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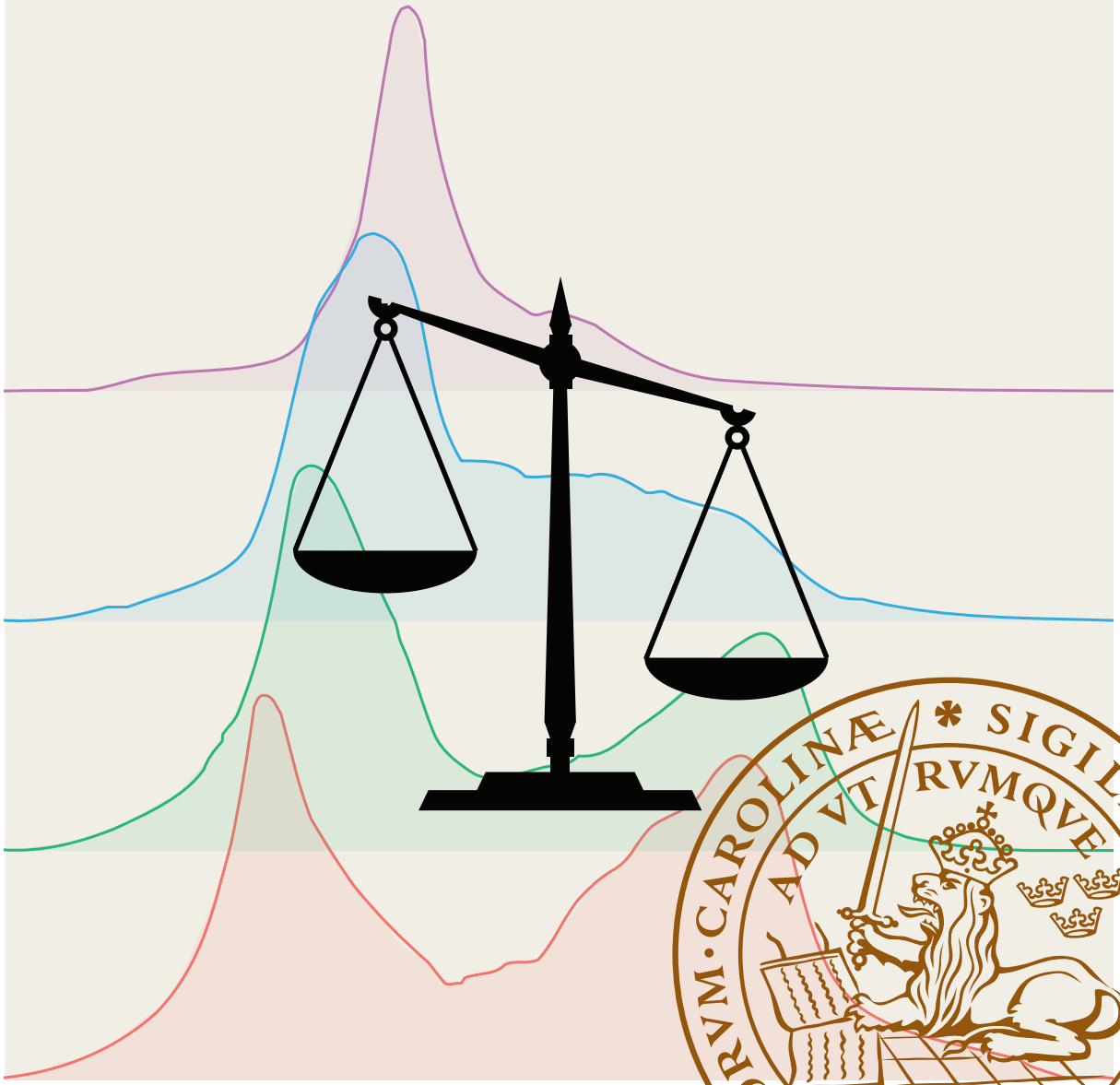

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Virus-host interactions in HIV-1 and HIV-2 infections

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Emil Johansson

LUND UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on May 17th at 13.00 in Agardh Lecture Hall, Department of Translational Medicine, CRC, Jan Waldenströms gata 35, Malmö.

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Abstract:

Human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) are the two causative agents of AIDS. HIV-1 is responsible for the HIV pandemic, while HIV-2 is primarily confined to West Africa. Although HIV-1 and HIV-2 share several characteristics, such as route of transmission, is HIV-2 less pathogenic than HIV-1. The asymptomatic disease stage in treatment naïve individuals is approximately twice as long among in people living with HIV-2 (PLWH2) compared to in people living with HIV-1 (PLWH1). The underlying mechanisms behind this difference are unknown but may be attributed to a more effective immune response in HIV-2 compared to in HIV-1 infection. Moreover, although most PLWH2 display low to undetectable plasma viral loads (pVL), in general they still do progress towards AIDS in the absence of antiretroviral treatment – albeit at a slower rate.

The objectives of this thesis were to characterise virus-host interactions in PLWH1 and PLWH2, and to investigate the associations of these interactions with different traits of disease progression. More specifically to study: 1) the impact of HIV-2 viraemia on $CD8^+$ T cell and B cell phenotypes, and plasma proteomes; and 2) associations between plasma proteome signatures and HIV disease progression.

To do this, we immunophenotyped $CD8^+$ T-cells and B-cells using flow cytometry and bioinformatics. We observed that both viraemic and aviraemic HIV-2 infection promoted $CD8^+$ T-cells exhaustion and induced an expansion of hyperactivated B-cells as well as high levels of the T helper 1 cell-associated transcription factor T-bet. We also utilised a novel analysis pipeline of data-independent acquisition mass-spectrometry (DIA-MS) to determine the proteome in blood plasma. The analysis showed alterations of plasma proteins that were associated with frequencies of terminally exhausted $CD8^+$ T-cells and hyperactivated B-cells. Next, we used the same DIA-MS approach on archived plasma collected within three years of the estimated date of HIV-1 or HIV-2 infection. The analysis indicated that increased release of proteins from sigmoid colon and spleen tissue was associated with depletion of $CD4^+$ T-cells, and that the expression profile of ten specific proteins, found to be associated with $CD4^+$ T-cell loss, could distinguish faster from slower disease progression.

In summary, increased understanding of how HIV-host interactions dictate viraemia and disease progression rate provides important insights about HIV-1 and HIV-2 pathogenesis that may open up new directions for developing HIV therapeutic and prophylactic strategies.

Key words: HIV-1, HIV-2, viraemia, disease progression, immune perturbations, plasma proteomics

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Aims of this doctoral thesis

The main aim of this doctoral thesis was to study virus-host interactions in HIV-1 and HIV-2 infections, and their potential association with disease progression. The specific aims for each study were:

Paper I: To investigate the immunophenotype of $CD8^+$ T-cell populations in people living with HIV-1 or HIV-2, and in HIV seronegative individuals, and determine the associations between $CD8^+$ T-cell populations and viraemia or disease progression.

Paper II: To study the immunophenotype of B-cell populations in people living with HIV-1 or HIV-2, and in HIV seronegative and determine the associations between B-cell populations and viraemia or disease progression.

Paper III: To characterise the blood plasma proteome in people living with HIV-1 or HIV-2, and in HIV seronegative individuals, and to determine associations between protein expression and HIV viraemia or pathogenic properties of B- and T-cells. Furthermore, to explore if inferred protein perturbations were associated with different cell types and tissues.

Paper IV: To characterise the blood plasma proteome in people living with chronic HIV-1 or HIV-2 infection, and in HIV seronegative individuals, and to determine the associations between disease progression rate and inferred tissue or protein perturbations.

Abstract

Human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2) are the two causative agents of AIDS. HIV-1 is responsible for the HIV pandemic, while HIV-2 is primarily confined to West Africa. Although HIV-1 and HIV-2 share several characteristics, such as route of transmission, is HIV-2 less pathogenic than HIV-1. The asymptomatic disease stage in treatment naïve individuals is approximately twice as long among in people living with HIV-2 (PLWH2) compared to in people living with HIV-1 (PLWH1). The underlying mechanisms behind this difference are unknown but may be attributed to a more effective immune response in HIV-2 compared to in HIV-1 infection. Moreover, although most PLWH2 display low to undetectable plasma viral loads (pVL), in general they still do progress towards AIDS in the absence of antiretroviral treatment – albeit at a slower rate.

The objectives of this thesis were to characterise virus-host interactions in PLWH1 and PLWH2, and to investigate the associations of these interactions with different traits of disease progression. More specifically to study: 1) the impact of HIV-2 viraemia on $CD8^+$ T cell and B cell phenotypes, and plasma proteomes; and 2) associations between plasma proteome signatures and HIV disease progression.

To do this, we immunophenotyped $CD8^+$ T-cells and B-cells using flow cytometry and bioinformatics. We observed that both viraemic and aviraemic HIV-2 infection promoted $CD8^+$ T-cells exhaustion and induced an expansion of hyperactivated B-cells as well as high levels of the T helper 1 cell-associated transcription factor T-bet. We also utilised a novel analysis pipeline of data-independent acquisition mass-spectrometry (DIA-MS) to determine the proteome in blood plasma. The analysis showed alterations of plasma proteins that were associated with frequencies of terminally exhausted $CD8^+$ T-cells and hyperactivated B-cells. Next, we used the same DIA-MS approach on archived plasma collected within three years of the estimated date of HIV-1 or HIV-2 infection. The analysis indicated that increased release of proteins from sigmoid colon and spleen tissue was associated with depletion of $CD4^+$ T-cells, and that the expression profile of ten specific proteins, found to be associated with $CD4^+$ T-cell loss, could distinguish faster from slower disease progression.

In summary, increased understanding of how HIV-host interactions dictate viraemia and disease progression rate provides important insights about HIV-1 and HIV-2 pathogenesis that may open up new directions for developing HIV therapeutic and prophylactic strategies.

Populärvetenskaplig sammanfattning

Två typer av humant immunbristvirus (hiv) har hittills identifierats, hiv-1 och hiv-2. Hiv-1 och hiv-2 är de två virus som kan orsaka sjukdomen aids (acquired immunodeficiency syndrome [*eng*], förvärvat immunbristsyndrom [*sv*]). Aids uppkommer när immunförsvar hos en hiv-infekterad individ försvagats så till den grad att det inte längre kan skydda individen mot vanligt förekommande smittämnen som är ofarliga för friska individer. Vid en hiv-infektion försvagas immunförsvaret genom att hiv infekterar och dödar en viktig typ av immuncell, CD4 T-cellen, som bland annat orkestrerar immunsvar mot virus. En annan bidragande faktor är att hiv förändras snabbare än immunförsvaret hinner med, vilket resulterar i en kronisk infektion där immunförsvaret gradvis utmattas.

Trots att hiv-1 och hiv-2 är två nära besläktade virus, så skiljer de sig åt i flera viktiga aspekter. Hiv-1 har spridits över hela världen medan hiv-2 framför allt är spritt i Västafrika. Hiv-2 är dessutom mindre patogent än hiv-1, och det tar ungefär dubbelt så lång tid att utveckla aids vid hiv-2-infektion jämfört med vid hiv-1-infektion. En anledning till detta kan vara att immunförsvaret hämmar hiv-2 mer effektivt än hiv-1. En annan skillnad är att hiv-2-infekterade personer oftast har väsentligt högre virusnivåer i blodet jämfört med hiv-1-infekterade personer om de inte får behandling med virushämmande läkemedel.

Målet med min avhandling har varit att studera hur hiv-1 och hiv-2-infektioner påverkar immunförsvaret och kompositionen av proteiner i blodet (proteinprofilen), och hur detta kan kopplas till olika virusnivåer samt hur snabbt hiv-infekterade individer utvecklar sjukdom. Resultaten från mina studier har jag sammanfattat i fyra arbeten. De tre första arbetena visar att hiv-2-infekterade personer med icke-detekterbara virusnivåer i blodet dels hade utmattade immunceller, och dels att även andra celler och vävnader, förutom de i blodet, påverkades av hivinfektionen. Detta tyder på att kronisk hiv-2-infektion kan leda till försämrat immunsvar och vävnadsskada även hos personer där hiv-2 inte detekteras i blodet. I det fjärde arbetet letade vi efter samband mellan proteinprofilen och hastigheten med vilken studiepersonerna utvecklade sjukdom, och identifierade tio specifika proteiner som kunde kopplas till snabbare eller långsammare utveckling av aids.

Sammantaget ger resultaten i dessa studier en ökad förståelse kring hur olika virus-värd-interaktioner påverkar sjukdomsförloppet hos hiv-infekterade personer. Denna typ av kartläggning är viktigt för utveckling av nya och befintliga terapeutiska eller profylaktiska behandlingar av hiv.

List of papers

Paper I
Scharf L, Pedersen C.B, **Johansson E**, Lindman J, Olsen L.R, Buggert M, Wilhelmson S, Månsson F, Esbjörnsson J, Biague A, Medstrand P, Norrgren H, Karlsson A.C*, Jansson M* and the SWEGUB CORE group. **Inverted CD8 T-Cell Exhaustion and Co-Stimulation Marker Balance Differentiate Aviremic HIV-2-Infected From Seronegative Individuals**. *Front Immunol*. 2021 Oct 12;12:744530. doi: 10.3389/fimmu.2021.744530. (*Shared senior authorship).

Paper II
Johansson E, Kerkman P. F, Scharf L, Lindman J, Szojka Z. I, Månsson F, Biague A, Medstrand P, Norrgren H, Buggert M, Karlsson A. C, Forsell M.N.E*, Esbjörnsson J*, Jansson M*, and the SWEGUB CORE group. **Hierarchical Clustering and Trajectory Analyses Reveal Viremia-Independent B-Cell Perturbations in HIV-2 Infection**. *Cells*. 2022 Oct 6;11(19):3142. doi: 10.3390/cells11193142. (*Shared senior authorship).

Paper III
Johansson E, Jamirah Nazziwa J, Freyhult E, Hong M, Lindman J, Neptin M, Karlson S, Rezeli M, Biague A.J, Medstrand P, Månsson F, Norrgren H, Jansson M*, Esbjörnsson J* and the SWEGUB CORE group. **Immunopathology in aviraemic HIV-2 infection is associated with elevated macrophage and endothelium derived plasma proteins**. *Manuscript*. 2023 (*Shared senior authorship).

Paper IV
Johansson E, Nazziwa J, Freyhult E, Hong M, Neptin M, Karlson S, Rezeli M, da Silva Z, Biague A.J, Lindman J, Palm A, Medstrand P, Månsson F, Norrgren H, Jansson M*, Esbjörnsson J* and the SWEGUB CORE group. **Distinct plasma protein profiles distinguish faster and slower disease progression in HIV-1 and HIV-2 infections**. *Manuscript*. 2023 (*Shared senior authorship).

Abbreviations

Ab	Antibody
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
BCR	B-cell receptor
CA	Capsid
CCR	CC chemokine receptor
CD	Cluster of differentiation
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCR	CXC chemokine receptor
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DIA-MS	Data-independent acquisition mass-spectrometry
DNA	Deoxyribonucleic acid
env	Envelope gene
Env	Envelope protein
gag	Group antigen gene
GALT	Gut associated lymphoid tissue
GPR	G-protein coupled receptor
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
Ig	Immunoglobulin
IL	Interleukin
IN	Integrase
INF	Interferon

MA	Matrix
MS	Mass-spectrometry
NC	Nucleocapsid
Nef	Negative Regulatory Factor
NF-κB	Nuclear factor kappa B
PAMP	Pathogen-associated molecular patterns
PD-1	Programmed Death 1 Receptor
PLWH	People living with HIV
pol	Polymerase gene
PR	Protease
pVL	Plasma viral load
Rev	Regulator of expression of viral proteins
RNA	Ribonucleic acid
RT	Reverse Transcriptase
SIV	Simian immunodeficiency virus
SU	Surface unit
SPVL	Set-point viral load
Tat	Trans-activator of HIV gene expression
T-bet	T-box transcription factor TBX21
TCR	T-cell receptor
TIGIT	T cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domains
TM	Transmembrane unit
Vif	Viral infectivity factor
Vpr	Viral protein R
Vpu	Viral protein U
Vpx	Viral protein X

Introduction

HIV-1 and HIV-2 and epidemiology

The two known types of human immunodeficiency virus (HIV), HIV-1 and HIV-2, emerged after zoonotic transmission of simian immunodeficiency virus (SIV) from primates to humans (Figure 1) (1). The existing HIV-1 strains can be divided into four groups: Main (M), Non-M and non-N (N), Outlier (O), and Pending (P) (2, 3). HIV-1 group M and N resulted from transmission of SIV from chimpanzees (SIVcpz) to humans, while group O and P originated from SIV in gorillas (SIVgor) (3). Nine separate transmission events of SIV from sooty mangabeys (SIVsmm) gave rise to HIV-2 groups A-I (4). HIV-1 group M and O and HIV-2 group A and B are the only HIV groups to have spread significantly in the human population, while the remaining groups have been identified in less than 20 individuals each (3).Following the transmission of HIV-1 group M and HIV-2 group A and B, HIV-1 group M gave rise to the global HIV pandemic while HIV-2 has been primarily restricted to West Africa (5). According to UNAIDS, approximately 84 million individuals have been infected by HIV-1 since the start of the pandemic, and approximately half have succumbed to HIV and AIDS-related illnesses (6). HIV-2 has been found to be less pathogenic and transmissible than HIV-1 (7, 8), but still approximately 1-2 million individuals have been estimated to be infected by HIV-2 (9).

HIV-1 group M is believed to have been transmitted from chimpanzees to humans in Southeast Cameroon and thereafter established its spread in Kinshasa, the Democratic Republic of Congo (DRC), during the 1920s (10), with subsequent rapid spread during the 1960s (10, 11). The high mutation rate of the reverse transcriptase, the high virus turnover, and the high genetic recombination rates of HIV-1 has given rise to nine subtypes (A-D, F-H, J, K) and several circulating recombinant forms that have been dispersed globally (12). Virus strains are divided into subtypes based on sequence similarity, where the genetic variation within a subtype is approximately 15-20% and the variation between subtypes is approximately 25–35% (12). Subtype B is believed to have arisen from a single strain that first spread to Haiti during the 1960s, and thereafter spread globally (13). A recent study identified the DRC city Kinshasa as the origin of subtypes A and D, Mbuji-Mayi as the origin of subtype C, and Matadi as the origin of subtypes H and J (14). A recent meta-analysis of global prevalence of HIV-1 subtypes revealed that today subtype C is the most prevalent subtype (46%), followed by subtype B (12%), subtype A

(10%), CRF02_AG (8%), CRF01_AE (5%), subtype G (5%), and subtype D (3%), and subtypes F, H, J, and K (<1%) (15).

The two endemic HIV-2 groups, A and B, are predicted to have been transmitted to humans from sooty mangabeys in the Taï forest in Ivory Coast during the 1940s (16-18). Although both group A and B are endemic, group A represents >90% of all HIV-2 infections. The remaining groups, believed to have been transmitted to humans in Ivory Coast, Liberia, and Sierra Leone, have only been detected in one or two individuals (16). Although HIV-2 group A and B are believed to have been transmitted to humans in Ivory Coast, Guinea-Bissau is the country that has reported the highest HIV-2 prevalence (16, 19-30). Similar to HIV-1 group M, the prevalence of HIV-2 is believed to have increased rapidly during the 1960s (17). In line with the high HIV-2 prevalence in Guinea-Bissau, phylogenetic analyses have suggested that Guinea-Bissau as a hub for HIV-2 transmission in West Africa (16, 31). Outside of Africa, Portugal and France, both with previous colonial ties to West Africa, are the two countries with the highest HIV-2 burden (31, 32). Despite the lack of evidence-based recommendations for preferred treatment regimens and criteria for treatment failure (9), HIV-2 incidence has been reported to decrease in both Guinea-Bissau and Senegal (20, 24-26, 28, 33). Given the observed reduced HIV-2 incidence, and the fact that the prevalence of HIV-2 is higher among older than younger adults (older or younger than 34 or 44 years (20, 34)), it has been suggested that HIV-2 might disappear in approximately 100 years (35).

The Guinea-Bissau HIV pandemic

As Guinea-Bissau is the country with the highest HIV-2 prevalence, as well as an increasing in HIV-1 prevalence (19, 20, 25, 26, 34), it has provided the chance to study the impact of HIV-1 and HIV-2 infections in the same ethnic groups. Three large cohorts have been established to monitor the HIV epidemic in Guinea-Bissau since 1988: the “Caió cohort” (a community-based cohort in the rural village Caió in north-western Guinea-Bissau) (34); the “Bissau HIV cohort” (a community-based cohort in the suburbs of Bissau, belonging to the Bandim Health Project) (20, 36, 37); and the “Guinea-Bissau police cohort” (an occupational cohort of police officers in Guinea-Bissau) (38-40).

In Guinea-Bissau, HIV-2 group A is the most prevalent HIV-2 group (16). During the 1980s, the HIV-2 prevalence was found to be up to 20% among individuals older than 40 years (21). It is not known why Guinea-Bissau became the epicentre of the HIV-2 epidemic, but iatrogenic medical procedures during the War of independence against Portugal 1963-1974 is believed to have been one of the key drivers (17, 21, 41, 42). However, in line with the low transmission rate of HIV-2 and the lower plasma viral load of HIV-2 infected individuals comparted to HIV-1 infected individuals (43, 44), the HIV-2 prevalence has decreased over time (19, 20, 25, 26, 33, 34). In addition to viral factors, the increased prevalence of HIV-1 and reduced

high-risk sexual behaviour following the Independence war has also been suggested to be associated with the reduced HIV-2 incidence (16, 45).

HIV-1 is believed to have been introduced into Guinea-Bissau during the end of the 1970s, likely in association with an observed migration wave following the end of the independence war in 1974 (46). A phylogeographic analysis suggested that HIV-1 was initially introduced to the capital Bissau, and subsequently spread to the rural areas of the country (46). The epidemic has been shown to be dominated by the recombinant form CRF02_AG, sub-subtype A3, and A3/CRF02_AG recombinant form, where A3/CRF02_AG infection has been associated with a faster time to death compared to sub-subtype A3 (46-49). Similar to the predicted increase in HIV-2 prevalence during the independence war 1963-1974, HIV-1 prevalence, but not HIV-2 prevalence, was found to increase during the civil war 1998-1999 (24, 25). Although HIV-1 was found to increase almost exponentially during the 1990s, later studies have found that HIV-1 prevalence and incidence has stabilised or potentially even decreased since the mid-00s (19, 20, 24, 25, 34, 50).

HIV structure, genome and replication cycle

Structure

Both HIV-1 and HIV-2 are spherical, approximately 100 nm diameter in size, enveloped viruses (Figure 1). The lipid bilayer enveloping the virion contains viral envelope glycoproteins (Env). The Env complex consists of an outer surface unit (SU), Gp120 in HIV-1 and Gp125 in HIV-2, and a transmembrane unit (TM), Gp41 in HIV-1 and Gp36 in HIV-2, associated into trimers (51). In addition to Env proteins the virions contain host proteins that are part of the producer cell surface plasma membrane proteome (52). It has been suggested that HIV-1 and HIV-2 differ in Env incorporation, where mature HIV-1 virions would contain fewer intact Env trimers compared to mature HIV-2 virions (53), but the underlying mechanism behind the difference in frequency of dissociated SU is still unknown. The inner lipid layer of the virion membrane is layered by the myristoylated virus matrix (MA) protein, through interaction with inositol phospholipids (54). Within the virion, a conical icosahedral capsid, consisting of the virus capsid (CA) protein, which encapsulate the nucleocapsid (NC)-virus RNA complex. Within the capsid also other viral proteins are contained, including viral enzymes Reverse Transcriptase (RT) and Integrase (IN) which are required for the intracellular steps of the replication cycle following infection of a target cell (55). Both HIV-1 and HIV-2 contains two copies of approximately 10 kilobase pair long single-stranded positive-sense viral RNAs (55).

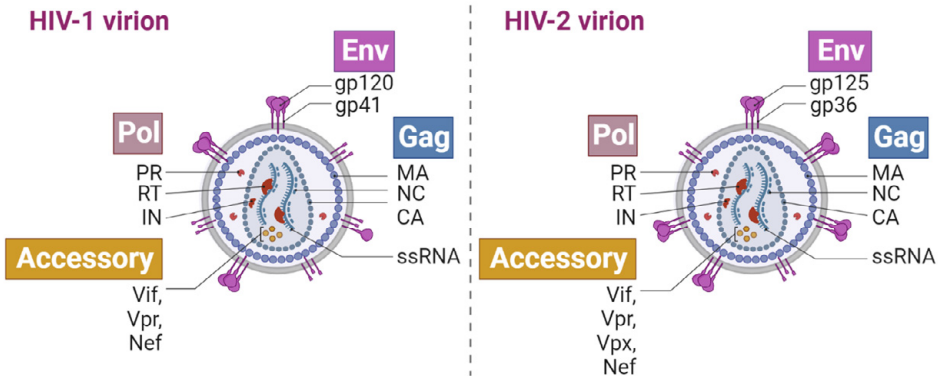

Figure 1. Schematic representation of HIV-1 and HIV-2 structure.

