy)	Yes [29 – 33, 35, 58 - 62]	Yes [21, 50, 51,53]
Hybrid Infection+vaccination	• increased binding, neutralizing and ADCC potential	Therapeutic [63]	Yes [54, 55, 64]

Table 1. Induction of Fc-mediated function and correlations for HIV and SARS-CoV-2. [1] NA: not available

These various results ascertain the potential participation of additional Ab functions. However, it is not yet clear to what extend and how they exactly participate to protection. Additional studies will be necessary to decipher the precise contribution of Fc-mediated function, how and to what extend they participate to protection and how we need to drive the immune response to efficiently induce such additional functional Ab response.

Fc-mediated function detected *in vitro*

In order to better understand the role of Fc-mediated function, we need to characterize them *in vitro*. However, these functions involve complex mechanism with Abs interacting with effector cells that make their detection highly challenging and *in vitro* assays are difficult to develop [57].

Two Fc-mediated inhibitory functions have been mainly analyzed *in vitro*: antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). For these two Fc-mediated functions, the Ab need to recognize an infected target cell via its Fab domain on one hand, and to bind via its Fc domain to a specific Fc-receptor expressed on the surface of an effector cell (dendritic cells or macrophages) [Figure 1]. This cross binding then lead either to the lysis of the infected target cell (ADCC) or to the engulfment and digestion of virus by phagocytosis (ADCP) [65, 66].

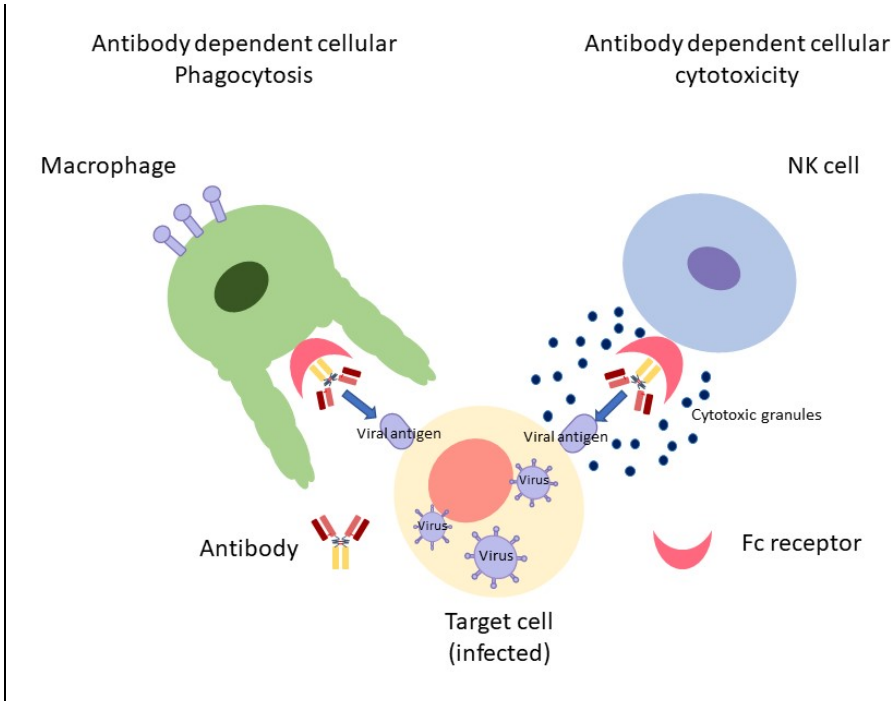

Figure 1. Fc-mediated functions: antibody dependent cellular phagocytosis (ADCP) and antibody dependent cellular cytotoxicity (ADCC).

A profusion of assays has been developed to allow *in vitro* measurement of the ADCC and ADCP functions, mainly for HIV and more recently for SARS-CoV-2 [Table 2]. These assays sometimes record different events, as FcR binding or capture of beads coated with antigens. However, these assays cannot exactly recapitulate the intrinsic interaction leading to *in vivo* effector functions. In the HIV field, only a few assays analyzing infected cell lysis with primary cells and replication competent virus have been developed. Such type of assays still needs to be implemented for SARS-

CoV-2. Noteworthy, assays analyzing cell lysis on primary cells do not necessarily give comparable results to that analyzing binding or using cell lines. The former may be more physiologically relevant but are time consuming and difficult to standardize. The question of the relevance of these different *in vitro* assays and their correlates with *in vivo* protection is questioned [57].

	Target cell	Effector cell	Antigen/Virus	Readout	Ref
HIV	▪ CEM.NKR.CCR5 1. HIV-infected 2. Reporter virus-infected 3. Antigen-pulsed ▪ Primary CD4+ T cell	Coated FcR Cell line Primary cell	Antigen-Coated beads Pseudovirus Infectious virus	Binding to FcR GzB uptake Expression of Luc in infected cells Target cell death Virus production and release	[63,66-70]
SARS-CoV-2	Reporter-only cell line (S-protein expressed)	Cell line Primary cell	Antigen-Coated beads Pseudovirus	Binding to FcR Expression of Luc in infected cells	[54,71-73]

Table 2. *In Vitro* ADCC assays for HIV and SARS-CoV-2

Conclusions

How can we ascertain the role of Fc-mediated function in protection?

The challenge now is to prove that the numerous associations between Fc-mediated function and protection are indeed relevant. This question is crucial to demonstrate that Abs displaying Fc-mediated function are worthy to induced by vaccination.

To answer this question, passive transfer of HIV specific Abs displaying non-neutralizing Fc-mediated functions have been performed in animal models. These experiments have shown decreased virus load or numbers of founder viruses and reduce virus burden following experimental virus challenge [42–45, 74]. However, sterilizing protection was not observed with these experiments. Moreover, modification of the fucosylation site in the heavy chain of Abs aiming to increase the Fc-mediated function did not show improvement of protection in challenge experiments with the NHP model [75]. The main concern of these experiments is the relevance of the NHP model for the investigation of Fc-mediated function of human Abs. Bias may be observed due to biophysical and functional differences of IgG

subclasses and Fc receptors and their possible triggering of Fc functions between human and NHP [76].

The use of passive transfer of Abs for these studies has other caveat for analyzing Fc-mediated Ab functions. Indeed, passive transfer of Ab allow high Ab concentration in the periphery, but the concentration of Ab in tissues is limited. It is nonetheless in tissues that the Fc-mediated function may be of major interest [77]. It is in tissues that antigen-presetting cells bearing Fc receptor mainly persist; thus, it is in tissues that we may expect to detect protective Fc-mediated inhibitory function.

Noteworthy, recent studies show that HIV-1 has evolved to escape recognition by NnAbs mediating ADCC. The "closed" conformation adopted by Env allows escaping from NnAb recognition and Vpu auxiliary protein, by reducing Ab recognition of infected cells, renders HIV resistant to ADCC [78][79]. This escape mechanism indirectly highlights the potential in vivo protective activity of ADCC.

Additional studies are desperately needed to characterize the inhibitory steps leading to virus destruction by Fc-mediated Ab *in vivo*. This crucial information will help to ascertain the role of Fc-mediated Ab functions in HIV and now in SARS protection and guide us for further vaccine strategy developments [46,80].

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4.5. Host-Vaccine interaction

A project supported by HIP (HVTN Initiative Project) have been developed in collaboration with the College of Health and Allied Sciences (UDSM-MCHAS, Tanzania), and the Medical Center of the University of Munich (LMU, Germany). This project aims to compare the induction of HIV-specific antibody response and their function between individuals with Caucasian and African ethnicity. For this project, the phase IIA HVTN 204 clinical trial was chosen because the same vaccine protocol was conducted in both United States and South Africa. We have gained access to historical samples (serum and cells) from 137 vaccination volunteers at two time point: visit 2 (V2) collected before first vaccine injection and visit 10 (V10) collected after volunteers received three immunization and one boost.

Total and HIV specific antibody IgG and IgA, neutralization, Fc-mediated function (antibody dependent cytotoxicity, ADCC), and FcR polymorphism were determined. The statistical analysis of the results was determined in an ethnicity aspect, grouping the volunteers in 3 groups: Caucasian, African living in USA, and African from SA only.

These results are currently being prepared for submission, so they are presented in manuscript form.

4.5.1. Manuscript

Title: Deciphering the Antibody response induce by HVTN 204

Li-Yun Lin[1,*], Mkunde Chachage[2, 3, 4,*], Thomas Ferte[5,*], Celso Casteano[6], Augusta Horvath[4], Michael Hoelscher[4,7], Kathrin Held[4,7], Julie Lucas[1], Géraldine Laumond[1], Sylvie Schmidt[1], Edna Viegas[6], Christof Geldmacher[4,7], Edouard Lhomme[5,8], Christiane Moog [1,8$]

[1] UMR_S 1109 INSERM, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France

[2] NIMR-Mbeya Medical Research Center (MMRC), Mbeya, Tanzania

[3] University of Dar es Salaam -Mbeya College of Health and Allied Sciences (UDSM-MCHAS), Mbeya Tanzania

[4] Division of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich (LMU), Germany

[5] Univ. Bordeaux, Department of Public Health, Inserm Bordeaux Population Health Research Centre, Inria SISTM, F-33000 Bordeaux, France

[6]Instituto Nacional de Sau´de (INS), Maputo, Mozambique

[7] German Center for Infection Research (DZIF), partner site Munich, Munich, Germany

[8] Vaccine Research Institute (VRI), Créteil, France

[*]Contribute equally

[$] corresponding authors

Abstract (150 words)

One recurrent question is whether the vaccine needs to be adapted to HIV clade, geographic and/or ethnicity background. Recent attempt to reproduce and/or enhance the Thai RV144 vaccine efficacy in South Africa failed. The reasons for this misleading outcome may be numerous.

In this study, we performed an in-depth characterization of the Ab response induced by HVTN204 vaccine trial carried out with identical immunization regiment in USA and South Africa. Multivariate Ab response analysis was performed according to Fc receptor (FcR) polymorphism, sex, age, ethnicity and geographic location.

We found that Africans displayed higher total Igs and induced in part distinct HIV-specific antibody responses after HVTN204 vaccination compared to Caucasian. Additional studies of immunological differences between ethnic groups and regions need to be undertaken, especially with new vaccine platforms inducing high HIV specific Ab response to better decipher the potential impact of ethnicity on HIV vaccine efficacy.

Introduction

There is solid evidence that intrinsic factors such as genetics, sex, age, and geographic region of origin strongly influence vaccine responses. Correlates with these factors have been largely investigated for the classic available vaccines used

worldwide as hepatitis B, Yellow fever, tetanus, etc.[1] These studies show that, for most of the vaccines, females induce a higher antibody (Ab) response than males. Moreover, MHC polymorphism dependency was observed. However, the effect on genetics might be confounded with differences observed with geographic regions, in addition to other factors such as preexisting immunity and adjoining infections.

Since HIV vaccine still under development, the role of ethnicity on HIV vaccine response and efficacy was largely questioned.[2,3] Especially after the low but significant 31% decreased risk of acquisition in the RV144 Thai vaccine trial, the potential benefit of such type of vaccine in Africa was rise.[4] Can this protection be translated to Africa? Indeed, Africa has the highest infection rate and yet only few phase III HIV vaccine trials were undertaken. HVTN705/Imbokodo vaccine trial was initiated in Africa in order to decipher whether the Thai protection could be reproduced.[5] Unfortunately, HVTN705/Imbokodo vaccine trial did not strictly reproduced the RV144 vaccine trial.[4] Numerous changes were introduced in vaccine protocol as a different envelop, different adjuvant, modified vaccination schedule etc. Moreover, the population targeted was at higher risk of infection with new incoming HIV strains distinct to the infectious strain circulating in Thailand during RV144 vaccine trial. The outcome of this vaccine trial was disappointing and therefore stopped prematurely. The deviations made with the

HVTN705/Imbokodo vaccine trial hampered any possible conclusions on the involvement of ethnicity and geographic location in HIV vaccine efficacy.

In order to address the possible effect of ethnicity and geographic location on HIV immune response, we compared the HIV specific response induced by HVTN 204 vaccine trial performed with exactly the same protocol on volunteers living in US and South African. For this study, we added the confounding variables of sex, age, race in addition to geographic localization. We also add the polymorphism of Fc receptors (FcR) frequencies of SNPs as these genes differ significantly between ethnic groups.[6] These FcR polymorphisms were previously described to modify HIV-1 protection or disease outcome. Indeed, the polymorphism of FcRIIA rs1801274 (GG and GA genotype for H and R at position 131) and sr10800309 (AA genotype) were associated with the rate of infection and HIV control respectively.[7,8]

By multivariable analysis, we identify a stronger increase of global IgG in Africans and a higher total IgA increase in male and volunteers with rs10800309 GG and AG FcRIIA genotype. These factors need to be considered for further improvement of vaccine efficacy. Still, the overall Ab response induced by vaccination was similar indicating that the difference of intrinsic genetic and surrounding environment engenders only minor variations for this vaccine trial.

Additional comparative studies should be undertaken with various vaccine trial in different geographic regions to indorse these results.

Material and Method

Volunteer and samples

Historical HVTN204 samples (serum and cells) from 137 vaccination volunteers at two time point: visit 2 (V2) collected before first vaccine injection and visit 10 (V10) collected after volunteers received three immunization and one boost, were tested. The age, race, location, gender, and sexual orientation of samples are as listed (Table 1).

Total and HIV- specific Ab detection by ELISA

96-well plates were coated overnight with a sheep anti-human IgG/ IgA (1 µg/mL in carbonate buffer, Binding Site) for the detection of total IgG and IgA. Plates were coated with MN/LAI, TH023/LAI, 92TH023 V1V2 and ConS (0.5µg/mL in carbonate buffer for HOV-specific IgGs) to detect HIV specific Abs (Table 2). Plates were washed and saturated with PBS contain 10% milk (1h, at 37 °C), then washed again and incubated with the diluted sera (2 h, at 37 °C). After washing, plates were incubated a secondary goat anti-human IgG-HRP (HorseRadish Peroxidase) or anti-human IgA-HRP added (1 h at 37 °C, 0.2 µg/mL in PBS, Southern Biotech). IgG and IgA were detected by addition of TMB (3,3′, 5,5′ TetraMethylBenzidine) substrate. The reaction was stopped by 25 µL of 1M

H2SO4 per well for 30 minutes. The optical density (OD) was read at 450 nm (reference 650 nm).

Cells

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood received from healthy volunteers at the Blood Transfusion Center (EFS) in Strasbourg using Ficoll-Hypaque sedimentation.

HIV-1 neutralization assay

Pseudoviruses used in Neutralization assay were produced by cotransfecting 293T cells with HIV-1 env expression plasmid and the env-deficient HIV-1 backbone plasmid (pSG3ΔEnv).

The neutralizing antibodies against HIV-1 were tested in TZM-bl neutralization assay as described previously.[9] Serial dilution of sera began at 1 : 20 dilution and the capacity of individuals' sera to neutralize murine leukemia virus (MuLV) was assessed as control. Two easy to neutralize tier-1 HIV-1 strain, SF162NW and MW965.26 were tested.

The inhibitory reciprocal dilution 50% (IRD50) was defined as the sample's dilution that can cause 50% reduction relative luminescence units and the IRD50> 20 will be considered as positive.

Luciferase antibody-dependent cellular cytotoxicity assay

The ADCC assay was performed using purified PBMCs as effector cells and the CEM.NKR.CCR5 cell line was infected with HIV-1 CE1176 (subtype C) expressing the Renilla luciferase reporter gene as previously described.[10]

Serial fold diluted samples (beginning at 1/50 for sera) were added to the 96 well flat bottom plates. 4 day-post infection, PBMCs were mixed with HIV-1 infected CEM.NKR.CCR5 cells at 30/1 effector/target ratio and incubated for 5 hours in the presence of fold dilution of sera in the medium contains RPMI-1640, 10% FCS, and 50 IU interleukin-2 (R&D Systems).

Five hours later, Viviren substrate (Promega) was added to reveal the luminescence intensity generated by living target cells. not lysed by ADCC-mediating Abs present in the volunteers' samples. The percentage of target cell lysis (decreased living cells) in the presence of Abs was normalized to control without Abs using the following formula:

$$\% \text{ of lysis} = \left(\frac{\text{RLU of infected target cells with effectors-RLU of infected target cells with effectors and samples}}{\text{RLU of infected target cells with effectors}} \right) * 100$$

The AUC was calculated as the integrated background-subtracted net activity over a range of dilutions using the trapezoidal method and was truncated above zero.

FcR

Genomic DNA was extracted from 3× 10^6 PBMCs with the Qiagen QIAamp DNA Mini Kit (Qiagen, Hilden, Germany), following manufacturer's instructions. The rs1801274 (H131R) in FCGR2A (FcγRIIa) and rs10800309 in FCGR2A (FcγRIIa) were genotyped with custom Taqman assays (Thermo Fisher Scientific, Waltham, MA, USA) following manufacturer's instructions.

Statistical model (UBx)

New multivariable and univariable models were created by University of Bordeaux, Department of Public Health, Inserm Bordeaux Population Health Research Centre.

Results:

Total Immunoglobulins

First, we analyzed the humoral immune response in the sera from the HVTN 204 vaccine volunteers irrespective of their geographic origin (Table 1). We observed significant intrinsic differences of Ig levels between African and Caucasians. Indeed, Africans showed higher total IgG and IgA responses (Figure 1). Notably, Africans who live in USA displayed intermediate total Ig lower that African living in South Africa African but higher than the Caucasian in USA. On contrary African show lower background of HIV specific Ab response (Abs binding to viral envelopes (table 2)) at V2 (Figure 2) before vaccination than Caucasians.

In order to get read of these intersect Ig variations, we determined the vaccine induced Ig response by subtracting the Ig response before vaccination (V2) from that observed after vaccination (V10).

HIV specific Ab responses

The HIV-specific Ab response induced in this vaccine trial was mainly observed against ConS, a subtype C consensus env (***Figure 2***) **(Supplementary *Figure 1*)**. The Abs induced against the other envs was low (**(Supplementary *Figure 1*)**. Interestingly, a decreased HIV specific Ab was detected in Africans for the MN/LAI, TH023/LAI, two env that have an unfolded env trimer. Interestingly to, Caucasian demonstrated higher ratio of HIV specific Ab ratio to total IgG than Africans, due to their lower total IgG. If we compare the HIV specific antibody with pre-immune result, Africans have higher delta due to the low pre-immune background. As for the difference between pre- and post- immune, there is not significantly difference among three groups **(Supplementary *Figure 1*)**.

The subclass HIV specific Abs were tested for Abs directed against CH54. Both South Africa Africans and USA Caucasians have anti-CN54 IgA, IgG1 and IgG3 induced after vaccination. There are no significant differences of these Abs according to ethnicities or locations in pre-immune samples as well as following vaccination (***Figure 3***).

Neutralization

The capacity of this vaccine to induce functional neutralizing Abs was tested. For this study, we used the conventional highly validated neutralizing assay on TZM-bl cell line against two easy-to-neutralize tier 1 viruses: MW965.26 and SF162. We found a significantly higher neutralization titer against HIV-1 MW965.26 in sera from Caucasians at V10 compared to Africans living in US (p=0.027) and Africans living in South Africa (p=0.007) (***Figure. 4)***. Neutralization against SF162 was not significantly different against HIV-1 SF162. Noteworthy, a significant difference was also observed for the background neutralizing activity between Caucasian and Africans (p=0.04) (***Figure. 4)***. Consequently, when analyzing the Delta of neutralizing activity (V10-V2) referring to vaccine response, the differences between ethnicity and location was no more significant. (**Supplementary *Figure 1***) (***Figure 5 and 6***) We also tested Tier 2, more difficult-to-neutralized viruses. However, we did not detect such Tier 2 neutralization, as previously described by GJ Churchyard et al.[11]

The correlations between all these HIV-specific Ab immune responses were analyzed as unavailable and multivariate analysis (***Figure 5***). High correlation (red square) was observed for all IgG binding against all envs except env ConS at V10. This env IgG specific response negatively correlated with Total IgG at V2 and 10 (blue rectangle).

Antibody dependent cellular cytotoxicity (ADCC)

We searched for ADCC in HVTN samples using the CEMLuc assay. Unfortunately, no induction of ADCC activity against the clade C CE1176 virus could be detected following vaccination (***Supplementary Figure 2***). Therefore, on the impact of ethnicity and location on ADCC response could not be analyzed in this vaccine trial.

FcR polymorphism

As FcRIIA rs1801274 and sr10800309 were associated with the rate of infection and HIV control respectively[7,8], we defined the frequency of these polymorphism in the volunteer unrolled in HVTN 204 according to ethnicity. We found a similar distribution of rs 1801274 gene in Caucasians and Africans. Interestingly, a statistically significant difference was found for FcyRIIA rs 10800309 gene between Caucasians and Africans (***Table. 3***). The AA genotype frequency of the rs 10800309 FcyRIIA polymorphism was around 10% for Caucasians and 2% Africans (p =0.00342).

To further investigate the genetic impact of vaccine-induced Ab, the univariate analysis was performed for each polymorphism **(*Supplementary Figure 3, 4).*** For rs1801274, a higher total IgG and a lower 92TH023 V1V2 specific IgG was detected for the AA compared to the GG genotypes. For rs10800309, a higher total IgG and IgA amount was detected at V10 for the AA compared to the GG genotypes. The immunoglobulin response to vaccination in HVTN 204 cohort

including the functional Abs responses are listed in the supplementary table 1 (***Supplementary Table 1***).

Multivariate analysis of vaccine induced response

The univariate and multivariate and analysis for HVTN204 vaccine response was performed. The linear regression of the vaccine response depending on the patients' FcR polymorphism sex, ethnicity and location were also analyzed for better understanding which factor impacts HVTN204 vaccine outcome (***Figure 6) (Supplementary Figure 5).*** The univariate analysis showed the only total IgG differ between locations (South Africa versus US) in response to this vaccine trial.

The multivariate analysis considering FcR polymorphism, gender, and age in addition to ethnicity and location confirmed the increased total IgG in South Africa versus US already evidenced in the univariate analysis. Moreover, we observed an increased delta response of total IgA for the rs10800309 phenotype GG and AG, and in male compared to female. We also evidenced difference of delta response for IgG3, IgG1 and IgA directed to CN54 env according to rs1801274, sex and ethnicity. Finally, a decreased delta response for total IgG and IgA according to the rs1801274 Ag and GG genotype was detected.

This analysis of the Ab response induced by the HVTN024 vaccine showed some intriguing differences appealing to strengthening the complementary investigations.

Discussion

With our increased knowledge of the immune factors involved in vaccine efficacy, analysis of the impact of ethnicity and geographic localization turns out to be highly pertinent. The role of the genic background on vaccines efficacy has been addressed previously but the relevance of this question builds up for HIV vaccine. Indeed, only one vaccine RV144 showed limited (31%) efficacy. Minimal influence of ethnicity or geographic localization may therefore drastically impact on vaccine efficacy. Besides, vaccine studies in Africa, continent with the highest incidence rate, were only carried out recently. Our knowledge of HIV vaccine outcome in Africa is therefore very unsatisfactory. Previous analysis of humoral immune response showed higher total IgA, IgG1, IgG2 IgG and IgA in people of African origin when compared to Caucasian or Hispanic populations living in the same country.[12-18] In these studies, the geographic origin with possible outcomes of preexisting immunity or adjoining infections could not analyzed as they used people living in the same country.

In order to gain insight into possible contribution geographic location and ethnicity in HIV vaccine response, we compared the humoral response induced

by the HVTN024 vaccine trial performed with identical vaccine protocol in South Africa and USA. We found higher total IgG and IgA in Africans compared to Caucasian. This result support previous studies analyzing Ig in Africans versus Caucasians of the same country. Interestingly, the immune responses of African living in USA had intermediate Ig levels compared to African living in SA Africans. This point to an additional impact of geographic location on total Ig level. Noteworthy, the background level of HIV specific immune response also significantly differed with higher inherent non-specific binding detected in Caucasians compared to Africans. This highly suggest a distinct basal Ig profile with higher HIV cross-reactive Ab in Caucasians. Whether this basal cross-reactivity is due to adjoining infections or to genetic factors, and whether this basal cross-reactivity participate in protection need additional investigations. Interestingly to, the HIV specific Ig level was also significantly higher in Caucasians for 3 of the 4 HIV sequences studied. However, when analysis analyzing the net vaccine response by the Delta score, where the background level is subtracted from the vaccine response, they were no significant differences of vaccine responses detected between Caucasians and Africans.

The FcR polymorphism analyzed in this study showed significant differences of FcR distribution. These differences may affect FcR-mediated inhibitory functions and consequently vaccine efficacy in addition to other confounding factors as age and sex.

Multivariable analysis taking into account these different confounding factors revealed so far increased total IgG in response to vaccine in peoples from South Africa. This increase may correspond to a boosted general activation of the IgG response following HIV immunization in Africans living in South Africa. Moreover, IgA was increased with a specific rs10800309 genotype and in Male compared to female further indicating genetic differences associated with the overall IgA response. Therefore, although the delta HIV specific response not significantly different between Caucasian and African, the inherent background difference of the total Ig response in addition to genetic ethnicity related variation, may significantly modulate the HIV vaccine response. These differences may be further amplified if improved HIV-specific humoral response is induced.

Conclusion

In conclusion, the difference of humoral immune response induced HVTN204 vaccine according to the genetic and ethnicity background support the investigation of additional vaccine induced humoral response. In particular, analysis of genetic and ethnicity effects on new improved vaccines able to induce high and sustained levels of HIV-specific Abs should be explored. Results of such study may give additional insights for future “specific-design vaccine strategies”, customized according to ethic or countries specificities, as what is currently envisaged for personalized medicine.

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Contribution

LYL performed the experiments and contributed to the writing. JL, GL, and SS assisted experiments. TF and EL performed all the analysis and garneted all the igures and tables. CM corrected, amended and edited the paper. All authors provided feedback on the report.

Conflict of interest

The authors declare no conflict of interest.

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	Overall (N=137)
Age	
Mean (SD)	28.3 (8.46)
Median [Min, Max]	25.0 [19.0, 50.0]
Race	
black	88 (64.2%)
white	49 (35.8%)
Location	
SA	76 (55.5%)
USA	61 (44.5%)
Sex	
Female	76 (55.5%)
Male	61 (44.5%)
Sexual orientation	
bi or homosexual	15 (10.9%)
heterosexual	122 (89.1%)

Table 1. Detail information of the 137 HVTN204 volunteers' age, race, geographical location, gender, and sexual orientation.

Abbreviation	Type	Description
ConS	Gp140	consensus envelope antigens
MN/LAI	Gp160	A hybrid oligomeric gp160 Env with gp120 derived from HIV-1 MN and gp41 derived from HIV-1 LAI.
TH023/LAI	Gp160	Recombinant Env with gp120 from HIV-1 92TH023 linked to gp41 from LAI, with a deletion in the immunodominant region.
92TH023 V1V2	gp70	a murine leukemia virus gp70 scaffold containing HIV-1 gp120 variable regions 1 and 2 (gp70-V1V2) from HIV-1 isolates 92TH023
CN54	gp140	Recombinant Env trimer containing gp120+gp41 ectodomain from HIV-1 CN54

Table 2. Information of envelope glycoproteins used in the ELISA binding experiments.

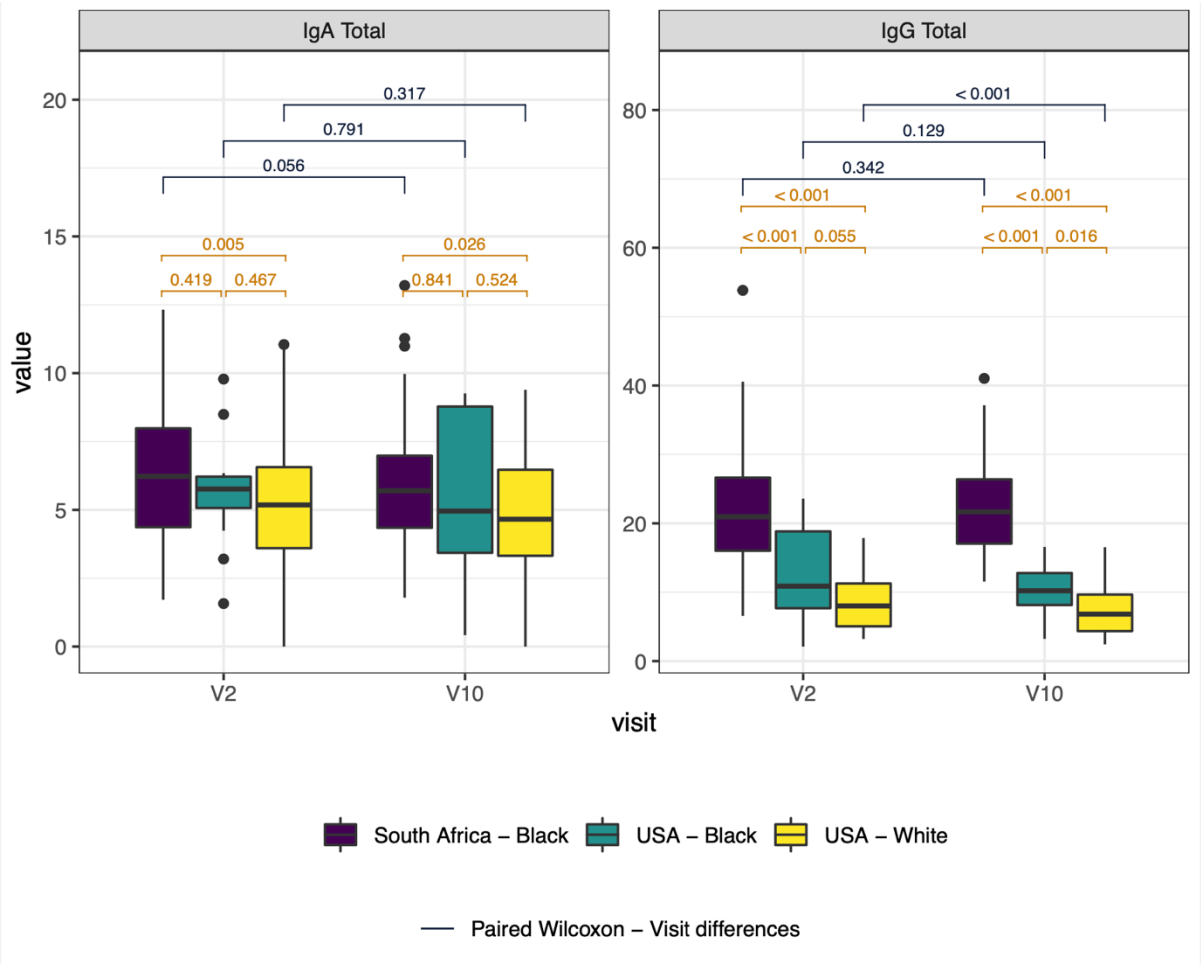


Figure 1. Total IgG and total IgA of the HVTN204 samples.

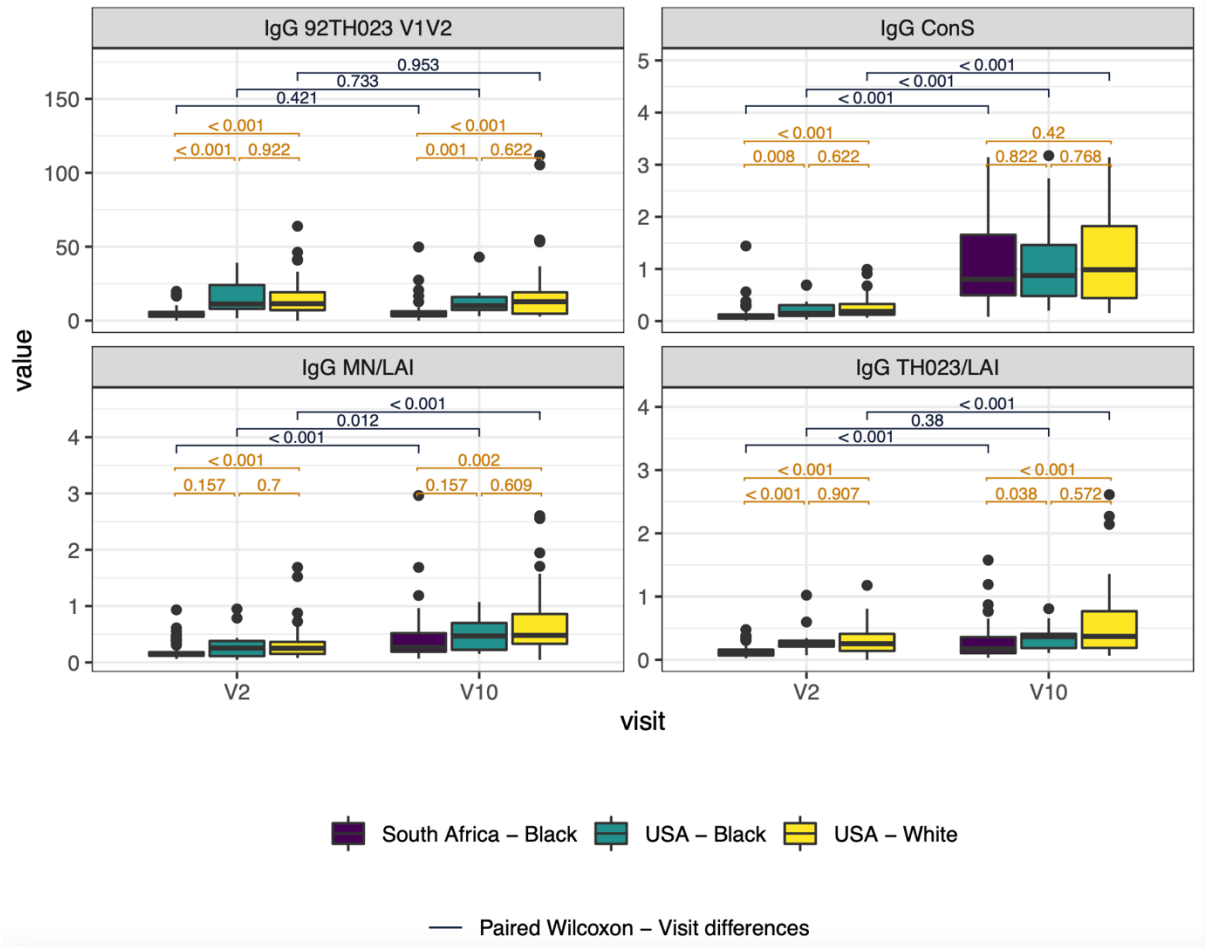


Figure 2. The HIV-specific antibody response before and after vaccination. The capacity of HIV-specific Abs to bind to several viral envelopes: ConS, MN/LAI, TH023, and V1V2.

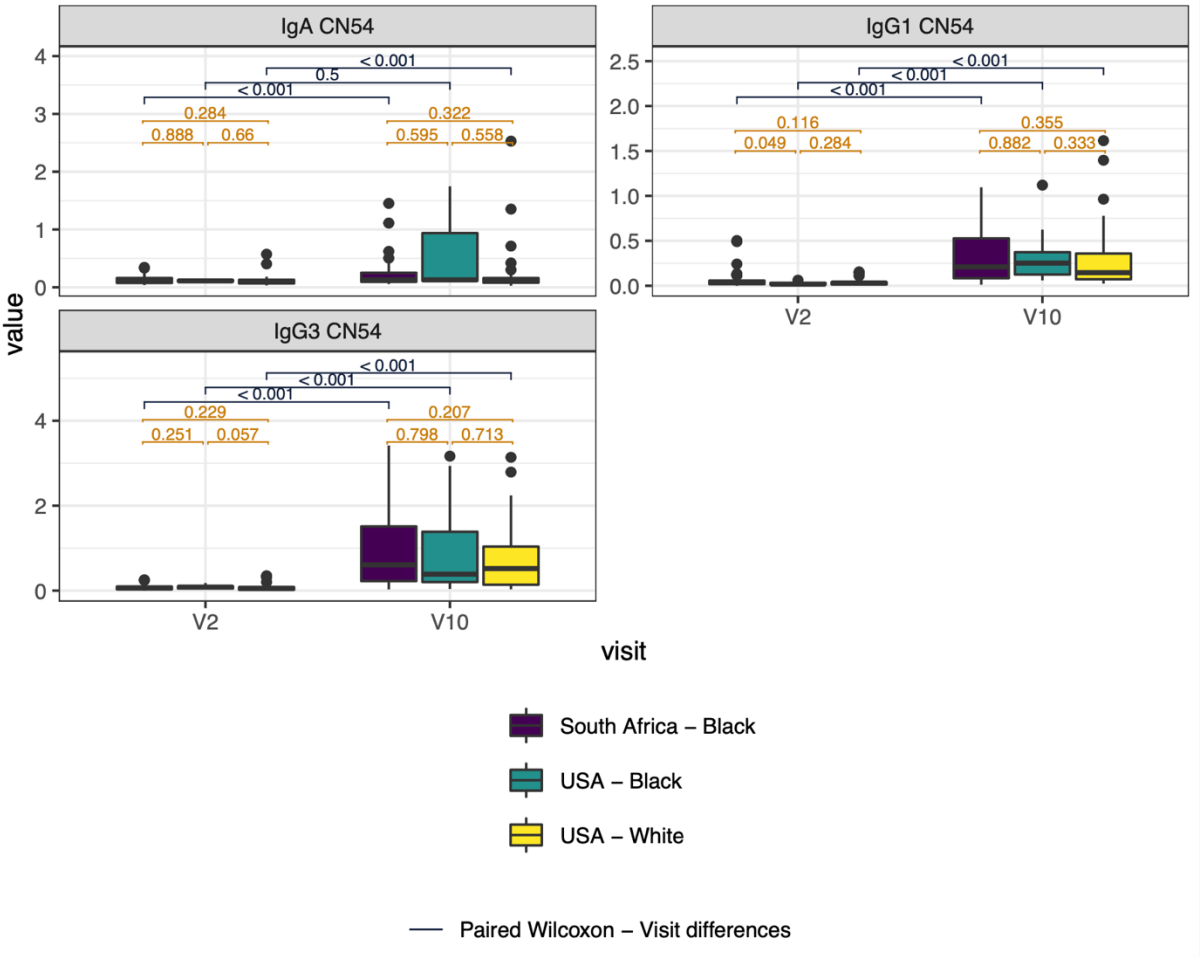


Figure 3. Subclass HIV specific antibody (CN54). The capacity of HIV-specific IgA, IgG1 and IgG3 to bind to CN54.

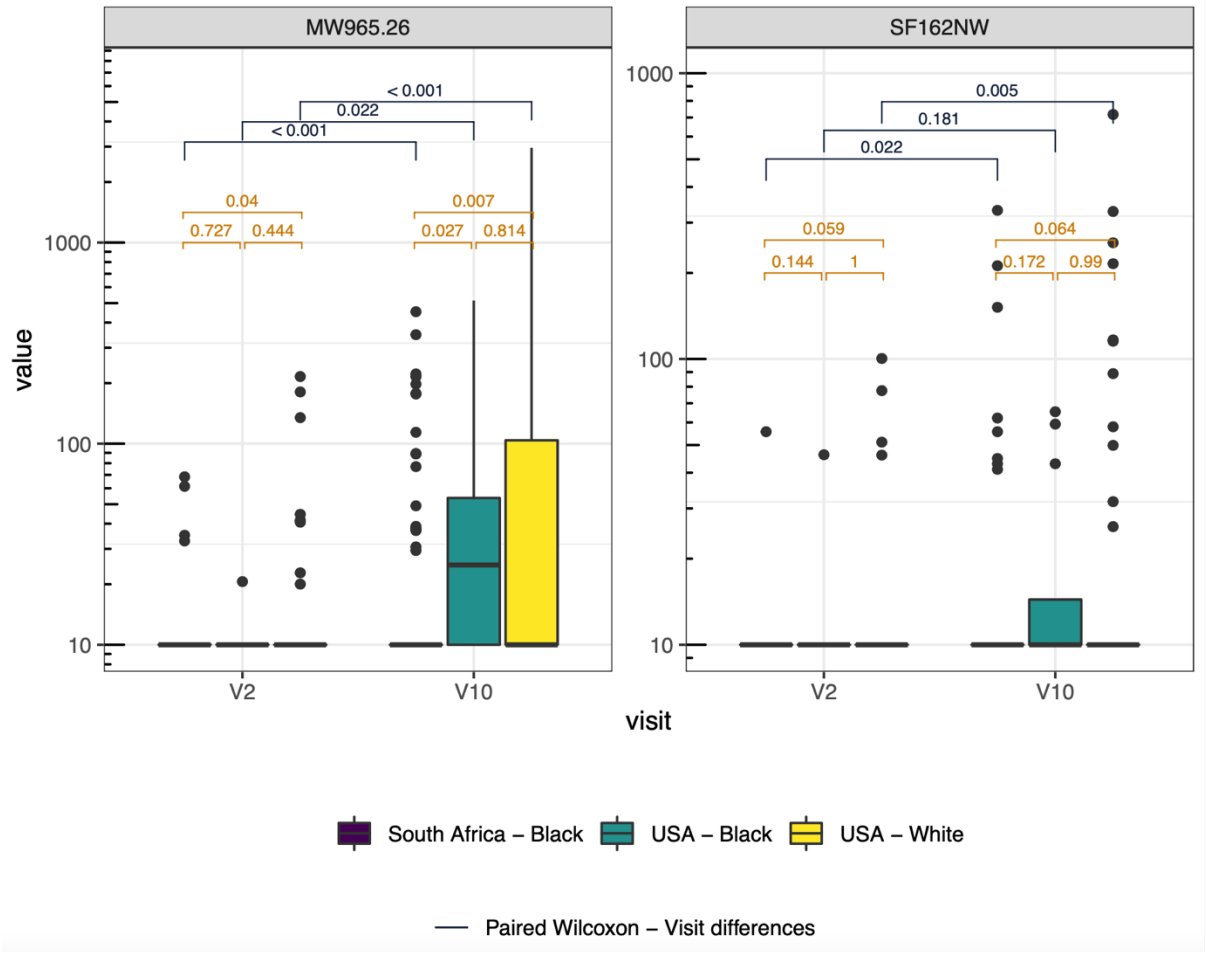


Figure 4. Neutralization ability against tier 1 HIV pseudovirus MW965.26 and SF162. Neutralization against easy-to-neutralize tier 1 HIV pseudoviruses were detected by TZM-bl neutralizing assay.

	Black (N=88)	**White** (N=49)	**P-value**
Age (years)			
Mean (SD)	26.8 (7.38)	31.0 (9.65)	0.015
Median [Min, Max]	25.0 [19.0, 50.0]	27.0 [19.0, 50.0]	
Location			
SA	76 (86.4%)	0 (0%)	<0.001
USA	12 (13.6%)	49 (100%)	
Sex			
Female	51 (58.0%)	25 (51.0%)	0.476
Male	37 (42.0%)	24 (49.0%)	
rs10800309			
AA	2 (2.3%)	5 (10.2%)	**0.00342**
AG	32 (36.4%)	27 (55.1%)	
GG	54 (61.4%)	17 (34.7%)	
rs1801274			
AA	14 (15.9%)	14 (28.6%)	0.221
AG	51 (58.0%)	25 (51.0%)	
GG	23 (26.1%)	10 (20.4%)	

Table 3. FcR polymorphism frequency (rs1801274 and rs10800309) of the HVTN 204 volunteers. Comparisons are performed by Wilcoxon (quantitative) or Fisher (qualitative) test.

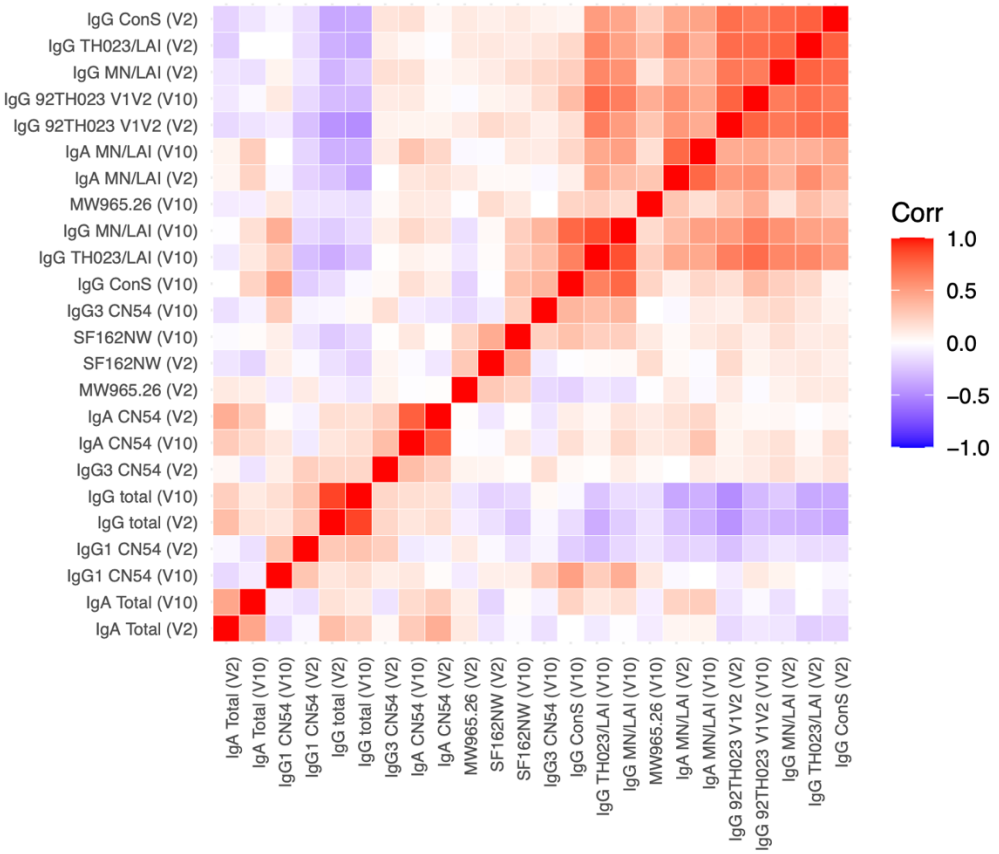


Figure 5. Correlation between V2 and V10.