Genome

The HIV-1 and HIV-2 genomes (Figure 2) share approximately 50% nucleotide sequence similarity (56), but have some key differences reflecting their different SIV origin (3).

The HIV-1 genome is approximately 9.7 kilobase pair long and encodes three structural genes, *gag*, *pol* and *env*, as well as the six accessory proteins *vif*, *vpr*, *vpu*, *tat*, *rev*, and *nef* (51). The HIV-2 genome is approximately 10.5 kilobase pair long (51), and encodes the same proteins as HIV-1, with the exception that HIV-2 has retained *vpx* and have not acquired *vpu* (57). The two terminal ends of the HIV genome consist of two long terminal regions (LTRs) that are required for the initiation of the reverse transcription and serves as the promotor region during the transcription of the integrated HIV provirus (58, 59). Importantly for the transcription of HIV-1 proviruses, the LTR regions contains two to three nuclear factor kappa B (NF-κB) binding sites that facilitates efficient transcription in activated cells (60). In contrast, HIV-2 LTRs have been found to contain commonly only one NF-κB binding site, possibly leading to reduced proviral transcription (61). The *gag* gene encodes for the Gag polyprotein, consisting of MA, CA, NC, and p6, as well as the two spacer peptides p2 and p1 located upstream and downstream of NC, respectively (62). Similarly, *pol* encodes for the Pol polyprotein which contains the three viral enzymes Protease (PR), RT, and IN (51). As previously mentioned, one of the key differences between the HIV-1 and HIV-2 genomes is the presence

of *vpu* in the HIV-1 genome instead of *vpx*, which also resulted in an overlap between *vif* and *vpr* in the HIV-1 genome (57). Further, in the HIV-2 genome *nef* overlaps with *gp36*, while *nef* and *gp41* are separated by two nucleotides in the HIV-1 genome (51). In addition, a putative antisense gene, called *antisense protein* (*asp*), has been suggested to overlap with *env* in the HIV-1 group M strains, but not in the remaining HIV-1 groups, SIVs, or HIV-2 (63-65). Interestingly, a recent study found that the presence of *asp* was approximately 85% in highly prevalent HIV-1 group M subtypes (A, B, C, G and CRF01_AE), compared to 45% presence in low-prevalent subtypes (D, F, J, H and K) (63). Although both antibodies and $CD8^+$ T-cells targeting ASP have been detected in infected individuals (65), it is still not known if this putative protein has a function *in vivo*.

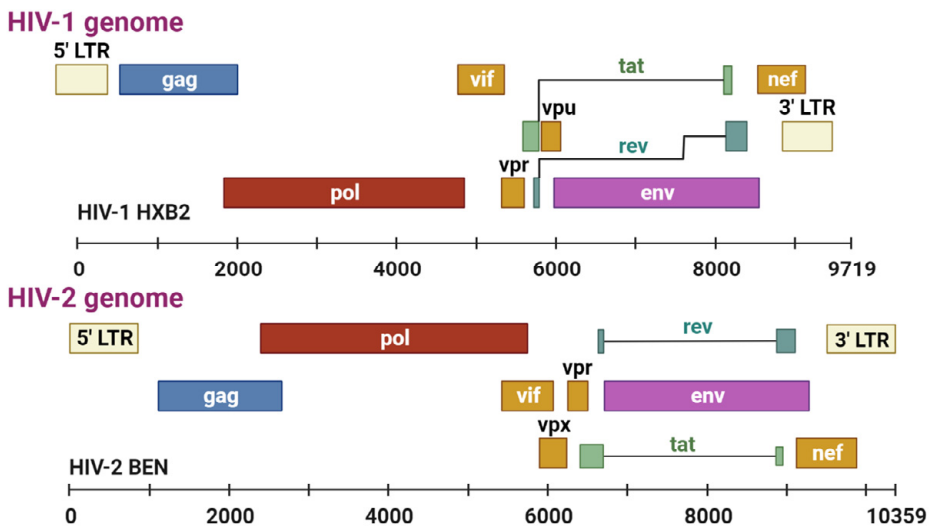

Figure 2. HIV-1 and HIV-2 genome organisation. The figure was adopted from (51).

Replication cycle

As the majority of HIV research has been performed on HIV-1, the following chapter will in large describe the HIV-1 replication cycle (Figure 3). However, key steps where HIV-2 differ from HIV-1 will be highlighted.

Virus entry

Virus entry is mediated through the interactions between Env trimers and the main receptor CD4 and a co-receptor, typically CCR5 or CXCR4. As is typical for type I fusion proteins (66), Env exists as heterodimeric trimers, consisting of Gp120 and Gp41, at the virus membrane (67). Interaction with CD4 is mediated by Gp120, while fusion to the plasma membrane of the infected cell is mediated by the fusion peptide in Gp41 (67). Following binding of Gp120 to CD4, Gp120 undergoes a conformational change that exposes the co-receptor binding site (the so-called bridging site and the V3 loop). Binding of the co-receptor stabilises the Env-receptor complex and brings the virus and cellular membranes closer together. Together, the conformational changes of Gp120 induced by CD4 and co-receptor binding will induce the dissociation of Gp120 from Gp41, and the fusion peptide in Gp41 to be inserted into the cellular plasma membrane (67, 68). Insertion of the fusion peptide will bring the virus and cellular membranes close enough to fuse and release the virus capsid into the cytoplasm.

However, more research is still needed to completely understand the events taking place during Env-binding and virus fusion. For example, the mechanisms behind CD4-independent infection, which has been reported to be more common among HIV-2 viruses compared to HIV-1 viruses (69), are still largely unknown. Coreceptor use of HIV-2 is also distinct from HIV-1 in the ability of HIV-2 to use alternative coreceptors, such as CCR2b, CXCR6 and GPR15 (69). Still, it has previously been reported that also HIV-2 isolates are highly dependent on either CCR5 or CXCR4 for replication in peripheral blood mononuclear cells (PBMC) (70). Moreover, the subcellular location of the entry event is still debated (71).

Uncoating, reverse transcription and nuclear transport

Capsid uncoating and reverse transcription were initially believed to occur in the cytoplasm shortly after the fusion of virus and cellular membranes (72). However, initial assumptions of the subcellular location of virus capsid uncoating and reverse transcription have recently been challenged by the introduction of novel advanced microscopic methods (72). Recent findings indicate that the capsid remains intact and thereby prevents detection of cDNA by cellular restriction factors (72). A central pore in the capsid hexamer has been postulated to mediate import of dNTPs into the capsid and enable encapsulated cDNA synthesis (73). Irrespective of the site of uncoating, the reverse transcription is initiated by a host transfer RNA ($tRNA_{Lys}$) bound to the virus RNA (59). The reverse transcription is performed by the viral RT enzyme. The high mutation rate of the RT (approximately 0.1 mutations/10 000 base pair) is partly responsible for the high evolutionary rate of HIV (10, 31, 74). Another key driver of HIV diversity is the frequent occurrence of recombination events during reverse transcription (up to 10 switches per cDNA synthesis event) (75-78). Recombination occurs when the virion contains two non-identical RNA strands, incorporated into the virion during budding from a cell

infected by two non-identical virus variants, and the RT jumps between the two strands during the synthesis of the negative cDNA strand (78).

Through interactions with cellular proteins, intact capsids have been shown to be transported to nuclear pores, transported into the nucleus, and subsequently disassembled to release the pre-integration complex (PIC) (79, 80). However, more research is still needed to determine if uncoating occurs in the cytoplasm, at the nuclear pore while allowing for the import of the PIC into the nucleus (81), or inside the nucleus (79). Following the import of the PIC into the nucleus, the cDNA is integrated into the host genome. The integration is catalysed by IN, which binds to the nascent cDNA to form the DNA-IN complex known as the intasome (82). The IN removes two nucleotides from the 3' ends of the cDNA strands to create two reactive 3′-hydroxyl ends, which are used by IN to perform the strand transfer and integrate the cDNA into the host genome and create the provirus (83).

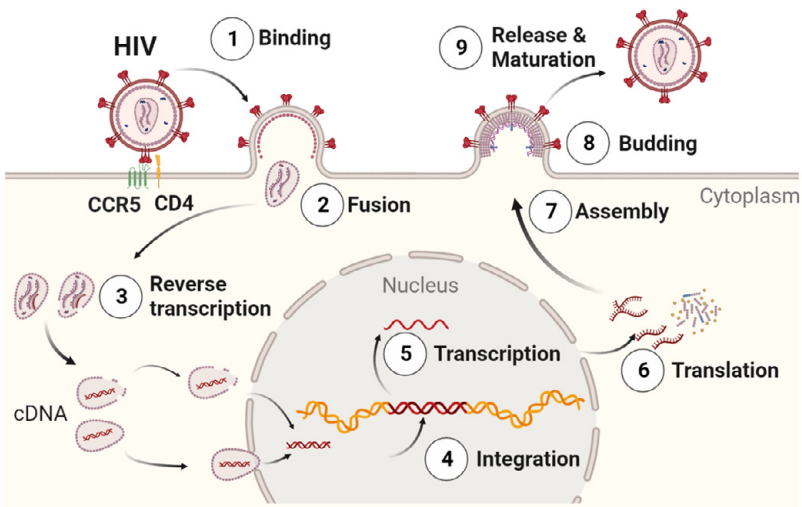

Figure 3. Schematic overview of key steps in the HIV replication cycle. The key steps include: 1) Binding of HIV to CD4 and a coreceptor. 2) Structural rearrangements of the Env proteins facilitates the fusion of the virus and host membrantes and the release of the virus capsid into the cytoplasm. 3) In the cytoplasm the virus RNA is reverse transcribed by the viral Reverse Transcriptase into cDNA. The cDNA is thereafter transported into the nucleus. 4) In the nucleus, the cDNA is integrated by the viral Integrase into the cellular genome. 5) The provirus is thereafter transcribed into mRNA that subsequently are exported into the cytoplasm. 6) In the cytoplasm the mRNA is either translated into virus proteins, or bound to Gag/Gag-Pol polyproteins for packaging into nascent viruses. 7) The Gag/Gag-Pol polyproteins are transported to the plasma membrane and assemble to form the new viruses. 8) Gag/Gag-Pol multimerisation induces membrane curvature and eventually budding of the nascent virus. 9) Following the budding, the viral Protease digest the Gag/Gag-Pol polyproteins and the mature, infectious virus is formed (84).

Transcription, assembly, budding and maturation

Following integration, the provirus can either remain transcriptionally silent, i.e. latent, or be actively transcribed. The initiation of provirus transcription is regulated by the balance of cellular transcription activators or repressors binding to the 5'-LTR. Key elements described to be required to initiate proviral include NK-κB, Sp1 and TATA-box binding protein (TBP) (58). These proteins bind to the 5'-LTR and recruit the RNA polymerase II (RNAPII)-dependent transcriptional machinery. During active virus transcription, Tat increases virus transcription 1000-fold (85), by interacting with positive transcriptional elongation factor b (P-TEFb), and stop RNAPII from pausing at the 5'-LTR to allow an efficient transcriptional elongation (58).

Following the initiation of proviral transcription, several different spliced variants of the proviral transcripts are produced. The different HIV transcripts are typically divided into three groups: Unspliced transcripts that encodes Gag and Gag-Pol polyproteins and are represent the genomic RNA (gRNA) packaged into new virions; singly spliced transcripts encoding the Env, Vif, Vpr, and Vpu proteins; and the multiply spliced transcripts that encode the Rev, Tat, and Nef proteins (86). During early transcription, multiply spliced transcripts are transported out of the nucleus, while the instability sequence elements (INS) containing unspliced and singly spliced transcripts are retained in the nucleus. After translation, Tat and Rev are transported into the nucleus where Tat will increase virus transcription and Rev will mediate the transport of unspliced and singly spliced transcripts into the cytoplasm. Nef will target CD4, CD28, MHC class I, and SERINC5 for degradation (57, 87). In addition, HIV-1 Nef and HIV-2 Env mediate the degradation of tetherin (57). The translation of the singly spliced transcripts produces Vif, Vpr, Vpu, and Env. Vif and Vpu increases viral replication by counteracting cellular restriction factors, by targeting these proteins for proteasomal degradation (57, 88-91). Vpr induces G2/M cell-cycle arrest of infected cells and increases proviral transcription (92). Although Vpr was initially believed to be involved in the transportation of the PIC to the nucleus, further studies did not support a role for Vpr in this process (72). Env is translated as Gp160 in the endoplasmic reticulum (ER), where it is extensively glycosylated (93). As the Gp160 trimers are transported through the Golgi apparatus, the oligosaccharide side chains are modified and Gp160 is proteolytically cleaved by the cellular proteas furin into Gp120 and Gp41 (93). Translation of the unspliced transcripts will produce the Gag and Gag-Pol polyproteins required for the assembly and maturation of nascent viruses (55).

MA in the Gag and Gag-Pol polyproteins are myristoylated following translation, and thereafter target the polyproteins to the plasma membrane through their interactions with inositol phospholipids (54). Unspliced gRNA dimers are bound to the NCs through the interaction of the packaging signal, Psi (Ψ), in the 5'-LTR region (94). At the plasma membrane, Gag and Gag-Pol multimerisation leads to membrane curvature and eventually budding (95). The exact mechanism of Env incorporation into the budding virus is still not known, but it has been shown that interactions between the cytoplasmic tail of Gp41 and MA is important for the

trapping of Env in the budding virus (96-98). Budding is mediated through the interaction of p6 and the ESCRT machinery (95).

Following, and during, budding of the nascent virion, PR cleaves Gag and Gag-Pol polyproteins and releases the individuals proteins. The separation of the individual Gag and Pol proteins drives the maturation of the virus, which is characterised by the formation of the mature capsid.

HIV-1 and HIV-2 pathogenesis

HIV infection is characterised by a progressive deterioration of the immune system, eventually leading to a collapse of the immune system and a subsequent increased susceptibility to opportunistic infections (99). HIV infection in individuals not receiving treatment is typically divided into three different stages: the acute infection stage, the asymptomatic stage, and finally the AIDS stage (Figure 4). As the majority of HIV research has focused on HIV-1 (9, 41), this chapter will broadly describe HIV-1 pathology and highlight key steps where HIV-2 infection is known to differ.

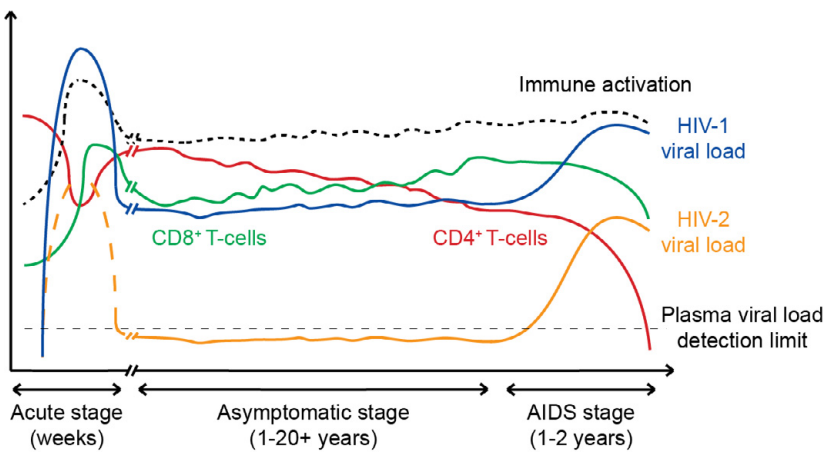

Figure 4. Schematic view of general HIV disease progression in treatment naive individuals. As plasma viral load dynamics during acute HIV-2 infection has not been studied, hypothesised dynamics are represented by a dashed line.

Transmission and acute infection stage

Sexual transmission represents the most common route of HIV transmission globally (100), but HIV can also be transmitted through contact with HIV contaminated blood, e.g., through sharing of injection equipment, transfusion of non-heat inactivated blood from PLWH or from an HIV infected mother to her child during delivery. Despite the presence of a highly variable population of viruses in infected individuals, onward sexual infection is typically established by a single transmitted founder (T/F) virus (101). The mechanisms regulating the epithelial translocation of HIV are still not known, but it is believed to either occur due to epithelial damage during intercourse, or that the viruses are captured by dendritic cells (DC) as the DCs sample the mucosa apical surface of the mucosa for incoming pathogens (102). The DCs are believed to transport viruses from the site of infection to neighbouring lymphoid tissue, where they will infect $CD4^+$ T-cells. However, it is still not known if this occurs through *cis*- or *trans*-infection. *Cis*-infection occurs when DCs becomes productively infected, and thereafter spreads newly produced viruses to $CD4^+$ T-cells through close contact sites known as immunological synapses (103). However, *cis*-infection is not believed to be a major pathway of *in vivo* mediated infections since myeloid cells express high levels of the restriction factor SAMHD1, which makes them poorly permissive to HIV-1 infection (104). Instead, *trans*-infection is believed account for most of the DC-mediated infections *in vivo*. *Trans*-infection is initiated by the binding of C-type lectins, such as Siglec-1 (105-107), to glycoproteins at the surface of the HIV particle without infecting the DC. The virus particle is thereafter either endocytosed and stored in the DC or remain at the surface of the DC. When the DC later on comes into close contact with $CD4^+$ T-cells, typically in nearby lymphoid tissue, the viruses released from the DCs can then infect the $CD4^+$ T-cells through the immunological synapse (102). In support for the role of *trans*-infection *in vivo*, a subset of DCs in the vaginal mucosa has been shown to have the exclusive ability to capture HIV particles, but not be infected by them (108). In addition, Siglec-1^+ DCs in cervical biopsies have been shown to have intracellular HIV-1-containing compartment (109). In addition to the DC-mediated infection in lymphoid tissue, $CD4^+$ T-cells can also be infected at the site of HIV entry, either as tissue resident cells or as $CD4^+$ T-cells infiltrating an inflammatory site.

Following sexual transmission, initial HIV replication typically occurs locally, and plasma viral load is undetectable. This stage, termed the eclipse stage, typically lasts for 6-10 days (110, 111), and is characterised by increasing plasma levels of pro-inflammatory cytokines and the establishment of the HIV reservoir (112). The rapid increase in plasma levels of pro-inflammatory cytokines, such as type 1 and type 2 IFNs, is typically referred to as a cytokine storm, and has been shown to occur during many types of infections (113). In the case of acute HIV infection, the initiation of the cytokine storm occurs as a result of the sensing of an increasing amount of HIV by the innate immune system. Resistance to type 1 IFN by T/F

viruses is known to be a key determinant for successful transmission (114), which highlights the importance of the early cytokine response during HIV transmission. Although the early cytokine response is likely to be protective against transmission and replication, the failure to control the replication leads to a pathogenic activation of the innate immune system and a stronger cytokine response during acute infection has been shown to correlate with increased disease progression rate (115). The uncontrolled HIV replication leads to an exponential increase of the plasma viral load and further systemic dissemination of HIV. Fiebig *et al*. divided the acute infection phase into five stages, referred to Fiebig I-V, following the sequential emergence of detectable pVL, positive CA ELISA test, and HIV specific antibodies (116). Following the systemic dissemination of HIV, HIV replication is established throughout the secondary lymphoid organs, in particular the gut associated lymphoid tissue (GALT). The GALT contains high numbers of activated $CD4^+$ T-cells, and the extensive replication taking place before the onset of the adaptive immune response is believed to deplete the GALT of approximately 80% of all $CD4^+$ T-cells within the three first weeks of infection (117-119). The depletion of $CD4^+$ T-cells occurs both due the cytopathic effect of HIV, but also due the massive death of bystander $CD4^+$ T-cells that are not infected. The exact mechanisms behind the rapid depletion of bystander $CD4^+$ T-cells are not known, but a highly pro-inflammatory cell death pathway called pyroptosis is believed to be a key reason (120). In addition to the depletion of $CD4^+$ T-cells, the high amount of damage associated molecular patterns (DAMPs) and pro-inflammatory cytokines causes both a collapse of germinal centres and loss of gut epithelial integrity (121, 122). The loss of epithelial integrity leads to increased microbial translocation, which further contributing to chronic systemic inflammation (123, 124). The pVL increases exponentially during the systemic replication, prior to the mounting of an adaptive immune response, and reaches the so-called peak VL. Following the mounting of an adaptive immune response, the pVL decreases until it reaches as steady-state level referred to as set-point VL (SPVL). The SPVL has been reported to vary up 1,000,000-fold between PLWH (125), due to a combination of both viral and host genetics factors (125-129), and has been found to be associated with disease progression rate (129-134). In parallel to the reduction of the pVL, the $CD4^+$ T-cell count in blood is restored to almost pre-infection levels, but not in GALT (117-119). The mounting of an adaptive immune response and the subsequent reduction in viral replication marks the end of the acute infection stage and the beginning of the early asymptomatic disease stage.

So far, only one adult HIV-2 acutely infected individual has been identified (135), which has prevented the study of differences between acute HIV-1 and HIV-2 infections. However, as the pVL of HIV-2 infected AIDS patients is lower compared to HIV-1 infected AIDS patients (5, 136-138), it is likely that acute HIV-2 infection is associated with lower VL compared to HIV-1 infection. In addition, HIV-2 viruses have been shown to induce a stronger type 1 IFN response in myeloid cells compared to HIV-1 viruses (139). As resistance to type I interferon has been shown

to be a key determinant of HIV-1 T/F virus transmission fitness, it is likely that initial HIV-2 replication is inhibited more potently by the innate immune response compared to HIV-1. In addition, DC-mediated HIV-2 *trans*-infection of $CD4^+$ T-cells has been shown to be less effective compared to HIV-1 *trans*-infection (140). Further, HIV-2 has been shown to be more susceptible to type I IFN-inducible restriction factors (57), further suggesting that acute HIV-2 infection could be better managed by the innate immune system compared to HIV-1.

The asymptomatic stage

The asymptomatic disease stage follows the acute infection stage. The length of the asymptomatic disease stage can vary greatly, from less than a year to more than 20 years, and is determined by a combination of both host and virus genetic factors (141). During the asymptomatic disease stage, the $CD4^+$ T-cell count gradually decreases as a consequence of virus replication and the chronic inflammation (125). The immune system is capable of maintaining the pVL at a relatively stable level around the SPVL during the asymptomatic disease stage, although a gradual increase is typically observed (134). The ongoing virus replication causes persistent stimulation of the innate and adaptive immune system, and eventually leads to immunosenescence (142). In addition to the exhaustion of the HIV-specific immune cells, the chronic inflammation causes a generalised exhaustion of both B- and T-cells (143, 144). The general immune activation increases gradually during the asymptomatic disease stage, until the exhaustion of immune cells and severe loss of $CD4^+$ T-cells causes the immune system to collapse, and the infected individual becomes susceptible to opportunistic pathogens.

Although PLWH1 and PLWH2 have similar disease trajectories, the asymptomatic disease stage is approximately twice as long among PLWH2 compared to PLWH1 (145). In line with the prolonged asymptomatic disease stage, the SPVL has been estimated to be 10-30x lower among PLWH2 compared to PLWH1 (5, 136, 138). Similar to PLWH1, the SPVL in PLWH2 has been found to be associated with disease progression (44). However, other reports have reported that general immune activation, rather than pVL, is associated with disease progression among PLWH2 (146, 147). Interestingly, the group have observed that PLWH2 can progress to immunodeficiency even in the absence of detectable viraemia (148). The underlying mechanisms behind $CD4^+$ T-cell decline in aviraemic PLWH2 are not well understood, but $CD4^+$ T-cell loss is closely related to general immune activation and exhaustion (146, 147, 149).

Interestingly, the group has previously found the time to AIDS and HIV/AIDS-related death following HIV-1 infection was approximately 50% longer among individuals with a preceding HIV-2 infection compared to individuals infected with only HIV-1 (150, 151). The $CD4^+$ T-cell decline was found to be similar among people living with HIV-1 and HIV-2 dual-infection (PLWH-D) and PLWH-1, but the extrapolated $CD4^+$ T-cell levels at estimated time of seroconversion was found

to be significantly higher among PLWH-D (150). This suggests an inhibition of early HIV-1 replication and initial $CD4^+$ T-cell decline, rather than a continuous inhibition of HIV-1 by HIV-2. However, the underlying mechanism behind this are still unknown, but both the innate and adaptive immune system have been proposed to play a role (152-157). Previous studies have suggested that inhibition of HIV-1 disease progression by HIV-2 could be attributed to cross reactive T-cells (156, 158), cross-reactive antibodies (152, 157), HIV-2 induced release of beta-chemokines that can compete for CCR5 binding (154, 155), as well as inhibition of intracellular HIV-1 replication steps in dual-infected cells (159, 160).