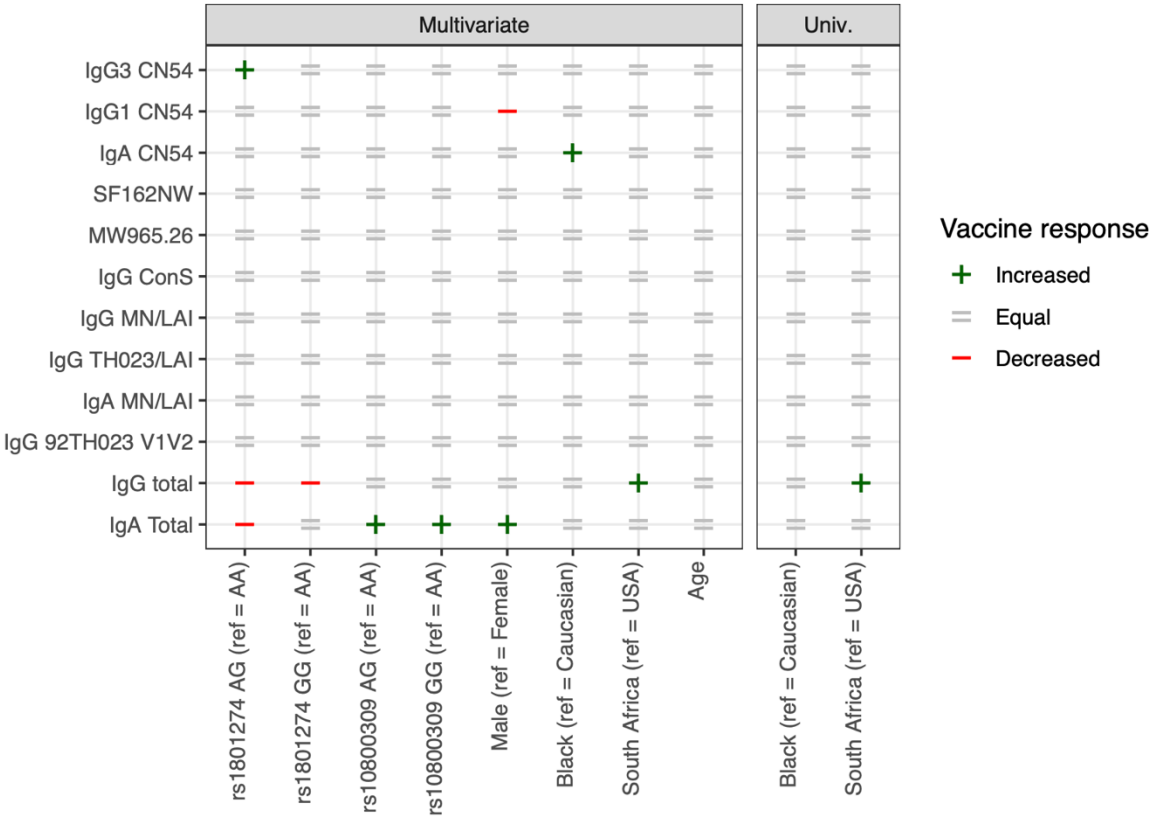
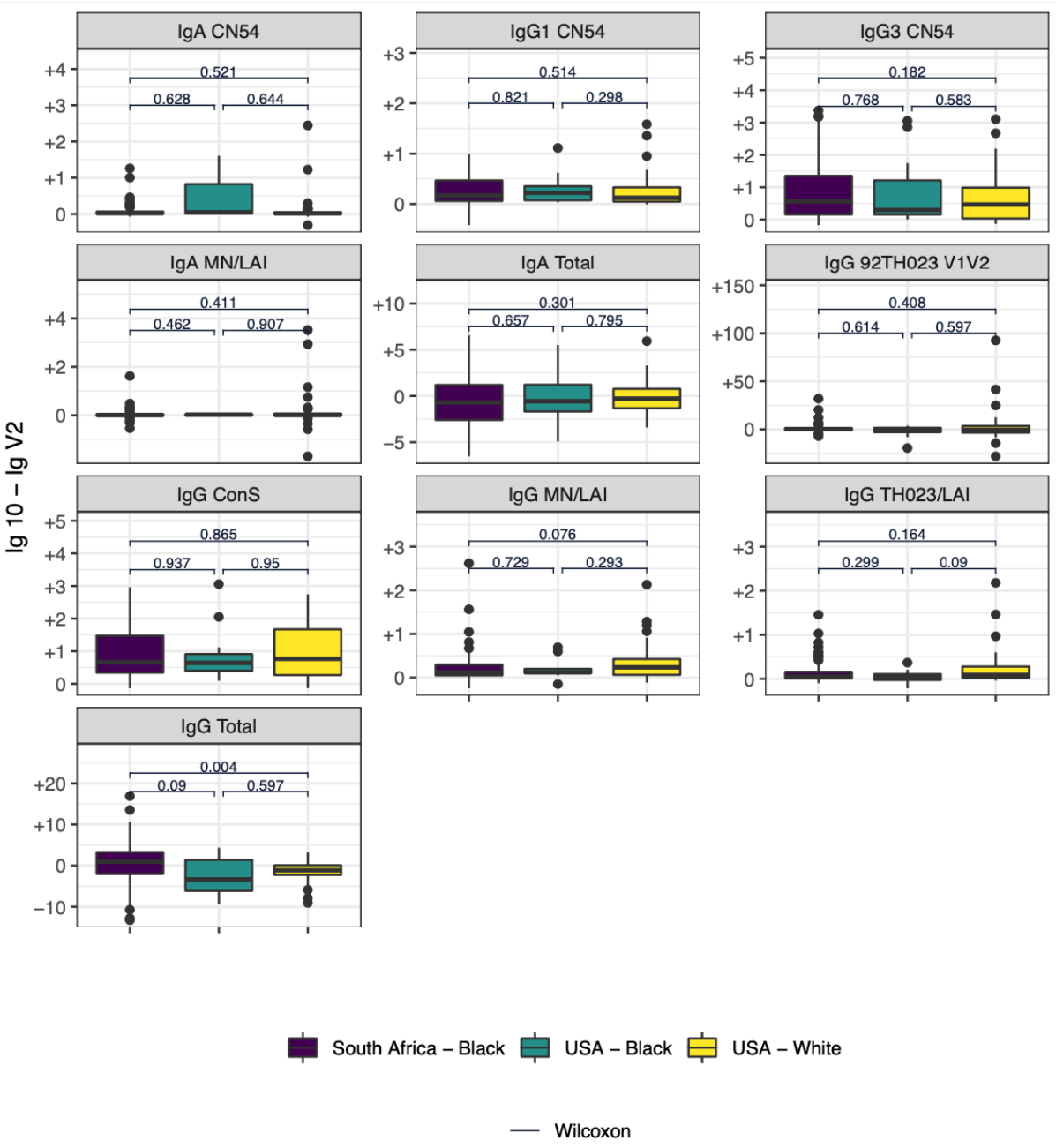
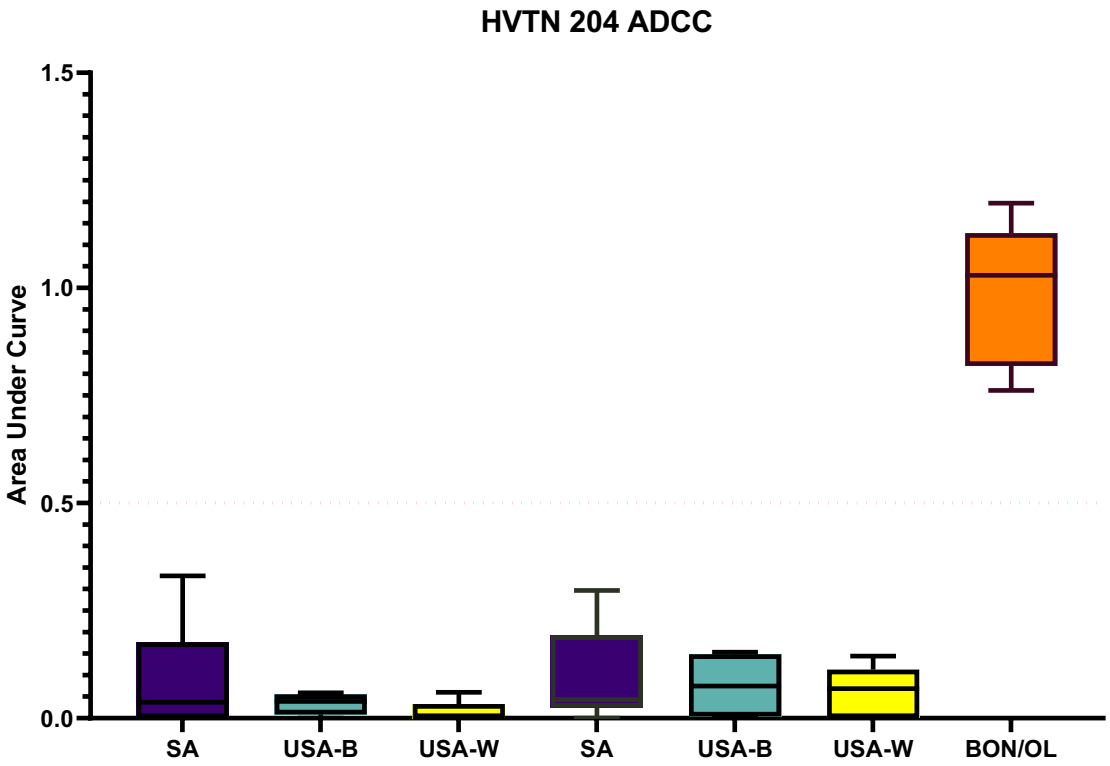


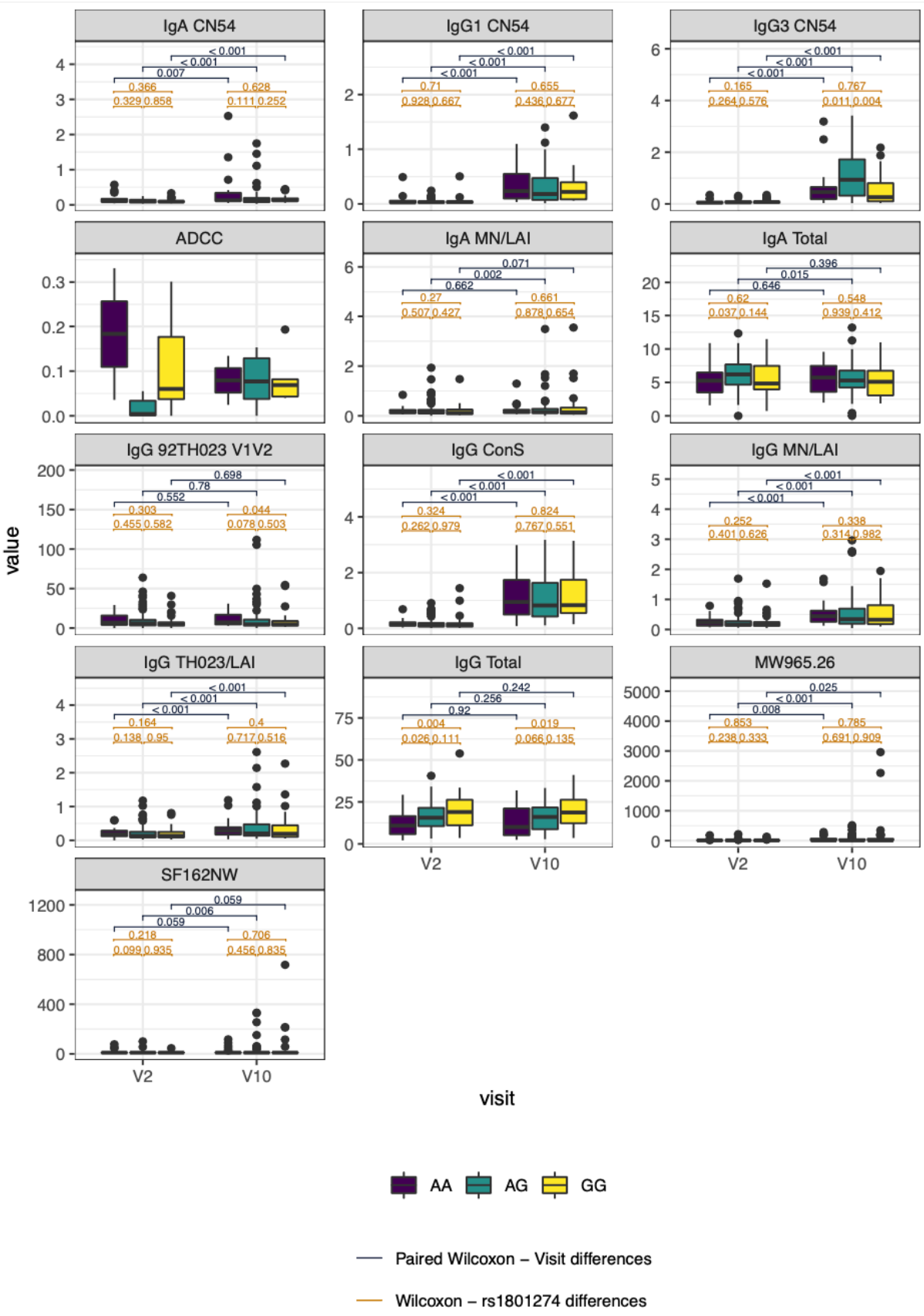
Figure 6. The multivariate and Univariate analysis for HVTN204 vaccination outcome.



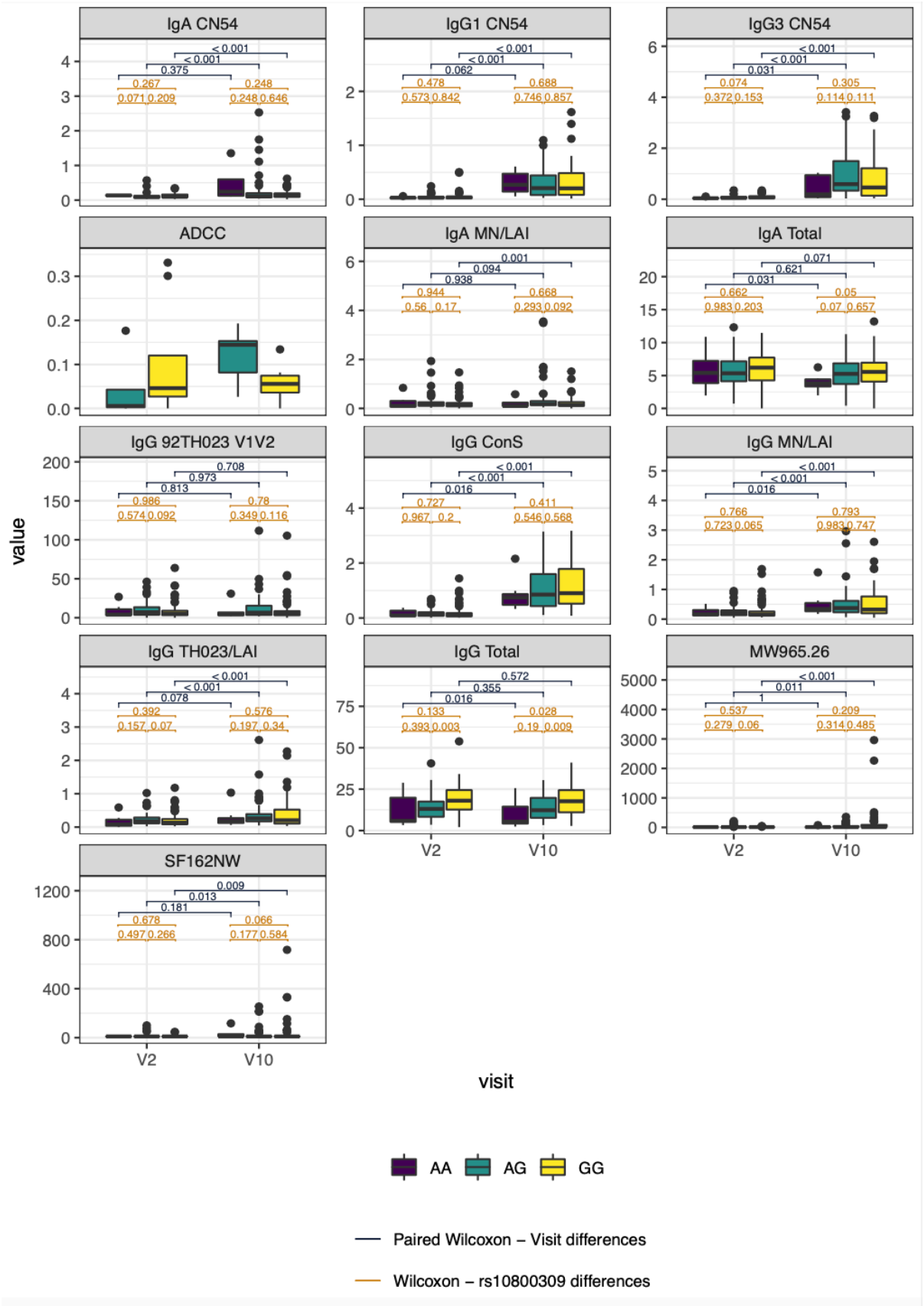
Supplementary Figure 1. The delta difference between each ethnicity groups. (Delta = Ig V10 - Ig V2)



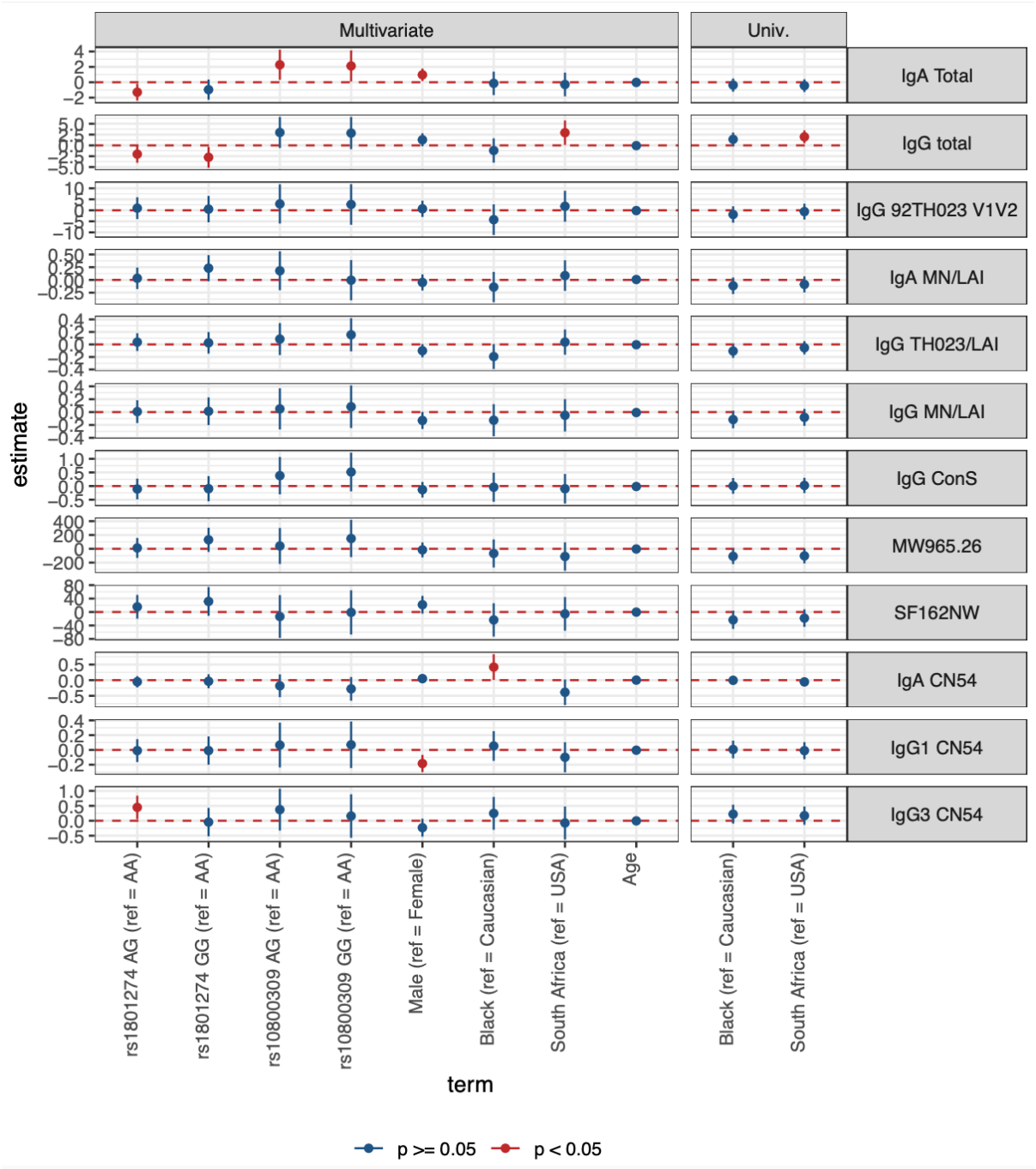
Supplementary Figure 2. ADCC of virus CE1176 (subtype C). No ADCC activity was detected against CE1176.



Supplementary Figure 3. Univariate comparison – rs1801274.



Supplementary Figure 4. Univariate comparison – rs10800309



Supplementary Figure 5. Linear regression of the vaccine response depending on the patient's characteristics.

Result

	V2	V10	p-value	p-value (BH)	test
	(N=142)	(N=142)			
IgA Total			0.078	0.102	student
Mean (SD)	5.87 (2.50)	5.50 (2.28)			
Median [Min, Max]	5.64 [0, 12.3]	5.30 [0, 13.2]			
Missing	5 (3.5%)	5 (3.5%)			
IgG Total			0.181	0.200	student
Mean (SD)	16.3 (9.00)	15.8 (8.79)			
Median [Min, Max]	15.2 [2.10, 53.8]	15.7 [2.43, 41.0]			
Missing	5 (3.5%)	5 (3.5%)			
IgG 92TH023 V1V2			0.200	0.200	student

Mean (SD)	9.59 (10.2)	10.8 (15.6)			
Median [Min, Max]	5.90 [0, 63.9]	5.30 [0, 112]			
Missing	5 (3.5%)	5 (3.5%)			
IgA MN/LAI			0.058	0.083	student
Mean (SD)	0.228 (0.276)	0.304 (0.494)			
Median [Min, Max]	0.138 [0, 1.93]	0.156 [0, 3.55]			
Missing	5 (3.5%)	5 (3.5%)			
IgG TH023/LAI			< 0.001	< 0.001	student
Mean (SD)	0.212 (0.197)	0.380 (0.413)			
Median [Min, Max]	0.145 [0, 1.18]	0.237 [0.0322, 2.61]			
Missing	5 (3.5%)	5 (3.5%)			

IgG MN/LAI			< 0.001	< 0.001	student
Mean (SD)	0.257 (0.243)	0.522 (0.490)			
Median [Min, Max]	0.170 [0.0463, 1.69]	0.361 [0.0468, 2.96]			
Missing	5 (3.5%)	5 (3.5%)			
IgG ConS			< 0.001	< 0.001	student
Mean (SD)	0.184 (0.206)	1.13 (0.844)			
Median [Min, Max]	0.114 [0.00935, 1.44]	0.880 [0.0821, 3.17]			
Missing	5 (3.5%)	5 (3.5%)			
MW965.26			0.009	0.015	student
Mean (SD)	15.8 (26.2)	89.1 (323)			
Median [Min, Max]	10.0 [10.0, 216]	10.0 [10.0, 2960]			

Missing	5 (3.5%)	5 (3.5%)			
SF162NW			0.005	0.010	student
Mean (SD)	12.3 (11.6)	31.1 (79.3)			
Median [Min, Max]	10.0 [10.0, 100]	10.0 [10.0, 717]			
Missing	5 (3.5%)	5 (3.5%)			
IgA CN54			< 0.001	0.002	student
Mean (SD)	0.122 (0.0792)	0.240 (0.359)			
Median [Min, Max]	0.101 [0.0317, 0.572]	0.127 [0.0267, 2.53]			
Missing	37 (26.1%)	37 (26.1%)			
IgG1 CN54			< 0.001	< 0.001	student
Mean (SD)	0.0478 (0.0704)	0.311 (0.306)			

	Result				
Median [Min, Max]	0.0320 [0, 0.503]	0.201 [0.0117, 1.62]			
Missing	27 (19.0%)	27 (19.0%)			
IgG3 CN54			< 0.001	< 0.001	student
Mean (SD)	0.0753 (0.0653)	0.858 (0.878)			
Median [Min, Max]	0.0620 [0, 0.348]	0.520 [0.0290, 3.42]			
Missing	8 (5.6%)	8 (5.6%)			
ADCC			0.191	0.200	wilcoxon
Mean (SD)	0.0806 (0.115)	0.0818 (0.0582)			
Median [Min, Max]	0.0374 [0, 0.331]	0.0722 [0, 0.193]			
Missing	129 (90.8%)	129 (90.8%)			

Supplementary Table 1. Description of immunoglobulin evolution after vaccination in HVTN 204 cohort. BH = Benjamini-Hockberg correction.

5. General Conclusion

In order to fight against the HIV world pandemic, anti-viral agents are available. Their high potency allowed them to decrease HIV until it was undetectable and almost abolished HIV transmission. This finding of U=U: Undetectable = Untransmittable provides hope to get read of the HIV epidemic, with the instauration the 90-90-90 treatment target by UNAIDS: 90% of all people with diagnosed HIV infection will receive sustained treatment, and 90% of all people receiving treatment will have viral load suppression. Unfortunately, this target is too long in coming because even the first goal of diagnosis of 90% of all people with HIV infection is not forthcoming. People with unknown infection will therefore continue to transmit HIV, drastically limiting HIV eradication. This comes to the unavoidable conclusion that the development of a safe and cost-effective vaccine, even if it is a difficult task, is the best strategy to consider for the HIV epidemic. After more than 30 years of research dedicated to HIV vaccine development, the requirements associated with protection are becoming apparent. Yet, we still do not know how to reach these requirements.

5.1. The protective Ab response induced early after infection

Induction of broadly Nabs by vaccination is considered to be one of the main targets to achieve. The currently characterized bNAbs all show drastic maturation features almost impossible to induce with the currently known vaccine strategies. With this problem in mind, we have searched for bNAbs induced early after infection.

Unexpectedly, we found some NAbs in sera collected early after infection. However, these Nabs were directed against T/F viruses and not against the classical Tier 2 viruses tested before. Later, we found a shift to NAbs directed against the classical non-T/F strains reconsolidating previously published results.[133] We could also detect ADCC early after infection. Noteworthy, we did not find a correlation between neutralizing activity and ADCC.[447] Additional viruses should be tested for both neutralization and ADCC activities in order to complete the characterization of the breadth of HIV-specific Ab response induced during the acute phase of the infection (PRIMO cohort). Moreover, the epitope recognized by these Abs should be identify. What is the specificity these Abs that allow neutralization of T/F viruses? This information may also give additional hints on the uniqueness of T/F viruses. Currently, contradictory results have been published on T/F viruses. The analysis of these

strains in the context of their neutralizing capacity of early induced HIV specific Abs may help to unravel their specific features.

The information of NAbs against T/F viruses induced early after infection give new hope of inducing such Abs by vaccination. Moreover, the early Fc-mediated functions detected suggest that functional Abs induced by vaccination may possibly provide help for decreased risk of HIV infection.

5.2. New Vaccine candidates and new concepts

Recently, Human clinical studies using bNAbs in passive transfer showed effectiveness fortreatment of HIV infection. These results support the potential role of such Abs for prevention, postexposure prophylaxis, and treatment of acute and chronic infection. We now know that induction of bNAbs will be challenging, since such Abs need long maturation and continued stimulation to be induced (Figure 54). Modified HIV env protein structures, new vectors and new delivery pathways are in development to improve Ab maturation.

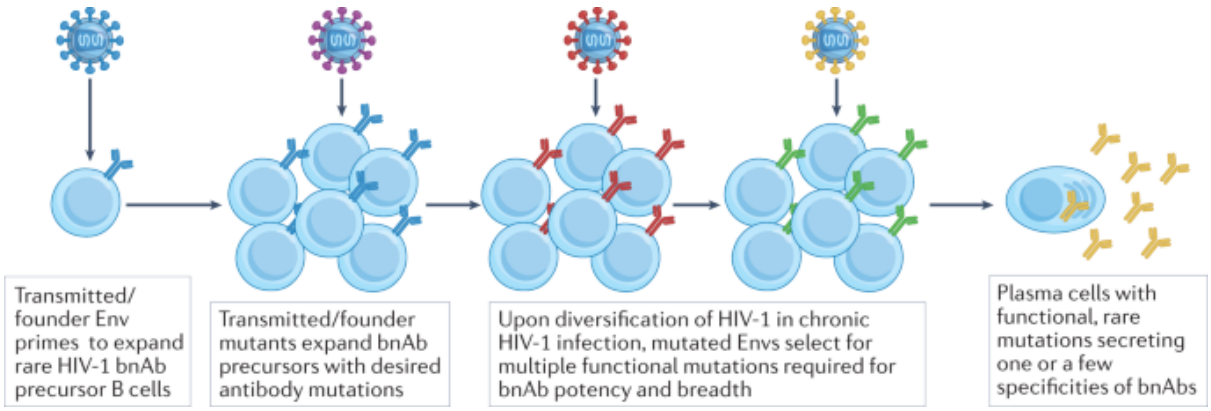


Figure 54. The model of bNAb development in human. The bnAb development needs a tortuous maturation pathway requiring the stimulation of multiple rare events by repeated evolving viruses.[211]

We tested the capacity of new vaccine candidates to induce bNAbs. In collaboration with EHVA, different new directions have been tested as new modified env, VSV vector and OP that all showed some benefit. These encouraging results gave new hits for immunogenicity

improvement. Our subsequent immunization strategy will skillfully combine our newly developed immunogens to promote complementary immune response enhancement. Noteworthy, the immune responses detected were high in small animal models but decreased when tested in NHP models. This variation of immune response strengthens the necessity to perform vaccine trials in humans. This is currently under investigation with the VRI06 vaccine trial. The env immunogen targeting CD40 that showed interesting immunogenicity in animal models is now tested in clinical trial. The in-depth characterization of the humoral response induced in human will help for future design of HIV protective vaccine development.

5.3. Impact of Ethincity and Geographic location on vaccine response?

Our increased interest in the Fc-mediated function of Abs in vaccine strategies directed our attention to complementary immune factors as FcR polymorphism or FcR expression on the surface of various effector immune cells. Indeed, FcR features directly impact Ig affinities. These affinities are also related to Ig isotypes, strengthening the necessity to analyze isotype switch following vaccination. Noteworthy, these parameters are related to genetic background variations outlined with ethnicity. The question of the involvement of polymorphism in Fc-mediated function is becoming central in our group and brought to the writing of two reviews. These papers summarized the current knowledge of Fc-mediated functions in infection and vaccine outcomes. In addition, a project was developed to estimate the contribution of genetic background in humoral immune response development and function. We got the opportunity to compare the humoral response induced by the HVTN204 vaccine trial according to ethnicity. By multivariate analysis, subtitle differences could be observed according to ethnicity in addition to the other effects related to age, sex, and geographic origin. This again points to a complex interplay of confounding factors involved in immune response induction. It strongly suggests that these confounding factors should be taken into account for future vaccine design.

In conclusion, my thesis's results gave additional insights into the humoral response that should lead to an improved HIV vaccine strategy. Increased knowledge on the consequence of subtitle modification of epitope recognition, isotype switch, genetic background etc., is still required

Future vaccine strategies will need to take the different factors highlighted in my thesis into account. My current results, support aa new vaccine strategy based the combination of of immnuogens that I have shown to demonstrated beneficial improved. According to my results, I propose the usean envelope trimer based on T/F virus sequences and to modify then as germline (GT) envs and chemically stabilized (CL) env or by focusing on CD4 binding site epitope with the KI/KIKO constructs.. The (GT+CL) T/F envelope will be expressed on VSV vector and used as prime and, the modified KI: KIKO envelope will be fused on a CD40 Ab to improve antigen presentation and used as boost.. New adjuvant such as 3M052 my be applied to support mucosa immune activation. Moreover, a more personalized tailored vaccine strategy may be envisioned in the future. The new upcoming vaccine strategies will need to take into account the human genetic and geographic background. in order to improve the efficacy of the so difficult-to-develop HIV protective vaccines.

6. Bibliographie

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7. Annexes

7.1. Coronaviruses pandemics: Can neutralizing antibodies help?

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Coronaviruses pandemics: Can neutralizing antibodies help?

Phuoc-Bao-Viet Tong[a], Li-Yun Lin[a], Tuan Hiep Tran[b,c,*]

[a] INSERM U1109, Fédération Hospitalo-Universitaire (FHU) OMICARE, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, Strasbourg, France
[b] Faculty of Pharmacy, PHENIKAA University, Yen Nghia, Ha Dong, Hanoi 12116, Viet Nam
[c] PHENIKAA Research and Technology Institute (PRATI), A&A Green Phoenix Group JSC, No.167 Hoang Ngan, Trung Hoa, Cau Giay, Hanoi 11313, Viet Nam

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ABSTRACT

For the first time in *Homo sapiens* history, possibly, most of human activities is stopped by coronavirus disease 2019 (COVID-19). Nearly eight billion people of this world are facing a great challenge, maybe not "to be or not to be" yet, but unpredictable. What happens to other major pandemics in the past, and how human beings went through these hurdles? The human body is equipped with the immune system that can recognize, respond and fight against pathogens such as viruses. Following the innate response, immune system processes the adaptive response by which each pathogen is encoded and recorded in memory system. The humoral reaction containing cytokines and antibodies is expected to activate when the pathogens come back. Exploiting this nature of body protection, neutralizing antibodies have been investigated. Learning from past, in parallel to SARS-CoV-2, other coronaviruses SARS-CoV and MERS-CoV who caused previous pandemics, are recalled in this review. We here propose insights of origin and characteristics and perspective for the future of antibodies development.

1. Introduction

In full social distancing crisis, far from lab work, in looking anxiously at the red dots growing every day in the graphic map of coronavirus disease 2019 (COVID-19) pandemic [1], we wonder how were the other major pandemics and how antibodies, natural or artificial, can fight the diseases.

Several pandemics have occurred throughout history, some had more effects on human's life and/or on economics than the others. As scientists, a key step in vaccines research is to learn from the past. By that mean, what we are doing now might be cornerstone preparing us for future pandemics.

In this review, we look for the diversity and variability of coronaviruses, including SARS-CoV, MERS-CoV and SARS-CoV-2, which cause pandemics from 21th century. The origins, impacts and molecular structure with insights of vulnerable sites of viruses who are targets of antibodies and its humoral responses will be described.

2. Overview of antibody and immunization

In response to pathogens including viruses, human body has evolved its immune system to protect it from invasion. Following the virus invasion, antibodies (Abs) are produced after a series of immune signaling and these Abs are able to recognize a diverse array of antigens (Ag) [2]. More specifically, the paratopes (Ag-binding sites) of Abs bind epitopes on virion-associated Ags. During an Ab response, B-cells which express Ag receptors are clonally expanded [3]. Antibodies structurally are composed of heavy (μ, α, γ, δ, ε) chains that are linked by disulfide bonds with light chains (κ, λ). In the progress of B-cell development, immunoglobulin heavy (IgH) chain gene recombination typically occurs before immunoglobulin light (IgL) chain gene recombination [4].

Neutralizing antibodies (nAbs) can inhibit the viral infection via following the viral replication cycle. Attachment is the first critical step blocked by Abs by interfering with the virion-receptor binding. Moreover, Abs may induce the aggregation of viral particles which cause a reduction of individual penetration. In post-attachment step, Abs on the virion possibly dampen virus endocytosis internalization leading to the lysosomal degradation. The Abs also block fusion of virion when they intercalate between viruses and cell membrane. The next stage of interference is to uncoat or appropriate intracellular localization of core or capsid. Lastly, Abs might bind virion surface then inhibit the metabolic events that blocks the replication of viruses even after internalization [3,5,6].

The approach to nAbs design relies on the identification of antigens; in other words, the epitopes are the central of quests. However, the variable regions of the antigen induce the largest fraction of the antibodies whereas broadly nAbs represent only a minor proportion of the response. The major challenges related to both sides were previously

* Corresponding author at: College of Pharmacy, Phenikaa University, Viet Nam.
E-mail address: hiep.trantuan@phenikaa-uni.edu.vn (T.H. Tran).

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Annex 1. Coronaviruses pandemics: Can neutralizing antibodies help?

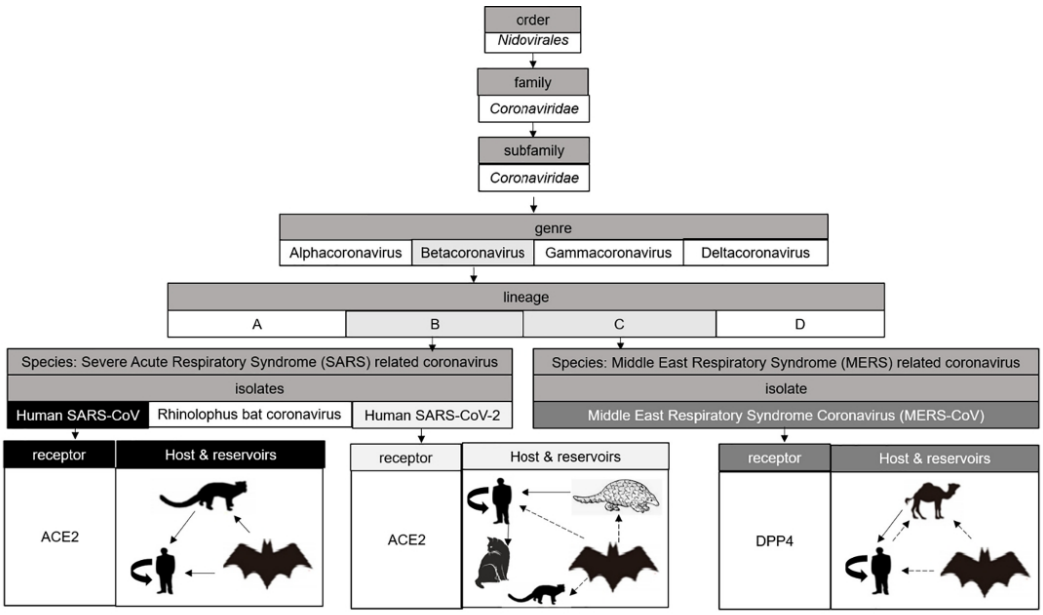

Fig. 1. Isolates of coronaviruses discussed in this review and their receptors, host and reservoirs. SARS-CoV and SARS-CoV-2 from the lineage B use ACE2 as receptor. MERS-CoV from the lineage C enters into host cells by binding DPP4. SARS-CoV has the masked palm civets as an intermediate host in which the virus has adapted from the Chinese Horseshoe bat reservoir to ultimately infect humans [13]. SARS-CoV-2 has bats and pangolins as natural reservoir and can infect ferrets or domestics animals, with a high susceptibility in cats [14]. MERS-CoV has the origin from bats [15] but maybe this virus had an adaptation through camels before its emergence into human [16].

described: viral antigen and the generation of antibodies to these sites. In the detail, the antigen concerns are (1) epitope masking or shielding by glycans or protein loops; (2) transient exposure during the entry process or via other mechanisms such as viral "breathing;" (3) the size of conserved epitopes is small resulting in the limitation of interaction, (4) epitopes are constrainedly accessible, (5) the mutability of epitopes. Regarding antibody production, (1) the need for extensive somatic mutations and focused evolution; (2) the use of specific germline allelic variants and HCDR3s of particular length and structure; and (3) the molecular mimicry of host molecules are mentioned [6].

3. Coronaviruses

Coronaviruses are a group of related viruses in the family *Coronaviridae* and subfamily *Coronavirinae*, order *Nidovirales*. In the subfamily *Coronavirinae*, coronaviruses include 4 genres: alphacoronavirus, betacoronavirus, gammacoronavirus and deltacoronavirus (Fig. 1). Coronaviruses infect a wide variety of hosts including many species of birds, mammals and humans [7]. Alphacoronaviruses and betacoronaviruses circulate among mammals, gammacoronaviruses and deltacoronaviruses infect birds and mammals. Within betacoronaviruses, there are 4 lineages: lineage A contains human coronaviruses HKU1 and OC43, lineage B to which SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) belongs, lineage C belongs to MERS-CoV (Middle East Respiratory Coronavirus Syndrome) and the lineage D has the bat coronaviruses HKU4 and HKU5 who are close to MERS-CoV.

The first human coronaviruses 229E (HCoV-229E) and OC43 (HCoV-OC43) were isolated in the 1960s and are now classified respectively as alphacoronaviruses and betacoronaviruses. As these viruses were not very pathogenic and often associated with colds [8], this family of viruses attracted little interest from scientists until the 2000s. In November 2002, SARS-CoV, first reported in Guangdong province, China, became the first highly pathogenic coronavirus that emerged in the human population. This virus was responsible for an epidemic of severe acute respiratory syndromes that started in China before spreading rapidly over the world with around 8000 infected people and with a mortality rate of around 10%, depending on patients' age [9]. However, this coronavirus from animal origin was initially unable to use the human angiotensin 2 converting enzyme (ACE2) as receptor [10–12]. It has been suggested that the masked palm civet (*Paguma larvata*) may be an intermediate host in which the viruses have adapted to ultimately infect humans. A recent study suggests that Chinese Horseshoe bats in the family *Rhinolophidae* may be the natural reservoir for SARS-CoV. One of these two viral isolates in this study, WIV1, was able to recognize the human ACE2 receptor and to replicate in certain human cell lines, suggesting that this virus can directly infect humans without adaptation [13].

4. SARS-CoV: from 2002 to 2003

SARS-CoV, first reported in 2002, belongs to the SARS-related coronavirus species that also includes many bat viruses.

Coronaviruses are spherical enveloped viruses with a diameter of 80 to 120 nm [17]. The viral capsid formed by the nucleoprotein (N) and the genome is contained in the envelope and is of helical symmetry. Three structural proteins are embedded on the surface of particles, the membrane protein (M), the envelope protein (E) and the protein spike (S). They give this aspect of crown in electron microscopy that inspired the name of this viral family.

The S protein of coronaviruses (~1255 amino acids) is a highly N-glycosylated type I transmembrane protein, from 180 to 200 kDa, that plays a major role in viral entry [18]. It insures a double function in viral entry by binding the cellular receptor before conformational changes and proceeding to the fusion of the viral envelope with the membranes of the target cells. S protein has a long N-terminal domain, a short C-terminal domain and assembles into homotrimers on the surface of the viral particle [19]. S protein has a decisive role in cellular tropism and for pathogenicity [20].

S protein of SARS-CoV is composed of two functionally distinct subunits: the globular S1 subunit (~aa 12–680) allows receptor recognition, whereas the S2 subunit (~aa 681–1255) facilitates membrane fusion and anchors S into the viral membrane. S1 is organized in four distinct domains A–D. Domains S1A and S1B may be used as a receptor-binding domain (RBD, aa 318–510) containing the highly conserved receptor-binding motif (RBM, aa 424–494) [21]. Moreover, RBD contains 3 functional glycosylation sites located at amino acids 318, 330 and 357, which are necessary for S expression but do not affect ACE2 binding [22]. S1B forms an extended loop on the viral membrane-distal side and is a hypervariable region [20]. S2 contains the fusion peptides (FP1 and FP2) [23], two heptad repeat regions (HR1, aa ~889–972 and HR2, aa ~1142–1193) and the well conserved transmembrane domain [24].

The mechanism of interactions with peptidases (aminopeptidase APN, ACE2, DPP4) as a cellular receptor for most coronaviruses is not known. Indeed, the binding of coronaviruses to their receptor is not enough and S protein on the surface of the virus must undergo proteolytic maturation. Coronaviruses do not use the catalytic activity of peptidases serving as receptors for this maturation but enter after the action of proteases located close to the receptors. The binding of SARS-CoV to its ACE2 receptor is followed by internalization and decrease in ACE2 enzyme activity on the cell surface, which may partly explain the severity of SARS-CoV infections [25].

4.1. Anti-S1 & RBD antibodies

Neutralizing Abs can fight against viral infections by blocking binding to cellular receptors or by interfering with viral fusion. Besides, in the case of enveloped viruses, the Abs can recruit effector cells or the complement, thus allowing the destruction of the infected cells or the lysis of the viral particles [6]. The S1 domain contains most of the epitopes recognized by nAbs during infection. The RBD located in this S1 domain would be the most important target for nAbs against SARS-CoV, MERS-CoV and the novel coronavirus SARS-CoV-2 [26–29]. More specifically, certain secondary structures such as extended loops seem to be particularly immunogenic.

RBD of SARS-CoV is composed of 193 amino acids (N318-V510) within S protein. Five regions on the S glycoprotein of SARS-CoV (residues 274–306, 510–586, 587–628, 784–803 and 870–893), in which three first regions belong to S1 subunit in the CTD2 and CTD3 (C-terminal domain) and two later belong to HR1 domain of the S2 subunit, were predicted to be associated with a robust immune response to SARS-CoV [30]. Several specific-nAbs for SARS-CoV were discovered; unfortunately none of them are under clinical trial [31] (Fig. 2).

The human single-chain variable region fragment (scFv) antibody 80R blocked ACE-RBD interaction (epitope aa 324–503) [32] but some 80R-escape variants were found with the mutations mostly locating at lysine D480 [33]. The target epitope of 80R is not conserved in SARS-CoV-2 then it does not affect this novel virus [34]. Another nAb generated from a non-immune scFv library, named 256, could bind to an epitope of RBD but did not inhibit RBD binding. 256 is weak but specific to D480A-muted strains of 80R-escape variants. Some engineered broad nAbs, fm6 and fm39, also showed a high affinity to D480A-muted strains [33]. m396 (epitope aa 482–491) from human antibody fab library was cross-reactive [35] and used the D95 of m396 to form a salt bridge with R395 or an electrostatic interaction with D408 of SARS-CoV RBD [34]. m396 potently neutralized GD03 strain isolated from the second outbreak which resisted neutralization by 80R and S3.1. m396 also neutralized isolates from the first SARS-CoV outbreak (Urbani, Tor2) and from palm civets (SZ3, SZ16) [36]. Another human monoclonal antibody from scFv libraries CR3014 (epitope aa 318–510) showed potent effects on SARS-CoV neutralization; however, this virus can escape CR3014 upon P426L mutation in the S glycoprotein [37]. Same as 80R, m396 and CR3014 RBD-specific SARS-CoV antibodies failed to bind the S protein of SARS-CoV-2 [34]. CR3022, always from scFv libraries, could bind noncompetitively the SARS-CoV RBD (epitope aa 318–510) and had a synergistic neutralizing effect with CR3014 on SARS-CoV, even with the escaped P426L-muted variants [37].

By using Xenomouse in which mouse immunoglobulin genes were replaced by human immunoglobulin genes, 19 neutralizing mAbs bound S1 were found. 18 of them, 1B5 [38], 3A7, 3C7, 3F3, 3H12, 4A10, 4E2, 4G2, 5A5, 5A7, 5D3, 5D6, 5E4, 6B1, 6B5, 6B8, 6C1 and 6C2 bound RBD (aa 318–510) to avoid virus binding to the ACE2 receptor. The last one, 4D4, bound an epitope (aa 12–261) located on the N-terminal of RBD and inhibited post-binding event but not the RBD binding. Truncation of the first 300 amino acids of S1 blocked the trimerization and the fusion of S protein [39]. Synergistic effects in some SARS-CoV strains of 4D4 with other mAbs targeting S1 or S2 proteins such as 3C7 (S1), 1F8 (HR1) or 5E9 (HR2) were also reported [38,40]. The tri-combination of 3C7, 3H12 and 4D4 could effectively neutralize escape variants.

Other neutralizing human monoclonal Abs from transgenic mice were also reported. Ab 201 interfered with ACE2 binding by targeting S1 protein at the epitope aa 490–510. In contrast to 201, Ab 68 bound epitope aa 130–150 at the N-terminal of RBD but did not affect ACE2 binding [41].

F26 family of monoclonal Abs generated from mice (F26G9, F26G10, F26G18 and F26G19) showed neutralizing effect against SARS-CoV [42]. F26G18 binding RBD at the epitope aa 460–476 showed the most potent effect [43]. F26G19 (epitope aa 486–492 on RBD [44]) or 80R could also bind SARS-CoV by forming salt bridge R426 (RBD)-D56 or D480 (RBD)-R162, respectively [34].

SARS-CoV mouse antibody 240CD had a nanomolar affinity for the SARS-CoV-2 RBD but did not significantly block ACE-2 receptor binding [45]. As 240CD, CR3022 also has high affinity to SARS-CoV-2 and moreover, CR3022 had cross-neutralizing activity with this novel coronavirus [34].

The effects of neutralizing human monoclonal antibodies, S3.1, S215.13 [46] and S230.15, from Epstein-Bar virus transformation of human B cells were observed. As m396, S230.15 had potent inhibitory activity against isolates from the first, second SARS-CoV outbreaks and from palm civets (SZ3, SZ16) [36].

4.2. Anti-S2 antibodies

In contrast to RBD, the fusion domains are more difficult to access due to the tight folding of viral glycoproteins or the excessively transient exposure during the fusion stage. This is why few epitopes are described in these regions [6]. Interestingly, the S2 specific mAbs can neutralize pseudotyped viruses which expressing different S proteins containing RBD sequences of various clinical isolates [47]. The S2 protein is highly conserved. No mutation in HR1 was reported in an analysis of the amino acid sequences of the S protein from 94 SARS-CoV clinical isolates. Only few mutations in HR2, at amino acids K1163 or Q1183 for example, were observed in this study [47].