Effect on tissue pathology

In addition to the depletion of $CD4^+$ T-cells, the HIV infection impacts several different bystander cells and increases the risk of comorbidities in PLWH (161). As previously mentioned, the extensive depletion of $CD4^+$ T-cells in the GALT leads to release of DAMPs and proinflammatory cytokines that both induces lymphoid tissue fibrosis, myeloid cell activation, and damage the gut mucosa (123, 124, 162). Impairment of the gut epithelial integrity has been suggested to be driven through interactions between epithelial cells with viruses, virus proteins, cytokines, and cytotoxic lymphocytes (163-167). In addition to the increased epithelial cell death, the loss of T helper 17 (Th17) cells in PLWH reduces IL-22-mediated epithelial regeneration (168-170). Further, Th17 cells have been found to be essential for the control of extracellular microbes, and the depletion of this cell type contribute to microbial overgrowth (168). LPS levels, a marker of microbial translocation, have been shown to be inversely correlated with CD4% in both PLWH1 and PLWH2 (123). In addition, clearance of microbial products is decreased in PLWH due the reduction of Kupffer cells (liver resident macrophages), both through direct infection and due to microbial translocation and chronic inflammation (171, 172). The activated Kupffer cells are also believed to promote a proinflammatory environment in the liver, which increases the risk of liver fibrosis (171-173), both in PLWH with or without Hepatis B or C co-infections (174, 175). In addition to the engagement of cells in the gut and liver, monocyte and macrophage activation have been attributed reduced function of several other tissues, such as the spleen and cardiovascular system (176). Within the spleen, HIV replicates in macrophages, as well as DCs and T-cells, and induces morphological changes that disrupt the function of the spleen (177-179). Microbial translocation, myeloid cell activation, and liver and spleen pathology have all been linked to the induction of a hypercoagulable state in PLWH (176, 180). The combination of monocyte activation and hypercoagulation are believed to be key drives of cardiovascular and tissue damage in PLWH (99, 181-184).

The AIDS stage

Following the exhaustion and depletion of essential immune compartments, an increase in pVL is often seen, which further decrease the $CD4^+$ T-cell levels. Once the $CD4^+$ T-cell count falls below a certain level, typically ~200 cells/μL or <14% $CD4^+$ T-cells of the total lymphocyte count (CD4%) (185, 186), the immune system is unable to control otherwise non-pathogenic pathogens, and opportunistic infections are observed (125). Identification of the appropriate $CD4^+$ T-cell level that should be used for the diagnosis of AIDS has been extensively studied, and $CD4^+$ T-cell levels below 200 cells/μL or <14% have been reported to be strong predictors for the development life-threatening opportunistic infections (186-189). CD4% has been reported to be a more stable marker of $CD4^+$ T-cell decline compared to absolute $CD4^+$T-cell count, especially in countries with high pathogenic burden (187, 188, 190, 191). A range of different AIDS-defining diseases have been described, such as pneumonia, candidiasis, Kaposi's sarcoma, and pulmonary tuberculosis (192, 193). Interestingly, AIDS onset has been described to occur at higher $CD4^+$ T-cell count in PLWH2 compared to PLWH1 (145, 193), but the underlying mechanisms are still not known. Without treatment, most PLWH succumb to the opportunistic infections within two years following AIDS onset (193, 194).

HIV-1 and HIV-2 immune responses

The immune system is typically divided into two parts, into the innate and the adaptive immune system. Innate immune responses are typically initiated through the recognition of conserved damage- and pathogen-associated molecular patterns (DAMPs and PAMPs) by germline-encoded pattern recognition receptors (PRR). In contrast, the receptors used by the adaptive immune responses are created through genetic rearrangement, which allow them to recognise a wide range of different proteins and structures (195). In addition to this division, the immune system consists of an ever-growing number of cell subtypes with specific functions (196). Following an infection of an individual, the various cell types coordinate the mounting of an immune response, followed by the equally important subduing of the immune response (197, 198). However, during an HIV infection, and other chronic infections, virus replication and antigen exposure persists and a chronic inflammatory state is instead established (99). The following chapter will describe important aspects of both protective and pathogenic immune responses occurring in PLWH.

Innate immune responses

The innate immune system is made up by both constitutive and inducible mechanisms, that are both required for the maintenance of host homeostasis and pathogen/damage surveillance (198). The constitutive mechanisms can be further divided into physical and chemical barriers, such as the skin and mucus layer, and molecularly defined mechanisms that can prevent microbes prior to the engagement by the inducible factors. These mechanisms include for example secreted antimicrobial peptides and lectins, as well as restriction factors like SAMHD1, APOBEC3s, TRIM5α, and tetherin (198). The restriction factors play an important role in the inhibition of HIV replication, and their expression is commonly increased by type I IFN signalling (199, 200). HIV-1 and HIV-2 (and the SIV strains they are derived from) have evolved different ways of evading the interferon stimulated restriction factors, which has been suggested to partly explain their difference in pathogenicity (201). During an acute HIV infection, the virus overcome the constitutive immune mechanisms and trigger the inducible factors. The inducible factors include different classes of PRRs (202). These receptors have primarily been studied in innate and adaptive immune cells, but they are also expressed in several non-immune cells. These include endothelial cells, epithelial cells, and fibroblasts, where they also play an important role in the initiation and maintenance of an immune response (202). Following the binding PRRs to PAMPs, such as HIV cDNA, a signalling cascade is initiated that upregulates the expression of several genes involved in proinflammatory and antiviral responses. These include intracellular antiviral restriction factors, chemokines, and proinflammatory cytokines such as type I interferons and tumour necrosis factor (TNF) (203). The mode of activation by the innate immune system shapes the subsequent response, as well as the ensuing adaptive immune response (197). Due to the potent proinflammatory response induced by PRRs, several negative feedback loops exist to control the duration of the PRRs response (203). The importance of the negative regulation of these processes are highlighted by the hyperactivation of the innate immune responses in several diseases including chronic antigen stimulations, such as autoimmune diseases, e.g. systemic lupus erythematosus (SLE), and chronic virus infections, e.g. HIV infection (202).

Tissue resident macrophages and dendritic cells continuously surveil the mucosal surface and underlying tissue for the presence of DAMPs and PAMPs (204). The binding of DAMPs or PAMPs to PRRs will result in a rapid release of chemokines and proinflammatory cytokines (203). This will induce the activation of vascular endothelial cells and promote recruitment of additional immune cells to the site of infection. Neutrophils are among the first responders following the detection of DAMPs and PAMPs, but monocytes, dendritic cells, and natural killer (NK) cells are also recruited (205). Circulating monocytes egress from the blood stream and differentiate into macrophages or monocyte derived DCs (MDDCs) (206). Neutrophils and macrophages can potently inhibit pathogen spread in the tissue

through phagocytosis of opsonised pathogens and the release of inhibitory factors such as proteases and reactive oxygen species (ROS) (207). Although these factors play an important role in the inhibition of pathogen replication and spread, their release also damages tissue and bystander cells (207). Uncontrolled activation of these cell types is therefore associated with extensive tissue damage, and PLWH have been shown to have increased risk of e.g. liver, lymphoid and myocardial tissue fibrosis (172, 173, 208-210). Further, the proinflammatory state of PLWH1 and PLWH2 has been found to induce the expansion of activated monocytes with an inflammatory profile (211, 212).

Several different types of DCs have been described, but the most commonly studied subset are conventional DCs (cDCs), plasmacytoid DCs (pDCs), and MDDCs (213). These different DC cell types have distinct expressional profiles, ontogeny, and play different roles in the mounting of an immune response (214). Activation of pDC results in the release of large amounts of type I interferons (215), which further drives the activation of the innate and adaptive immune systems. In addition, type I interferons induce the upregulation of several ISG, several of which are intracellular restriction factors that inhibit various intracellular steps of the HIV replication cycle (216). pDCs and MDDC are believed to act as antigen presenting cells (APC) at the sites of inflammation, while cDCs are believed to be the primary DC subset that migrates to lymphoid tissue and activate T-cells (214). During HIV-1 and HIV-2 infections, pDCs and MDDCs have been shown to be progressively depleted from peripheral blood during disease progression (211, 217). DCs are the most efficient APCs, although other cell types such as macrophages and B-cell can also act as APC. Following the sensing of a PAMP by a DC, they will transition into a professional APC (214). The professional APCs bridge the innate and adaptive immune response by presenting pathogen-derived peptides to T-cells. The transcriptional program of the dendritic cells is shaped by a combination of cytokine signalling and mode of PRR stimulation (213), which in turn influence the activation signals provided to T-cells during the subsequent antigen presentation. This will shape the adaptive immune response mounted to the invading pathogen (197).

The recruitment of NK cells also plays an important role in innate immune response towards viruses (218). As indicated by their name, they can efficiently induce apoptosis in infected cells. This is performed through two mechanisms, either through the release of cytotoxic proteins such as granzyme and perforin, or through death receptor-induced apoptosis (219). In addition to their apoptosis-mediating role, they also secrete chemokines and proinflammatory cytokines, such as IFN-γ. NK cell activation and effector function depends on the summative interaction of activating and inhibitory receptors with the target cells (220). The inhibitory receptors bind to MHC class I receptors and prevent NK cell mediated killing. Infected or malignant cells often have reduced MHC class I expression and upregulated expression of exhaustion or senescence associated receptors that can interact with the stimulating receptors on the NK cells, which results in killing of the target cell (220). In addition, antibodies binding to virus proteins expressed on

the surface of infected cells can interact with CD16 on the NK cells, and mediate antibody dependent cellular cytotoxicity (ADCC) (221). Interestingly, increased NK cell response skewed towards increased ADCC activity has been associated with a reduced risk of HIV-1 acquisition (218, 222). In addition, certain MHC class I HLA-C alleles, the target of several inhibitory NK cell receptors (223), have been associated with reduced risk of HIV-1 seroconversion, further suggesting that NK cells can play an important role in preventing the establishment of an HIV infection (224). The downregulation of HLA-C protein expression by HIV-1 Vpu and HIV-2 Vif prevent the binding of the inhibitory receptors and can induce NK-cell mediate lysis of infected cells (223, 225).

Although immunological memory and antigen specificity was initially believed to be an exclusive hallmark of the adaptive immune response, this dogma has been challenged by a growing number of observations of memory-like and antigen specific-responses among different innate immune cell types (226, 227). Trained immunity has primarily been studied in monocytes and macrophages, but memory-like traits among subsets of NK cells has gained interest due to their role in maintenance of viral infections and malignancies (226). Interestingly, a subset of both human and murine NK cells has been found to shown antigen-specificity towards human and murine cytomegalovirus (CMV), and these cells can undergo rapid expansion and faster degranulation upon a second challenge (226-229). Although HIV-specific NK cells have been observed in humanized mice vaccinated with HIV-1 Gp120 (230, 231), the contribution of antigen specific NK cells in PLWH are still unknown.

Adaptive mediated immune responses

B- and T-cells are responsible for the adaptive immune response towards HIV (195). In contrast to the innate immune cells, B- and T-cells do not recognise their antigens using germline encoded receptors. Instead, the genes encoding their receptors undergoes somatic assembly of variable (V), diversity (D), and joining (J) gene segments (232). Several different sets of V, D, and J segments exist, and an extreme diversity of the T-cell receptor (TCR) and B-cell receptors (BCR) repertoire is created by the random arrangement of single VDJ (TCR β and δ chains and BCR heavy chain chains) and VJ (TCR α and γ chains and BCR light chains) segments in individual cells (233). The diversity is further increased by endonuclease trimming followed by the addition of random non-template encoded nucleotides at the recombination ends between the segments (232). Following the exposure of naïve B- or T-cells to their cognate antigen, they will undergo rapid proliferation and expand the size of the clonal population. The activated cells can then undergo differentiation towards several different fates to generate both various effector cells, which target the existing pathogen, as well as memory cells, that can rapidly respond to the next exposure to the same pathogen (234).

T-cell mediated immune responses

Following maturation of T-cells in the thymus, naïve T-cells circulate between secondary lymphoid organs and the blood until they encounter an activated DC presenting the peptide sequence that they are specific to. The PRR-mediated activation of the DCs induces the upregulation of MHC molecules and co-stimulatory receptors and increases the release of proinflammatory cytokines. Activation of $CD4^+$ T-cells require presentation of peptides on MHC class II molecules, while $CD8^+$ T-cells require cross-presentation of the peptides on MHC class I molecules. The naïve T-cells are primed by the activated DC through the signalling induced by these three factors. The subsequent proliferative response and subset commitment by the naïve T-cell is determined by the cumulative signalling induced by the three factors and by the DC subset (197).

The activation of naïve $CD8^+$ T-cells results in proliferation and differentiation into effector cells (T_{eff}) that can efficiently eliminate infected cells. The massive activation of HIV-specific $CD8^+$ T-cells during acute HIV infection largely coincides with the decrease in HIV viraemia (235, 236). In line with the lower SPVL of PLWH2, HIV-2 infection has been found to induce a broader and more potent HIV-2-specific $CD8^+$ T-cell response compared to HIV-1 infections (5, 156, 237, 238). Interestingly, a more heterogenous $CD8^+$ TCR repertoire in PLWH2 has been suggested to enhance the potential of the $CD8^+$ T-cells to recognise emerging virus variants (239), and possibly limiting viral diversification (240). In the case of viral clearance, the majority of activated $CD8^+$ T-cells die by apoptosis, while a small subset differentiates into long-lived memory cells (Tmem). The Teff and Tmem subsets are believed to differentiate from the same activated progenitor cells, driven by expression networks maintained by T-bet or Eomes, respectively (241). However, during chronic HIV infections the persistent antigen exposure prevent efficient differentiation into Teff and Tmem cells (241). Instead, the activated $CD8^+$ progenitor cells differentiate into exhausted cells (Tex), with distinct transcriptomic profile, progressive loss of effector function, elevated levels of inhibitory receptors, such as PD-1, CTLA-4, and TIGIT, and reduced ability to differentiate into long-lived memory cells (241). Both HIV-1 and HIV-2 infection have been found to be associate with progressive increase in activated and exhausted T-cells (146, 148, 242, 243). This is believed to be part of the normal response of the immune system to maintain control of virus replication, for parallel reduced risk of bystander cell and tissue damage (244). The reduced effector function of Tex cells depends partly on the binding of the inhibitory receptors, such as PD-1, to their ligands on APC, such as PD-L1. However, the hampering of the effector functions can be overcome in cells with intermediate PD-1 levels through the administration of PD-1L-binding antibodies (245, 246), suggesting that the exhausted cells can exist on a spectrum between intermediate and terminally exhausted (247). Intriguingly, antibodies binding to inhibitory receptors/ligands have been shown to increase the effector function of HIV-specific $CD8^+$ T-cells both *ex vivo* and *in vivo* (248). Early progenitor cells of Tex cells (Tex^{prog}) are believed to be maintained by the

expression of TCF-1 and TOX. Depending on the activation signals received, this population can subsequently differentiate into an polyfunctional effector population (Tex^{eff}) or a terminally exhausted population (Tex^{term}) (247). Similar to the Teff, the Tex^{eff} express high levels of the transcription factor T-bet and intermediate levels of PD-1. The Tex^{term} express high levels of the transcription factor Eomes and PD-1. Administration of PD-L1 targeting antibodies has been found to induce the expansion and differentiation of Tex^{prog} into Tex^{eff} (249, 250). The maintenance of the Tex^{prog} and Tex^{eff} populations have been found to be crucial for the control of chronic viral infections in mice (251), but their importance in humans are still being elucidated.

$CD4^+$ T-cells can differentiate into several different subsets that promote diverse types of immune responses. These subsets include Th1 cells, which are important for the mounting of an efficient $CD8^+$ T-cells response, and Th2 and Tfh cells that are important for efficient antiviral B-cell responses (252). Th1 cell differentiation is promoted in the presence of type I interferons and IL-12, and they are characterised by the expression of the master regulator transcription factor T-bet and by their secretion of IFN-γ and TNF (253). Th2 differentiation is dependent on IL-2 and IL-4 and they are characterised by the expression of the transcription factor GATA3 and production of IL4, IL5, and IL-13 (252). Tfh cell differentiation requires IL-6 and IL-21, which in turns upregulate the transcription factor STAT3, and they are characterized by high expression of the receptors PD-1, CXCR5, and ICOS (254). Th1 and Th2 cells promote the licensing of DCs, which in turn promote the activation and differentiation of $CD8^+$ T-cells and B-cells. The Tfh migrate into germinal centres and provide crucial support to B-cells undergoing affinity maturation. Similar to $CD8^+$ T-cells, the $CD4^+$ T-cells become exhausted in chronically infected individuals, although the process has been less extensively studied in this T-cell population (143). Due to their role as a key the coordinator of the adaptive immune response, the progressive loss and exhaustion of this population eventually causes a collapse of the immune system (143). Further, the HIV reservoir has been found to be enriched among $CD4^+$ cells expressing exhaustion markers (255). The extensive exhaustion of memory $CD4^+$ T-cells, the main target cells of HIV replication, has therefore been suggested to contribute to the maintenance of the latent reservoir. In line with this, administration of antibodies blocking CTLA4 and/or PD-1 has been seen to increase cell bound RNA in $CD4^+$ T-cells, but not in plasma (256, 257). This suggests that immune checkpoint blockers (ICBs) might both help induce the latent reservoir, and improve the effector functions of HIV specific $CD4^+$ T-cell and $CD8^+$ T-cells (248). However, due to the high risk of adverse effects associated with ICBs, this therapy is so far only used to treat PLWH1 that have been diagnosed with leukaemia (258, 259).

B-cell mediated immune responses

Following the rearrangement of the V(D)J segments and the creation of a functional BCR, immature/transitional B-cells leave the bone marrow and migrate to the spleen to mature into naïve B-cells (260). The naïve B-cells thereafter circulate between secondary lymphoid organs (SLOs) until they either encounter their cognate antigen or die, typically within six week (261). Although B-cells can encounter and become activated by soluble antigens in blood and SLO, the most efficient way has been shown via DCs, macrophages, or follicular DC (FDCs) presentation of antigen to the B-cells in the SLO (205). The DC are capable of capturing antigen in the periphery and migrate to the SLO; the SLO resident macrophages sample the lymphatic fluid passing through the SLO and bind e.g. immune complexes and opsonised viruses; and the FDC can bind antigen through complement and Fcγ receptors (262). Once the naïve B-cell encounter its cognate antigen, it can go through either T-cell-dependent or T-cell-independent activation. During a primary HIV infection, Th cells provide help at the border between the B-cell follicles and T-cell zone of the SLO, which induce the B-cells to differentiate into short-lived plasmablasts that provide rapid production of low affinity antibodies (263). High affinity antibodies are produced following affinity maturation of the B-cells, which requires the activated B-cell to re-enter the follicle and either establish a new germinal centre (GC) or to enter an existing one (264). In the dark zone of the GC the B-cells proliferate rapidly and undergo activation-induced cytidine deaminase (AID)-induced somatic hypermutations (SHM). The B-cells then compete for the binding of the cognate antigen, presented on FDCs, using the BCR. The B-cell with the highest affinity BCR will bind the cognate antigen and present it to Tfh cells in the light zone of the GC (265). B-cells receiving help from Tfh cells can either re-enter the dark zone to go through another cycle of affinity maturation, or differentiate into memory B-cells (MBCs) or plasmablasts/plasma cells (266). In addition to SHM, affinity maturation can also include isotype class switching (265, 267). The five different antibody isotypes, IgM, IgD, IgG, IgA and IgE, have unique structural features that regulate the function of the antibodies (268). For example, the pentameric IgM can efficiently activate the complement cascade upon antigen binding, while IgGs show enhanced Fc-receptor (FcR)-mediated effector functions, such as ADCC. Class-switching therefor represent another way of adapting the immune response towards the incoming pathogen.

In PLWH, class switching to IgG1 and IgG3 increases both the neutralisation capacity and FcR-mediated effector function of the anti-HIV antibodies (268). Importantly, in the RV144 vaccine trial, the only HIV-1 vaccine trial to have shown evidence of efficacy, protection against HVI-1 acquisition was attributed to mounting of IgG1 and IgG3 antibodies targeting the V1/V2 region and increased ADCC activity (269). Class switching to IgG1/IgG3 has been shown to be regulated by the T-bet, the Th1 master regulator transcription factor (270). T-bet expression in B-cells has been shown to be required for the clearance of viral infections in mice (271), and plays a key role in antiviral responses in human too (270). In addition to

B-cells and Th1-cells, T-bet is also expressed in DC, $CD8^+$ T-cells, and NK cells as part of a multi-cell type orchestrated Th1-associated antiviral immune response. In addition to their role in infections, T-bet expressing B-cells have also been suggested as the source of autoantibodies (272). As molecular mimicry is a well-established immune evasion mechanism by several pathogens, peripheral B-cell tolerance is commonly decreased during virus infection to allow self-reactive B-cell to undergo somatic hypermutations and increase their affinity for foreign antigens (273-275). This has been shown to be especially important in PLWH, where neutralising antibodies (nAbs) have been suggested to often arise from self-reactive antibodies (276-278).

Following the clearance of an invading pathogen, the frequency of pathogen-specific B-cells gradually decreases (279). However, in chronic infections such as HIV, the persistent exposure to antigens and proinflammatory cytokines drives exhaustion of B-cells (144). Within PLWH1 and PLWH2, an expansion of hyperactivated atypical MBC (atMBC) has been observed (144, 242, 280-283). The atMBCs can be identified by their downregulation of memory markers, such as CD21 and CD27, upregulation of inhibitory receptors, such as Fcrl4, Fcrl5, and CD95, and expression of T-bet (284-286). T-bet expressing memory B-cells were recently shown to dominate the HIV-specific B-cell response (287), but their role in protective HIV immune responses is still not known (288). The production of high-affinity antibodies is dependent on B-cells receiving help from Tfh during affinity maturation. However, the exhausted Tfh in PLWH1 has been shown to provide inadequate help, which hinders the development of nAbs (289). In addition, the expansion of Th1 cells and the cytokine milieu in the follicles has been suggested to promote extrafollicular B-cell responses (288). In line with the importance of GCs for affinity maturation, HIV-1-specific extrafollicular T-bet expressing B-cells have been shown to have lower BCR mutation frequencies and neutralisation capacity compared to HIV-1-specific GC B-cells (288). More research is needed to define if, and under which circumstances, T-bet expressing B-cells provide an immunological benefit to the host.

Understanding how broadly nAbs (bnAbs), nAbs that capable of neutralising a broad range of HIV strains, are developed in PLWH is vital for development of both prophylactic and therapeutic vaccines. The emergence of bnAbs in PLWH1 is very rare, and commonly take several years to develop (290). In contrast, the antibody response among PLWH2 has been found to be broader and more potent compared to PLWH1 (152, 153, 291-295). In PLWH1, bnAbs have been found to often originate from naïve B-cells with uncommon precursor traits (290). Extensive research is ongoing to try to stimulate the activation and sequential affinity maturation of these rare naïve B-cells, for the purpose to trigger vaccine-elicited HIV-specific bnAbs (296, 297).

Treatment

The first antiretroviral drug used to inhibit HIV-1 replication, azidothymidine (AZT), was introduced in 1987 (298). However, it was not until the introduction of combination triple-drug antiretroviral therapy (ART) in the mid-1990s that the treatment resulted in sustained reduction of pVL and increased $CD4^+$ T-cell count (299, 300). Initially, the three-drug regimen consisted of two nucleoside RT inhibitors (NRTIs) and one protease inhibitor (PI) (299, 300). In addition to these two classes, several additional classes of ART have been developed. These include non-nucleotide RT inhibitors (NNRTIs), IN strand transfer inhibitors (INSTIs), and CA inhibitor (301). The NRTIs are nucleotide analogues that are incorporated into nascent cDNA during reverse transcription and prevent the addition of additional nucleotides. In contrast, NNRTIs, PIs, INSTI, and CA inhibitors bind directly to their target protein and introduces structural changes of the protein, which inhibits their function. As available antiretroviral drugs have been developed for HIV-1 proteins, they are therefore typically less effective, or not effective at all, against HIV-2 (301). HIV-2 viruses have been found to be intrinsically resistant towards all NNRTIs, as well as some PIs (9, 301). In addition, the $CD4^+$ T-cell recovery has been shown to be slower among PLWH2 compared to PLWH1, especially for individuals with <200 $CD4^+$ T-cells/µl (302, 303).

Despite the success of ART in inhibiting virus replication and disease progression, PLWH need to receive lifelong ART treatment since the latently infected cells are not killed. Following treatment cessation, viral rebound is typically detected within a few months (304). Extensive research is now focused on achieving viral control following ART treatment interruption (305). However, the only individuals cured from HIV-1 so far have received CCR5Δ32/Δ32 haematopoietic stem cell (HSC) transplantation, as part of their treatment of leukaemia (306-308).