Some S2 epitopes inducing nAbs were reported. A peptide containing aa 1055–1192 can elicit neutralizing activity [48]. Two other proteins Trx-F3 and Trx-F9 containing linear antigenic determinants (Leu 803 to Ala 828 and Pro 1061 to Ser 1093, respectively) on the S2 domain were identified by using sera from convalescent SARS-CoV patients. Trx-F3 was capable of inducing nAbs in some animals [49].

Some human mAbs anti-HR1 (1F8, 1D12, 2A12, 2B12, 4A4, 4F9,

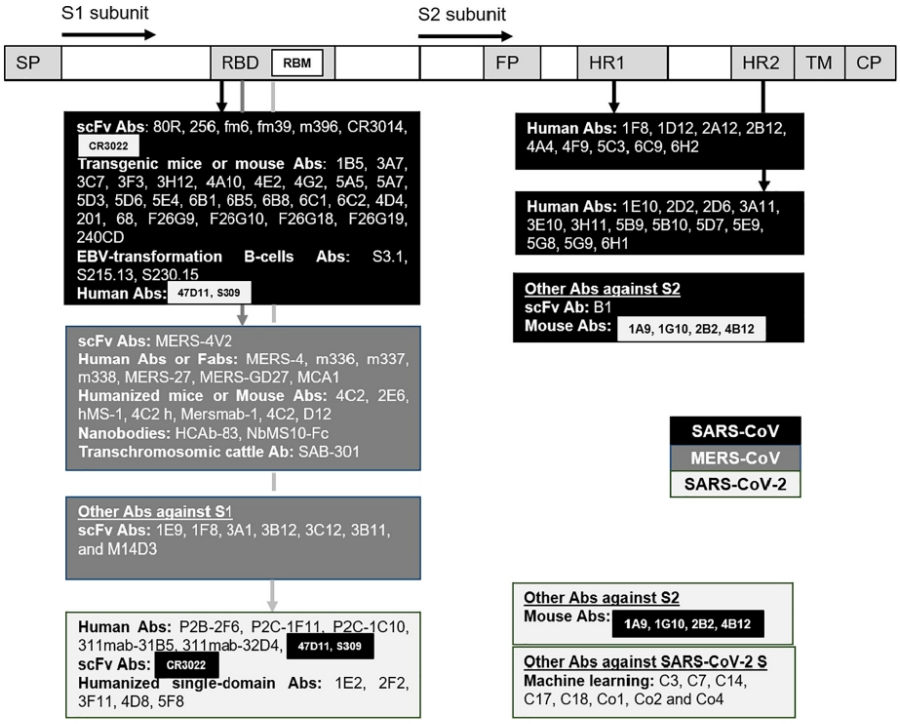

Fig. 2. S protein of SARS-CoV, MERS-CoV, SARS-CoV-2 with its subdomains are the target of antibodies. The antibodies cited in this review have different origins or techniques, and some of them have specific targets such as the receptor binding domain (RBD) containing the receptor binding motif (RBM), the heptad repeat regions (HR1 and HR2). Some antibodies could bind SARS-CoV and SARS-CoV-2. Background color: Black for SARS-CoV, dark grey for MERS-CoV, grey for SARS-CoV-2. SP: Signal peptide, FP: Fusion peptide, TM: Transmembrane domain, CP: Cytoplasm domain.

5C3, 6C9, 6H2) and anti-HR2 (1E10, 2D2, 2D6, 3A11, 3E10, 3H11, 5B9, 5B10, 5D7, 5E9, 5G8, 5G9, 6H1) were reported. With these Abs, the authors showed that the combination of HmAbs targeting different regions of the S protein would likely increase the broad neutralization against different isolates [47].

A human scFv antibody, named B1, showed a high affinity to an epitope (aa 1023–1189) on S2 protein. This antibody also showed potent neutralizing activities against SARS-CoV in vitro [50]. B1, 1F8 and 5E9 nAbs against epitopes on SARS-CoV S2 also showed effectiveness in neutralization [51].

The protective immunity by the time in patients after SARS-CoV natural infection was observed. After 6 years, the humoral immunity continuously decreased and eventually disappeared in most infected individuals. The IgG Ab could be an indicator of neutralizing Ab for the humoral response to SARS-CoV infection [52].

5. MERS-CoV: from 2012 to present

MERS-CoV, a zoonotic virus, belonging to lineage C in the genre betacoronavirus of the family *Coronaviridae*, caused the Middle East respiratory syndrome, in 2012. As of August 11, 2016, the virus had infected 1791 patients, with a mortality rate of 35.6% [53]. The natural reservoir of MERS-CoV is assumedly bats whereas intermediate host is possibly dromedary camels [54–57]. BtCoV-HKU4 and BtCoV-HKU5 bat viruses have been shown to be the closest phylogenetically even if these viruses are not direct ancestors [15]. The first transmission of a bat virus to camels for an adaptation before its emergence into human was suggested. nAbs anti-MERS-CoV could accordingly be found in camels. Moreover, the viruses circulating in dromedaries and in humans are very close suggesting that the dromedary is a reservoir of the virus [58,59]. The genomic structures of bat, human and camel MERS-CoVs are similar but their genomic sequences are different [16].

Structurally, MERS-CoV is a spherical, enveloped, single-stranded, positive sense RNA beta-coronavirus [60]. MERS-CoV utilizes its S protein to mediates cell internalization via binding with the receptor dipeptidyl peptidase 4 (DPP4) on the surface of cells instead of the receptor ACE2 of SARS-CoV and SARS-CoV-2. S protein is therefore the most exposed and immunogenic viral protein [61]. The association of MERS-CoV S protein is similar to that of SARS-CoVs including: the distal subunit S1 containing the RBD and the membrane-anchored subunit S2 containing a putative fusion peptide, transmembrane domain and two heptad repeat regions HR1 and HR2. This S protein is also the target to develop nAbs, particularly the RBD [62].

Using the fragment containing residues 358–588 of S protein, the neutralization against MERS-CoV of induced Abs were observed [63]. Other studies, also approaching RBD, reported the generated Abs with the epitopes aa 377–662 or aa 377–588 of MERS-CoV RBD. The latter elicited the strongest effect which effectively neutralized MERS-CoV infection [64,65]. The epitope aa 736–761 also induced nAbs [66].

Using a novel panning strategy, seven anti-S1 scFvs Abs, named 1E9, 1F8, 3A1, 3B12, 3C12, 3B11, and M14D3, which bind one or several of these three different epitopes (aa 21–358, 349–751 and

349–590) were identified. They neutralized MERS-CoV infection at nanomolar of concentration [67].

Other Abs, such as MERS-4 and its variant MERS-4 V2, were intriguingly discovered to bind RBD and compete with DPP4 but from outside. MERS-4 Fab and MERS-4V2 scFv interact with β5-β6, β6-β7, and β7-β8 loops of RBD resulting in the inhibition of MERS-CoV infection [68,69].

Three human monoclonal Abs, m336, m337, and m338 bind RBD of MERS-CoV at extremely low concentration, 4.2, 9.3, and 15 nM, respectively. m336, that neutralized 50% of both pseudotyped and live MERS-CoV at 0.005 and 0.07 μg/mL, respectively, suggested the prophylaxis and therapy of MERS-CoV infection [70].

In another study, using MERS-RBD to immunize mice, two monoclonal nAbs 4C2 and 2E6 recognizing an epitope that partially overlaps the receptor-binding footprint in MERS-CoV RBD were identified. The 4C2 could further reduce the number of viral particles in MERS-CoV infected mice [71].

Recently, other nAbs, such as human mAbs or Fabs (MERS-27, MERS-GD27, or MCA1), humanized mAbs (hMS-1, 4C2 h), mouse mAbs (Mersmab1, 4C2, or D12), single-domain antibodies (nanobodies Nbs) HCAb-83 or NbMS10-Fc and transchromosomic cattle antibody SAB-301 recognizing epitopes on the RBD have been demonstrated to neutralize pseudotyped and/or live MERS-CoVs [31]. Only SAB-301 is under phase I clinical trial [72].

Despite the efforts of scientists to find anti-MERS-CoV antibodies, no vaccine has been found yet and the virus continues to circulate in human beings and other species.

6. SARS-CoV-2: from 2019 to present

The novel SARS-CoV-2 coronavirus, first appeared in Wuhan, China, in December 2019, is creating a pandemic over the world with the number of confirmed cases reached 3,529,408, of which 248,025 were dead up to the 4th May 2020 [1]. Phylogenetic analysis of SARS-CoV-2 demonstrated similarity with SARS-CoV and bat-derived SARS-like coronaviruses (SL-CoVs) with 79.6% and 88% sequence identity, respectively. They belong to lineage B of the beta coronavirus genus [73,74]. SARS-CoV-2 seems to be more contagious but less pathogenic than SARS-CoV [75]. COVID-19 is a self-limiting disease in > 80% of patients. Same as Spanish influenza viruses, SARS-CoV and SARS-CoV-2 induce a “cytokine storm” but to different degrees. The difference of some conserved interferon antagonists and of inflammasome activators explains their abilities to modulate antiviral and proinflammatory responses.

Along with the race of finding therapeutic treatment, nAbs and vaccine development are also important to control the spread in the long run. SARS-CoV-2 entries the host via the binding of its spike S protein to the ACE2 receptor - sharing receptor, but with higher affinity than SARS-CoV S [76], suggesting a basis for the greater human-to-human transmission of SARS-CoV-2 [51,77].

S protein of SARS-CoV-2 composed of 1273 amino acids [76] uses its N-terminal S1 subunit to bind ACE2 receptor with a better affinity than SARS-CoV S glycoprotein for entry [78]. Effectively, S1 subunit divides into an N-terminal domain (NTD) and a receptor-binding domain (RBD). The latter is necessary for viral binding and a potential target for nAbs. During infection, SARS-CoV-2 first binds the host cell through interaction between its S1-RBD and ACE2, triggering conformational changes in the S2 subunit that is indispensable for virus fusion and entry into the target cell [79,80]. Some recent studies also confirmed that RBD is a conformational epitope [78]. Antibodies binding RBD may sterically hinder binding to the nearby peptide S14P5 of ACE2 receptor, thereby abolishing virus infection [34].

SARS-CoV-2 nAbs could be detected in patients from 10 to 15 days after symptoms onset and the positive rate for IgG reached close to 100% around 20 days [81,82] with the highest level during day 31–40 since onset. Some patients (5.7%) had neutralizing Abs titers under the detectable level (ID50: < 40) [83]. The level of IgG antibodies was different between gender, age and clinical classification. The average IgG antibody level in female patients was higher than in male patients [84]. Patient over 40 years old developed higher levels of SARS-CoV-2 specific nAbs than the younger persons. Patients with a worse clinical classification had a higher antibody titer [83]. This remark is useful to select a research candidate and to save research time. The passive antibody therapy, such as plasma fusion containing polyclonal antibodies from COVID-19 neutralized patients has been tested. This method was tested as an option to treat other viruses such as influenza, Ebola or SARS-CoV [85–89]. The lack of human sera, and the possibility of contamination with other infectious agents limit this strategy. However, several groups have reported some positive results demonstrating the potential of this approach. After one dose of 200 mL of convalescent plasma derived from recently recovered donors with the neutralizing antibody titers above 1:640, the patients with SARS-CoV-2 positive revealed an improvement. Among ten patients, seven patients were virus-negative post transfusion [90]. Whereas in another study, among 5 patients received transfusion with convalescent plasma with a neutralization titer > 40, 3 have been discharged from the hospital (length of stay: 53, 51, and 55 days), and 2 are in stable condition at 37 days after transfusion [91]. More studies might brighter this approach but evaluation in clinical trials are also still far from a bold conclusion.

6.1. Effect of cross-reactive antibodies on SARS-CoV-2 pandemic

Due to the high similarity of S proteins from SARS-CoV and MERS-CoV [73], their specific cross-nAbs were tested against SARS-CoV-2 infection in the COVID-19 outbreak. Serum Abs from recovered SARS-CoV patients could efficiently cross-neutralize SARS-CoV-2 but with lower efficiency as compared to SARS-CoV [92]. Cross-reactive Abs against SARS-CoV-2 S protein mostly target non-RBD regions [93]. Using simulation technique, the binding of five Abs against SARS-CoV, six Abs anti-MERS-CoV to RBD of SARS-CoV-2 was predicted with Rosetta antibody-antigen docking protocols. The amino acid position 445–449 (VGGNY) and 470–486 (TEIYQAGSTPCNGVEGF) were found to be conserved in SARS-CoV-2. Moreover, in addition to the amino acid positions 71–77 (GTNGTKR) in the NTD region of the S protein, aa 445–449 and 470–486 are potential for further development [94].

The difference between RBD of SARS-CoV and SARS-CoV-2 is located at the C-terminus residues. This change has an important impact on the cross-reactivity of nAbs. This difference was observed using bioinformatic approaches of epitope analysis. The antibody epitope score of SARS-CoV-2 is higher than SARS-CoV. Moreover, compared with the conserved regions, the non-conserved regions had a significantly higher antibody epitope score indicating that non-conserved regions of spike proteins are much more antigenic. The non-conserved regions also showed significantly higher surface epitope accessibility scores suggesting an easier accessibility for antibody recognition of non-conserved regions. The divergence of spike proteins is considered as a major change in the antibody epitopes. The search for SARS-CoV-2 requires more effort than simply screening SARS-CoV antibodies [95].

Antibody response to RBD is viral species-specific. Effectively, none of the found SARS-CoV-2 antibodies nor the infected plasma cross-reacted with RBDs from either SARS-CoV or MERS-CoV. In a study, 206 monoclonal antibodies specific to the RBD SARS-CoV-2 were identified in eight patients. These mAbs are different in: antibody heavy and light chains, antibody clones, CDR3 length… which lead to different binding and neutralizing capacities. ACE2 is out-competed with almost 100% efficacy by some mAbs such as P2B-2F6 and P2C-1F11. Interestingly the latter and a moderate antibody P2C-1C10 seems to target the different epitopes, and they could be combined for synergistic antiviral effect [96]. CR3022, a SARS-CoV RBD-specific antibody, can bind strongly with a kd of 6.3 nM to an epitope on RBD that does not overlap with the SARS-Cov-2 ACE-2 binding site [34]. Despite its strong binding, CR3022 could not neutralize SARS-CoV-2 [97].

S1 is a specific antigen for SARS-CoV-2 diagnostics [98]. The S1 subunit of SARS-CoV or SARS-CoV-2 has four core domains S1A through S1D. The human 47D11 antibody binds the S1B of both viruses, without competing with S1B binding to ACE2 receptor expressed at the cell surface, and showed cross-neutralizing activity by an unknown mechanism that is different from receptor binding interference [99]. An immunogenic domain in the S2 subunit of SARS-CoV S (aa 1029–1192) was highly conserved in several strains of SARS-CoV-2. Four murine monoclonal Abs, 1A9, 1G10, 2B2 and 4B12, against this S2 subunit of SARS-CoV can also cross-reactive with the S protein of SARS-CoV-2. Interestingly, 1A9 can strongly bind the S2 subunit of SARS-CoV-2 through a novel epitope (aa 1111–1130) and can detect S protein in SARS-CoV-2 during infection [100]. This epitope also overlaps with one of two cytotoxic T-lymphocyte epitopes (aa 884–891 and 1116–1123) of SARS-CoV S2 subunit [101]. 1A9 is therefore suggested to induce both humoral and cellular immune responses against SARS-CoV and SARS-CoV-2.

In a serologic cross-reactivity test, Khan et al. found out that 4 out of 5 showed high IgG seroreactivity across the 4 common human coronaviruses but all showed low IgG seroreactivity to SARS-CoV-2, SARS-CoV, and MERS-CoV [102]. The weak cross-immunity against SARS-CoV-2 from others betacoronaviruses, such as HCoV-OC43 and HCoV-HKU1, could restraint the transmission of SARS-CoV-2 but a resurgence is possible in the future [103]. Moreover, spike- and non-spike specific CD4 + T cell responses were detectable not only in SARS-CoV-2 infected patients but also in uninfected individuals. If there is an absence of antibody cross-reactivity, T lymphocyte cross-reactivity present in 50% of cases will be responsible for the epidemiological evolution of SARS-CoV-2 infection [104].

6.2. Anti-SARS-CoV-2 specific antibodies

Up to this moment, only few tests of specific Abs against SARS-CoV-2 have been reported. 311mab-31B5 and 311mab-32D4 human monoclonal Abs could strongly and specifically bind the RBD protein. These mAbs could efficiently block SARS-CoV-2-ACE2 interaction and neutralize pseudovirus entry into host cells ectopically expressing ACE2 [105].

Peptides S14P5 and S21P2 in the two distinct peptide pools S14 and S21 from SARS-CoV-2 S library were strongly detected in COVID-19 patients but not in SARS-CoV patients by using pools of overlapping linear peptides and functional assays [78]. With the data from antibodies depletion assays, researchers indicated that S14P5 and S21P2 were necessary for SARS-CoV-2 neutralization. Moreover, pool S51 contains very conserved fusion peptide in coronavirus [106,107] and is partially overlapped in the sera of SARS-CoV and SARS-CoV-2 patients. These results suggested that S51 may be a potential pan-coronavirus epitope. Sera from recalled SARS-CoV patients could neutralize SARS-CoV, but not the SARS-CoV-2 pseudotyped lentiviruses [78].

In an effort to screen a set of B cell and T cell epitopes of SARS-CoV toward to the spike S and nucleocapsid (N) proteins of SARS-CoV-2, 27 epitope-sequences were identical within SARS-CoV-2 proteins among 115 T cell epitopes. However, 19 out of 27 epitopes are associated with five distinct MHC alleles (at 4-digit resolution): HLA-A*02:01, HLA-B*40:01, HLA-DRA*01:01, HLA-DRB1*07:01, and HLA-DRB1*04:01. For B cell epitopes, they found 49 identical match epitope-sequences that have potential for developing effective vaccines to combat the SARS-CoV-2 [108]. Based on the sequence of the spike glycoprotein, seven epitope residue/regions (491–505, 558–562, 703–704, 793–794, 810, 914, and 1140–1146) in the surface glycoprotein were predicted to be associated with a robust immune response to SARS-CoV-2 [30]. Other candidate epitopes need to be confirmed [95,108].

Using the memory B cells from a survivor who was SARS-CoV infected in 2003, one nAb anti-RBD named S309 was found to bind to SARS-CoV-2 without interfering ACE2 binding. Besides, S309 could recognize a N343-glycan epitope that is distant from the RBM of SARS-CoV-2. Interestingly, N343-glycan of SARS-CoV-2 corresponds to SARS-CoV N330 and they are highly conserved. S309 potently neutralized both pseudotyped SARS-CoV and SARS-CoV-2 and also the authentic SARS-CoV-2 [109].

Using machine learning approaches with the data from other virus outbreaks, some synthetic nAbs named C3, C7, C14, C17, C18, Co1, Co2 and Co4 showed a potential to against SARS-CoV-2. The authors also confirmed that the mutations of Methionine and Tyrosine could increase the affinity of antibody-target binding [110].

19 potential immunogenicity B-cell epitopes, including 2 epitopes located within the RBD region were reported using in silico analysis. 17 of them have > 14 amino acids. The B-cell epitopes which had highest score in this study is the 1052-FPQSAPH-1058 located at position 1052aa of S protein. 499 T-cell epitopes bound 34 most popular HLA alleles in the Chinese population were also found. Around 30 candidate vaccine peptides in which 5 peptides located within the RBD region and 17 of them contained both B- and T-cell epitopes, were designed [111]. These vaccine candidates are theoretically able to induce either specific humoral or cellular immune against SARS-CoV-2.

A panel of five humanized single domain antibodies (sdAbs) or nanobodies, 1E2, 2F2, 3F11, 4D8 and 5F8, was recently discovered. These sdAbs bound SARS-CoV-2 tightly but not SARS-CoV, except for 5F8 could bind both viruses but with weaker affinity to SARS-CoV. They also showed neutralization activity against both pseudotyped and authentic SARS-CoV-2. 1E2, 3F11 and 4D8 completely prevented SARS-CoV-2 RBD-ACE2 binding but this effect of 2F2 and 5F8 was only partial. Interestingly, the fusion of the human IgG1 Fc to these sdAbs improved their neutralization activity by 10- to 80-fold [112].

Due to the lack of repairing mechanism of RNA virus replicase complex, SARS-CoV-2 mutations frequently occur during viral replication [111]. The genetic drifts of SARS-CoV-2 are a selective evolution toward less immunogenicity for host immune surveillance by T- or B-cells. The latter appearing strains are less immunogenic than earlier ones [113]. Antigenic drift is also reported in the COVID-19 pandemic. The highly prevalent 23403A > G (p.D614G) variant in the European population may result in vaccine mismatches with little protection to that group of patients [114,115].

Though SARS-COV-2 genome has a much lower mutation rate and genetic diversity than SARS, some of its mutations attract the special attention of scientists. Single amino acid mutation R408I in RBD can reduce the affinity of ACE2 receptor binding [115] that leads to a low or ineffective vaccine for the future epidemic. Effectively, sequence alignment showed that this 408R is strictly conserved in SARS-CoV-2, SARS-CoV. 408R located at the interface between RBD and ACE2, but positioned relatively far away from ACE2, does not have direct interaction with ACE2. 408R can form a hydrogen bond with the 90 N of ACE2. This hydrogen bond is suggested to contribute to the high binding affinity of ACE2 binding [115].

7. Discussion

Science, with new advances, somehow might find the therapy to protect human beings from COVID-19. Among these, plasma therapy composing of antibodies and humoral immune components has been doing great and being one of the first solutions. Because of that, the quest of an antibody always becomes a “must-do-first” when human population facing new pandemic. However, only one lesson could be obvious is we can never get the answer for every pandemic at ones.

Some coronaviruses can infect birds, bats and other species, some are phylogenetically similar to known pathogenic human coronaviruses. The search for the reservoir has resulted in the vast expansion of the library of known coronaviruses which suggests that additional emergence events are possible.

Pandemics will create the urgent need for vaccines around the world simultaneously. But it is not because of this urgency that we can license a vaccine when its benefits and side effects are not clear.

Researching a vaccine for influenza viruses, HIV or SARS-CoV-2 is always challenging. Firstly, although the immunogen for protection of a virus, glycoproteins gp120 or gp41 of HIV or S protein of SARS-CoV-2 for example, can be quickly detected, but the immune response needs to be optimized with a good antigen design. Secondly, any drug has side effects immediately or in long-term, directly by the composition of the medication or indirectly by the response of the body to the medication. Pre-clinical experiences with vaccine candidates for SARS-CoV and MERS-CoV are typical examples in aggravating lung disease, either directly or by antibody-dependent enhancement [116–118]. Thirdly, in natural acquired infection, the time point of the detection of nAbs can be easily observed, from 10 to 14 days post-infection in SARS-CoV-2 case for example [119,120], but the potential duration of immunity response is not clear. Therefore, the use of singe-dose or several doses of vaccines needs to be confirmed. Moreover, once a vaccine has been approved, that does not mean that virus research and monitoring can stop. Indeed, influenza and HIV viruses have been reported to have high mutations, making it difficult to find broadly neutralizing vaccines. In the actual pandemic of SARS-CoV-2, drift variants have been reported and that can affect COVID-19 vaccine development [114,115]. No vaccine is available against any coronavirus [121]. Monoclonal antibodies cocktails including multiple epitopes targeting Abs could be taken into account to broaden spectrum of therapy.

Vaccine development is a long and expensive process. From identifying a virus to producing vaccines to market, it takes us a few years. If pandemic gets end before vaccines are approved, the research of vaccine candidates under development need to be continued and ready for clinical trials, in order to get emergency authorization when an outbreak recurs. This statement draws on experience from Ebola pandemic in which vaccine was still under development when the Ebola outbreak ended in 2016. Ebola vaccine is recently approved [122–124] and already used in the recent outbreak in the Democratic Republic of Congo [125].

Studying and understanding the antigen-antibody mechanism of a virus can be used as a precondition to accelerate the studying another virus during an outbreak. In the COVID-19 pandemic, some nAbs studies were based on the research of previous viruses such as SARS, MERS, Ebola and HIV. Through machine learning approaches with the data composed of HIV gp41-antibodies complexes and of 13 more different virus types, some potential nAbs against SARS-CoV-19 were found [110]. This case shows the usefulness of this review of host-antibody interactions from coronaviruses pandemics for young or mature scientists working on vaccine research.

The key findings in coronavirus antibodies investigation could reveal S protein and its subunits as major frame for antibody generation in which RBD seems to be the most efficient peptides. A lot of effort has been tried but still plenty of gaps to fill up. Other approaches and therapies are also needed to protect us from coronavirus infection.

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7.2. Conformational Stabilization of Gp41-Mimetic Miniproteins Opens Up New Ways of Inhibiting HIV-1 Fusion

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Conformational Stabilization of Gp41-Mimetic Miniproteins Opens Up New Ways of Inhibiting HIV-1 Fusion

Mario Cano-Muñoz [1,2,*], Julie Lucas [2], Li-Yun Lin [2], Samuele Cesaro [1,†], Christiane Moog [2] and Francisco Conejero-Lara [1,*]

[1] Departamento de Química Física, Instituto de Biotecnología y Unidad de Excelencia de Química Aplicada a Biomedicina y Medioambiente (UEQ), Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain; samuele.cesaro91@gmail.com

[2] INSERM U1109, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, 67084 Strasbourg, France; julie.lucas@etu.unistra.fr (J.L.); li-yun.lin@etu.unistra.fr (L.-Y.L.); c.moog@unistra.fr (C.M.)

* Correspondence: mariocano@ugr.es (M.C.-M.); conejero@ugr.es (F.C.-L.); Tel.: +34-958242371 (F.C.-L.)

† Present address: Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy.

Abstract: Inhibition of the HIV-1 fusion process constitutes a promising strategy to neutralize the virus at an early stage before it enters the cell. In this process, the envelope glycoprotein (Env) plays a central role by promoting membrane fusion. We previously identified a vulnerability at the flexible C-terminal end of the gp41 C-terminal heptad repeat (CHR) region to inhibition by a single-chain miniprotein (named covNHR-N) that mimics the first half of the gp41 N-terminal heptad repeat (NHR). The miniprotein exhibited low stability, moderate binding to its complementary CHR region, both as an isolated peptide and in native trimeric Envs, and low inhibitory activity against a panel of pseudoviruses. The addition of a disulfide bond stabilizing the miniprotein increased its inhibitory activity, without altering the binding affinity. Here, to further study the effect of conformational stability on binding and inhibitory potency, we additionally stabilized these miniproteins by engineering a second disulfide bond stapling their N-terminal end, The new disulfide-bond strongly stabilizes the protein, increases binding affinity for the CHR target and strongly improves inhibitory activity against several HIV-1 strains. Moreover, high inhibitory activity could be achieved without targeting the preserved hydrophobic pocket motif of gp41. These results may have implications in the discovery of new strategies to inhibit HIV targeting the gp41 CHR region.

Keywords: fusion inhibitor; calorimetry; coiled-coil; envelope glycoprotein; N-terminal domain; antiviral therapy; gp41

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1. Introduction

The HIV/AIDS pandemic is still very active and continues to be one of the world's largest pandemics to date with more than 40 million people currently living with HIV still representing a worldwide health issue [1,2]. What is more striking is the upsurge in HIV infections over different populations around the world, such as the outbreak in China's students, where the number of newly diagnosed college students has seen an annual growth rate ranging from 30 to 50% over the past several years [3]. All this together with the fact that HIV newly infects 1.8 million people each year, makes the development of an HIV vaccine a global health priority [4]. However, almost 40 years after the discovery of HIV as the causative agent of AIDS we still do not have a licensed vaccine. Progress has been hindered by the extensive genetic variability of HIV and our limited understanding of the immune responses required to protect against HIV acquisition [5].

Annex 2.Conformational Stabilization of Gp41-Mimetic Miniproteins Opens Up New Ways of Inhibiting HIV-1 Fusion

This emphasizes the importance of therapeutics to treat the infection. Despite modern Highly Active Antiretroviral Therapy (HAART) having helped to reduce the number of deaths, the absence of an effective vaccine combined with the growing emergence of multi-resistant HIV variants to several of these drugs urges the development of some new anti-HIV compounds directed against the different stages of the virus life cycle, and in particular against the entry of HIV into the cell [1,6].

In order to enter the target human cell, the virus must fuse its membrane with that of the cell. This fusion process is mediated by the Envelope glycoprotein (Env), a non-covalently associated trimer of heterodimers composed of two glycoprotein subunits, gp120 and gp41 [7]. CD4 receptor and co-receptor (CCR5 or CXCR4) binding to gp120 triggers a series of conformational changes that ultimately cause the adoption of a more energetically favorable conformation called the 6 helix bundle (6HB) formed by the N-terminal heptad repeat (NHR) and C-terminal heptad repeat (CHR) regions of gp41. In this 6HB structure, three CHR regions associate externally over an inner helical coiled-coil NHR trimer in an antiparallel fashion. This energetically favorable interaction between NHR and CHR brings viral and host-cell membranes into close proximity promoting fusion and eventually causing infection. Consequently, compounds that interact with either CHR or NHR interfere with this key process and thereby constitute HIV fusion inhibitors [8–10]. For this reason, gp41 has become a very attractive target for the development of potential HIV-1 inhibitors.

Different kinds of fusion inhibitors have been described and classified into two major categories regarding whether they interact with the NHR or CHR regions in gp41. Class-1 inhibitors target the exposed hydrophobic grooves of the NHR helical trimer and comprise a variety of molecules including CHR peptide mimetics, artificial D-peptides, natural products and small-molecule compounds and antibodies [11–15]. On the other hand, class-2 inhibitors target the CHR region and generally encompass NHR peptide mimetics that have traditionally been regarded as low activity anti-HIV compounds. This limited potency may be due to the low solubility and the tendency of NHR peptides to aggregate in solution. These problems can be alleviated by engineered stabilized protein constructs that mimic exposed trimeric NHR grooves. However, they have certain advantages, such as their activity against strains resistant to CHR inhibitors [16,17]. Despite these promising therapeutic approaches, the only FDA-approved fusion inhibitor of AIDS/HIV is T20 (enfuvirtide), a CHR-derived peptide whose clinical use has been limited by its short half-life [18] (proteolysis-sensitive and rapid renal filtration) requiring, therefore, high dosage injections at least twice a day. Moreover, the continuous and expensive treatment generates the appearance of T20-resistant viruses. Nevertheless, compounds that are able to interfere with the formation of the gp41 6HB continue to be very attractive targets for drug design strategies [19,20].

Recently, we have developed several protein molecules called covNHR which consist of a single polypeptide chain with three helical regions that fold as an antiparallel trimeric bundle with a structure highly similar to the NHR gp41 region [21,22]. These proteins can be produced recombinantly by expression in *E. coli* with high yields, without any post-translational modification, are easy to purify, very stable and highly soluble [23]. The NHR binding surface has been described as composed of four different hotspots, namely an N-terminal polar pocket (NTP), a shallow middle pocket (MP), a deep and prominent hydrophobic pocket (HP), which has been widely used as a drug discovery target [14], and finally a C-terminal pocket (CTP) adjacent to the HP [23] (Figure 1C). In a recent study, we designed, produced and characterized two single-chain covNHR miniproteins each encompassing only two consecutive pockets out of the four pockets of the NHR groove [24]. Each miniprotein mimics the N- and the C-terminal half of NHR, respectively, and they were called covNHR-N (harboring only NTP and MP) and covNHR-C (exposing only the HP and the CTP). These miniproteins folded autonomously and represent subdomains of NHR, with very different intrinsic stability. Although both covNHR miniproteins could bind their respective complementary CHR peptides with similar affinity, the covNHR-C protein could not bind its target in soluble prefusion Env spikes and did not show any HIV-1 inhibitory activity in vitro. This is probably due to the HP and CTP binding motifs

being engaged in a tryptophan clasp involving the side chains of Trp623 (CTP motif), Trp628 and Trp631 (HP motif) that locks Env in its pre-fusion conformation [25,26]. On the other hand, covNHR-N could bind its target in soluble prefusion Env spikes and showed moderate HIV-1 inhibitory activity in vitro. However, it proved to be quite unstable and required to be stabilized by engineering a disulfide bond connecting one of its two loops to its C-terminal end. The stabilized versions of covNHR-N with one disulfide bond (called covNHR-N-SS) showed a similar affinity towards its complementary CHR peptide and similar capability to bind to soluble prefusion Env spikes. Strikingly, these stabilized variants showed improved inhibitory potency against different HIV strains.

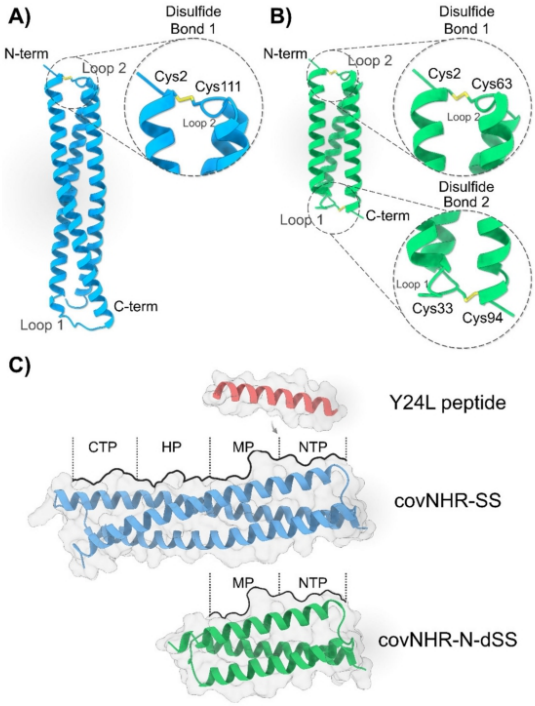

Figure 1. Design of covNHR miniproteins. (**A**,**B**) Ribbon models of covNHR-SS (**A**, blue) and covNHR-N-dSS (**B**, green) showing the location of the residues chosen for mutations to form disulfide bonds. Cysteines are shown in sticks and colored in yellow. (**C**) Model of the structure of the proteins and peptides involved in this study depicted in molecular surface and colored green (covNHR-N-dSS), blue (covNHR-SS) and red (Y24L peptide, gp41 residues 638–661). CovNHR is a highly accurate mimic of the full trimeric gp41 NHR coiled-coil, as such, its binding surface is also composed of four different hotspots: CTP (C-terminal Pocket), HP (Hydrophobic Pocket), MP (Middle Pocket) and NTP (N-terminal Pocket), see details in the text.

Here, we have furtherly increased the stability of covNHR-N by engineering an additional internal disulfide bond stapling the other end of the molecule to test the hypothesis that an increase in stability can lead to a substantial improvement of the affinity to its complementary CHR peptide accompanied by a subsequent improvement in anti-HIV inhibitory potency. This stabilizing strategy has also been implemented in the complete covNHR parent molecule. Therefore, both protein molecules, called covNHR-N-dSS and covNHR-SS, respectively, (Figure 1) were designed, biophysically characterized and tested

for direct Env binding upon several variants. Their capacity to inhibit different HIV-1 pseudoviruses and primary isolates, including T20-resistant strains, was also assessed. The results provide valuable knowledge to the development of protein-based antivirals and reveal new ways to exploit the vulnerability of the gp41 CHR region.

2. Results

2.1. Design and Stabilization of CovNHR Miniproteins

The initial designs of the parent molecules in this study, covNHR and covNHR-N, contain, respectively, the four and two (NTP and MP) of the pockets described in gp41 (Figure 1 and Figure S1). In order to design the new proteins covNHR-SS and covNHR-N-dSS, we used the crystallographic structure of covNHR in complex with C34 peptide (PDB ID: 6R2G, previously determined by our group [22]) as a template. In our previous study, we had achieved a considerable stabilization of over +20 °C in covNHR-N ($T_m \approx 41$ °C) by engineering a disulfide bridge that connected the first loop of the miniprotein with its C-terminal end (G33C/R94C mutations) resulting in a new variant called covNHR-N-SS [24]. This "staple" strategy was recreated in this study in order to achieve even further stabilization of the molecules. To accomplish that, Disulfide by Design [27], a web-based tool for disulfide engineering in proteins, was used to predict pairs of residues that will likely form a disulfide bond if mutated to cysteines. We found one possibility for disulfide bond creation connecting different structural elements of the protein by X-Cys mutations. For covNHR-N-dSS, residues Ala2 at the N-terminus and Leu64 in loop 2 fulfilled the strict geometric constraints that disulfide bonds usually require and were also mutated to Cys. Accordingly, covNHR-N-dSS contains mutations A2C/L63C and G33C/R94C. The equivalent disulfide bond connecting the N-terminus and loop 2 was also engineered for covNHR-SS, which in this case contains mutations A2C/L111C (Figure 1).

In order to validate and assess in silico the stability and dynamic behavior of the newly designed miniproteins we conducted all-atom explicit-solvent molecular dynamics (MD) simulations of the free molecules. Root mean square fluctuation (RMSF) values, which are a measurement of the average atomic mobility of the residues in the protein, showed an increase in the stability (i.e., lower values) in the sites where X-Cys mutations were placed (Figure 2A,B). CovNHR-SS showed decreased RMSF values in the vicinity of the mutated sites compared to covNHR (Figure 2A), which helped to decrease the overall residue mobility of the N-terminal end and the second loop, two hotspots for protein instability. A similar scenario occurred with covNHR-N-dSS if we compare its RMSF values with those of the covNHR-N parent molecule (Figure 2B), in this case, the four hotspots in the protein chain, the N- and C-term as well as the first and second loops were stabilized by the addition of the two disulfide bonds. Moreover, the mean RMSF values for each new protein were lower than their respective parent molecule (Figure 2A,B).

Figure 2C,D shows the time evolution of backbone root mean square deviations (RMSD) of the miniproteins. All the molecules reached equilibrium within the first 5 ns of MD simulation. However, the disulfide-stabilized miniproteins reached a more stable plateau and maintained it throughout the entire simulation time while their parent molecules showed a less stable profile. This is also supported by the fact that the mean RMSD values for the newly engineered miniproteins were also lower than those from their parent molecules. These results indicate that the engineered disulfide bonds reduce the overall conformational fluctuations of the new proteins compared to their parent molecules.

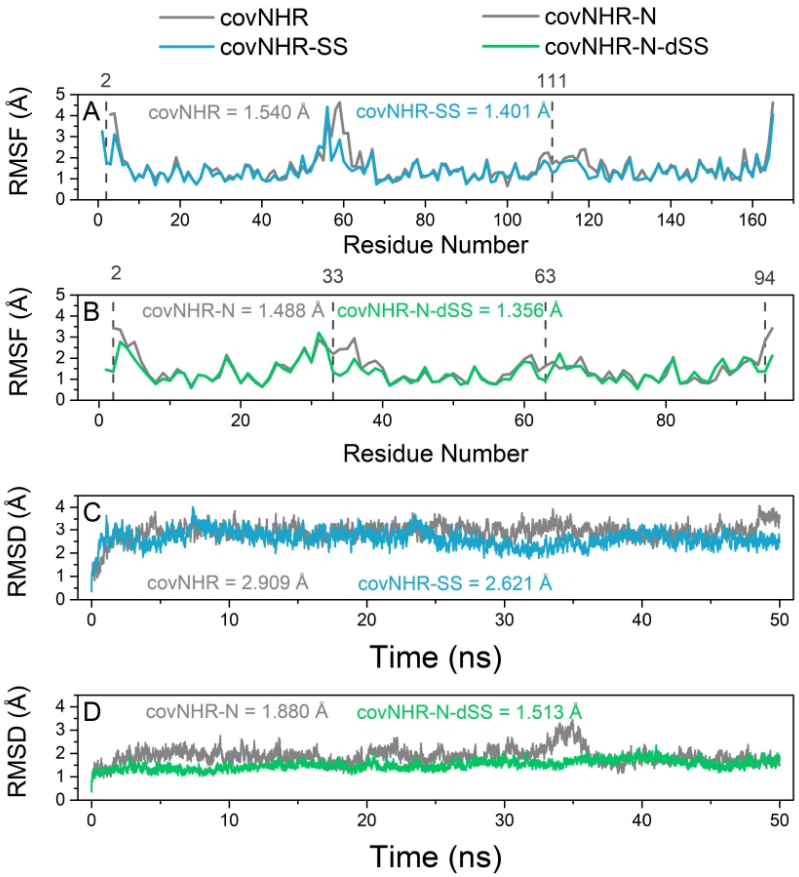

Figure 2. Molecular dynamics simulations analysis of the two miniproteins: covNHR-SS (blue) and covNHR-N-dSS (green) compared with their parent molecule covNHR and covNHR-N, respectively (grey). (**A**) Root mean square fluctuation (RMSF) per residue for covNHR and covNHR-SS. (**B**) RMSF per residue for covNHR-N and covNHR-N-dSS. (**C**) Evolution of mean backbone root mean square deviation (RMSD) for covNHR and covNHR-SS. (**D**) Evolution of RMSD for covNHR-N and covNHR-N-dSS. The locations of residues forming the disulfide bonds are highlighted with grey dashed lines and with their residue number shown above in A and B. Mean values for the parameters throughout the entire simulation time are also displayed in each panel with the same color code used above.

2.2. Biophysical Characterization of CovNHR Variants

Both disulfide-bonded mutants covNHR-N-dSS and covNHR-SS could be expressed and produced recombinantly in *E. coli* with good yields even higher than those of their respective parent molecule. All purification steps were made in the presence of 10 mM β-mercaptoethanol, and a final oxidation step was carried out by extensive dialysis with buffer without a reducing agent. The formation of the disulfide bonds was confirmed in both mutants using Ellman's assay (Thermo Fisher, Waltham, MA, USA). The protein purity was assessed by SDS-PAGE, and the identity of each protein variant was confirmed by mass spectrometry analysis.

The structure and stability of the miniproteins were characterized using various biophysical techniques (Figure 3). The two miniproteins showed far-UV circular dichroism (CD) spectra typical of a mostly α-helical structure (Figure 3A,B); covNHR-SS has a similar α-helical structure content to its parent molecule covNHR [22]. On the other hand, covNHR-N-dSS has higher negative ellipticity values than covNHR-N and covNHR-N-SS, its parent molecules [24], showing a higher α-helical structure percentage (72.4% against 64% and 67.1%, respectively). This indicates that the newly formed disulfide bridge is stabilizing the antiparallel helical bundle. At the same time, mixtures between each protein and the Y24L peptide, containing the NTP and MP binding motifs (Figure 1C), showed an increase in negative ellipticity relative to the theoretical ellipticities of the spectra calculated as the sum of the spectra of the free molecules (Figure 3A,B). This indicates the acquisition of the helical conformation for the Y24L peptide as a consequence of binding onto the NHR groove of both miniproteins. The ellipticity increase was similar in both complexes indicating a comparable acquisition of helical structure. This was not the case for covNHR-N, which showed a higher relative ellipticity increase when binding to Y24L peptide, as a consequence of the protein acquiring a more ordered and helical structure when bound to Y24L [24].

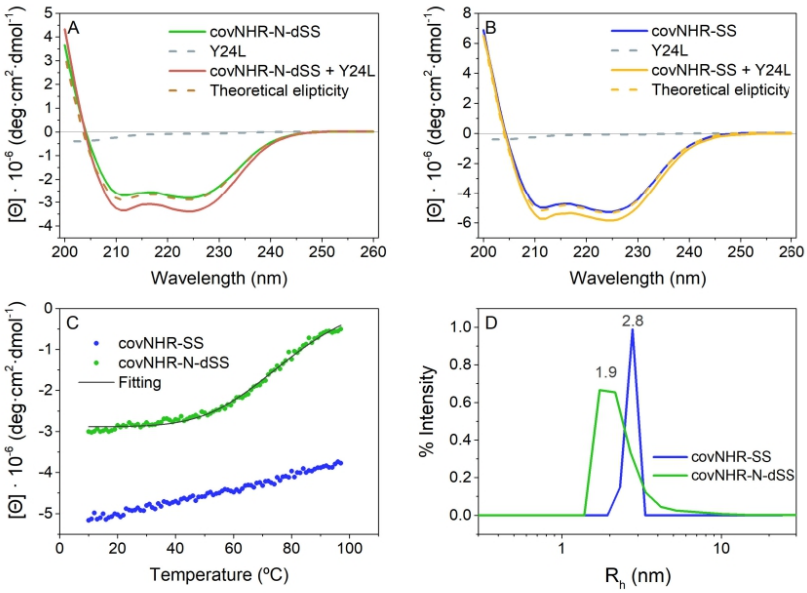

Figure 3. Secondary structure and thermal stability of covNHR-N-dSS (green) and covNHR-SS (blue). (**A**) Far UV CD spectra of free covNHR-N-dSS (green, solid line) and (**B**) covNHR-SS (blue, solid line) and in a (1:2) mixture with their complementary CHR peptide, Y24L (red and yellow lines, respectively). Y24L peptide alone is shown in dashed grey lines in both panels. The red and yellow dashed lines represent the theoretical sum of the spectra of the free molecules. (**C**) Thermal unfolding of covNHR-N-dSS (green symbols) and covNHR-SS (blue symbols) followed by monitoring the CD signal at 222 nm. The grey solid line corresponds to the best fitting carried out using a two-states unfolding model. (**D**) Particle size distributions measured by dynamic light scattering with solutions of covNHR-N-dSS (green) and covNHR-SS (blue). All experiments were carried out at pH 7.4 in 50 mm sodium phosphate.

Thermal denaturation experiments of covNHR-SS and covNHR-N-dSS indicated strong stabilization of the proteins by the disulfide bonds. The melting temperature (T_m)

of covNHR-N-dSS was −79 °C (Figure 3C), a strong increase of +38 °C compared with covNHR-N without any disulfide bridge and an increase of +18 °C compared with the single disulfide bonded covNHR-N-SS [24]. Regarding covNHR-SS, no unfolding transition was observed even after heating up to 98 °C, confirming its extremely high thermostability. The thermal stability of covNHR-SS was estimated by differential scanning calorimetry (DSC) −124 °C (Figure S2), compared to the 105 °C of the covNHR protein under the same conditions [22]. The denaturation peak showed a sharp drop on the high-temperature side of the peak suggesting thermally induced aggregation similar to what happens to covNHR parent molecule. The thermal stability is thus increased by about 18–20 °C with each disulfide bond engineered in both miniproteins.

Both protein variants are highly soluble at physiological pH and the particle sizes of both proteins were assessed by dynamic light scattering (DLS) (Figure 3D). CovNHR-N-dSS showed an apparent hydrodynamic radius (R_h) of 1.9 nm and covNHR-SS exhibits a R_h of 2.8 nm similar to their respective parent molecules and to their theoretical R_h. This demonstrates that both variants are monomeric at physiological pH in 50 mM sodium phosphate buffer.

2.3. Binding of the CHR Peptide to CovNHR Miniproteins

To characterize in detail the thermodynamics of binding of the miniproteins to the complementary CHR peptide, we performed isothermal titration calorimetry (ITC) analysis (Figure 4 and Figure S3 and Table 1) by direct titration of the protein solutions with peptide Y24L, corresponding to gp41 residues 638–661, at the second half of CHR. Previously, we determined the interaction between Y24L and covNHR, which happened to be moderately tight (K_d = 90 ± 7 nM at 25 °C) [23]. CovNHR-SS exhibited a similar K_d of 116 ± 11 nM at the same temperature. The binding enthalpies and heat capacities are also very similar for these two protein variants (−1.6 vs. −1.7 $kJ{\cdot}K^{-1}{\cdot}mol^{-1}$, Table 1) [23]. This indicates that the presence of the disulfide bond at the N-terminus of the complete covNHR protein has a small influence on the binding thermodynamics to the CHR peptide and suggests that the presence of a continuous NHR coiled-coil structure already provides strong conformational stability to the N-domain for a competent binding capability at the NTP and MP pockets.

Table 1. Thermodynamic parameters of binding of gp41 CHR peptide Y24L to covNHR miniproteins measured by ITC.