Materials and methods

Study cohort and sample collection

All four studies in this thesis have been performed using samples collected from participants in an occupational cohort in Guinea-Bissau (39, 40, 145). The cohort includes police officers recruited from the Guinea-Bissau police force, from both rural and urban areas of the country. The cohort was established in February 1990 and study participants were included until September 2009, and individuals followed up until September 2011. Following inclusion in the cohort, blood samples were collected for serology testing and general health check-up was performed. At enrolment, each individuals donated a blood sample for serology testing and received a general health check-up. Follow-up visits were scheduled at 12-18-month intervals. In 2013, 2015 and 2017 additional blood samples were collected from previously enrolled police officers in the capital Bissau, but not from other regions of the country. The cohort, currently studied within a network of scientist in Sweden and Guinea-Bissau named "Sweden Guinea-Bissau Cohort Research (SWEGUB) group, is unique since it contains a large number of HIV-1 and HIV-2 seroconverting individuals, i.e. individuals that were seronegative at enrolment and then tested positive for HIV-1 and/or HIV-2 at follow-up visits. The estimated date of infection was determined as the date between the last seronegative and the fist seropositive sample. In addition, the cohort also contain seroprevalent individuals, i.e. individuals that tested positive for HIV-1 or HIV-2 at inclusion. Following a seropositive sample, absolute $CD4^+$ T-cell count and CD4% were determined using flow cytometry, and blood plasma were stored for subsequent analyses. To allow the comparison of seronegative and seropositive individuals, longitudinal $CD4^+$ T-cell dynamics was studied in age and sex matched HIV seronegative individuals each time a new seroprevalent or seroconverting individual was identified. Following a seropositive sample, each individual received counselling and were informed about ART (following the introduction of ART) and referred to the Guinea-Bissau National HIV treatment program initiated in 2005.

In **papers I-III** blood samples obtained in 2017 from PLWH1, PLWH2 and PLWHD, as well as HIV seronegative individuals, were analysed. In **paper IV**, archived blood plasma samples obtained between 1993 and 2008 from PLWH1 and PLWH2, as well as HIV seronegative individuals, were used to analyse the plasma proteome using MS-DIA.

Ethical considerations

The studies performed in this thesis have been approved by the ethical committees at both Lund University and the Ministry of Health in Guinea-Bissau. Prior to 2011 oral consent was required, but at the following visits written consent was also received.

The study participants received pre-test counselling, and it was voluntary to receive the test result. As the cohort was established before the national ART program was started, the participants diagnosed before the program was started would not be able to receive effective treatment. For this reason, some participants decided not to know their test results. The individuals that were informed about their positive results received post-test counselling. However, after the establishment of the national ART program in 2005, almost all individuals chose to receive their results. All individuals that were notified about a positive HIV test result received post-testing counselling and were also referred to the national ART program. However, the health care system in Guinea-Bissau still faces a lot of challenges at all stages of the HIV treatment continuum (309), and many individuals that known their HIV status do not receive successful treatment (310).

Flow cytometry

In **paper I** and **II** we immunophenotyped the $CD8^+$ T-cells and B-cells, respectively. Whole blood samples were collected using Cyto-Chex BCT tubes (Streck) and the samples were stored for up to 14 days before they were labelled by antibodies and analysed using a BD Fortessa instrument (BD Biosciences). Prior to antibody labelling, the whole blood samples were divided into two portions and labelled by two separate antibody panels, for phenotyping of B-cells and T-cells, respectively.

Raw data was exported from the Fortessa and compensated using the FlowJo™ Software (BD Life Sciences). For the FlowSOM analyses in **paper I** and **II**, $CD14^-CD19^-CD3^+CD8^+$ T-cells and $CD3^-CD14^-CD19^+$ B-cells were exported, respectively. A combination of hierarchical and Boolean gating was performed in FlowJo to manually determine the frequency of populations of interest.

Viral load determination

Plasma samples were collected from each participant using EDTA vacutainer tubes (BD Biosciences) during the 2017 sampling and an in-house developed RT-qPCR protocols were used to determine the pVL of PLWH1 and PLWH2 (311). The quantification limit was 75 RNA copies/ml for both HIV-1 and HIV-2 (311), and PLWH2 with viral loads below this limit were classified as aviraemic. ART treated individuals with a pVL below 1000 copies/ml were classified as successfully treated

and those with pVL above 1000 copies/ml were classified as suboptimally treated (312).

Multiplexing assays for protein detection

In **paper I**, absolute plasma concentrations of IP-10, soluble CD14 (sCD14), and b2-microglobulin (B2M) were determined using the Magnetic Luminex assay (R&D Systems Inc.) on the Bio-Plex 200 platform (Bio-Rad Laboratories Inc.). In **paper II** we utilised a proximity ligation assay approach to determine the relative concentration of 92 plasma proteins using the Olink® Immuno-oncology panel (Olink Proteomics). The relative concentrations are reported in the arbitrary unit Normalized Protein eXpression (NPX) in a Log2 scale.

Data-independent acquisition mass spectrometry

The top 14 most abundant plasma proteins (albumin, alpha-1-acid glycoprotein, alpha-1-antitrypsin, alpha-2-macroglobulin, apolipoprotein A1, fibrinogen, haptoglobin, and transferrin, and the kappa and lambda light chains of immunoglobulin (Ig) G, IgA, IgM, IgD, and IgE) constitute approximately 95% of all plasma proteins. We therefore decided to deplete these proteins to increase the detection of less abundant plasma proteins (313, 314), using the High Select™ Top14 Abundant Protein Depletion Mini Spin Columns (ThermoFisher Scientific). The remaining proteins were thereafter denatured, reduced, alkylated, and finally digested by Trypsin. For each sample, one µg of digested peptides was injected onto the Dionex Ultimate 3000 Rapid Separation liquid chromatography (RSLC) nano Ultra-performance LC system coupled to an Orbitrap Exploris 480 MS with FAIMS Pro interface (Thermo Scientific). The raw peptide precursor and fragment ion intensities data was exported from the Spectronaut (Biognosys) software. Using the raw data, the relative protein concentration was determined using the *iq* package in R (315). Proteins with more than 20% missingness were excluded from further analysis (316), which is the consensus level in the field. As missing values will prevent normalisation of the data, the missing values were replaced with imputed values. At imputation the missing values were replaced with a random value between 1, so that all values would remain positive once transformed to a logarithmic scale, and the lowest detected value, which was assumed to represent the detection limit of the instrument. The imputed dataset was thereafter normalised by the variance stabilizing normalization algorithm (317).

We chose the MS-DIA approach to analyse the archived plasma samples in **paper IV** since we hypothesised that this method was less sensitive to structural modifications of proteins compared to e.g. antibody based approaches. Since the samples have been

stored for up to 30 years and used for several projects, the multiple freeze-thaw cycles may have impacted the structural integrity of the plasma proteins, as well as increase the rate of protein degradation. However, as the proteins are denatured and digested by trypsin prior to mass spectrometry analysis, we hypothesised that this method is less sensitive to freeze-thaw cycles compared to structure-dependent methods. In addition, even if the proteins are partially degraded due to the freeze-thaw cycles, the mass spectrometer will still be able to detect the peptides.

Statistical analyses and bioinformatics

General statistics

The analysis of variance (ANOVA) or Kruskal-Wallis tests were used for comparisons of multiple groups when the data was either parametric or non-parametric, respectively. Pearson correlation test or Spearman rank test were used for parametric and non-parametric data, respectively. In **paper IV** we compared the relative protein intensity between different HIV status groups using a linear regression model with sample age as a covariate. Dunn´s multiple comparisons test was used to correct for multiple testing in **paper I** and **II**, while the Benjamini-Hochberg (BH) procedure was used in **paper III** and **IV**.

Kaplan-Meier analysis

In **paper IV** we performed a Kaplan-Meier analysis to compare the time to AIDS onset between two clusters of PLWH (318). AIDS onset was determined as CD4% of ≤14% (185). A log-rank test was performed to compare the two groups. Individuals that did not reach AIDS during follow-up, or that were lost during follow-up, were right censored at their last visit.

Simplified Presentation of Incredibly Complex Evaluations

In **paper I**, we used the simplified presentation of incredibly complex evaluations (SPICE) method to study the expression, or the lack of expression, of proteins on the $CD8^+$ T-cells (319). Although this user-friendly method allows a comprehensive analysis of the different markers combinations, the expression of proteins was only assessed in a binary fashion in our analysis.

FlowSOM

Flow cytometry data has historically been analysed primarily using a manual gating strategy where cells are either regarded as expressing or not expressing a protein of

interest. However, this approach has several limitations. One limitation is that this approach typically does not take in account the variation of expression of each protein, but rather assumes a binary expression pattern. Although this type of analysis is suitable for the detection of e.g. CD4 or CD8 on T-cells, it is less suitable when studying activation or differentiation of cells where the expression of several proteins increases or decreases gradually (320-322). In addition, the increased dimensionality of flow cytometry experiments (up to 50 in new spectral flow cytometers) makes analysis of one to two proteins at a time too time consuming. To address these limitations, several different groups have developed different types of clustering algorithms to analyse high-dimensional flow cytometry data.

For **paper I** and **II**, we utilised the FlowSOM algorithm to perform unsupervised hierarchical cluster analysis of the flow cytometry data (320, 323). When performing hierarchical clustering, the number of clusters in the data is not pre-determined. Instead, the data is clustered a certain number of times, 100 times in our two studies, and the clustering algorithm is used to determine the optimal number of clusters for the dataset (323).

Dimensionality reduction

When analysing high-dimensional data, presenting the data in one or two dimensions becomes very time consuming and increases the risk of missing important and novel information. As the dimensionality reduction can be performed in an unsupervised manner (324), it can be used to identify clusters of cells that express a combination of markers that have not been previously studied. We utilised the uniform manifold approximation and projection (UMAP) technique to reduce the multidimensionality of the flow cytometry data down to two dimensions (325). The strength of UMAP over the previously dominant dimensionality reduction technique t-distributed stochastic neighbour embedding (t-SNE) is that it scales better when using datasets with a large sample size (324). In addition, it preserves the global data structure better than t-SNE (i.e. separate clusters that are closer to each other are more likely to be more similar on a UMAP plot compared to a t-SNE plot), which makes it easier to visualise the continuity of the cell subsets during processes such as exhaustion and differentiation (324). This makes results from pseudotime trajectory inference analyses, where an ongoing differentiation process is modelled, more easily visualised (321).

Similar to UMAP, a principal component analysis (PCA) can be done to reduce the dimensionality of a dataset (326). In **paper II** we performed a PCA to compare the composition of the B-cell compartment (of FlowSOM clusters) between HIV status groups. In **paper III** and **IV**, we performed PCA analyses to identify technical aspects, such as sample age and preparation order, that could influence the relative protein intensity of all proteins within each individual.

Pseudotime trajectory analysis

As previously mentioned, protein expression typically changes in a gradual way during activation or differentiation of cells. As the FlowSOM clustering algorithm can identify cells that only differ in subtle protein expression changes, the clusters can be used to recreate a differentiation trajectory. In **paper II**, we used the Slingshot algorithm to infer B-cell differentiation (321, 322). Based on literature, we defined transitional B-cell as the least differentiated population detected and specified this as the starting population for the pseudotime trajectory. The Slingshot algorithm then generated a minimum spanning tree, based on the gradual increase or decrease of protein expression in each cluster, to determine the pseudotime trajectories found in the dataset.

Kyoto Encyclopedia of Genes and Genomes pathway analysis

To reduce the complexity of the mass spectrometry data in **paper IV**, and to gain mechanistic insights into the impact of HIV-1 and HIV-2 infections, we performed a Kyoto encyclopaedia of genes and genomes (KEGG) pathway analysis. The KEGG pathway database contains a collection of manually drawn pathways, based on experimental evidence. Although commonly used to analyse intracellular protein or gene expression, KEGG pathway analysis is also used when studying changes in the blood plasma proteome (327).

Analysis of disease progression

In **paper IV**, we used CD4% at time of sample donation and CD4% midpoint as markers of disease progression (328). The CD4% midpoint was defined as the midpoint on a regression line fitted to the longitudinal CD4% measurement.

As previously mentioned, CD4% has been found to be a more reliable marker of disease progression in countries with a high pathogenic burden (187, 190), and has therefore been the preferred marker of disease progression within the Guinea-Bissau police cohort (39, 40).

Generation of tissue engagement signatures

To produce tissue engagement signatures, we used a previously published list of tissue-specific transcriptional signature dataset (329). The proteins that overlapped between the datasets in **paper III** and **IV**, and a previously published tissue-specific transcriptional signature dataset were used to create the tissue engagement signatures used for our studies (329).

Identification of cell type enhanced proteins

To improve our understanding of the impact of HIV-1 and HIV-2 infections on different cell types, we utilised the information from the Human Protein Atlas (HPA) to identify plasma proteins that were cell type enriched (330, 331). According to the HPA, the cell type enriched genes have an expression in 1-10 cell types that is at least four times higher compared to the remaining 78-69 cell types (based on single cell RNA sequencing data).

Main findings and discussion

The overarching aim of this doctoral thesis was to investigate virus-host interactions in HIV-1 and HIV-2 infections, with particular focus on; 1) studies on the impact of viraemia on B and T cell perturbations, and the plasma proteome, in HIV-2 infection; and 2) identification of blood plasma signatures associated with HIV disease progression.

The impact of viraemia on B- and T-cell perturbations, and the plasma proteome, in HIV-2 infection

A large fraction of PLWH2 was initially believed to be able to control their infection without ART. A study found that the mortality rate of aviraemic PLWH2 was similar to that of the control population, suggesting that that aviraemic HIV-2 infection had limited impact on the infected host (44). However, the SWEGUB CORE group recently reported that the majority of HIV-2 infected individuals most likely will progress to AIDS in the absence of ART (145). In line with this, previous studies have reported that aviraemic PLWH2 harbour PBMCs expressing HIV-2 RNA and CA expression in colon tissue, which indicate that active HIV-2 replication is still ongoing (350, 351). We therefore hypothesized that HIV-2 replication is ongoing in both viraemic and aviraemic PLWH2, and that the replication would induce both immunopathology and tissue damage. To evaluate the impact of viraemia in HIV-2 infections, we performed an in-depth characterisation of immunopathologies and plasma proteome profiles in viraemic and aviraemic PLWH2 using flow cytometry and DIA-MS. As a comparison, we also included PLWH1 and HIV seronegative individuals.

Main findings

- In **paper I**, unsupervised hierarchical cluster analysis showed a cluster of activated and exhausted $CD8^+$ T-cells that distinguished viraemic PLWH1 from successfully treated PLWH1 and viraemic PLWH2 from treatment naïve aviraemic PLWH2.

- The in **paper I** identified $CD8^+$ T-cell cluster was associated with pVL, CD4% and plasma levels of inflammation markers, including IP-10, soluble CD14 (sCD14), and beta-2 microglobulin (B2M).
- In **paper I**, both viraemic and aviraemic HIV-2 infection skewed the $CD8^+$ T-cells towards exhaustion and reduced responsiveness, i.e elevated expression of TIGIT and reduced expression of CD226.
- In **paper II**, unsupervised hierarchical cluster analysis showed an expansion of T-bet^{high} hyperactivated and T-bet^+ proliferating memory-like B-cells in both viraemic and aviraemic PLWH2.
- In **paper II**, pseudotime trajectory inference analysis showed that HIV-1 and HIV-2 infection promoted terminal B-cell differentiation towards T-bet-associated hyperactivation.
- In **paper III**, both viraemic and aviraemic PLWH2 had elevated tissue engagement signatures, although the changes tended to be more obvious in viraemic individuals.
- In **paper III**, blood plasma profiling suggested that macrophage and endothelial cell engagement was associated with HIV immunopathology in PLWH2.

$CD8^+$ T-cell activation

In **paper I**, we investigated the impact of HIV-1 and HIV-2 viraemia on the activation and exhaustion state of $CD8^+$ T-cells by analysing the expression of differentiation markers (CD45RO, CCR7), a co-stimulation marker (CD226), activation markers (CD38, HLA-DR), inhibitory receptors (2B4, PD-1, TIGIT), and the exhaustion-associated transcription factor Eomes. Unsupervised hierarchical cluster analysis indicated a cluster of activated and exhausted memory $CD8^+$ T-cells with high expression of the exhaustion markers 2B4, PD-1, TIGIT and Eomes. The frequency of this cluster was higher in viraemic PLWH1 compared to PLWH1 on ART, in viraemic PLWH2 compared to aviraemic PLWH2, and in aviraemic PLWH2 compared to HIV seronegative individuals. Further in-depth analysis of $CD8^+$ T cells expressing CD38, HLA-DR, 2B4, PD-1, and TIGIT suggested that the frequencies of highly exhausted memory cells coexpressing all five markers could distinguish aviraemic PLWH2, but not successfully treated PLWH1, from HIV seronegative individuals. Furthermore, the frequency of these cells was associated with CD4%, pVL, and general inflammation in PLWH2 (IP-10, sCD14 and B2M.

Finally, we investigated the relationship between the two receptors CD226 and TIGIT in PLWH2. As previously described, these two receptors compete for the binding of PVR, and reduced CD226 expression and increased TIGIT expression has been associated with reduced co-stimulation responsiveness and increased

exhaustion (332, 333). We found that both viraemic and aviraemic HIV-2 infection induced a clear skewing of the CD226/TIGIT axis, and a significant expansion of $CD226^{-}TIGIT^{+}$ cells and reduction of the $CD226^{+}TIGIT^{-}$ memory $CD8^{+}$ T-cells were observed among both viraemic and aviraemic PLWH2 compared to HIV seronegative individuals.

Taken together, these findings suggest that although $CD8^{+}$ T-cell activation and exhaustion is associated with viraemia and inflammation in PLWH1 and PLWH2, aviraemic PLWH2 also display signs of immunopathology. These results are in line with previous findings showing that aviraemic PLWH2 harboured elevated frequencies of activated and exhausted $CD4^{+}$ T-cells (148). The analysis of the expression of both co-stimulatory receptors and inhibitory receptors allowed us to dissect the transition of the $CD8^{+}$ T-cells from activation towards exhaustion. Both viraemic and aviraemic HIV-2 infection was associated with a transition of $CD8^{+}$ T-cells from the expression of the activation marker 2B4 and the co-stimulatory receptors CD226 towards exhaustion, as defined by the loss of the co-stimulatory receptor CD226 and upregulation of the inhibitory receptors PD-1 and TIGIT. The expression of CD226 is needed for the activation of $CD8^{+}$ T-cells by non-professional APCs (334), and the loss of CD226 on bulk $CD8^{+}$ T-cells can contribute to loss of $CD8^{+}$ T-cell responsiveness to other infections and malignant cells (335-338). The association between the frequency of terminal exhausted $CD8^{+}$ T-cells and the three inflammation markers suggest that the chronic inflammation in aviraemic PLWH2 drives the expansion of the exhausted $CD8^{+}$ T-cells.

Taken together, these results suggest that HIV-2 infection induces the propagation of exhausted $CD8^{+}$ T-cells also in aviraemic PLWH2. The underlying mechanisms behind this are still unknown, but they might include low-level replication in tissue (339, 340).

B-cell activation

In **paper II**, we immunophenotyped the B-cells, in the same study participants as in **paper I**, to gain a broader understanding of the impact of aviraemic and viraemic HIV-2 infections on the adaptive immune system. In this study, we focused on the expression of the Th1-associated transcription factor T-bet in B-cells, which has previously been reported to be expressed by the majority of the HIV-1 Env-specific B-cells (287). Moreover, the expansion of virus-specific T-bet expressing B-cells has been reported to be driven by HIV-1 antigen exposure (286, 287). However, T-bet expression in B-cells from PLWH2 had not been studied before.

We identified twelve different B-cell clusters using unsupervised hierarchical cluster analysis, representing different differentiation stages and activation statuses. A principal component analysis of the cluster frequencies suggested that both viraemic and aviraemic HIV-2 infection induced a statistically significant reorganisation of the B-cell compartment. Viraemic and aviraemic PLWH2 displayed elevated frequencies of a cluster containing hyperactivated $T\text{-}bet^{high}$ B-cells and a cluster containing proliferating $T\text{-}bet^{+}$ B-cells, as well as reduced

frequencies of resting naïve-like B-cells. The elevated frequency of the cluster of hyperactivated T-bet^{high} B-cells in PLWH2 was associated with lower CD4%, and higher pVL as well as plasma levels of the Th1-associated cytokines IL-12, IL-18, TNF-α, IFN-γ, CXCL9, and IP-10. A trajectory inference analysis suggested that viraemic HIV-1 and HIV-2 infections in particular, but also aviraemic HIV-2 infection, promoted terminal differentiation of B-cells into activated T-bet expressing B-cells.

Taken together, and in line with the results from **paper I**, we observed that both viraemic and aviraemic HIV-2 infection induce B-cell perturbations. Although the expansion of T-bet expressing B-cells has been reported to be dependent of viraemia in PLWH1 (287), we observed an increase of this population among aviraemic PLWH2. This contrasted with PLWH1 on successful ART, which did not display increased frequencies of T-bet expressing B-cells. The functional role of T-bet expressing B-cells *in vivo* are still not fully known, but it has been suggested to play an important role in the clearance of viral infections (270, 286, 341). However, in the context of chronic inflammation, e.g. during chronic infections or autoimmune diseases, these cells have been associated with elevated levels of autoantibodies, and their role in protective and pathological immune responses are still to be elucidated (270, 342-344). As HIV-specific B-cells were not labelled in our study, the contribution of the HIV-specific response in relation to the expansion of non-HIV-specific T-bet expressing B-cells was not assessed. However, the HIV-specific B-cells have been reported to represent as little as approximately one percent of the class-switched memory B-cells (287). Taken together with the fact that the frequency of the T-bet expressing B-cells in our study far exceeded this, the expansion observed by us is likely due to non-HIV-specific activation. The observed correlation between the plasma levels of Th1-associated pro-inflammatory cytokines and the frequency of hyperactivated T-bet^{high} B-cells may also suggest that the expansion is due to the heightened inflammatory state of PLWH. Indeed, *in vitro* experiments have shown that CD40 ligation and Th1-associated cytokine mediated signalling can rescue potentially autoreactive B-cells from BCR-mediated TLR9 signalling-induced apoptosis and promote the expression of T-bet in these cells (270, 342). BCR-mediated TLR9 signalling can occur through binding of e.g. protein-DNA complexes, such as histones, released from dying cells. The induced cell death is believed to act as a peripheral tolerance mechanism to reduce autoreactivity (342). However, the ability of Th1-associated cytokines to reverse this mechanism is believed to be important during infections when pathogen DNA/RNA is produced (270, 274, 286). Still, the contribution of the T-bet expressing B-cells to protective B-cell responses needs to be further investigated. A recent study suggested that T-bet expressing MBCs were primarily located outside of the GC, and the BCR mutation frequency of HIV-specific T-bet^{+} MBC was lower compared to HIV-specific GCBCs (288). In addition, the neutralisation breadth of cloned BCRs from T-bet^{+} MBC was lower compared to GCBC, and the frequency of extra-GC T-bet^{+} MBC were inversely correlated with the plasma neutralisation

breadth of the study participants (288). However, influenza infection in mice and humans has been shown to give rise to different subpopulations of T-bet expressing B-cells, with distinct migratory and functional attributes (345). Thus, this suggest that T-bet expression in B-cells may vary according to compartment, and more research is needed to elucidate the role of the subpopulations in effective antiviral responses.

Taken together, our results suggest that both viraemic and aviraemic HIV-2 infection induces a proinflammatory milieu that promotes the expansion of T-bet expressing B-cells. Further research is needed to determine if these cells provide an immunological benefit to the host, or if they represent an immunopathology-associated population.

Plasma proteome profiling

In **paper III**, we characterised the blood plasma proteome of the study participants included in **paper I** and **II**. Previously, in PLWH1, the persistent high levels of both pro-inflammatory cytokines and viral proteins have been shown to contribute to fibrotic remodelling, endothelial and epithelial dysfunction, and hypercoagulation (209, 346, 347). However, less is known about the impact of HIV-2 infection on the plasma proteome profile, particularly in aviraemic HIV-2 infection. To study the impact of both HIV-1 and HIV-2 infection on the proteome, we utilised publicly available databases to define plasma signatures suggestive of specific tissue engagement, and to define cell type enhanced proteins. Although both viraemic and aviraemic PLWH2 displayed signatures of colon and heart tissue engagement, viraemic infection was associated with engagement of more tissues. Increased plasma signatures of spleen and lung engagement were associated with elevated frequencies of exhausted $CD8^+$ T-cells in PLWH2. In depth analysis of tissue and cell type enriched proteins identified 19 proteins that were associated with $CD8^+$ T-cell and B-cell immunopathology. Further analysis suggested that the most common cellular origins of these proteins were macrophages and endothelial cells.