Protein	Peptide	Temperature (°C)	K_d (nM)	ΔH_b ($kJ{\cdot}mol^{-1}$)	n	ΔC_{pb} ($kJ{\cdot}K^{-1}{\cdot}mol^{-1}$)
covNHR-SS	Y24L	10	80 ± 8	−30.4 ± 1.0	0.75	−1.70 ± 0.21
		15	85 ± 9	−43.5 ± 0.8	0.77	
		20	91 ± 4	−52.0 ± 1.2	0.8	
		25	116 ± 11	−57.7 ± 0.6	0.83	
covNHR-N-dSS	Y24L	10	61 ± 8	−60.7 ± 0.4	0.76	−2.26 ± 0.11
		15	94.3 ± 2.4	−72.1 ± 2.1	0.77	
		20	149 ± 6	−84.0 ± 1.3	0.78	
		25	243 ± 10	−90 ± 4	0.89	

Uncertainties in the parameters correspond to standard errors of the fittings.

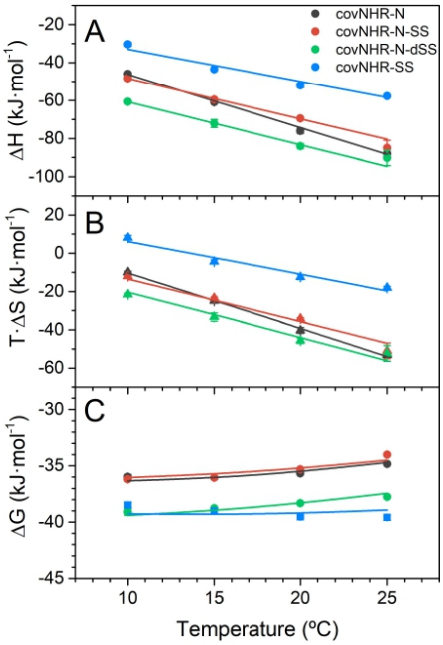

Figure 4. Thermodynamic parameters of Y24L peptide binding to the covNHR proteins. Data have been calculated from the parameters of Table 1 and data from [24], measured by isothermal titration calorimetry (ITC) (**A**) Binding enthalpies; (**B**) binding entropies and (**C**) binding Gibb's energies. The symbols correspond to the values derived from experimental data and the lines represent the temperature dependencies of each parameter according to the binding heat capacity changes.

On the other hand, the affinity of covNHR-N for Y24L peptide was determined to be much lower (K_d = 790 ± 20 nM) and with more negative binding enthalpy and heat capacity as a result of a considerable entropy penalty associated to structural stabilization of covNHR-N upon interaction with the CHR peptide [24]. This binding entropy cost was not reduced in the singly disulfide-bonded covNHR-N-SS [24], which showed very similar binding parameters although slightly reduced binding heat capacity (Figure 4). In marked contrast, the disulfide bond at the N-terminus in covNHR-N-dSS confers a remarkably higher affinity to Y24L (Table 1 and Figure 4), about a 4.5-fold increase. The thermodynamic magnitudes (Table 1, Figure 4A,B) showed significantly more negative binding enthalpy, partially compensated by a higher entropy cost. These magnitudes indicate a tighter interaction in the complex produced by the new disulfide bond. In fact, the binding affinities and Gibb's energies are close to those measured for the complete covNHR proteins at low temperatures, although they decrease rapidly with temperature due to a more negative heat capacity change.

2.4. Binding to Envelope Proteins

We investigated the influence of stabilization by disulfide bonding on the interaction of covNHR-SS and covNHR-N-dSS with their target gp41 CHR region in various Env proteins, both in uncleaved and cleaved trimeric pre-fusion conformations (Table S1). ELISA experiments showed that the two miniproteins bind efficiently to all Envs (Figure 5), comparable to each parent molecule [24], except for a slightly enhanced binding of covNHR-N-dSS to gp140 CN54 Env compared to covNHR-N-SS.

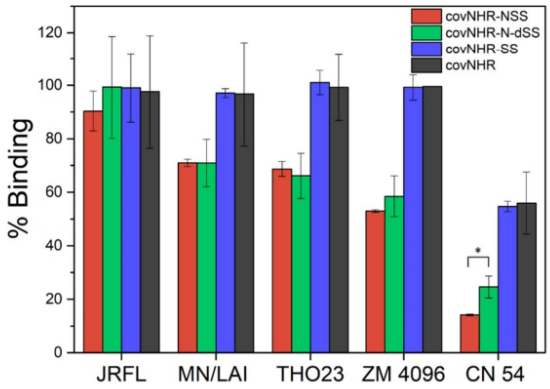

Figure 5. Binding of covNHR miniproteins to different soluble Envs (Table S1) measured by ELISA. Miniproteins binding to Env was detected using anti-6X Histag Ab as primary antibody. Background binding was measured without Env and subtracted from the data; 100% positive control was measured with wells directly coated with a His-tagged Env. Data correspond to mean ± S.D. values of three independent measurements. * $p < 0.05$, difference between covNHR-N-dSS and covNHR-N-SS.

Gp41 mimetics encompassing the four NHR pockets, i.e., covNHR and covNHR-SS, displayed higher levels of binding than the miniproteins harboring only two pockets, covNHR-N-SS and covNHR-N-dSS, except for the structurally folded trimeric JRFL Env, in good agreement with their higher affinity for CHR. These and our previous results [24] demonstrate that the C-terminal part of CHR in a prefusion-like Env conformation is accessible to interaction by the covNHR miniproteins encompassing the MP and NTP. However, stabilization by disulfide bonds has a small influence on the capability of each protein to bind the CHR region in pre-fusion stabilized Envs.

2.5. HIV-1 Inhibition

The inhibitory activities of the miniproteins against HIV-1 infection in vitro were analyzed using the conventional TZM-bl assay (Figure 6). In these studies, we used different HIV-1 pseudovirus strains: two easy to neutralize pseudoviruses (SF162 and MW956.26) [28], two pseudoviruses with pNL4.3 backbone, (pNL4.3 XCS and pNL4.3 DIM) displaying mutations conferring resistance to T20 [22], and one difficult to neutralize primary isolate (CE1176) [28].

The IC_{50} values were compared with those of the parent covNHR proteins (Table 2). The two proteins containing the four NHR binding pockets, covNHR and covNHR-SS, display similar IC_{50} values in the low nanomolar range meaning that the addition of the N-terminal disulfide bond did not significantly modify the inhibitory capacity of the entire NHR groove mimetic. This is consistent with the fact that both proteins show very similar binding to the CHR target, as shown above.

The inhibitory activity of covNHR-N (containing only two pockets) was very poor as a result of its low affinity for its CHR target [24]. However, conformational stabilization by one disulfide bond in covNHR-N-SS and two disulfide bonds in covNHR-N-dSS increased strongly and progressively the inhibitory activity (Figure 6 and Table 2). These observed activity increments do not appear to be a result of an increase in target binding affinity but rather a consequence of the improved conformational stability of the miniproteins. In fact, the first disulfide bond in covNHR-N-SS did not alter the affinity for the Y24L peptide and the three miniproteins show a similar capacity to bind CHR of prefusion trimeric Envs in ELISA experiments.

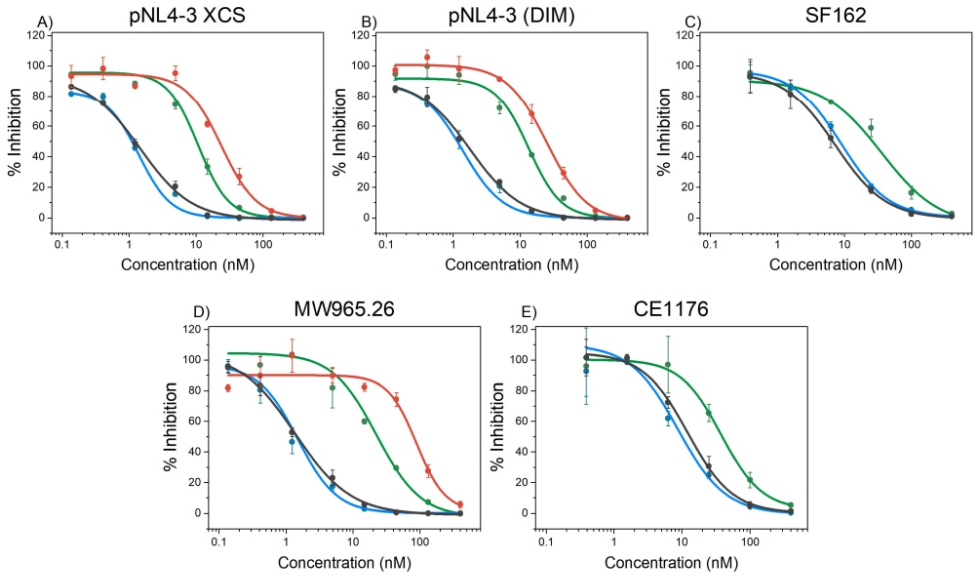

Figure 6. HIV-1 inhibitory activity of the miniproteins implemented in this study on different HIV-1 strains. In vitro inhibition of different HIV-1 strains infection of TZM-bL cells by fusion inhibitors, covNHR (black), covNHR-SS (blue), covNHR-N-SS (red) and covNHR-N-dSS (green), added at different concentrations. The different HIV-1 strains are (**A**) pNL4-3 XCS (pseudovirus designed for resistance to T20); (**B**) pNL4-3 (DIM) (pseudovirus designed for resistance to T20); (**C**) SF162 (pseudovirus strain); (**D**) MW965.26 (pseudovirus strain) and (**E**) CE1176 (primary isolate). Data are the mean ± S.D. of three independent measurements. Continuous lines correspond to non-linear regression curves using a sigmoidal Hill function as implemented in Origin software (Originlab, Northampton, MA, USA).

Table 2. In vitro HIV-1 inhibition by covNHR miniproteins.

Pseudovirus	covNHR-N-SS	covNHR-N-dSS	covNHR	covNHR-SS
pNL4-3 XCS [a]	25.6 ± 2.4	11 ± 4 **	1.3 ± 0.2	1.4 ± 0.1
pNL4-3 (DIM) [a]	24.8 ± 2.0	13 ± 1.3 **	1.6 ± 0.1	1.4 ± 0.2
SF162	n.d.	36 ± 12	8.0 ± 1.3	8.9 ± 0.9
MW965.26	96 ± 12	23 ± 3 ***	1.5 ± 0.3	2.0 ± 0.5
CE1176 [b]	n.d	37.9 ± 1.1	10.8 ± 1.3	8.8 ± 1.6

Inhibitory activity (IC_{50} nM ± S.D. of triplicates) was measured with the standard TZM-bl assay using different pseudoviruses; [a] T20-resistant strains. [b] Primary isolate. ** $p < 0.01$; *** $p < 0.001$, differences between covNHR-N-dSS and covNHR-N-SS.

Strikingly, covNHR-N-dSS exhibits IC_{50} values of 1–3 tens of nanomolar, only 4- to 10-fold higher than covNHR and covNHR-SS, despite the fact that the former miniprotein does not harbor the HP and CTP pockets and has a consequently a much lower affinity for the CHR region.

Compared to T20, the stabilized miniproteins show consistently higher and broader inhibitory activity against the same virus strains, including T20-resistant strains [24]. Additionally, no cytotoxicity, monitored by microscopic examination, was detected even with the highest concentration of the miniproteins used in the assay.

3. Discussion

The NHR region has traditionally been considered as a low stability domain in gp41 with a strong tendency to aggregate, and therefore, NHR-based peptides are poor HIV-1 inhibitors. However, this highly preserved region remains to be an attractive target for fusion inhibition and vaccine design. Different approaches to stabilize an exposed NHR coiled-coil trimer have generally involved inter-helical tethering [29], fusion to foldon domains [9], or partial association with CHR sequences [30]. In these studies, a substantial correlation between coiled-coil stability and inhibitory activity has been repeatedly reported but no clear explanation for this correlation has been provided. We have previously demonstrated that using an innovative design and engineering approach, highly stable mimics of a fully exposed NHR region can be produced in a single-chain form [21–24,31]. Within the NHR coiled coil, its N-terminal region has also been described as an intrinsically unstable sub-domain compared to that of the C-terminal domain or the entire NHR [24,32]. Here we show how the versatility of our single-chain design allows for simple and effective conformational stabilization by disulfide bond engineering. Each disulfide bond is formed spontaneously in the correct configuration and thermally stabilizes the proteins by about 20 °C, consistently with a strong reduction in the conformational entropy of folding [33].

Despite the additive global stabilizing effect of each disulfide bond, only the addition of the second disulfide bond at the N-terminus has a significant influence on the binding affinity to the CHR peptide. The thermodynamics of binding suggest tighter interactions of the doubly disulfide bonded variant with the CHR peptide, possibly due to a reduced conformational dynamics at the peptide-protein complex interface, as a result of a local stabilization effect produced by the disulfide bond as suggested by the MD simulations. Nevertheless, both disulfide bonds contribute to marked increases in HIV-1 inhibitory activity. Disulfide bond stabilization also produced strong increases in anti-HIV-1 activity in other NHR coiled-coil trimer mimetics [10,34]. However, this high affinity, stability and inhibitory activity has, to the best of our knowledge, never been seen before for a NHR gp41 construct lacking the HP, which has been in the last decades the main focus of attention to interfere with the NHR-CHR interaction of gp41 and thereby inhibit fusion and infection [35–37]. For instance, stabilized NHR constructs IZN17, IZN23 and IZN36, all containing the HP but differing in the sequence extension of NHR towards the N-terminus, were reported to have similar inhibitory activities, suggesting that the antiviral activity of this class of chimeric NHR peptides is recapitulated in the HP region [10].

In marked contrast, we show here that the HP is not necessary to achieve a potent and broad inhibitory activity in the tens of nanomolar range for an NHR based construct, such as covNHR-N-dSS. The inhibitory activity is relatively close (only 4- to 10-fold lower) to the activity of the complete NHR mimetic covNHR, as well as other NHR mimetics, with IC_{50} values in the low nM range, even for a difficult to neutralize primary virus isolate (Figure 6). This is surprising because the binding interface of the complete covNHR with its complementary CHR region is much larger and can bind long CHR peptides that also include the HP binding motif, such as C34, with sub-pM affinity [22], whereas the affinity of covNHR-N-dSS for its complementary CHR region, encompassing only the MP and NTP, is only in the high nM range. This suggests that not every CHR binding motif is equally accessible to covNHR for inhibition. It has also been reported that inhibition potency of 5-helix constructs is kinetically restricted by the rate of association of the inhibitor to its target in CHR, which is only transiently exposed during fusion [38,39]. However, all NHR-based inhibitors used in previous studies contain the HP and interact with the HP binding motif, which is engaged in a tryptophan clamp stabilizing the prefusion Env conformation, and therefore, needs Env activation to become accessible [25,26]. On the other hand, accessibility of covNHR-N-dSS to its targeted CHR region may be facilitated by its small size reducing steric impediments and by the high flexibility of the CHR C-terminus [40]. In fact, a recent cryo-EM structure of full-length Env localizes the connection between the CHR end and the membrane-proximal external region (MPER) at a flexible and disordered polar segment composed of residues E^{654}KNEQE^{659} [41]. This segment is actually part of the binding

motif of the NTP and is engaged in a water-mediated network of hydrogen bonds in the gp41 post-fusion 6HB structure [22]. Both the NTP and the NTP-binding motif are as highly preserved as the HP and HP-binding motif (Figure S4), suggesting chief importance of this interaction in gp41 function. It is, therefore, possible that this flexible segment connecting CHR and MPER constitutes the primary target of covNHR-N-dSS in the virion context. This would explain why a stabilization of the miniprotein favoring this conformationally constrained interaction acts so strongly to increase inhibition potency.

Our results suggest two different and probably complementary modes of fusion inhibition targeting gp41 CHR. First, inhibitors containing the HP, which mainly act by targeting the HP binding motif and require its release and exposure by Env activation. Second, inhibitors targeting the C-terminal part of CHR, immediately upstream of the MPER, such as the covNHR-N miniproteins described here, which due to their small size and the higher accessibility and flexibility of their target, are less sterically restricted, and therefore, can achieve potent activities with less stringent requirements in binding affinity.

Our covNHR miniproteins have advantages over peptide-based fusion inhibitors, such as T20. First, they can be produced in recombinant form by *E. coli* expression with high yields. They spontaneously fold with the correct mimetic structure without any posttranslational modification. They are monomeric, highly soluble and stable, and can be lyophilized and reconstituted in standard buffers without structure or activity loss. All these features facilitate the scaling up of production and storage. Second, as potential antivirals, due to their polypeptidic nature, such as T20, they would need to be administered by intravenous injection but, because of their folded structure and high stability, it is highly likely that they will have a higher resistance to proteolytic degradation and longer life in the bloodstream, allowing a considerable reduction of the dosage and/or the frequency of injection, which are among the main drawbacks of T20 treatment.

These results shed light on the design of new inhibitors encompassing the N-terminal subdomain of NHR gp41 traditionally less investigated, proving the potency of the stabilized gp41's NHR mimetics and opening up new ways of inhibiting HIV-1 by engineering new modifications increasing the stability of this region, as well as by improving the already high binding affinity for its target by adding, for instance, new motifs targeting the nearby MPER region.

4. Materials and Methods

4.1. Molecular Dynamics Simulations

All-atom molecular dynamics simulations were performed using YASARA Structure (v.17.12.24) [42] with explicit solvent (TIP3P water, the solvent density was equilibrated to a final value of 0.997 g/mL) in a periodic box with a size 10 Å larger than the protein in every dimension. In order to describe long-range electrostatics, the Particle Mesh Ewald (PME) [43] method was used with a cutoff distance of 8 Å at physiological conditions (0.9% NaCl, pH 7.4), constant temperature (298 K) using a weakly-coupled Berendsen thermostat and constant pressure (1 bar). Ewald summation was used to assign amino acids charge according to their predicted side chain pK_a and was neutralized by adding counterions (NaCl) [44]. The AMBER14 [45] force field was used together with multiple time step integration where intra-molecular forces were calculated every 2 fs and inter-molecular forces every 2.5 fs. The structures were initially energy-minimized using first steepest descent without electrostatics to remove steric clashes and conformational stress and subsequently relaxed by steepest descent minimization and simulated annealing (time step 2 fs, atom velocities scaled down by 0.9 every 10th step) until convergence was reached, i.e., the energy improved by less than 0.05 kJ mol^{-1} per atom during 200 steps. The minimized system was slowly heated up during an equilibration phase until the target temperature and density was reached. Every system was simulated for a minimum of 50 ns and coordinates were saved every 10 ps, yielding 5000 time points for each trajectory.

4.2. Protein and Peptide Samples

The NHR and CHR gp41 sequences used in this work are described in Figure S1. The reference gp41 sequence was taken from the full gp160 precursor glycoprotein of the HIV-1 BRU isolate (Swiss-Prot entry sp|P03377|ENV_HV1BR). CovNHR miniproteins were computationally designed using YASARA software. The DNA encoding the protein sequences were synthesized and cloned into pET303 expression vectors (Thermo Fisher Scientific, Waltham, USA). To facilitate purification by Ni- Sepharose affinity chromatography, the protein sequences were histidine tagged at the C terminus with the sequence GGGGSHHHHHH. The covNHR proteins were produced and purified following the protocol previously described [21]. Synthetic CHR peptides, both N-acetylated and C- amidated, were acquired from Genecust (Luxembourg), with a purity >95%. Protein and peptide concentrations were determined by UV absorption measurements at 280 nm using the extinction coefficients calculated according to their respective amino acid sequences with the ExPasy ProtParam server (https://web.expasy.org/protparam/ accessed on 2 February 2022) [46].

4.3. Circular Dichroism

CD spectra were recorded in a Jasco J-715 spectropolarimeter (Jasco, Tokyo, Japan) equipped with a Peltier thermostatic cell holder. Measurements of the far-UV CD spectra (260–200 nm) were made with a 1-mm path-length quartz cuvette at a protein concentration of ~15 μM. Spectra were recorded at a scan rate of 100 nm/min, 1-nm step resolution, 1-s response, and 1-nm bandwidth. The resulting spectra were usually the average of five scans and the percentage of the α-helical structure was estimated from the far-UV CD spectra as described elsewhere [47]. In thermal melting experiments, the CD signal was monitored as a function of temperature at 222 nm. Each spectrum was corrected by baseline subtraction using the blank spectrum obtained with the buffer and finally, the CD signal was normalized to molar ellipticity ([θ], in $deg \cdot dmol^{-1} \cdot cm^2$). The interaction experiments with CHR peptides were carried out at a 1:2 molar ratio between the proteins and the corresponding peptide.

4.4. Dynamic Light Scattering

The particle sizes of the covNHR proteins were assessed by DLS measurements using a DynaPro MS-X instrument (Wyatt, Santa Barbara, CA, USA). Dynamics software (Wyatt Technology Corporation, Santa Barbara, CA, USA) was used in data collection and processing. Sets of DLS data were measured at 25 °C with an average number of 50 acquisitions and an acquisition time of 10 s.

4.5. Isothermal Titration Calorimetry

ITC measurements were carried out in a Microcal VP-ITC calorimeter (Malvern Instruments, Worcestershire, UK). The protein solutions were typically titrated with 25 injections of 5 μL of the peptide solution at 480 s intervals. Protein concentration in the cell was ~20 μM, while the ligands in the syringe were typically at ~300 μM. The experiments were carried out in 50 mM sodium phosphate buffer, pH 7.4. As a blank, an independent experiment with only buffer in the calorimeter's cell was performed with the same peptide solution to determine the corresponding heats of dilution. The experimental thermograms were baseline corrected and the peaks were integrated to determine the heats produced by each ligand injection. Finally, each heat was normalized per mole of added ligand. The resulting binding isotherms were fitted using a binding model of identical and independent sites, allowing the determination of the binding constant, K_b, the binding enthalpy, ΔH_b, and the binding stoichiometry, n, for each interaction. From these values, the Gibbs energy and entropy of binding could be derived as $\Delta G_b = -RT \cdot \ln K_b$ and $T \cdot \Delta S_b = \Delta H_b - \Delta G_b$. Binding heat capacities were determined from the slope of the dependences of the binding enthalpies measured at different temperatures (ranging from 10 to 25 °C).

4.6. Binding to HIV-1 Envelope Spikes

The capacity of the covNHR proteins to bind soluble HIV-1 envelope proteins (Env) was determined by ELISA. Briefly, 96-well ELISA plates (Maxisorp, Nunc) were coated at 4 °C overnight with various Envs (Table S1) in 0.1 M bicarbonate buffer (pH 9.6). After saturation with 2% BSA, 0.05% Tween in PBS for 1.5 h at 25 °C, 0.01 µM of covNHR molecules, corresponding to the protein concentrations that allowed detecting optical density changes within a linear range, (100 µL diluted in 1% BSA 0.05% Tween solution) were added and incubated for 2 h at room temperature. The plate was then washed five times and covNHR binding was detected with 100 µL anti-6X His-tag antibody conjugated to horseradish peroxidase (HRP) (Abcam, Cambridge, UK) at 1/dilution incubated for 1 h at room temperature. Antibody binding was then revealed with tetramethylbenzidine (TMB) substrate buffer, the reaction was stopped with 1 M H_2SO_4 and optical density was read at 450 nm with a Molecular Device Plate Reader equipped with SoftMax Pro 6 program. Background binding was measured in plates without Env and subtracted from the data. The percentage of binding was calculated using the readings with wells coated with His-tagged Env incubated with PBS buffer instead of covNHR molecules as a control for 100% binding.

4.7. HIV-1 Inhibitory Assays

The inhibition of HIV replication was determined using the conventional TZM-bl assay measured as a function of reductions in Tat-regulated Firefly luciferase (Luc) reporter gene expression [48]. Pseudoviruses expressing different Env were tested for HIV inhibitory potential [28]. The IC_{50}, the concentration (in nM) of inhibitor inducing a 50% decrease in relative luminometer units (RLU), corresponding to a 50% decrease in virus replication was calculated by non-linear regression using a sigmoidal Hill function, as implemented in Origin software (Originlab, Northampton, MA, USA).