Taken together, these results suggest that similar to HIV-1 infection, HIV-2 infection induces bystander cell engagement. In line with **paper I** and **II**, several of the immunopathology-associated proteins were differentially expressed in aviraemic PLWH2 compared to HIV seronegative individuals, further supporting our previous findings that aviraemic PLWH2 display signs of continuous pathologic processes (145, 148, 243, 281, 348, 349). The observed signs of macrophage engagement are in line with previous studies reporting activation of myeloid cells throughout HIV-2 disease progression (211, 217). In addition to activation of macrophages, release of proteins could also be a sign of increased macrophage cell death. Since both HIV-1 and HIV-2 can infect macrophages, it is possible that the observed increase of macrophage-originating proteins is associated with ongoing HIV replication in macrophages. The expression of Vpx allows HIV-2 to counteract the restriction factor SAMHD1, which increases the permissibility of myeloid cells (350). However, the increased reverse transcription in myeloid cells have also been

linked to increased sensing of intracellular cDNA in DCs, resulting in increased type 1 IFN signalling (139). In addition to activation of macrophages as a result of direct infection, macrophages have also been reported to be activated by several indirect pathways. These include e.g. elevated levels of pro-inflammatory cytokines, tissue damage, and increased microbial translocation (351). Of note, the SWEGUB CORE group has previously noted that decreasing CD4% was associated with elevated plasma LPS levels in both PLWH1 and PLWH2 (123). The combination of systemic inflammation and increased LPS levels could therefore further explain the observed increased engagement of macrophages.

Although certain HIV-1 and HIV-2 strains have been reported to have the ability to infect endothelial cells (352), the observed engagement of endothelial cells most likely involve an indirect pathway (346, 347). Endothelial cell activation has been well studied in PLWH1, particularly in the cardiovascular research field (346, 347), but less is known about the impact of HIV-2 infection. The association of the endothelial derived proteins with immunopathology-associated $CD8^+$ T-cell and B-cell populations suggest that this could be due to the chronic inflammation. Indeed, chronic inflammation is a well-known mechanism of endothelial cell damage and activation.

Taken together, these results further support the findings in **paper I** and **II** that aviraemic PLWH2 displays signs of immune perturbations and pathogenic activities. The attenuated tissue and bystander cell types engagement profile of aviraemic PLWH2 compared to viraemic PLWH2 likely reflects the lower degree of immune activation and HIV-2 replication induced death of infected cells, which are known causes of bystander cell death and tissue damage (120, 346).

Conclusions from the investigation of the impact of HIV-2 viraemia on immunophenotypes and the plasma proteome profile

In conclusion, **paper I-III** showed that both viraemic and aviraemic PLWH2 display signs of disease progression. The underlying mechanisms behind disease progression in the absence of detectable viraemia is still largely unknown. However, the study of progressing HIV-1 viraemic controllers (HIV-1 VCs, i.e. ART naïve aviraemic individuals), and so-called immunological non-responders (i.e. PLWH1 on ART with virological suppression with persistently low or declining $CD4^+$ T cell counts), have implicated disruption of lymphopoiesis as a potential mechanism for viraemia independent disease progression (353, 354). This mechanism includes both effects directly on the hematopoietic stem and progenitor cells (HSPC), as well as reduced thymic output (353-358). Progressing HIV-1 VCs have been reported to both harbour lower frequencies of circulating $CD34^+$ hematopoietic progenitor cells (HPCs) and to have HPCs with reduced lymphopoietic potential in comparison to non-progressing HIV-1 VCs (353). In addition, both slowly progressing PLWH2 and immunologically responding PLWH1 have been found to have a higher thymic

output compared to chronically progressing PLWH2 and immunologically non-responding PLWH1, respectively (355-359). Reduced lymphopoietic potential of HSPCs and reduced thymic output have been attributed to both direct binding of HIV-1 and HIV-1 proteins to the HSPCs and thymocytes, and to the elevated plasma concentration of pro-inflammatory cytokines (354). Taken together, these results indicate that HIV-1 both induces exhaustion of existing T-cells, as well as impairs the replenishment of the senescent T-cells by *de novo* produced naïve T-cells. Although the lymphopoietic potential of HSPCs in PLWH2 has not been investigated, $CD4^+$ T-cell decline in both PLWH1 and PLWH2 has been associated with a lower thymic output (356, 359), Furthermore, both PLWH1 and PLWH2 below the age of 45 years have been found to have reduced thymic output compared to HIV seronegative individuals (356, 359), suggesting that both HIV-1 and HIV-2 infection induces premature aging of the immune system. A combination of reduced thymic output, as reported by others (355-359), and exhaustion of the immune system, as observed both in **paper I-II** and in additional papers by us and others (146, 148, 283, 348, 349), could explain the slow but progressive depletion of $CD4^+$ T-cells in aviraemic PLWH2.

Although direct binding of HIV-1 and HIV-2 proteins to the HSPCs and thymocytes can impact lymphopoiesis, this is unlikely to drive the exhaustion in PLWH2 due to the low viraemia. Instead, the heightened inflammatory state of the PLWH2 is more likely to drive the exhaustion and aging of the immune system (146, 354). Still, the underlying mechanism behind the pro-inflammatory state of aviraemic PLWH2 are still not fully known. A potential explanation could be that low-level replication in tissue, which does not result in sufficient release of viruses into plasma for the detection by RT-qPCR, drives systemic inflammation. In line with this, previous studies have reported the detection of HIV-2 RNA and CA expression in PBMCs and colon tissue, respectively, in aviraemic PLWH2 (339, 340). Furthermore, the increased ability of DCs to sense HIV-2 cDNA compared to HIV-1 cDNA could potentially explain the heightened inflammatory state in aviraemic PLWH2 (139). In line with this, DCs sensing HIV-2 cDNA have been reported to release IP-10 (139), a cytokine we found to correlated with the expansion of pathogenic $CD8^+$ T-cell and B-cell populations in **paper I** and **II**, respectively. In addition, a recent study found that IP-10 was part of a group of proteins that could distinguish immunological responders from immunological non-responders (360). In addition to HIV replication-mediated inflammation, microbial translocation has also been associated with systemic immune activation in PLWH1 (124). In agreement with this, plasma LPS levels have been found to be inversely associated with $CD4^+$ T-cell levels during HIV-2 disease progression (123). Similarly, colon epithelial dysfunction has been reported to be distinguish immunological non-responders from immunological responders (361). Importantly, our findings in **paper III,** that aviraemic HIV-2 infection is associated with an engagement of multiple tissues and cell types further highlights the systemic impact of aviraemic HIV-2 infection.

In conclusion, our results show that aviraemic HIV-2 infection, potentially due to low-level tissue replication, triggers chronic immune activation in PLWH2. The chronic immune activation could in turn drive exhaustion of immune cells and HSPCs, which eventually could lead to a collapse of the immune system.

Identification of blood plasma signatures associated with HIV disease progression

A large variation in time from infection to AIDS onset has been reported among PLWH (7, 44, 137, 145, 362). To determine protein expression patterns that distinguish faster from slower progressors, we profiled plasma proteomes of PLWH1 and PLWH2, after three years of infection.

Main findings

- Increased leakage of sigmoid colon enhanced proteins distinguished PLWH1 from PLWH2 and HIV seronegative individuals.
- The level of sigmoid colon engagement was associated with CD4% and CD4% midpoint.
- Hierarchical cluster analysis showed that the plasma levels of 10 specific proteins distinguished two groups of PLWH that differed significantly in their time to AIDS onset.

In **paper IV** we characterised the plasma proteome of PLWH1, PLWH2, and HIV seronegative participants from the same occupational cohort as the study participants in **paper I-III**. A KEGG pathway analysis suggested that HIV-1 and HIV-2 infections induced similar reorganisations of the plasma proteome in the two infections. As the analysis of plasma signatures of tissue and cell type engagement could be used to identify proteins associated with immunopathology, we next investigated if this approach could be used to identify a plasma signature associated with faster or slower HIV disease progression. We found that signatures of increased protein leakage from sigmoid colon and increased secretion of proteins from spleen, two known HIV replication sites (363), was associated with disease progression. Although both HIV-1 and HIV-2 infection induced increased proteins from the spleen, the leakage of proteins from the colon sigmoid was higher in PLWH1 compared to both PLWH2 and HIV seronegative individuals. In addition, and in line with **paper III**, we found that both HIV-1 and HIV-2 infections were associated with engagement of several tissues and cell types. Furthermore, we identified 10 proteins whose plasma levels could be used to distinguish faster from slower HIV progressors.

Conclusions from the identification of blood plasma signatures associated with HIV disease progression

In conclusion, **paper IV** showed that HIV-1 and HIV-2 infections induce a similar reorganisation of the plasma proteome. However, in line with the faster disease progression rate of PLWH1 (7, 44, 145, 362), these changes appeared to be more obvious among PLWH1 compared to PLWH2. Thus, in **paper IV**, using the archived plasma samples and study participants with estimated data of infection we uniquely could analyse, side-by-side, the reorganisation of the plasma proteome in PLWH1 and PLWH2 after three years of infection. A comparison not possible to do in **papers I-III** due to different length of infection duration and survival bias. Thus, of particular interest, the results obtained in **paper IV** suggest that HIV-1 infection induces more leakage of proteins from the sigmoid colon, possibly due to increased cell death. These results are in line with previous reports that HIV-2 infection is associated with reduced impact on the gastrointestinal epithelium integrity compared to HIV-1 infection (340). In addition, the negative association between sigmoid colon protein leakage and CD4% are also in line with a previous report from our cohort showing that plasma LPS levels were inversely associated with CD4% in both PLWH1 and PLWH2 (123).

In addition to the impact of HIV infection on the sigmoid colon, we found that proteins released from both potential target cells and bystander cells were associated with disease progression. The systemic impact of HIV-1 on bystander cells have been attributed to both direct interactions with viruses and virus proteins, and to elevated levels of proinflammatory cytokines (209, 346, 347, 354, 364). However, as previously mentioned, in the context of HIV-2 infection, the impact is most likely driven by immune activation (146). In line with this, two previous studies have found inflammatory markers predictive of HIV-2 disease progression (147, 365). In our study, we identified ten proteins that could be used to distinguish faster from slower HIV progressors. Further analysis is required to determine if these ten proteins can distinguish faster from slower progressors independently of the HIV type.

In conclusion, plasma signatures indicative of increased engagement of target and bystander cells was associated with faster HIV disease progression rate. These findings motivate both further research on the role of bystander cells during HIV disease progression, as well as investigation of the potential for plasma markers of bystander cell engagement to serve as biomarkers for disease progression and treatment success.

Overall conclusion and future perspectives

The overall aim of this thesis was to compare virus-host interactions in HIV-1 and HIV-2 infections, and their associations with disease progression. In line with previous studies from us and others (123, 148, 217, 243, 281, 283, 348, 349), **paper I-III** showed that PLWH2 display similar signs of immunopathology and disease progression as PLWH1, despite low pVL and slower disease progression trajectory. In **paper IV**, we observed engagement of several tissues and cell types already within the first three years past the estimated date of infection, further highlighting the need for treatment as early as possible. We hypothesise that the enhanced ability of the innate immune system to sense HIV-2 compared to HIV-1 is responsible for the control of HIV-2 replication (139, 366), but also for the chronic immune inflammation observed in these individuals. The chronic inflammatory state may gradually exhaust the lymphopoietic potential of the host (353, 354), despite the low pVL. In line with this, several publications have reported a high fraction of PLWH2 act as immunological non-responders following ART initiation (302, 303, 367), a phenomenon found to be associated with reduced lymphopoietic potential of HSPCs and reduced thymic output (353-361). As the fraction of HIV-2 infected immunological non-responders has been reported to be inversely correlated with the CD4% at treatment initiation (303), it is important for PLWH2 to receive ART treatment as early as possible. However, the most recent HIV survey in Guinea-Bissau indicated that only 10% of PLWH2 received ART (310). Taken together with our findings, this clearly highlights the need to improve all areas of the treatment continuum in Guinea-Bissau, from increased testing to maintained retention of individuals within treatment programs (309), to prevent further HIV spread and HIV infection-related death.

Future perspectives

$CD8^+$ T-cells

In **paper I** we found that both HIV-1 and HIV-2 infections promote the transition of $CD8^+$ T-cells, from co-receptor stimulation receptive $2B4^+PD\text{-}1^-TIGIT^-CD226^+$

memory cells to exhausted $2B4^+PD1^+TIGIT^+CD226^-$ memory cells. Although this paper provided important insight into the exhaustion process in both viraemic and aviraemic PLWH2, new surface markers and transcription factors have been identified that can delineate the exhaustion process in higher resolution. Analysing the expression of additional surface markers, such as $Ly108^+$, CXCR5, CXCR6, CX_3CR1, Tim-3, KLRG1, and Lag3, and transcription factors, such as TOX, TCF-1, and T-bet, would allow us to determine in more depth the different exhausted $CD8^+$ T-cell subpopulations in PLWH1 and PLWH2 (241, 247). Recent studies have suggested that the maintenance of Tex^{prog} population with proliferative capacity is important for the maintenance of viral control during chronic infections (247), but the frequency of this population has not been investigated in PLWH2. Investigating the prevalence, polyfunctionality, and proliferative capacity of the different exhausted $CD8^+$ T-cell populations in PLWH1 and PLWH2 could therefore provide important insight into the difference between HIV-1 and HIV-2 pathogenicity. Further, analysis of HIV specific T-cells, identified using the activation induced marker (AIM) assay, would allow us to determine the frequency and functionality of exhausted HIV-specific $CD8^+$ T-cells among PLWH1, viraemic PLWH2, and aviraemic PLWH2. In addition, available longitudinal samples collected from PLWH2 with persistent virological control and samples collected before and after loss of virological control will provide novel insight into the underlying mechanism behind the emergence of viraemia in PLWH2. A study of PLWH1 has previously found that expression of the antiproliferative transcription factor KLF2 in HIV-specific $CD8^+$ T-cell was associated with loss of virological control (368), but this has not been assessed in PLWH2.

B-cells

In **paper II** we observed an expansion of hyperactivated T-bet expressing B-cells in both PLWH1 and PLWH2. In contrast to a previous report where the expansion of T-bet expressing B-cells was viraemia-dependent in PLWH1 (287), we observed an expansion of these B-cells even in aviraemic PLWH2. In PLWH1, the T-bet expressing B-cells were found to dominate the HIV-1-specific B-cell response (287). However, as we did not identify the HIV-2-specific B-cells we do not know to which extent these cells contributed to the HIV-2 specific response. Future studies where HIV-specific B-cells in PLWH1 and PLWH2 would be immunophenotyped, including additional transcription factors, activation markers and inhibitory receptors, could provide important insight into how the exhaustion state of the B-cell compartment is associated with the previously mentioned difference in the breadth and potency of anti-HIV-1 and anti-HIV-2 B-cell responses (152, 153, 291-295, 311, 369). In addition, cloning of the BCR from available longitudinal samples would provide insight into both the differentiation pathways of the T-bet expressing B-cells, as well as information about ongoing affinity maturation and neutralisation capacity of the different B-cell subpopulations. A recent study suggested that the majority of

T-bet expressing B-cells accumulate in the extrafollicular regions of lymph nodes in PLWH1, where they underwent limited affinity maturation, and do not contribute to effective control of HIV-1 replication (288). However, the contribution of such B-cells to HIV-2 control has so far not been examined. Cloning and sequencing of the BCR would permit examination of the hypermutation frequency and neutralisation capacity of the T-bet expressing B-cell in PLWH2. Extrafollicular accumulation of memory B-cells has been suggested to be a consequence of the hyperinflammatory state of PLWH1 (288, 289). As HIV-2 infections are associated with reduced inflammation compared to HIV-1 infections, it is possible that more efficient affinity maturation is ongoing in PLWH2. In line with this, previous studies have described gradual increase in Env-specific antibody titres in PLWH2 during disease progression (369, 370). Studies of HIV-2 neutralisation sensitivity has focused on Env, which has been suggested to have a more open configuration compared to HIV-1 Env, which in turn could make HIV-2 more sensitive to neutralising antibodies (294). However, the BCR sequence motifs that are associated with HIV-2 neutralisation has not been investigated so far. In PLWH1, bnAbs have been found to commonly derive from BCR clones with uncommon sequence motifs, such as SHMs outside the complement determining regions (CDRs), the regions with the largest impact on antigen binding, as well as long CDR3 loops (290, 371). Whether the same is true for HIV-2 bnAbs is not known. Thus, future studies of the sequence motifs will provide important insight into effective HIV-targeting antibodies. In addition to investigation of BCR sequences from PLWH2, *in vitro* studies of the antigenicity of HIV-1 and HIV-2 could provide important insight into effective anti-HIV antibody responses. HIV-2 particles have been suggested to have a higher Env spike density compared to HIV-1 (53). As higher antigen density has been shown to be associated with more efficient affinity maturation and production of neutralising antibodies (273), it is possible that HIV-2 is more antigenic compared to HIV-1. In a recent publication, a human tonsil organoid system was established where *de novo* adaptive B-cell responses were mounted towards novel antigens (372). This model system could therefore be used to compare the antigenicity of HIV-1 and HIV-2, as well as investigate their potential in activating the rare B-cell subsets that have been associated with HIV-1 bnAb production. In addition, this system could also enable the comparison of the impact of HIV-1 and HIV-2 infection on B-cell responses. HIV-1 Nef has previously been shown to induce dysregulation of B-cells in tonsil tissue (373), but the impact of HIV-2 infections on B-cell responses has not yet been investigated.

Plasma profiling

In **paper III** and **IV**, we utilized a novel analysis pipeline to profile the plasma proteome. Although plasma inflammation markers have long been used as markers of disease progression in PLWH1 and PLWH2 (115, 147, 149, 181, 188, 374-376), no one has so far taken an untargeted plasma profiling approach to identify signatures of tissue and cell type engagement in PLWH. As ongoing HIV replication

impacts several tissues and cell types, we have taken advantage of recent advances in the proteomics field to perform an in depth characterisation of the plasma proteome to investigate the presence of signatures of tissue and cell type engagement (329, 331, 377-379). However, although modern MS instruments can identify over 1000 plasma proteins (380), they are still far less sensitive compared to targeted approaches offered by companies such as Olink and SomaLogic that are able to quantify up to 7000 proteins (329, 377). Plasma proteome profiling using MS-based approaches will therefore be more limited by the number of identified proteins compared to studies using the more sensitive approaches offered by Olink and SomaLogic. Future studies where these large panels of proteins are quantified can therefore identify novel biomarkers of HIV disease progression, tissue damage, cell activation, and cell death. In addition, the potential identification of proteins exclusively associated with either HIV-1 or HIV-2 disease progression could provide novel insight into their differential pathogenicity. Further, as traditional inflammation markers can be upregulated following a wide range of infections and pathological conditions (113, 381), studies comparing the use of inflammation markers to tissue damage/cell type engagement markers could potentially identify more specific biomarkers for HIV replication.

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References

1. Jasinska AJ, Apetrei C, Pandrea I. Walk on the wild side: SIV infection in African non-human primate hosts—from the field to the laboratory. Frontiers in Immunology. 2023;13.
2. Robertson DL, Anderson JP, Bradac JA, Carr JK, Foley B, Funkhouser RK, et al. HIV-1 Nomenclature Proposal. Science. 2000;288(5463):55-.
3. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med. 2011;1(1):a006841.
4. van der Kuyl AC. Contemporary Distribution, Estimated Age, and Prehistoric Migrations of Old World Monkey Retroviruses. Epidemiologia. 2021;2(1):46-67.
5. Nyamweya S, Hegedus A, Jaye A, Rowland-Jones S, Flanagan KL, Macallan DC. Comparing HIV-1 and HIV-2 infection: Lessons for viral immunopathogenesis. Rev Med Virol. 2013;23(4):221-40.
6. UNAIDS. Global HIV & AIDS statistics — Fact sheet 2022 [Available from: https://www.unaids.org/en/resources/fact-sheet.
7. Marlink R, Kanki P, Thior I, Travers K, Eisen G, Siby T, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. Science. 1994;265(5178):1587-90.
8. Kanki PJ, Travers KU, S MB, Hsieh CC, Marlink RG, Gueye NA, et al. Slower heterosexual spread of HIV-2 than HIV-1. Lancet. 1994;343(8903):943-6.
9. Gottlieb GS, Raugi DN, Smith RA. 90-90-90 for HIV-2? Ending the HIV-2 epidemic by enhancing care and clinical management of patients infected with HIV-2. The Lancet HIV. 2018;5(7):e390-e9.
10. Worobey M, Gemmel M, Teuwen DE, Haselkorn T, Kunstman K, Bunce M, et al. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. Nature. 2008;455(7213):661-4.
11. Faria NR, Rambaut A, Suchard MA, Baele G, Bedford T, Ward MJ, et al. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. Science. 2014;346(6205):56-61.
12. Bbosa N, Kaleebu P, Ssemwanga D. HIV subtype diversity worldwide. Current Opinion in HIV and AIDS. 2019;14(3):153-60.
13. Gilbert MTP, Rambaut A, Wlasiuk G, Spira TJ, Pitchenik AE, Worobey M. The emergence of HIV/AIDS in the Americas and beyond. Proceedings of the National Academy of Sciences. 2007;104(47):18566-70.

14. Faria NR, Vidal N, Lourenco J, Raghwani J, Sigaloff KCE, Tatem AJ, et al. Distinct rates and patterns of spread of the major HIV-1 subtypes in Central and East Africa. PLoS Pathog. 2019;15(12):e1007976.
15. Hemelaar J, Elangovan R, Yun J, Dickson-Tetteh L, Fleminger I, Kirtley S, et al. Global and regional molecular epidemiology of HIV-1, 1990-2015: a systematic review, global survey, and trend analysis. Lancet Infect Dis. 2019;19(2):143-55.
16. Visseaux B, Damond F, Matheron S, Descamps D, Charpentier C. Hiv-2 molecular epidemiology. Infection, Genetics and Evolution. 2016;46:233-40.
17. Lemey P, Pybus OG, Wang B, Saksena NK, Salemi M, Vandamme A-M. Tracing the origin and history of the HIV-2 epidemic. Proceedings of the National Academy of Sciences. 2003;100(11):6588-92.
18. Wertheim JO, Worobey M. Dating the Age of the SIV Lineages That Gave Rise to HIV-1 and HIV-2. PLOS Computational Biology. 2009;5(5):e1000377.
19. da Silva ZJ, Oliveira I, Andersen A, Dias F, Rodrigues A, Holmgren B, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? AIDS. 2008;22(10):1195-202.
20. Olesen JS, Jespersen S, da Silva ZJ, Rodrigues A, Erikstrup C, Aaby P, et al. HIV-2 continues to decrease, whereas HIV-1 is stabilizing in Guinea-Bissau. AIDS. 2018;32(9):1193-8.
21. Poulsen A-G, Aaby P, Frederiksen K, Kvinesdal B, Mølbak K, Dias F, et al. PREVALENCE OF AND MORTALITY FROM HUMAN IMMUNODEFICIENCY VIRUS TYPE 2 IN BISSAU, WEST AFRICA. The Lancet. 1989;333(8642):827-31.
22. Poulsen AG, Aaby P, Gottschau A, Kvinesdal BB, Dias F, Mølbak K, et al. HIV-2 infection in Bissau, West Africa, 1987-1989: incidence, prevalences, and routes of transmission. J Acquir Immune Defic Syndr (1988). 1993;6(8):941-8.
23. Larsen O, da Silva Z, Sandström A, Andersen PK, Andersson S, Poulsen A-G, et al. Declining HIV-2 prevalence and incidence among men in a community study from Guinea-Bissau. AIDS. 1998;12(13).
24. Månsson F, Alves A, Silva ZJ, Dias F, Andersson S, Biberfeld G, et al. Trends of HIV-1 and HIV-2 prevalence among pregnant women in Guinea-Bissau, West Africa: possible effect of the civil war 1998 1999. Sex Transm Infect. 2007;83(6):463-7.
25. Månsson F, Biague A, da Silva ZJ, Dias F, Nilsson LA, Andersson S, et al. Prevalence and incidence of HIV-1 and HIV-2 before, during and after a civil war in an occupational cohort in Guinea-Bissau, West Africa. Aids. 2009;23(12):1575-82.
26. Norrgren H, Andersson S, Biague AJ, da Silva ZJ, Dias F, Nauclér A, et al. Trends and interaction of HIV-1 and HIV-2 in Guinea-Bissau, west Africa: no protection of HIV-2 against HIV-1 infection. AIDS. 1999;13(6):701-7.
27. Jespersen S, Månsson F, Lindman J, Wejse C, Medina C, da Silva ZJ, et al. HIV treatment in Guinea-Bissau: room for improvement and time for new treatment options. AIDS Research and Therapy. 2020;17(1):3.
28. Biague A, Månsson F, da Silva Z, Dias F, Nantote Q, Costa J, et al. High sexual risk taking and diverging trends of HIV-1 and HIV-2 in the military of Guinea Bissau. J Infect Dev Ctries. 2010;4(5):301-8.

29. Norrgren H, Cardoso AN, da Silva ZJ, Andersson S, Dias F, Biberfeld G, et al. Increased prevalence of HIV-2 infection in hospitalized patients with severe bacterial diseases in Guinea-Bissau. Scand J Infect Dis. 1997;29(5):453-9.
30. Norrgren H, Da Silva ZJ, Andersson S, Biague AJ, Dias F, Biberfeld G, et al. Clinical features, immunological changes and mortality in a cohort of HIV-2-infected individuals in Bissau, Guinea-Bissau. Scand J Infect Dis. 1998;30(4):323-9.
31. Faria NR, Hodges-Mameletzis I, Silva JC, Rodés B, Erasmus S, Paolucci S, et al. Phylogeographical footprint of colonial history in the global dispersal of human immunodeficiency virus type 2 group A. J Gen Virol. 2012;93(Pt 4):889-99.
32. Carvalho A, Valadas E, França L, Carvalho C, Aleixo M, Mendez J, et al. Population mobility and the changing epidemics of HIV-2 in Portugal. HIV Medicine. 2012;13(4):219-25.
33. Heitzinger K, Sow PS, Dia Badiane NM, Gottlieb GS, N'Doye I, Toure M, et al. Trends of HIV-1, HIV-2 and dual infection in women attending outpatient clinics in Senegal, 1990–2009. International Journal of STD & AIDS. 2012;23(10):710-6.
34. Tienen C, van der Loeff MS, Zaman SM, Vincent T, Sarge-Njie R, Peterson I, et al. Two distinct epidemics: the rise of HIV-1 and decline of HIV-2 infection between 1990 and 2007 in rural Guinea-Bissau. J Acquir Immune Defic Syndr. 2010;53(5):640-7.
35. Fryer HR, Van Tienen C, Van Der Loeff MS, Aaby P, Da Silva ZJ, Whittle H, et al. Predicting the extinction of HIV-2 in rural Guinea-Bissau. AIDS. 2015;29(18):2479-86.
36. Bandim Health Project [Available from: https://www.bandim.org/.
37. Jespersen S, Hønge BL, Oliveira I, Medina C, da Silva Té D, Correira FG, et al. Cohort Profile: The Bissau HIV Cohort—a cohort of HIV-1, HIV-2 and co-infected patients. International Journal of Epidemiology. 2014;44(3):756-63.
38. Esbjornsson J, Jansson M, Jespersen S, Mansson F, Honge BL, Lindman J, et al. HIV-2 as a model to identify a functional HIV cure. AIDS Res Ther. 2019;16(1):e25 - e31.
39. Mansson F, Biague A, da Silva ZJ, Dias F, Nilsson LA, Andersson S, et al. Prevalence and incidence of HIV-1 and HIV-2 before, during and after a civil war in an occupational cohort in Guinea-Bissau, West Africa. AIDS. 2009;23(12):1575-82.
40. Norrgren H, Andersson S, Naucler A, Dias F, Johansson I, Biberfeld G. HIV-1, HIV-2, HTLV-I/II and Treponema pallidum infections: incidence, prevalence, and HIV-2-associated mortality in an occupational cohort in Guinea-Bissau. J Acquir Immune Defic Syndr Hum Retrovirol. 1995;9(4):422-8.
41. de Silva TI, Cotten M, Rowland-Jones SL. HIV-2: the forgotten AIDS virus. Trends in Microbiology. 2008;16(12):588-95.
42. Campbell-Yesufu OT, Gandhi RT. Update on Human Immunodeficiency Virus (HIV)-2 Infection. Clinical Infectious Diseases. 2011;52(6):780-7.
43. Andersen MN, Hønge BL, Jespersen S, Medina C, da Silva Té D, Laursen A, et al. Soluble Macrophage Mannose Receptor (sCD206/sMR) as a Biomarker in Human Immunodeficiency Virus Infection. J Infect Dis. 2018;218(8):1291-5.

44. van der Loeff MF, Larke N, Kaye S, Berry N, Ariyoshi K, Alabi A, et al. Undetectable plasma viral load predicts normal survival in HIV-2-infected people in a West African village. Retrovirology. 2010;7:46.
45. Schmidt WP, Schim Van Der Loeff M, Aaby P, Whittle H, Bakker R, Buckner M, et al. Behaviour change and competitive exclusion can explain the diverging HIV-1 and HIV-2 prevalence trends in Guinea–Bissau. Epidemiology & Infection. 2008;136(4):551-61.
46. Esbjörnsson J, Mild M, Månsson F, Norrgren H, Medstrand P. HIV-1 molecular epidemiology in Guinea-Bissau, West Africa: origin, demography and migrations. PLoS One. 2011;6(2):e17025.
47. Palm AA, Esbjörnsson J, Månsson F, Kvist A, Isberg P-E, Biague A, et al. Faster Progression to AIDS and AIDS-Related Death Among Seroincident Individuals Infected With Recombinant HIV-1 A3/CRF02_AG Compared With Sub-subtype A3. The Journal of Infectious Diseases. 2013;209(5):721-8.
48. Palm AA, Esbjörnsson J, Månsson F, Biague A, da Silva ZJ, Norrgren H, et al. Cocirculation of several similar but unique HIV-1 recombinant forms in Guinea-Bissau revealed by near full-length genomic sequencing. AIDS Res Hum Retroviruses. 2015;31(9):938-45.
49. Wilhelmson S, Månsson F, Lopatko Lindman J, Biai A, Esbjörnsson J, Norrgren H, et al. Prevalence of HIV-1 pretreatment drug resistance among treatment naïve pregnant women in Bissau, Guinea Bissau. PLoS One. 2018;13(10):e0206406.
50. Rasmussen DN, Vieira N, Hønge BL, da Silva Té D, Jespersen S, Bjerregaard-Andersen M, et al. HIV-1 and HIV-2 prevalence, risk factors and birth outcomes among pregnant women in Bissau, Guinea-Bissau: a retrospective cross-sectional hospital study. Scientific Reports. 2020;10(1):12174.
51. Cristian Apetrei BH, Andrew Rambaut, Steven Wolinsky, J. Rodney Brister, Brandon Keele, and Christophe Faser. HIV Sequence Compendium 2019: Los Alamos National Laboratory, Theoretical Biology and Biophysics, Los Alamos, New Mexico. LA-UR-23-20934; 2019.
52. Burnie J, Guzzo C. The Incorporation of Host Proteins into the External HIV-1 Envelope. Viruses. 2019;11(1).
53. Palmer E, Goldsmith CS. Ultrastructure of human retroviruses. J Electron Microsc Tech. 1988;8(1):3-15.
54. Murphy RE, Saad JS. The Interplay between HIV-1 Gag Binding to the Plasma Membrane and Env Incorporation. Viruses. 2020;12(5):548.
55. Sundquist WI, Kräusslich HG. HIV-1 assembly, budding, and maturation. Cold Spring Harb Perspect Med. 2012;2(7):a006924.
56. Li G, Piampongsant S, Faria NR, Voet A, Pineda-Peña A-C, Khouri R, et al. An integrated map of HIV genome-wide variation from a population perspective. Retrovirology. 2015;12(1):18.
57. Sauter D, Kirchhoff F. Key Viral Adaptations Preceding the AIDS Pandemic. Cell Host Microbe. 2019;25(1):27-38.
58. Dutilleul A, Rodari A, Van Lint C. Depicting HIV-1 Transcriptional Mechanisms: A Summary of What We Know. Viruses. 2020;12(12):1385.

59. Hu WS, Hughes SH. HIV-1 reverse transcription. Cold Spring Harb Perspect Med. 2012;2(10).
60. Bosso M, Stürzel CM, Kmiec D, Badarinarayan SS, Braun E, Ito J, et al. An additional NF-κB site allows HIV-1 subtype C to evade restriction by nuclear PYHIN proteins. Cell Rep. 2021;36(12):109735.
61. Markovitz DM, Smith MJ, Hilfinger J, Hannibal MC, Petryniak B, Nabel GJ. Activation of the human immunodeficiency virus type 2 enhancer is dependent on purine box and kappa B regulatory elements. J Virol. 1992;66(9):5479-84.
62. Marie V, Gordon ML. The HIV-1 Gag Protein Displays Extensive Functional and Structural Roles in Virus Replication and Infectivity. International Journal of Molecular Sciences. 2022;23(14):7569.
63. Cassan E, Arigon-Chifolleau A-M, Mesnard J-M, Gross A, Gascuel O. Concomitant emergence of the antisense protein gene of HIV-1 and of the pandemic. Proceedings of the National Academy of Sciences. 2016;113(41):11537-42.
64. Miller RH. Human immunodeficiency virus may encode a novel protein on the genomic DNA plus strand. Science. 1988;239(4846):1420-2.
65. Gholizadeh Z, Iqbal MS, Li R, Romerio F. The HIV-1 Antisense Gene ASP: The New Kid on the Block. Vaccines (Basel). 2021;9(5).
66. Lozada C, Barlow TMA, Gonzalez S, Lubin-Germain N, Ballet S. Identification and Characteristics of Fusion Peptides Derived From Enveloped Viruses. Frontiers in Chemistry. 2021;9.
67. Chen B. Molecular Mechanism of HIV-1 Entry. Trends Microbiol. 2019;27(10):878-91.
68. Ozorowski G, Pallesen J, de Val N, Lyumkis D, Cottrell CA, Torres JL, et al. Open and closed structures reveal allostery and pliability in the HIV-1 envelope spike. Nature. 2017;547(7663):360-3.
69. Mörner A, Björndal Å, Albert J, KewalRamani VN, Littman DR, Inoue R, et al. Primary Human Immunodeficiency Virus Type 2 (HIV-2) Isolates, Like HIV-1 Isolates, Frequently Use CCR5 but Show Promiscuity in Coreceptor Usage. Journal of Virology. 1999;73(3):2343-9.
70. Mörner A, Björndal A, Leandersson AC, Albert J, Björling E, Jansson M. CCR5 or CXCR4 is required for efficient infection of peripheral blood mononuclear cells by promiscuous human immunodeficiency virus type 2 primary isolates. AIDS Res Hum Retroviruses. 2002;18(3):193-200.
71. Wilen CB, Tilton JC, Doms RW. HIV: cell binding and entry. Cold Spring Harb Perspect Med. 2012;2(8).
72. Müller TG, Zila V, Müller B, Kräusslich HG. Nuclear Capsid Uncoating and Reverse Transcription of HIV-1. Annu Rev Virol. 2022;9(1):261-84.
73. Jacques DA, McEwan WA, Hilditch L, Price AJ, Towers GJ, James LC. HIV-1 uses dynamic capsid pores to import nucleotides and fuel encapsidated DNA synthesis. Nature. 2016;536(7616):349-53.

74. Rawson JMO, Landman SR, Reilly CS, Mansky LM. HIV-1 and HIV-2 exhibit similar mutation frequencies and spectra in the absence of G-to-A hypermutation. Retrovirology. 2015;12(1):60.
75. Song H, Giorgi EE, Ganusov VV, Cai F, Athreya G, Yoon H, et al. Tracking HIV-1 recombination to resolve its contribution to HIV-1 evolution in natural infection. Nature Communications. 2018;9(1):1928.
76. Chen J, Powell D, Hu W-S. High Frequency of Genetic Recombination Is a Common Feature of Primate Lentivirus Replication. Journal of Virology. 2006;80(19):9651-8.
77. Zhuang J, Jetzt AE, Sun G, Yu H, Klarmann G, Ron Y, et al. Human Immunodeficiency Virus Type 1 Recombination: Rate, Fidelity, and Putative Hot Spots. Journal of Virology. 2002;76(22):11273-82.
78. Rawson JMO, Nikolaitchik OA, Keele BF, Pathak VK, Hu WS. Recombination is required for efficient HIV-1 replication and the maintenance of viral genome integrity. Nucleic Acids Res. 2018;46(20):10535-45.
79. Zila V, Margiotta E, Turoňová B, Müller TG, Zimmerli CE, Mattei S, et al. Cone-shaped HIV-1 capsids are transported through intact nuclear pores. Cell. 2021;184(4):1032-46.e18.
80. Li C, Burdick RC, Nagashima K, Hu WS, Pathak VK. HIV-1 cores retain their integrity until minutes before uncoating in the nucleus. Proc Natl Acad Sci U S A. 2021;118(10).
81. Francis AC, Melikyan GB. Single HIV-1 Imaging Reveals Progression of Infection through CA-Dependent Steps of Docking at the Nuclear Pore, Uncoating, and Nuclear Transport. Cell Host Microbe. 2018;23(4):536-48.e6.
82. Hare S, Gupta SS, Valkov E, Engelman A, Cherepanov P. Retroviral intasome assembly and inhibition of DNA strand transfer. Nature. 2010;464(7286):232-6.
83. Lusic M, Siliciano RF. Nuclear landscape of HIV-1 infection and integration. Nat Rev Microbiol. 2017;15(2):69-82.
84. Engelman A, Cherepanov P. The structural biology of HIV-1: mechanistic and therapeutic insights. Nature Reviews Microbiology. 2012;10(4):279-90.
85. Brigati C, Giacca M, Noonan DM, Albini A. HIV Tat, its TARgets and the control of viral gene expression. FEMS Microbiology Letters. 2003;220(1):57-65.
86. Jeang K-T. Multi-Faceted Post-Transcriptional Functions of HIV-1 Rev. Biology. 2012;1(2):165-74.
87. Hirao K, Andrews S, Kuroki K, Kusaka H, Tadokoro T, Kita S, et al. Structure of HIV-2 Nef Reveals Features Distinct from HIV-1 Involved in Immune Regulation. iScience. 2020;23(1):100758.
88. Zhao Y, Zhao K, Wang S, Du J. Multi-functional BST2/tetherin against HIV-1, other viruses and LINE-1. Frontiers in Cellular and Infection Microbiology. 2022;12.
89. Perez-Caballero D, Zang T, Ebrahimi A, McNatt MW, Gregory DA, Johnson MC, et al. Tetherin inhibits HIV-1 release by directly tethering virions to cells. Cell. 2009;139(3):499-511.

90. Compton AA, Emerman M. Convergence and divergence in the evolution of the APOBEC3G-Vif interaction reveal ancient origins of simian immunodeficiency viruses. PLoS Pathog. 2013;9(1):e1003135.
91. Uriu K, Kosugi Y, Ito J, Sato K. The Battle between Retroviruses and APOBEC3 Genes: Its Past and Present. Viruses. 2021;13(1):124.
92. Zhang F, Bieniasz PD. HIV-1 Vpr induces cell cycle arrest and enhances viral gene expression by depleting CCDC137. eLife. 2020;9:e55806.
93. Checkley MA, Luttge BG, Freed EO. HIV-1 envelope glycoprotein biosynthesis, trafficking, and incorporation. J Mol Biol. 2011;410(4):582-608.
94. Rein A. The heart of the HIV RNA packaging signal? Proceedings of the National Academy of Sciences. 2020;117(33):19621-3.
95. Lerner G, Weaver N, Anokhin B, Spearman P. Advances in HIV-1 Assembly. Viruses. 2022;14(3):478.
96. Buttler CA, Pezeshkian N, Fernandez MV, Aaron J, Norman S, Freed EO, et al. Single molecule fate of HIV-1 envelope reveals late-stage viral lattice incorporation. Nature Communications. 2018;9(1):1861.
97. Pezeshkian N, Groves NS, van Engelenburg SB. Single-molecule imaging of HIV-1 envelope glycoprotein dynamics and Gag lattice association exposes determinants responsible for virus incorporation. Proceedings of the National Academy of Sciences. 2019;116(50):25269-77.
98. Alfadhli A, Staubus AO, Tedbury PR, Novikova M, Freed EO, Barklis E. Analysis of HIV-1 Matrix-Envelope Cytoplasmic Tail Interactions. J Virol. 2019;93(21).
99. Deeks SG, Overbaugh J, Phillips A, Buchbinder S. HIV infection. Nature Reviews Disease Primers. 2015;1(1):15035.
100. Cohen MS. Preventing Sexual Transmission of HIV. Clinical Infectious Diseases. 2007;45(Supplement_4):S287-S92.
101. Keele BF, Giorgi EE, Salazar-Gonzalez JF, Decker JM, Pham KT, Salazar MG, et al. Identification and characterization of transmitted and early founder virus envelopes in primary HIV-1 infection. Proceedings of the National Academy of Sciences. 2008;105(21):7552-7.
102. Gonzalez SM, Aguilar-Jimenez W, Su R-C, Rugeles MT. Mucosa: Key Interactions Determining Sexual Transmission of the HIV Infection. Frontiers in Immunology. 2019;10.
103. Ganor Y, Zhou Z, Tudor D, Schmitt A, Vacher-Lavenu MC, Gibault L, et al. Within 1 h, HIV-1 uses viral synapses to enter efficiently the inner, but not outer, foreskin mucosa and engages Langerhans–T cell conjugates. Mucosal Immunology. 2010;3(5):506-22.
104. Laguette N, Sobhian B, Casartelli N, Ringeard M, Chable-Bessia C, Ségéral E, et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature. 2011;474(7353):654-7.

105. Izquierdo-Useros N, Lorizate M, Puertas MC, Rodriguez-Plata MT, Zangger N, Erikson E, et al. Siglec-1 Is a Novel Dendritic Cell Receptor That Mediates HIV-1 Trans-Infection Through Recognition of Viral Membrane Gangliosides. PLOS Biology. 2012;10(12):e1001448.

106. Puryear WB, Akiyama H, Geer SD, Ramirez NP, Yu X, Reinhard BM, et al. Interferon-Inducible Mechanism of Dendritic Cell-Mediated HIV-1 Dissemination Is Dependent on Siglec-1/CD169. PLOS Pathogens. 2013;9(4):e1003291.

107. Perez-Zsolt D, Raïch-Regué D, Muñoz-Basagoiti J, Aguilar-Gurrieri C, Clotet B, Blanco J, et al. HIV-1 trans-Infection Mediated by DCs: The Tip of the Iceberg of Cell-to-Cell Viral Transmission. Pathogens. 2022;11(1):39.

108. Rodriguez-Garcia M, Shen Z, Barr FD, Boesch AW, Ackerman ME, Kappes JC, et al. Dendritic cells from the human female reproductive tract rapidly capture and respond to HIV. Mucosal Immunology. 2017;10(2):531-44.

109. Perez-Zsolt D, Cantero-Pérez J, Erkizia I, Benet S, Pino M, Serra-Peinado C, et al. Dendritic Cells From the Cervical Mucosa Capture and Transfer HIV-1 via Siglec-1. Frontiers in Immunology. 2019;10.

110. Konrad BP, Taylor D, Conway JM, Ogilvie GS, Coombs D. On the duration of the period between exposure to HIV and detectable infection. Epidemics. 2017;20:73-83.

111. Rolland M, Tovanabutra S, Dearlove B, Li Y, Owen CL, Lewitus E, et al. Molecular dating and viral load growth rates suggested that the eclipse phase lasted about a week in HIV-1 infected adults in East Africa and Thailand. PLoS Pathog. 2020;16(2):e1008179.

112. Vanhamel J, Bruggemans A, Debyser Z. Establishment of latent HIV-1 reservoirs: what do we really know? J Virus Erad. 2019;5(1):3-9.

113. Fajgenbaum DC, June CH. Cytokine Storm. New England Journal of Medicine. 2020;383(23):2255-73.

114. Iyer SS, Bibollet-Ruche F, Sherrill-Mix S, Learn GH, Plenderleith L, Smith AG, et al. Resistance to type 1 interferons is a major determinant of HIV-1 transmission fitness. Proc Natl Acad Sci U S A. 2017;114(4):E590-e9.

115. Roberts L, Passmore JA, Williamson C, Little F, Bebell LM, Mlisana K, et al. Plasma cytokine levels during acute HIV-1 infection predict HIV disease progression. Aids. 2010;24(6):819-31.

116. Fiebig EW, Wright DJ, Rawal BD, Garrett PE, Schumacher RT, Peddada L, et al. Dynamics of HIV viremia and antibody seroconversion in plasma donors: implications for diagnosis and staging of primary HIV infection. Aids. 2003;17(13):1871-9.

117. McMichael AJ, Borrow P, Tomaras GD, Goonetilleke N, Haynes BF. The immune response during acute HIV-1 infection: clues for vaccine development. Nature Reviews Immunology. 2010;10(1):11-23.

118. Brenchley JM, Schacker TW, Ruff LE, Price DA, Taylor JH, Beilman GJ, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med. 2004;200(6):749-59.

119. Veazey RS, DeMaria M, Chalifoux LV, Shvetz DE, Pauley DR, Knight HL, et al. Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. Science. 1998;280(5362):427-31.

120. Doitsh G, Galloway NL, Geng X, Yang Z, Monroe KM, Zepeda O, et al. Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. Nature. 2014;505(7484):509-14.
121. Levesque MC, Moody MA, Hwang KK, Marshall DJ, Whitesides JF, Amos JD, et al. Polyclonal B cell differentiation and loss of gastrointestinal tract germinal centers in the earliest stages of HIV-1 infection. PLoS Med. 2009;6(7):e1000107.
122. Sankaran S, George MD, Reay E, Guadalupe M, Flamm J, Prindiville T, et al. Rapid Onset of Intestinal Epithelial Barrier Dysfunction in Primary Human Immunodeficiency Virus Infection Is Driven by an Imbalance between Immune Response and Mucosal Repair and Regeneration. Journal of Virology. 2008;82(1):538-45.
123. Nowroozalizadeh S, Månsson F, da Silva Z, Repits J, Dabo B, Pereira C, et al. Microbial translocation correlates with the severity of both HIV-1 and HIV-2 infections. J Infect Dis. 2010;201(8):1150-4.
124. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006;12(12):1365-71.
125. Coffin J, Swanstrom R. HIV pathogenesis: dynamics and genetics of viral populations and infected cells. Cold Spring Harbor perspectives in medicine. 2013;3(1):a012526.
126. Zhao L, Wymant C, Blanquart F, Golubchik T, Gall A, Bakker M, et al. Phylogenetic estimation of the viral fitness landscape of HIV-1 set-point viral load. Virus Evolution. 2022;8(1):veac022.
127. Blanquart F, Wymant C, Cornelissen M, Gall A, Bakker M, Bezemer D, et al. Viral genetic variation accounts for a third of variability in HIV-1 set-point viral load in Europe. PLoS biology. 2017;15(6):e2001855.
128. Yue L, Prentice HA, Farmer P, Song W, He D, Lakhi S, et al. Cumulative Impact of Host and Viral Factors on HIV-1 Viral-Load Control during Early Infection. Journal of Virology. 2013;87(2):708-15.
129. Bonhoeffer S, Fraser C, Leventhal GE. High Heritability Is Compatible with the Broad Distribution of Set Point Viral Load in HIV Carriers. PLOS Pathogens. 2015;11(2):e1004634.
130. Fraser C, Hollingsworth TD, Chapman R, de Wolf F, Hanage WP. Variation in HIV-1 set-point viral load: Epidemiological analysis and an evolutionary hypothesis. Proceedings of the National Academy of Sciences. 2007;104(44):17441-6.
131. Robb ML, Eller LA, Kibuuka H, Rono K, Maganga L, Nitayaphan S, et al. Prospective Study of Acute HIV-1 Infection in Adults in East Africa and Thailand. N Engl J Med. 2016;374(22):2120-30.
132. Mellors JW, Rinaldo CR, Jr., Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science. 1996;272(5265):1167-70.
133. O'Brien TR, Rosenberg PS, Yellin F, Goedert JJ. Longitudinal HIV-1 RNA levels in a cohort of homosexual men. J Acquir Immune Defic Syndr Hum Retrovirol. 1998;18(2):155-61.