4.8. Statistical Analysis

All statistical analyses were performed using the Prism 6 scientific software. Data were expressed as the mean ± SD of 3 experiments per group. An unpaired Student's *t*-test was used to compare the differences between two experimental groups. A *p*-value of 0.05 or less was considered to be statistically significant.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms23052794/s1.

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Supplementary information to:

Conformational stabilization of gp41-mimetic miniproteins opens up new ways of inhibiting HIV-1 fusion

Mario Cano-Muñoz[1,2,*], Julie Lucas[2], Lin Li-Yun[2], Samuele Cesaro[1,†], Christiane Moog[2] and Francisco Conejero-Lara[1,*]

[1] Departamento de Química Física, Instituto de Biotecnología y Unidad de Excelencia de Química Aplicada a Biomedicina y Medioambiente (UEQ), Facultad de Ciencias, Universidad de Granada, 18071 Granada, Spain; mariocano@ugr.es, conejero@ugr.es

[2] INSERM U1109, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg, 67084 Strasbourg, France; julie.lucas@etu.unistra.fr (J.L.); li-yun.lin@etu.unistra.fr (L.L.-Y.); c.moog@unistra.fr (C.M.)

* Correspondence: mariocano@ugr.es (M.C.-M.); conejero@ugr.es (F.C.-L.); Tel.: +34-958242371 (F.C.-L.)

† Present address: Department of Neurosciences, Biomedicine and Movement Sciences, Section of Biological Chemistry, University of Verona, Strada Le Grazie 8, 37134 Verona, Italy.

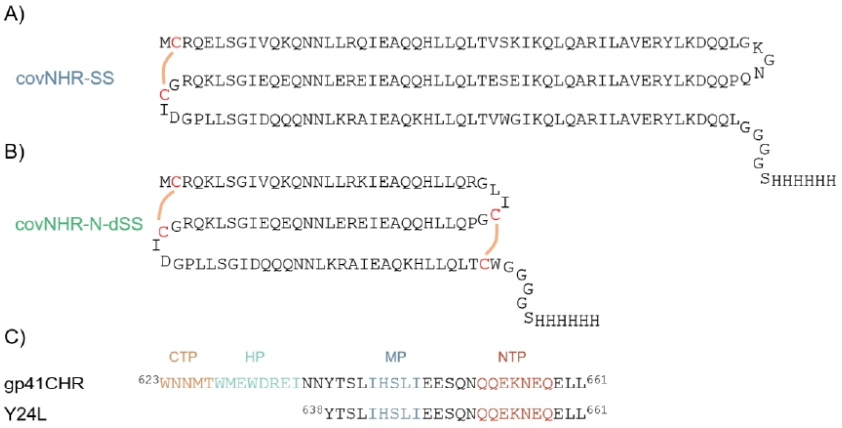

Figure S1. Sequences and topology of the covNHR miniproteins and peptides. Mutations in red were engineered in this work. A) covNHR-SS sequence. B) covNHR-N-dSS sequence C) CHR C34 and Y24L peptide sequences. Residues corresponding to the different binding pocket motifs are colored as follows: CTP in orange, HP in cyan, MP in blue and NTP in red.

Table S1. Description of envelope glycoproteins used in the ELISA binding experiments.

Abbreviation	Type	Description	Reference
JRFL	Gp140	A soluble uncleaved gp140 Env stabilized trimer derived from HIV-1 JRFL containing a C-terminal foldon sequence.	[1]
MN/LAI	Gp160	A hybrid oligomeric gp160 Env with gp120 derived from HIV-1 MN and gp41 derived from HIV-1 LAI.	[2]
THO23/LAI	Gp160	Recombinant Env with gp120 from HIV-1 92TH023 linked to gp41 from LAI, with a deletion in the immunodominant region.	[3]
ZM 4096	Gp140	Synthetic construct derived from gp140 sequence of the codon-optimized HIV-1 96ZM651.	[4]
CN54	Gp140	Recombinant Env trimer containing gp120+gp41 ectodomain from HIV-1 CN54.	[5]

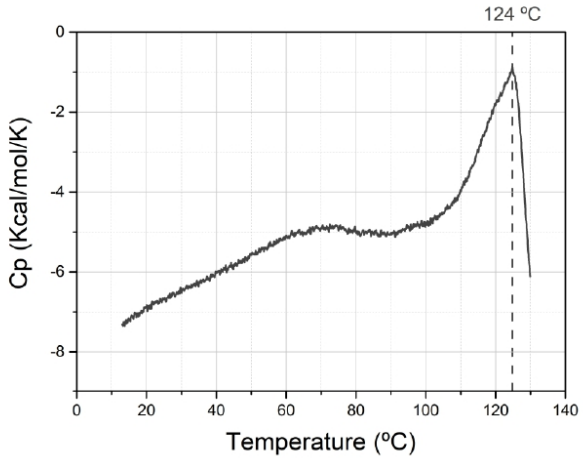

Figure S2. Differential scanning calorimetry thermogram of the thermally induced denaturation of covNHR-SS. The DSC thermogram was recorded at a scan rate of 2°C·min^{-1} at a concentration of 30 μM.

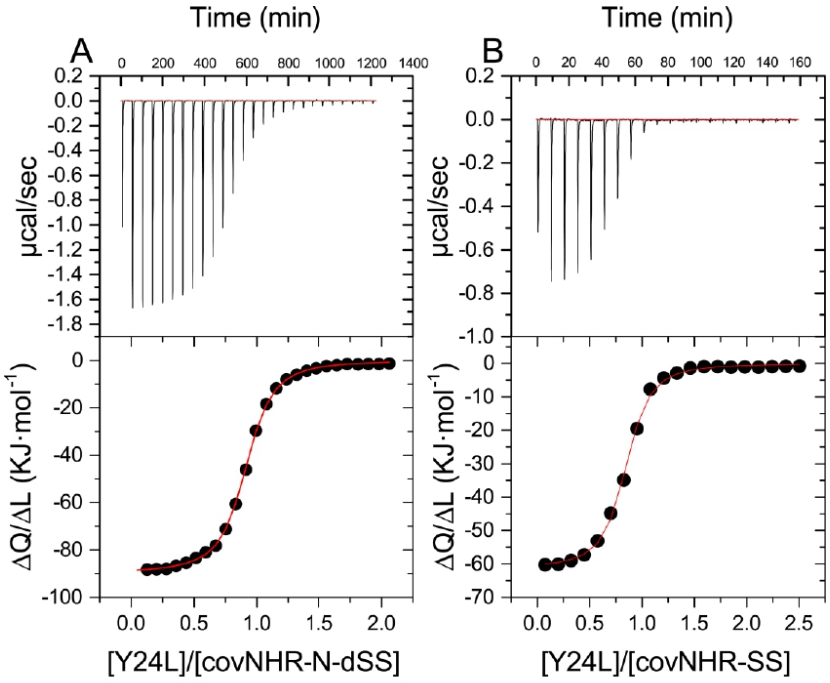

Figure S3. Isothermal titration calorimetry experiments of Y24L peptide binding to the covNHR proteins. **A)** Y24L binding to covNHR-N-dSS and B) to covNHR-SS. The experiments were measured at 25 °C by titration of 10 μM of each miniprotein in the cell with ~300 μM of Y24L peptide from the syringe. The upper panels show the experimental ITC thermograms and the lower panels the normalized binding isotherms. The symbols in the lower panels correspond to the

experimental heats normalized per mole of injected peptide and the lines represent the best fittings using a binding model of n identical and independent sites.

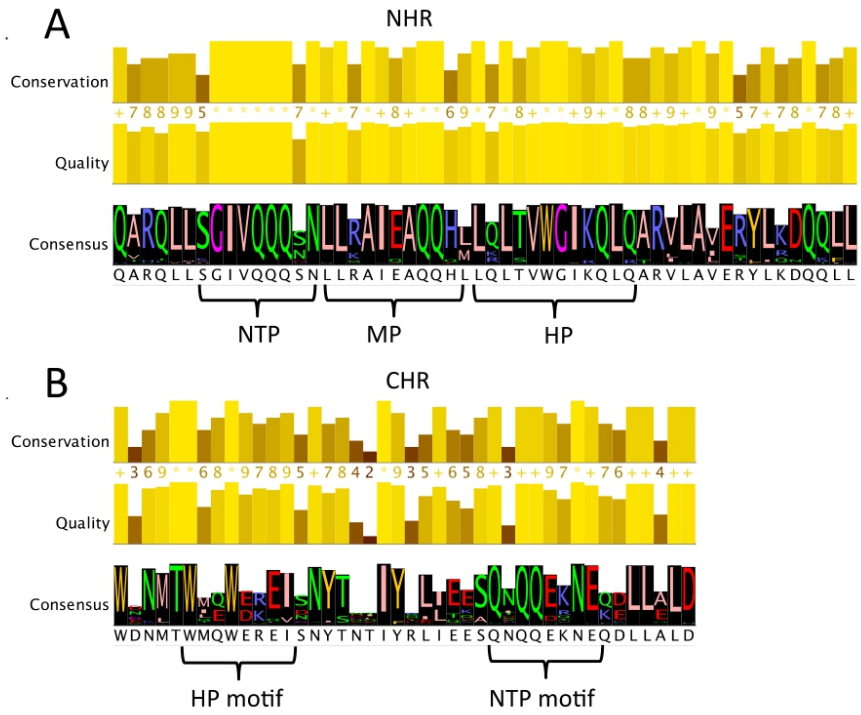

Figure S4. Sequence conservation and consensus sequence of NHR and CHR in gp41. The plots have been made with Jalview [6] using the 2018 Compendium sequence alignment [7] from Los Alamos Sequence Database (https://www.hiv.lanl.gov).

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8. Resume of thesis

Li-Yun LIN

Characterization of the protective antibody response induced following vaccination or infection.

Résumé

Le développement de traitement efficace n'a pas permis d'éradiquer la pandémie provoquée par le virus de l'immunodéficience humaine (VIH). Le développement d'un vaccin prophylactique efficace contre le VIH représente un des axes majeurs dans la lutte contre ce virus. Différentes stratégies vaccinales ont été développées afin d'induire une réponse immunitaire efficace contre le VIH. Cependant, de nombreuses questions demeurent. 1) Les anticorps (Abs) inhibiteurs protecteurs peuvent-ils être induits par vaccination ? 2) Cette réponse est-elle similaire partout dans le monde ou la variation ethnique ou géographique a-t-elle un impact sur la réponse immunitaire et sur la protection contre l'infection ? 3) Quel est le profil de la réponse Ab protectrice induite tôt après l'infection ?

En raison de la grande diversité des sous-types de VIH, l'induction d'Abs avec des fonctions inhibitrices dirigés contre un large spectre de virus sera nécessaire. Les Abs neutralisants (NAbs) sont des Abs capables de protéger les cellules contre les particules infectieuses, conduisant à l'inhibition de la réplication du VIH. Induire de tels Abs est considéré comme le Graal. Cependant, des Nabs à large spectre sont difficiles à induire en raison de la nécessité d'une longue et complexe maturation de la réponse humorale et de la différentiation des lymphocytes B en différents types et sous-types d'immunoglobulines (Ig). En plus de ces NAbs difficiles à induire, des Ab capables d'inhiber le VIH par la fonction médiée par le domaine Fc des Abs ont été décrits. Dans ce cas, les Abs qui reconnaissent le virus se lient également aux récepteurs Fc (FcR) à la surface des cellules immunitaires effectrices. Cette reconnaissance active des fonctions inhibitrices des Abs médiées par les FcR. De ce fait, le niveau d'inhibition du VIH par les Abs est également dépendant du génotype des FcR de l'hôte. En effet, un polymorphisme spécifique du FcγRIIA de génotype rs 10800309 s'est avéré significativement augmenté dans une cohorte de patients VIH qui contrôlent leur infection (cohorte contrôleurs). Ce polymorphisme s'accompagne d'une expression accrue du récepteur FcγRII à la surface des cellules dendritiques, suggérant un renforcement de la réponse immunitaire grâce à la régulation positive du FcRII. Nous proposons que ce polymorphisme pourrait servir de marqueur prédictif au contrôle du VIH par les Abs. Ainsi, aussi bien les types d'Ig que les polymorphismes des FcR à la surface des cellules effectrices contribueraient à la protection du VIH par les Abs.

Les approches vaccinales actuelles proposent d'induire des Nabs ainsi que des Abs inhibiteurs médiés par les FcR. Par conséquent, la quantité et la qualité des types et sous-types d'Ig induites, ainsi que des récepteurs Fc (FcR) devront être pris en considération. Comme ces facteurs varient selon l'ethnicité, les futurs vaccins devront peut-être adapter leurs stratégies à l'origine génétique de la population ciblée ainsi qu'aux VIH localement en circulation.

L'objectif de ma thèse a été d'analyser la réponse immunitaire humorale, en particulier la neutralisation et l'inhibition médiée par les domaines Fc des Abs, dans le cadre de nouvelles stratégies vaccinales, et de décrypter l'implication de l'origine génétique dans les fonctions inhibitrices des Abs induits.

Au cours de ma thèse, j'ai pu démontrer que des Nabs étaient induits lors de la phase aiguë de l'infection. Ces Ab neutralisaient de manière spécifique différents VIH transmis/fondateur (T/F), des virus isolés très tôt après leur transmission. Ces résultats sont différents de ceux précédemment publiés montrant l'absence de Nabs dans les sérums prélevés lors de la phase aiguë de l'infection. Cependant les virus testés étaient des virus primaires non T/F. La mise en évidence de NAbs induit tôt après l'infection est très prometteuse car elle redonne l'espoir induire de tels Abs par vaccination.

J'ai également analysé la réponse Ab induite par différents nouveau candidats vaccins. Ces essais vaccinaux ont été menés dans le cadre de collaboration/consortium Français VRI (LabEx) et Européens EHVA (Horizon 2020). Nous avons caractérisé la réponse Ab induite.

J'observe que,

1) la réponse Ab est très efficace lorsque l'antigène est couplé avec un anticorps dirigé contre le DC40. Cette construction permet de cibler les cellules dendritiques CD40+ et ainsi d'activer efficacement la réponse immune. Un essai de vaccination de phase I est en cours en France chez des volontaires à faible risque d'infection par le VIH. Les résultats de cet essai montrent que l'immunogène est bien toléré et qu'il induit une réponse Ab forte.

2) la présentation de l'enveloppe du VIH dans le contexte du vecteur viral VSV (vesicular stomatitis virus) a permis d'obtenir une réponse Ab forte et maintenue dans le temps dans un modèle de petits rongeurs (lapins).

3) L'infusion lente et maintenue de l'antigène par l'intermédiaire d'une pompe osmotique a permis d'augmenter la réponse Ab induite dans le modèle primate non-humain (NHP).

Enfin, j'ai participé à l'étude du rôle de l'ethnicité dans la réponse induite par vaccination. Pour cette étude, l'essai de phase IIA HVTN024 a été sélectionné car un protocole similaire de vaccination a été administré à des Africains et Caucasien vivant aux USA et à des Africains vivant en Afrique du Sud. J'observe que, à la fois l'ethnicité (Africains versus Caucasien) et la géographie (USA versus Afrique du Sud) impacte la réponse immune induite par vaccination.

Conclusion :

Ce travail de thèse a permis de caractériser de manière fine la réponse Ab induite par de nouvelles stratégies vaccinales actuellement en cours de développement. Il a fourni des indications sur les orientations futures et des approches vaccinales à venir. Les prochaines étapes consisteront à combiner nos immunogènes nouvellement développés afin d'augmenter la qualité et la quantité de la réponse Ab induite. Un effort particulier devra être entrepris afin améliorer la durabilité des Abs et leur reconnaissance d'un large spectre souches virales en circulation. L'identification des Nabs contre les souches T/F apporte également des informations sur les futures env qui devraient être analysées en tant que nouveaux immunogènes. L'identification de l'impact génétique sur la réponse immunitaire et des conséquences potentielles sur la protection vaccinale devrait nous orienter vers le développement de stratégies vaccination plus « personnalisées », en proposant des vaccinations individualisées directement adaptées à l'environnement de l'individu.

Résumé en anglais

According to data published by the World Health Organization (WHO) in 2021, 38.4 million people live with the human immunodeficiency virus (HIV), and 68% of them are Africans. Besides treatment, developmenting an effective prophylactic vaccine is crucial and urgent to combat the growing HIV pandemic. Different vaccine strategies have been developed to induce an effective specific immune response against HIV. However, many questions remain. 1) Can protective inhibitory antibodies (Abs) be induced by vaccination? 2) Is this response similar worldwide, or does ethnic or geographic variation impact immune response and protection against infection? 3) What is the profile of the protective Ab response induced early after infection?

To eradicate the HIV pandemic, an effective protective vaccine is needed. Yet, due to the great diversity of HIV subtypes, the induction of Abs with inhibitory functions against a broad spectrum of circulating viruses will be necessary. Neutralizing Abs (Nabs) are Abs capable of protecting cells against infectious particles, leading to the inhibition of HIV replication. Inducing such Abs is considered as the Holy Grail. However, Nabs inhibiting a broad spectrum of HIV are challenging to induce due to the requirement of long maturation of the humoral response and to the differentiation of B cells into immunoglobulin (Ig) different types and subtypes. In addition to these difficult-to-induce NAbs, Abs capable of inhibiting HIV through the Fc domain-mediated function of Abs have been described. In this case, Abs need to recognize the virus via the Fab domain and to bind to Fc receptors (FcRs) on the surface of effector immune cells via their Fc domain. This dual binding will activate FcR-mediated inhibitory functions. Consequently, the Ab inhibition level also relies on FcR genotype of the host. Interestingly, the specific polymorphism of FcyRIIA of genotype rs 10800309 was found to be significantly increased in a cohort of HIV patients who control their infection (HIV controller cohort). This polymorphism is accompanied by an increased expression of the FcɣRII receptor on the surface of dendritic cells, suggesting a reinforcement of the immune response thanks to the upregulation of FcRII. We, therefore, propose that this polymorphism may serve as a predictive marker of HIV control by Abs. Thus, both Ig types and FcR polymorphisms on the surface of effector cells could contribute to the protection of HIV by Abs. Current vaccine approaches propose to induce Nabs as well as FcR-mediated inhibitory Abs. Therefore, the types and subtypes of induced Igs, as well as the quantity and quality of Fc receptors (FcRs) expressed on immune cells should be considered. As these factors vary by ethnicity, future vaccines may need to tailor their strategies to genetic variations of the target population and adapt the immunogens to the local HIV strains in circulation.

The objective of my thesis was to analyze the humoral immune response, in particular the neutralization and inhibition mediated by the Fc domains of Abs, in the context of new vaccine strategies and to decipher the implication of the genetic origin in the inhibitory functions of induced Abs.

During my thesis, I demonstrated that some Nabs were induced already during the acute phase of the infection. These Abs specifically neutralized different HIV transmitted/founder (T/F) viruses isolated very early after their transmission. These results are different from those previously published showing the absence of Nabs in the sera collected during the acute phase of the infection. Noteworthy, the viruses previously tested were primary strains isolated during the chronic phase and not T/F primary viruses. The demonstration of induced NAbs against T/F strains early after infection is very promising because it reinstates the hope of inducing such Abs by vaccination.

I also analyzed the Ab response induced by different new vaccine candidates. These vaccine trials were conducted within the framework of a French (LabEx) VRI and European (Horizon 2020) EHVA collaboration/consortiums. We have characterized the Ab response induced following different immunization strategies.

I observe that:

1) the Ab response is very effective when the antigen is coupled with an antibody directed against DC40. This construction makes it possible to target dendritic cells and thus effectively activate the immune response. A phase I vaccination trial is underway in France sponsored by the VRI (LabEx) framework in volunteers at low risk of HIV infection. The preliminary results of this assay show that this immunogen is well tolerated and, indeed, induces a strong Ab response.

2) the presentation of the HIV envelope in the context of the viral vector VSV (vesicular stomatitis virus) made it possible to obtain a strong and maintained Ab response over time in a model of small rodents (rabbits)

3) The slow and sustained infusion of antigen via an osmotic pump increased the Ab response induced in the non-human primate (NHP) model

Finally, I participated in the study of the role of ethnicity in the response induced by vaccination. For this study, the phase IIA HVTN024 trial was selected because a similar vaccination protocol was administered to Africans and Caucasians living in the USA and to Africans living in South Africa. I observe that both ethnicity (Africans versus Caucasians) and geography (USA versus South Africa) impact the immune response induced by vaccination.

Conclusion:

This thesis work has enabled us to conduct an in-depth characterization of the Ab response induced by the new vaccine strategies currently under development. It provided insights into future directions and upcoming vaccine approaches. The next steps will combine our newly developed immunogens to increase the quality and quantity of the induced Ab response. A particular effort should be made to improve the durability and breath of the Abs induced. The identification of Nabs against T/F strains also provides information on future envs that should be analyzed as new immunogens. Identifying the genetic impact on the immune response and the potential consequences on vaccine protection should direct us towards developing more "personalized" vaccination strategies by offering individualized vaccinations directly adapted to the individual's environment.

Li-Yun LIN

Characterization of the protective antibody response induced following vaccination or infection.

Résumé

Différentes stratégies vaccinales ont été développées afin d'induire une réponse immunitaire efficace contre le VIH. Cependant, de nombreuses questions demeurent. 1) Quel est le profil de la réponse Ab protectrice induite tôt après l'infection? 2) Des anticorps (Abs) inhibiteurs protecteurs peuvent-ils être induits par vaccination? 3) Cette réponse est-elle similaire partout dans le monde ou la variation ethnique ou géographique a-t-elle un impact sur la réponse immunitaire et sur la protection contre l'infection ? Mon travail de thèse montre que, 1) une réponse Ab neutralisante est détectée très tôt après infection, 2) des immunisations avec une enveloppe exprimée dans le contexte du vecteur viral VSV (vesicular stomatitis virus) ou avec un antigène qui cible les cellules dendritiques via un anticorps dirigé contre le CD40 ont permis d'obtenir une réponse Ab forte et maintenue dans le temps. De plus L'infusion lente et prolongée de l'antigène par l'intermédiaire d'une pompe osmotique a permis d'augmenter la réponse Ab induite dans le modèle primate non-humain (NHP). 3) À la fois l'ethnicité (Africains versus Caucasien) et la géographie (USA versus Afrique du Sud) impacte sur la réponse immune induite et donc potentiellement sur la protection vaccinale. Ce travail de thèse a permis de caractériser de manière fine la réponse Ab induite par de nouvelles stratégies vaccinales et démontre que d'autres facteurs impactent la réponse immune induite. Il a fourni des indications sur les approches vaccinales à venir. Nos résultats devraient nous orienter vers le développement de stratégies vaccination plus « personnalisées », en proposant des immunisations individualisées directement adaptées à l'individu et à l'environnement.

Mots-clés : VIH-1, vaccin, infection, immunoglobulines, ethnicité, Ab fonctionnel

Abstract

Different vaccine strategies have been developed to induce an effective immune response against HIV. However, many questions remain. 1) What is the profile of the protective antibody (Ab) response induced early after infection? 2) Can protective inhibitory Abs be induced by vaccination? 3) Is this response similar around the world or does ethnic or geographic variation impact immune response and protection against infection? My thesis work shows that, 1) a neutralizing Ab response is detected very early after infection, 2) immunizations with an envelope expressed in the context of the viral vector VSV (vesicular stomatitis virus) or with antigen targeting dendritic cells via an antibody directed against CD40 allow to obtain a strong and maintained Ab response over time. In addition, the slow and prolonged infusion of the antigen via an osmotic pump made it possible to increase the Ab response induced in the non-human primate (NHP) model. 3) Both ethnicity (Africans versus Caucasians) and geography (USA versus South Africa) influence the induced immune response and therefore potentially vaccine protection. This thesis work allowed to finely characterize the Ab response induced by new vaccine strategies and demonstrated that other factors impact the induced immune response. It provided insights into future vaccine approaches. Our results should guide us towards the development of more "personalized" vaccination strategies, by offering individualized immunizations directly adapted to the individual and the environment.

Key words : HIV-1, vaccine, infection, immunoglobulins, ethnicity, functional Ab