134. Sabin CA, Devereux H, Phillips AN, Hill A, Janossy G, Lee CA, et al. Course of viral load throughout HIV-1 infection. J Acquir Immune Defic Syndr. 2000;23(2):172-7.
135. Besnier J-M, Barin F, Baillou A, Liard F, Choutet P, Goudeau A. Symptomatic HIV-2 primary infection. The Lancet. 1990;335(8692):798.
136. Andersson S, Norrgren H, da Silva Z, Biague A, Bamba S, Kwok S, et al. Plasma viral load in HIV-1 and HIV-2 singly and dually infected individuals in Guinea-Bissau, West Africa: significantly lower plasma virus set point in HIV-2 infection than in HIV-1 infection. Arch Intern Med. 2000;160(21):3286-93.
137. Popper SJ, Sarr AD, Travers KU, Gueye-Ndiaye A, Mboup S, Essex ME, et al. Lower human immunodeficiency virus (HIV) type 2 viral load reflects the difference in pathogenicity of HIV-1 and HIV-2. J Infect Dis. 1999;180(4):1116-21.
138. Berry N, Ariyoshi K, Jaffar S, Sabally S, Corrah T, Tedder R, et al. Low peripheral blood viral HIV-2 RNA in individuals with high CD4 percentage differentiates HIV-2 from HIV-1 infection. J Hum Virol. 1998;1(7):457-68.
139. Lahaye X, Satoh T, Gentili M, Cerboni S, Conrad C, Hurbain I, et al. The Capsids of HIV-1 and HIV-2 Determine Immune Detection of the Viral cDNA by the Innate Sensor cGAS in Dendritic Cells. Immunity. 2013;39(6):1132-42.
140. Kijewski SDG, Akiyama H, Feizpour A, Miller CM, Ramirez NP, Reinhard BM, et al. Access of HIV-2 to CD169-dependent dendritic cell-mediated trans infection pathway is attenuated. Virology. 2016;497:328-36.
141. Monaco DC, Ende Z, Hunter E. Virus-Host Gene Interactions Define HIV-1 Disease Progression. Curr Top Microbiol Immunol. 2017;407:31-63.
142. Appay V, Sauce D. Immune activation and inflammation in HIV-1 infection: causes and consequences. J Pathol. 2008;214(2):231-41.
143. Fenwick C, Joo V, Jacquier P, Noto A, Banga R, Perreau M, et al. T-cell exhaustion in HIV infection. Immunol Rev. 2019;292(1):149-63.
144. Moir S, Fauci AS. B-cell exhaustion in HIV infection: the role of immune activation. Curr Opin HIV AIDS. 2014;9(5):472-7.
145. Esbjornsson J, Mansson F, Kvist A, da Silva ZJ, Andersson S, Fenyo EM, et al. Long-term follow-up of HIV-2-related AIDS and mortality in Guinea-Bissau: a prospective open cohort study. Lancet HIV. 2018;6(1):e25-e31.
146. Sousa AE, Carneiro J, Meier-Schellersheim M, Grossman Z, Victorino RM. CD4 T cell depletion is linked directly to immune activation in the pathogenesis of HIV-1 and HIV-2 but only indirectly to the viral load. J Immunol. 2002;169(6):3400-6.
147. Nyamweya S, Townend J, Zaman A, Steele SJ, Jeffries D, Rowland-Jones S, et al. Are plasma biomarkers of immune activation predictive of HIV progression: a longitudinal comparison and analyses in HIV-1 and HIV-2 infections? PLoS One. 2012;7(9):e44411.
148. Buggert M, Frederiksen J, Lund O, Betts MR, Biague A, Nielsen M, et al. CD4+ T cells with an activated and exhausted phenotype distinguish immunodeficiency during aviremic HIV-2 infection. AIDS. 2016;30(16):2415-26.

149. Thiebaut R, Charpentier C, Damond F, Taieb A, Antoine R, Capeau J, et al. Association of soluble CD14 and inflammatory biomarkers with HIV-2 disease progression. Clin Infect Dis. 2012;55(10):1417-25.
150. Esbjornsson J, Mansson F, Kvist A, Isberg PE, Nowroozalizadeh S, Biague AJ, et al. Inhibition of HIV-1 disease progression by contemporaneous HIV-2 infection. N Engl J Med. 2012;367(3):224-32.
151. Esbjornsson J, Mansson F, Kvist A, Isberg PE, Biague AJ, da Silva ZJ, et al. Increased survival among HIV-1 and HIV-2 dual-infected individuals compared to HIV-1 single-infected individuals. AIDS. 2014;28(7):949-57.
152. Karlsson I, Tingstedt JL, Sahin GO, Hansen M, Szojka Z, Buggert M, et al. Cross-Reactive Antibodies With the Capacity to Mediate HIV-1 Envelope Glycoprotein-Targeted Antibody-Dependent Cellular Cytotoxicity Identified in HIV-2-Infected Individuals. J Infect Dis. 2019;219(11):1749-54.
153. Ozkaya Sahin G, Holmgren B, da Silva Z, Nielsen J, Nowroozalizadeh S, Esbjornsson J, et al. Potent intratype neutralizing activity distinguishes human immunodeficiency virus type 2 (HIV-2) from HIV-1. J Virol. 2012;86(2):961-71.
154. Akimoto H, Kaneko H, Sekigawa I, Hashimoto H, Kaneko Y, Yamamoto N. Binding of HIV-2 envelope glycoprotein to CD8 molecules and related chemokine production. Immunology. 1998;95(2):214-8.
155. Kokkotou EG, Sankale JL, Mani I, Gueye-Ndiaye A, Schwartz D, Essex ME, et al. In vitro correlates of HIV-2-mediated HIV-1 protection. Proc Natl Acad Sci U S A. 2000;97(12):6797-802.
156. Zheng NN, Kiviat NB, Sow PS, Hawes SE, Wilson A, Diallo-Agne H, et al. Comparison of Human Immunodeficiency Virus (HIV)-Specific T-Cell Responses in HIV-1- and HIV-2-Infected Individuals in Senegal. Journal of Virology. 2004;78(24):13934-42.
157. Weiss RA, Clapham PR, Weber JN, Whitby D, Tedder RS, O'Connor T, et al. HIV-2 antisera cross-neutralize HIV-1. AIDS. 1988;2(2):95-100.
158. Bertoletti A, Cham F, McAdam S, Rostron T, Rowland-Jones S, Sabally S, et al. Cytotoxic T cells from human immunodeficiency virus type 2-infected patients frequently cross-react with different human immunodeficiency virus type 1 clades. Journal of virology. 1998;72(3):2439-48.
159. Arya SK, Gallo RC. Human immunodeficiency virus (HIV) type 2-mediated inhibition of HIV type 1: a new approach to gene therapy of HIV-infection. Proceedings of the National Academy of Sciences. 1996;93(9):4486-91.
160. Mahdi M, Szojka Z, Mótyán JA, Tőzsér J. Inhibitory Effects of HIV-2 Vpx on Replication of HIV-1. J Virol. 2018;92(14).
161. Zicari S, Sessa L, Cotugno N, Ruggiero A, Morrocchi E, Concato C, et al. Immune Activation, Inflammation, and Non-AIDS Co-Morbidities in HIV-Infected Patients under Long-Term ART. Viruses. 2019;11(3):200.
162. Sanchez JL, Hunt PW, Reilly CS, Hatano H, Beilman GJ, Khoruts A, et al. Lymphoid Fibrosis Occurs in Long-Term Nonprogressors and Persists With Antiretroviral Therapy but May Be Reversible With Curative Interventions. The Journal of Infectious Diseases. 2014;211(7):1068-75.

163. Epple HJ, Allers K, Tröger H, Kühl A, Erben U, Fromm M, et al. Acute HIV infection induces mucosal infiltration with CD4+ and CD8+ T cells, epithelial apoptosis, and a mucosal barrier defect. Gastroenterology. 2010;139(4):1289-300.
164. Nazli A, Chan O, Dobson-Belaire WN, Ouellet M, Tremblay MJ, Gray-Owen SD, et al. Exposure to HIV-1 directly impairs mucosal epithelial barrier integrity allowing microbial translocation. PLoS Pathog. 2010;6(4):e1000852.
165. Maresca M, Mahfoud R, Garmy N, Kotler DP, Fantini J, Clayton F. The virotoxin model of HIV-1 enteropathy: involvement of GPR15/Bob and galactosylceramide in the cytopathic effects induced by HIV-1 gp120 in the HT-29-D4 intestinal cell line. J Biomed Sci. 2003;10(1):156-66.
166. Kam LY, Targan SR. Cytokine-based therapies in inflammatory bowel disease. Curr Opin Gastroenterol. 1999;15(4):302-7.
167. Lien K, Mayer W, Herrera R, Rosbe K, Tugizov SM. HIV-1 proteins gp120 and tat induce the epithelial-mesenchymal transition in oral and genital mucosal epithelial cells. PLoS One. 2019;14(12):e0226343.
168. Sandler NG, Douek DC. Microbial translocation in HIV infection: causes, consequences and treatment opportunities. Nat Rev Microbiol. 2012;10(9):655-66.
169. Brenchley JM, Paiardini M, Knox KS, Asher AI, Cervasi B, Asher TE, et al. Differential Th17 CD4 T-cell depletion in pathogenic and nonpathogenic lentiviral infections. Blood. 2008;112(7):2826-35.
170. Chege D, Sheth PM, Kain T, Kim CJ, Kovacs C, Loutfy M, et al. Sigmoid Th17 populations, the HIV latent reservoir, and microbial translocation in men on long-term antiretroviral therapy. Aids. 2011;25(6):741-9.
171. Balagopal A, Ray SC, De Oca RM, Sutcliffe CG, Vivekanandan P, Higgins Y, et al. Kupffer cells are depleted with HIV immunodeficiency and partially recovered with antiretroviral immune reconstitution. Aids. 2009;23(18):2397-404.
172. Zhang L, Bansal MB. Role of Kupffer Cells in Driving Hepatic Inflammation and Fibrosis in HIV Infection. Frontiers in Immunology. 2020;11.
173. Stabinski L, Reynolds SJ, Ocama P, Laeyendecker O, Ndyanabo A, Kiggundu V, et al. High prevalence of liver fibrosis associated with HIV infection: a study in rural Rakai, Uganda. Antivir Ther. 2011;16(3):405-11.
174. Han SH, Kim SU, Kim CO, Jeong SJ, Park JY, Choi JY, et al. Abnormal liver stiffness assessed using transient elastography (Fibroscan®) in HIV-infected patients without HBV/HCV coinfection receiving combined antiretroviral treatment. PloS one. 2013;8(1):e52720.
175. Tahiri M, Sodqi M, Lahdami FEZ, Marih L, Lamdini H, Hliwa W, et al. Risk factors for liver fibrosis among human immunodeficiency virus monoinfected patients using the FIB4 index in Morocco. World Journal of Hepatology. 2013;5(10):584.
176. Deeks Steven G, Tracy R, Douek Daniel C. Systemic Effects of Inflammation on Health during Chronic HIV Infection. Immunity. 2013;39(4):633-45.
177. Bellinger DL, Lorton D. Sympathetic Nerves and Innate Immune System in the Spleen: Implications of Impairment in HIV-1 and Relevant Models. Cells. 2022;11(4):673.

178. Falk S, Stutte HJ. The spleen in HIV infection — morphological evidence of HIV-associated macrophage dysfunction. Research in Virology. 1990;141(2):161-9.
179. Diaz LK, Murphy RL, Phair JP, Variakojis D. The AIDS autopsy spleen: a comparison of the pre-anti-retroviral and highly active anti-retroviral therapy eras. Modern pathology. 2002;15(4):406-12.
180. Caetano DG, Ribeiro-Alves M, Hottz ED, Vilela LM, Cardoso SW, Hoagland B, et al. Increased biomarkers of cardiovascular risk in HIV-1 viremic controllers and low persistent inflammation in elite controllers and art-suppressed individuals. Scientific Reports. 2022;12(1):6569.
181. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med. 2008;5(10):e203.
182. Luo L, Han Y, Song X, Zhu T, Zeng Y, Li T. CD16-expressing monocytes correlate with arterial stiffness in HIV-infected ART-naïve men. HIV Clin Trials. 2018;19(2):39-45.
183. Barska K, Kwiatkowska W, Knysz B, Arczyńska K, Karczewski M, Witkiewicz W. The role of the tissue factor and its inhibitor in the development of subclinical atherosclerosis in people living with HIV. PLoS One. 2017;12(7):e0181533.
184. Schechter ME, Andrade BB, He T, Richter GH, Tosh KW, Policicchio BB, et al. Inflammatory monocytes expressing tissue factor drive SIV and HIV coagulopathy. Sci Transl Med. 2017;9(405).
185. 1993 revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Recomm Rep. 1992;41(Rr-17):1-19.
186. Schneider E, Whitmore S, Glynn MK, Dominguez K, Mitsch A, McKenna MT. Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged< 18 months and for HIV infection and AIDS among children aged 18 months to< 13 years—United States, 2008. Morbidity and Mortality Weekly Report: Recommendations and Reports. 2008;57(10):1-12.
187. Taylor JM, Fahey JL, Detels R, Giorgi JV. CD4 percentage, CD4 number, and CD4:CD8 ratio in HIV infection: which to choose and how to use. J Acquir Immune Defic Syndr (1988). 1989;2(2):114-24.
188. Fahey JL, Taylor JM, Detels R, Hofmann B, Melmed R, Nishanian P, et al. The prognostic value of cellular and serologic markers in infection with human immunodeficiency virus type 1. N Engl J Med. 1990;322(3):166-72.
189. Masur H, Ognibene FP, Yarchoan R, Shelhamer JH, Baird BF, Travis W, et al. CD4 counts as predictors of opportunistic pneumonias in human immunodeficiency virus (HIV) infection. Ann Intern Med. 1989;111(3):223-31.
190. Anglaret X, Diagbouga S, Mortier E, Meda N, Vergé-Valette V, Sylla-Koko F, et al. CD4+ T-lymphocyte counts in HIV infection: are European standards applicable to African patients? J Acquir Immune Defic Syndr Hum Retrovirol. 1997;14(4):361-7.
191. Gupta V, Gupta S. Laboratory markers associated with progression of HIV infection. Indian J Med Microbiol. 2004;22(1):7-15.

192. Mocroft A, Youle M, Phillips AN, Halai R, Easterbrook P, Johnson MA, et al. The Incidence of AIDS-Defining Illnesses in 4883 Patients With Human Immunodeficiency Virus Infection. Archives of Internal Medicine. 1998;158(5):491-7.
193. Martinez-Steele E, Awasana AA, Corrah T, Sabally S, van der Sande M, Jaye A, et al. Is HIV-2- induced AIDS different from HIV-1-associated AIDS? Data from a West African clinic. AIDS. 2007;21(3):317-24.
194. Pantaleo G, Graziosi C, Fauci AS. The Immunopathogenesis of Human Immunodeficiency Virus Infection. New England Journal of Medicine. 1993;328(5):327-35.
195. Chaplin DD. Overview of the immune response. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S3-23.
196. Stubbington MJT, Rozenblatt-Rosen O, Regev A, Teichmann SA. Single-cell transcriptomics to explore the immune system in health and disease. Science. 2017;358(6359):58-63.
197. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. Nature Immunology. 2015;16(4):343-53.
198. Paludan SR, Pradeu T, Masters SL, Mogensen TH. Constitutive immune mechanisms: mediators of host defence and immune regulation. Nature Reviews Immunology. 2021;21(3):137-50.
199. Colomer-Lluch M, Ruiz A, Moris A, Prado JG. Restriction Factors: From Intrinsic Viral Restriction to Shaping Cellular Immunity Against HIV-1. Front Immunol. 2018;9:2876.
200. Doyle T, Goujon C, Malim MH. HIV-1 and interferons: who's interfering with whom? Nature Reviews Microbiology. 2015;13(7):403-13.
201. Sauter D, Kirchhoff F. Chapter 4 - Properties of Human and Simian Immunodeficiency Viruses. In: Ansari AA, Silvestri G, editors. Natural Hosts of SIV. Amsterdam: Elsevier; 2014. p. 69-84.
202. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. Cell. 2010;140(6):805-20.
203. Li D, Wu M. Pattern recognition receptors in health and diseases. Signal Transduct Target Ther. 2021;6(1):291.
204. Zuniga EI, Macal M, Lewis GM, Harker JA. Innate and Adaptive Immune Regulation During Chronic Viral Infections. Annual Review of Virology. 2015;2(1):573-97.
205. Qi H, Kastenmüller W, Germain RN. Spatiotemporal Basis of Innate and Adaptive Immunity in Secondary Lymphoid Tissue. Annual Review of Cell and Developmental Biology. 2014;30(1):141-67.
206. Wacleche VS, Tremblay CL, Routy JP, Ancuta P. The Biology of Monocytes and Dendritic Cells: Contribution to HIV Pathogenesis. Viruses. 2018;10(2).
207. Halper-Stromberg A, Jabri B. Maladaptive consequences of inflammatory events shape individual immune identity. Nature Immunology. 2022;23(12):1675-86.
208. Vannella KM, Wynn TA. Mechanisms of Organ Injury and Repair by Macrophages. Annual Review of Physiology. 2017;79(1):593-617.

209. Teer E, Dominick L, Mukonowenzou NC, Essop MF. HIV-Related Myocardial Fibrosis: Inflammatory Hypothesis and Crucial Role of Immune Cells Dysregulation. Cells. 2022;11(18):2825.
210. Zeng M, Smith AJ, Wietgrefe SW, Southern PJ, Schacker TW, Reilly CS, et al. Cumulative mechanisms of lymphoid tissue fibrosis and T cell depletion in HIV-1 and SIV infections. The Journal of Clinical Investigation. 2011;121(3):998-1008.
211. Cavaleiro R, Tendeiro R, Foxall RB, Soares RS, Baptista AP, Gomes P, et al. Monocyte and myeloid dendritic cell activation occurs throughout HIV type 2 infection, an attenuated form of HIV disease. J Infect Dis. 2013;207(11):1730-42.
212. Iannetta M, Isnard S, Manuzak J, Guillerme J-B, Notin M, Bailly K, et al. Conventional Dendritic Cells and Slan+ Monocytes During HIV-2 Infection. Frontiers in Immunology. 2020;11.
213. Rhodes JW, Tong O, Harman AN, Turville SG. Human Dendritic Cell Subsets, Ontogeny, and Impact on HIV Infection. Frontiers in Immunology. 2019;10.
214. Cabeza-Cabrerizo M, Cardoso A, Minutti CM, Costa MPd, Sousa CRe. Dendritic Cells Revisited. Annual Review of Immunology. 2021;39(1):131-66.
215. Beignon A-S, McKenna K, Skoberne M, Manches O, DaSilva I, Kavanagh DG, et al. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor–viral RNA interactions. The Journal of clinical investigation. 2005;115(11):3265-75.
216. Schoggins JW. Interferon-Stimulated Genes: What Do They All Do? Annual Review of Virology. 2019;6(1):567-84.
217. Cavaleiro R, Baptista AP, Soares RS, Tendeiro R, Foxall RB, Gomes P, et al. Major depletion of plasmacytoid dendritic cells in HIV-2 infection, an attenuated form of HIV disease. PLoS Pathog. 2009;5(11):e1000667.
218. Alrubayyi A, Rowland-Jones S, Peppa D. Natural killer cells during acute HIV-1 infection: clues for HIV-1 prevention and therapy. AIDS. 2022;36(14):1903-15.
219. Paul S, Lal G. The Molecular Mechanism of Natural Killer Cells Function and Its Importance in Cancer Immunotherapy. Frontiers in Immunology. 2017;8.
220. Ramírez-Labrada A, Pesini C, Santiago L, Hidalgo S, Calvo-Pérez A, Oñate C, et al. All About (NK Cell-Mediated) Death in Two Acts and an Unexpected Encore: Initiation, Execution and Activation of Adaptive Immunity. Frontiers in Immunology. 2022;13.
221. Wang W, Erbe AK, Hank JA, Morris ZS, Sondel PM. NK Cell-Mediated Antibody-Dependent Cellular Cytotoxicity in Cancer Immunotherapy. Frontiers in Immunology. 2015;6.
222. Zhao NQ, Vendrame E, Ferreira AM, Seiler C, Ranganath T, Alary M, et al. Natural killer cell phenotype is altered in HIV-exposed seronegative women. PLoS One. 2020;15(9):e0238347.
223. Bernard NF, Alsulami K, Pavey E, Dupuy FP. NK Cells in Protection from HIV Infection. Viruses. 2022;14(6).
224. Peterson TA, Kimani J, Wachihi C, Bielawny T, Mendoza L, Thavaneswaran S, et al. HLA class I associations with rates of HIV-1 seroconversion and disease progression in the Pumwani Sex Worker Cohort. Tissue Antigens. 2013;81(2):93-107.

225. Hopfensperger K, Richard J, Stürzel CM, Bibollet-Ruche F, Apps R, Leoz M, et al. Convergent Evolution of HLA-C Downmodulation in HIV-1 and HIV-2. mBio. 2020;11(4).
226. Mujal AM, Delconte RB, Sun JC. Natural Killer Cells: From Innate to Adaptive Features. Annual Review of Immunology. 2021;39(1):417-47.
227. Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, et al. Defining trained immunity and its role in health and disease. Nature Reviews Immunology. 2020;20(6):375-88.
228. Hammer Q, Rückert T, Borst EM, Dunst J, Haubner A, Durek P, et al. Peptide-specific recognition of human cytomegalovirus strains controls adaptive natural killer cells. Nat Immunol. 2018;19(5):453-63.
229. Min-Oo G, Lanier LL. Cytomegalovirus generates long-lived antigen-specific NK cells with diminished bystander activation to heterologous infection. J Exp Med. 2014;211(13):2669-80.
230. Nikzad R, Angelo LS, Aviles-Padilla K, Le DT, Singh VK, Bimler L, et al. Human natural killer cells mediate adaptive immunity to viral antigens. Science Immunology. 2019;4(35):eaat8116.
231. Paust S, Gill HS, Wang B-Z, Flynn MP, Moseman EA, Senman B, et al. Critical role for the chemokine receptor CXCR6 in NK cell–mediated antigen-specific memory of haptens and viruses. Nature Immunology. 2010;11(12):1127-35.
232. Jackson K, Kidd M, Wang Y, Collins A. The Shape of the Lymphocyte Receptor Repertoire: Lessons from the B Cell Receptor. Frontiers in Immunology. 2013;4.
233. Bassing CH, Swat W, Alt FW. The Mechanism and Regulation of Chromosomal V(D)J Recombination. Cell. 2002;109(2):S45-S55.
234. Adams NM, Grassmann S, Sun JC. Clonal expansion of innate and adaptive lymphocytes. Nature Reviews Immunology. 2020;20(11):694-707.
235. Koup R, Safrit JT, Cao Y, Andrews CA, McLeod G, Borkowsky W, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. Journal of virology. 1994;68(7):4650-5.
236. Sáez-Cirión A, Manel N. Immune Responses to Retroviruses. Annual Review of Immunology. 2018;36(1):193-220.
237. Duvall MG, Precopio ML, Ambrozak DA, Jaye A, McMichael AJ, Whittle HC, et al. Polyfunctional T cell responses are a hallmark of HIV-2 infection. Eur J Immunol. 2008;38(2):350-63.
238. Leligdowicz A, Onyango C, Yindom LM, Peng Y, Cotten M, Jaye A, et al. Highly avid, oligoclonal, early-differentiated antigen-specific CD8+ T cells in chronic HIV-2 infection. Eur J Immunol. 2010;40(7):1963-72.
239. Lopes AR, Jaye A, Dorrell L, Sabally S, Alabi A, Jones NA, et al. Greater CD8+ TCR heterogeneity and functional flexibility in HIV-2 compared to HIV-1 infection. J Immunol. 2003;171(1):307-16.

240. Jennes W, Camara M, Dièye T, Mboup S, Kestens L. Higher homologous and lower cross-reactive Gag-specific T-cell responses in human immunodeficiency virus type 2 (HIV-2) than in HIV-1 infection. J Virol. 2008;82(17):8619-28.
241. McLane LM, Abdel-Hakeem MS, Wherry EJ. CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annual Review of Immunology. 2019;37(1):457-95.
242. Honge BL, Petersen MS, Jespersen S, Medina C, Te DDS, Kjerulff B, et al. T-cell and B-cell perturbations identify distinct differences in HIV-2 compared with HIV-1-induced immunodeficiency. AIDS. 2019;33(7):1131-41.
243. Scharf L, Pedersen CB, Johansson E, Lindman J, Olsen LR, Buggert M, et al. Inverted CD8 T-Cell Exhaustion and Co-Stimulation Marker Balance Differentiate Aviremic HIV-2-Infected From Seronegative Individuals. Front Immunol. 2021;12:744530.
244. Speiser DE, Utzschneider DT, Oberle SG, Münz C, Romero P, Zehn D. T cell differentiation in chronic infection and cancer: functional adaptation or exhaustion? Nature reviews immunology. 2014;14(11):768-74.
245. Kahan SM, Wherry EJ, Zajac AJ. T cell exhaustion during persistent viral infections. Virology. 2015;479:180-93.
246. Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. Nature. 2006;443(7109):350-4.
247. Zander R, Cui W. Exhausted CD8(+) T cells face a developmental fork in the road. Trends Immunol. 2023.
248. Gubser C, Chiu C, Lewin SR, Rasmussen TA. Immune checkpoint blockade in HIV. eBioMedicine. 2022;76.
249. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. Nature. 2016;537(7620):417-21.
250. He R, Hou S, Liu C, Zhang A, Bai Q, Han M, et al. Follicular CXCR5-expressing CD8+ T cells curtail chronic viral infection. Nature. 2016;537(7620):412-6.
251. Utzschneider DT, Charmoy M, Chennupati V, Pousse L, Ferreira DP, Calderon-Copete S, et al. T Cell Factor 1-Expressing Memory-like CD8+ T Cells Sustain the Immune Response to Chronic Viral Infections. Immunity. 2016;45(2):415-27.
252. Luckheeram RV, Zhou R, Verma AD, Xia B. CD4⁺T Cells: Differentiation and Functions. Clinical and Developmental Immunology. 2012;2012:925135.
253. Swain SL, McKinstry KK, Strutt TM. Expanding roles for CD4+ T cells in immunity to viruses. Nature Reviews Immunology. 2012;12(2):136-48.
254. Crotty S. T Follicular Helper Cell Differentiation, Function, and Roles in Disease. Immunity. 2014;41(4):529-42.
255. Fromentin R, Bakeman W, Lawani MB, Khoury G, Hartogensis W, DaFonseca S, et al. CD4+ T cells expressing PD-1, TIGIT and LAG-3 contribute to HIV persistence during ART. PLoS pathogens. 2016;12(7):e1005761.

256. Wightman F, Solomon A, Kumar SS, Urriola N, Gallagher K, Hiener B, et al. Effect of ipilimumab on the HIV reservoir in an HIV-infected individual with metastatic melanoma. AIDS (London, England). 2015;29(4):504.
257. Lau JS, McMahon JH, Gubser C, Solomon A, Chiu CY, Dantanarayana A, et al. The Impact of Immune Checkpoint Therapy on the Latent reservoir in HIV Infected Individuals with Cancer on Antiretroviral Therapy. AIDS (London, England). 2021;35(10):1631.
258. Gay CL, Bosch RJ, McKhann A, Moseley KF, Wimbish CL, Hendrickx SM, et al. Suspected Immune-Related Adverse Events With an Anti-PD-1 Inhibitor in Otherwise Healthy People With HIV. J Acquir Immune Defic Syndr. 2021;87(5):e234-e6.
259. Sahin IH, Kane SR, Brutcher E, Guadagno J, Smith KE, Wu C, et al. Safety and Efficacy of Immune Checkpoint Inhibitors in Patients With Cancer Living With HIV: A Perspective on Recent Progress and Future Needs. JCO Oncol Pract. 2020;16(6):319-25.
260. Melchers F. Checkpoints that control B cell development. J Clin Invest. 2015;125(6):2203-10.
261. Kienzler A-K, Eibel H. Human B Cell Development and Tolerance. In: Ratcliffe MJH, editor. Encyclopedia of Immunobiology. Oxford: Academic Press; 2016. p. 105-21.
262. Cyster JG. B cell follicles and antigen encounters of the third kind. Nature Immunology. 2010;11(11):989-96.
263. Lam JH, Smith FL, Baumgarth N. B Cell Activation and Response Regulation During Viral Infections. Viral Immunol. 2020;33(4):294-306.
264. de Carvalho RVH, Ersching J, Barbulescu A, Hobbs A, Castro TBR, Mesin L, et al. Clonal replacement sustains long-lived germinal centers primed by respiratory viruses. Cell. 2023;186(1):131-46.e13.
265. Victora GD, Nussenzweig MC. Germinal Centers. Annual Review of Immunology. 2022;40(1):413-42.
266. Mesin L, Ersching J, Victora Gabriel D. Germinal Center B Cell Dynamics. Immunity. 2016;45(3):471-82.
267. Roco JA, Mesin L, Binder SC, Nefzger C, Gonzalez-Figueroa P, Canete PF, et al. Class-Switch Recombination Occurs Infrequently in Germinal Centers. Immunity. 2019;51(2):337-50.e7.
268. Lu LL, Suscovich TJ, Fortune SM, Alter G. Beyond binding: antibody effector functions in infectious diseases. Nat Rev Immunol. 2018;18(1):46-61.
269. Stephenson KE, Wagh K, Korber B, Barouch DH. Vaccines and Broadly Neutralizing Antibodies for HIV-1 Prevention. Annual Review of Immunology. 2020;38(1):673-703.
270. Knox JJ, Myles A, Cancro MP. T-bet(+) memory B cells: Generation, function, and fate. Immunol Rev. 2019;288(1):149-60.
271. Barnett BE, Staupe RP, Odorizzi PM, Palko O, Tomov VT, Mahan AE, et al. Cutting Edge: B Cell–Intrinsic T-bet Expression Is Required To Control Chronic Viral Infection. The Journal of Immunology. 2016;197(4):1017-22.

272. Myles A, Sanz I, Cancro MP. T-bet(+) B cells: A common denominator in protective and autoreactive antibody responses? Curr Opin Immunol. 2019;57:40-5.
273. Burnett DL, Schofield P, Langley DB, Jackson J, Bourne K, Wilson E, et al. Conformational diversity facilitates antibody mutation trajectories and discrimination between foreign and self-antigens. Proceedings of the National Academy of Sciences. 2020;117(36):22341-50.
274. Baumgarth N. How specific is too specific? B-cell responses to viral infections reveal the importance of breadth over depth. Immunol Rev. 2013;255(1):82-94.
275. Root-Bernstein R. Human Immunodeficiency Virus Proteins Mimic Human T Cell Receptors Inducing Cross-Reactive Antibodies. Int J Mol Sci. 2017;18(10).
276. Haynes BF, Fleming J, St. Clair EW, Katinger H, Stiegler G, Kunert R, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005;308(5730):1906-8.
277. Mouquet H, Scheid JF, Zoller MJ, Krogsgaard M, Ott RG, Shukair S, et al. Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. Nature. 2010;467(7315):591-5.
278. Roskin KM, Jackson KJL, Lee J-Y, Hoh RA, Joshi SA, Hwang K-K, et al. Aberrant B cell repertoire selection associated with HIV neutralizing antibody breadth. Nature Immunology. 2020;21(2):199-209.
279. Yoshida T, Mei H, Dörner T, Hiepe F, Radbruch A, Fillatreau S, et al. Memory B and memory plasma cells. Immunological Reviews. 2010;237(1):117-39.
280. Moir S, Ho J, Malaspina A, Wang W, DiPoto AC, O'Shea MA, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. J Exp Med. 2008;205(8):1797-805.
281. Johansson E, Kerkman PF, Scharf L, Lindman J, Szojka ZI, Mansson F, et al. Hierarchical Clustering and Trajectory Analyses Reveal Viremia-Independent B-Cell Perturbations in HIV-2 Infection. Cells. 2022;11(19).
282. Ponnan SM, Vidyavijayan KK, Thiruvengadam K, Hilda JN, Mathayan M, Murugavel KG, et al. Role of Circulating T Follicular Helper Cells and Stem-Like Memory CD4(+) T Cells in the Pathogenesis of HIV-2 Infection and Disease Progression. Front Immunol. 2021;12:666388.
283. Tendeiro R, Fernandes S, Foxall RB, Marcelino JM, Taveira N, Soares RS, et al. Memory B-cell depletion is a feature of HIV-2 infection even in the absence of detectable viremia. AIDS. 2012;26(13):1607-17.
284. Portugal S, Tipton CM, Sohn H, Kone Y, Wang J, Li S, et al. Malaria-associated atypical memory B cells exhibit markedly reduced B cell receptor signaling and effector function. Elife. 2015;4:e07218.
285. Sullivan RT, Kim CC, Fontana MF, Feeney ME, Jagannathan P, Boyle MJ, et al. FCRL5 Delineates Functionally Impaired Memory B Cells Associated with Plasmodium falciparum Exposure. PLoS Pathog. 2015;11(5):e1004894.
286. Knox JJ, Kaplan DE, Betts MR. T-bet-expressing B cells during HIV and HCV infections. Cell Immunol. 2017;321:26-34.

287. Knox JJ, Buggert M, Kardava L, Seaton KE, Eller MA, Canaday DH, et al. T-bet+ B cells are induced by human viral infections and dominate the HIV gp140 response. JCI Insight. 2017;2(8):e92943.
288. Austin JW, Buckner CM, Kardava L, Wang W, Zhang X, Melson VA, et al. Overexpression of T-bet in HIV infection is associated with accumulation of B cells outside germinal centers and poor affinity maturation. Sci Transl Med. 2019;11(520).
289. Cubas RA, Mudd JC, Savoye A-L, Perreau M, van Grevenynghe J, Metcalf T, et al. Inadequate T follicular cell help impairs B cell immunity during HIV infection. Nature Medicine. 2013;19(4):494-9.
290. Griffith SA, McCoy LE. To bnAb or Not to bnAb: Defining Broadly Neutralising Antibodies Against HIV-1. Frontiers in Immunology. 2021;12.
291. Bjorling E, Scarlatti G, von Gegerfelt A, Albert J, Biberfeld G, Chiodi F, et al. Autologous neutralizing antibodies prevail in HIV-2 but not in HIV-1 infection. Virology. 1993;193(1):528-30.
292. Kong R, Li H, Bibollet-Ruche F, Decker JM, Zheng NN, Gottlieb GS, et al. Broad and potent neutralizing antibody responses elicited in natural HIV-2 infection. J Virol. 2012;86(2):947-60.
293. Ozkaya Sahin G, Holmgren B, Sheik-Khalil E, da Silva Z, Nielsen J, Nowroozalizadeh S, et al. Effect of complement on HIV-2 plasma antiviral activity is intratype specific and potent. J Virol. 2013;87(1):273-81.
294. Shi Y, Brandin E, Vincic E, Jansson M, Blaxhult A, Gyllensten K, et al. Evolution of human immunodeficiency virus type 2 coreceptor usage, autologous neutralization, envelope sequence and glycosylation. J Gen Virol. 2005;86(Pt 12):3385-96.
295. de Silva TI, Aasa-Chapman M, Cotten M, Hue S, Robinson J, Bibollet-Ruche F, et al. Potent autologous and heterologous neutralizing antibody responses occur in HIV-2 infection across a broad range of infection outcomes. J Virol. 2012;86(2):930-46.
296. Leggat DJ, Cohen KW, Willis JR, Fulp WJ, deCamp AC, Kalyuzhniy O, et al. Vaccination induces HIV broadly neutralizing antibody precursors in humans. Science. 2022;378(6623):eadd6502.
297. Martinez-Murillo P, Tran K, Guenaga J, Lindgren G, Àdori M, Feng Y, et al. Particulate Array of Well-Ordered HIV Clade C Env Trimers Elicits Neutralizing Antibodies that Display a Unique V2 Cap Approach. Immunity. 2017;46(5):804-17.e7.
298. Fischl MA, Richman DD, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, et al. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. N Engl J Med. 1987;317(4):185-91.
299. Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, et al. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. N Engl J Med. 1997;337(11):725-33.

300. Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med. 1998;338(13):853-60.
301. Moranguinho I, Taveira N, Bártolo I. Antiretroviral Treatment of HIV-2 Infection: Available Drugs, Resistance Pathways, and Promising New Compounds. Int J Mol Sci. 2023;24(6).
302. Wittkop L, Arsandaux J, Trevino A, Schim van der Loeff M, Anderson J, van Sighem A, et al. CD4 cell count response to first-line combination ART in HIV-2+ patients compared with HIV-1+ patients: a multinational, multicohort European study. Journal of Antimicrobial Chemotherapy. 2017;72(10):2869-78.
303. Minchella PA, Adjé-Touré C, Zhang G, Tehe A, Hedje J, Rottinghaus ER, et al. Long-term immunological responses to treatment among HIV-2 patients in Côte d'Ivoire. BMC Infectious Diseases. 2020;20(1):213.
304. Deeks SG, Lewin SR, Ross AL, Ananworanich J, Benkirane M, Cannon P, et al. International AIDS Society global scientific strategy: towards an HIV cure 2016. Nature medicine. 2016;22(8):839-50.
305. Zhou C, Wu Y, Zhang Y, Wang Y, Wu H, Zhang T, et al. Factors associated with post-treatment control of viral load in HIV-infected patients: a systematic review and meta-analysis. International Journal of Infectious Diseases. 2023;129:216-27.
306. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. Nature. 2019;568(7751):244-8.
307. Hütter G, Nowak D, Mossner M, Ganepola S, Müßig A, Allers K, et al. Long-Term Control of HIV by CCR5 Delta32/Delta32 Stem-Cell Transplantation. New England Journal of Medicine. 2009;360(7):692-8.
308. Jensen B-EO, Knops E, Cords L, Lübke N, Salgado M, Busman-Sahay K, et al. In-depth virological and immunological characterization of HIV-1 cure after CCR5Δ32/Δ32 allogeneic hematopoietic stem cell transplantation. Nature Medicine. 2023;29(3):583-7.
309. Hønge BL, Jespersen S, Nordentoft PB, Medina C, da Silva D, da Silva ZJ, et al. Loss to follow-up occurs at all stages in the diagnostic and follow-up period among HIV-infected patients in Guinea-Bissau: a 7-year retrospective cohort study. BMJ Open. 2013;3(10):e003499.
310. Jensen MM, Byberg S, Jespersen S, Olesen JS, da Silva ZJ, Medina C, et al. The HIV care continuum of Guinea-Bissau; Progress towards the UNAIDS 90-90-90 targets for HIV-1 and HIV-2. Acta Trop. 2023:106887.
311. Ozkaya Sahin G, Mansson F, Palm AA, Vincic E, da Silva Z, Medstrand P, et al. Frequent intratype neutralization by plasma immunoglobulin a identified in HIV type 2 infection. AIDS Res Hum Retroviruses. 2013;29(3):470-8.
312. WHO Guidelines Approved by the Guidelines Review Committee. Consolidated Guidelines on the Use of Antiretroviral Drugs for Treating and Preventing HIV Infection: Recommendations for a Public Health Approach.Vol. 2016. Geneva: World Health Organization Copyright © World Health Organization (2016).

313. Pernemalm M, Lewensohn R, Lehtiö J. Affinity prefractionation for MS-based plasma proteomics. PROTEOMICS. 2009;9(6):1420-7.
314. Pieper R, Su Q, Gatlin CL, Huang ST, Anderson NL, Steiner S. Multi-component immunoaffinity subtraction chromatography: an innovative step towards a comprehensive survey of the human plasma proteome. Proteomics. 2003;3(4):422-32.
315. Pham TV, Henneman AA, Jimenez CR. iq: an R package to estimate relative protein abundances from ion quantification in DIA-MS-based proteomics. Bioinformatics. 2020;36(8):2611-3.
316. Smilde AK, van der Werf MJ, Bijlsma S, van der Werff-van der Vat BJC, Jellema RH. Fusion of Mass Spectrometry-Based Metabolomics Data. Analytical Chemistry. 2005;77(20):6729-36.
317. Huber W, von Heydebreck A, Sültmann H, Poustka A, Vingron M. Variance stabilization applied to microarray data calibration and to the quantification of differential expression. Bioinformatics. 2002;18 Suppl 1:S96-104.
318. T T. A Package for Survival Analysis in R 2023 [Available from: https://CRAN.R-project.org/package=survival [Accessed 2023-02-16].
319. Roederer M, Nozzi JL, Nason MC. SPICE: Exploration and analysis of post-cytometric complex multivariate datasets. Cytometry Part A. 2011;79A(2):167-74.
320. Quintelier K, Couckuyt A, Emmaneel A, Aerts J, Saeys Y, Van Gassen S. Analyzing high-dimensional cytometry data using FlowSOM. Nat Protoc. 2021;16(8):3775-801.
321. Melsen JE, van Ostaijen-Ten Dam MM, Lankester AC, Schilham MW, van den Akker EB. A Comprehensive Workflow for Applying Single-Cell Clustering and Pseudotime Analysis to Flow Cytometry Data. J Immunol. 2020;205(3):864-71.
322. Street K, Risso D, Fletcher RB, Das D, Ngai J, Yosef N, et al. Slingshot: cell lineage and pseudotime inference for single-cell transcriptomics. BMC Genomics. 2018;19(1):477.
323. Van Gassen S, Callebaut B, Van Helden MJ, Lambrecht BN, Demeester P, Dhaene T, et al. FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. Cytometry A. 2015;87(7):636-45.
324. Becht E, McInnes L, Healy J, Dutertre C-A, Kwok IWH, Ng LG, et al. Dimensionality reduction for visualizing single-cell data using UMAP. Nature Biotechnology. 2019;37(1):38-44.
325. Melville J. uwot: The Uniform Manifold Approximation and Projection (UMAP) Method for Dimensionality Reduction. R package version 0.1.11. 2021 [Available from: https://CRAN.R-project.org/package=uwot [accessed on 03-04-22].
326. Lê S, Josse J, Husson F. FactoMineR: An R Package for Multivariate Analysis. Journal of Statistical Software. 2008;25(1):1 - 18.
327. Wu X, Hasan MA, Chen JY. Pathway and network analysis in proteomics. J Theor Biol. 2014;362:44-52.
328. Palm AA, Lemey P, Jansson M, Mansson F, Kvist A, Szojka Z, et al. Low Postseroconversion CD4(+) T-cell Level Is Associated with Faster Disease Progression and Higher Viral Evolutionary Rate in HIV-2 Infection. mBio. 2019;10(1).

329. Arthur L, Esaulova E, Mogilenko DA, Tsurinov P, Burdess S, Laha A, et al. Cellular and plasma proteomic determinants of COVID-19 and non-COVID-19 pulmonary diseases relative to healthy aging. Nature Aging. 2021;1(6):535-49.
330. Atlas HP. [Available from: proteinatlas.org [Accessed 2023-02-08].
331. Karlsson M, Zhang C, Méar L, Zhong W, Digre A, Katona B, et al. A single-cell type transcriptomics map of human tissues. Science Advances. 2021;7(31):eabh2169.
332. Tauriainen J, Scharf L, Frederiksen J, Naji A, Ljunggren H-G, Sönnerborg A, et al. Perturbed CD8+ T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals. Scientific Reports. 2017;7(1):40354.
333. Yu X, Harden K, Gonzalez LC, Francesco M, Chiang E, Irving B, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol. 2009;10(1):48-57.
334. Johnston RJ, Yu X, Grogan JL. The checkpoint inhibitor TIGIT limits antitumor and antiviral CD8(+) T cell responses. Oncoimmunology. 2015;4(9):e1036214.
335. Weulersse M, Asrir A, Pichler AC, Lemaitre L, Braun M, Carrié N, et al. Eomes-Dependent Loss of the Co-activating Receptor CD226 Restrains CD8(+) T Cell Anti-tumor Functions and Limits the Efficacy of Cancer Immunotherapy. Immunity. 2020;53(4):824-39.e10.
336. Chiang EY, Mellman I. TIGIT-CD226-PVR axis: advancing immune checkpoint blockade for cancer immunotherapy. J Immunother Cancer. 2022;10(4).
337. Chew GM, Fujita T, Webb GM, Burwitz BJ, Wu HL, Reed JS, et al. TIGIT marks exhausted T cells, correlates with disease progression, and serves as a target for immune restoration in HIV and SIV infection. PLoS pathogens. 2016;12(1):e1005349.
338. Tauriainen J, Scharf L, Frederiksen J, Naji A, Ljunggren H, Sönnerborg A, et al. Perturbed CD8+ T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals. Sci Rep 7: 40354. 2017.
339. Soares RS, Tendeiro R, Foxall RB, Baptista AP, Cavaleiro R, Gomes P, et al. Cell-associated viral burden provides evidence of ongoing viral replication in aviremic HIV-2-infected patients. J Virol. 2011;85(5):2429-38.
340. Fernandes SM, Pires AR, Matoso P, Ferreira C, Nunes-Cabaco H, Correia L, et al. HIV-2 infection is associated with preserved GALT homeostasis and epithelial integrity despite ongoing mucosal viral replication. Mucosal Immunol. 2018;11(1):236-48.
341. Eccles JD, Turner RB, Kirk NA, Muehling LM, Borish L, Steinke JW, et al. T-bet+ Memory B Cells Link to Local Cross-Reactive IgG upon Human Rhinovirus Infection. Cell Rep. 2020;30(2):351-66 e7.
342. Sindhava VJ, Oropallo MA, Moody K, Naradikian M, Higdon LE, Zhou L, et al. A TLR9-dependent checkpoint governs B cell responses to DNA-containing antigens. The Journal of Clinical Investigation. 2017;127(5):1651-63.
343. Peng SL, Szabo SJ, Glimcher LH. T-bet regulates IgG class switching and pathogenic autoantibody production. Proc Natl Acad Sci U S A. 2002;99(8):5545-50.

344. Wang S, Wang J, Kumar V, Karnell JL, Naiman B, Gross PS, et al. IL-21 drives expansion and plasma cell differentiation of autoreactive CD11c(hi)T-bet(+) B cells in SLE. Nat Commun. 2018;9(1):1758.
345. Johnson JL, Rosenthal RL, Knox JJ, Myles A, Naradikian MS, Madej J, et al. The Transcription Factor T-bet Resolves Memory B Cell Subsets with Distinct Tissue Distributions and Antibody Specificities in Mice and Humans. Immunity. 2020;52(5):842-55.e6.
346. Dominick L, Midgley N, Swart LM, Sprake D, Deshpande G, Laher I, et al. HIV-related cardiovascular diseases: the search for a unifying hypothesis. Am J Physiol Heart Circ Physiol. 2020;318(4):H731-h46.
347. Perkins MV, Joseph SB, Dittmer DP, Mackman N. Cardiovascular Disease and Thrombosis in HIV Infection. Arterioscler Thromb Vasc Biol. 2023;43(2):175-91.
348. Bachle SM, Malone DF, Buggert M, Karlsson AC, Isberg PE, Biague AJ, et al. Elevated levels of invariant natural killer T-cell and natural killer cell activation correlate with disease progression in HIV-1 and HIV-2 infections. AIDS. 2016;30(11):1713-22.
349. Palm AA, Veerla S, Lindman J, Isberg P-E, Johansson E, Biague A, et al. Interferon Alpha-Inducible Protein 27 Expression Is Linked to Disease Severity in Chronic Infection of Both HIV-1 and HIV-2. Frontiers in Virology. 2022;2.
350. Hrecka K, Hao C, Gierszewska M, Swanson SK, Kesik-Brodacka M, Srivastava S, et al. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature. 2011;474(7353):658-61.
351. Herbein G, Varin A. The macrophage in HIV-1 infection: From activation to deactivation? Retrovirology. 2010;7(1):33.
352. Willey SJ, Reeves JD, Hudson R, Miyake K, Dejucq N, Schols D, et al. Identification of a subset of human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus strains able to exploit an alternative coreceptor on untransformed human brain and lymphoid cells. J Virol. 2003;77(11):6138-52.
353. Sauce D, Larsen M, Fastenackels S, Pauchard M, Ait-Mohand H, Schneider L, et al. HIV disease progression despite suppression of viral replication is associated with exhaustion of lymphopoiesis. Blood. 2011;117(19):5142-51.
354. Tsukamoto T. Hematopoietic Stem/Progenitor Cells and the Pathogenesis of HIV/AIDS. Frontiers in Cellular and Infection Microbiology. 2020;10.
355. Rb-Silva R, Nobrega C, Azevedo C, Athayde E, Canto-Gomes J, Ferreira I, et al. Thymic Function as a Predictor of Immune Recovery in Chronically HIV-Infected Patients Initiating Antiretroviral Therapy. Frontiers in Immunology. 2019;10.
356. Dion M-L, Poulin J-F, Bordi R, Sylvestre M, Corsini R, Kettaf N, et al. HIV Infection Rapidly Induces and Maintains a Substantial Suppression of Thymocyte Proliferation. Immunity. 2004;21(6):757-68.
357. Ferrando-Martinez S, De Pablo-Bernal RS, De Luna-Romero M, De Ory SJ, Genebat M, Pacheco YM, et al. Thymic Function Failure Is Associated With Human Immunodeficiency Virus Disease Progression. Clinical Infectious Diseases. 2017;64(9):1191-7.

358. Li T, Wu N, Dai Y, Qiu Z, Han Y, Xie J, et al. Reduced thymic output is a major mechanism of immune reconstitution failure in HIV-infected patients after long-term antiretroviral therapy. Clinical Infectious Diseases. 2011;53(9):944-51.

359. Gautier D, Beq S, Cortesão CS, Sousa AE, Cheynier R. Efficient thymopoiesis contributes to the maintenance of peripheral CD4 T cells during chronic human immunodeficiency virus type 2 infection. J Virol. 2007;81(22):12685-8.

360. Wan L-Y, Huang H-H, Zhen C, Chen S-Y, Song B, Cao W-J, et al. Distinct inflammation-related proteins associated with T cell immune recovery during chronic HIV-1 infection. Emerging Microbes & Infections. 2023;12(1):2150566.

361. Meyer-Myklestad MH, Medhus AW, Lorvik KB, Seljeflot I, Hansen SH, Holm K, et al. Human Immunodeficiency Virus–Infected Immunological Nonresponders Have Colon-Restricted Gut Mucosal Immune Dysfunction. The Journal of Infectious Diseases. 2020;225(4):661-74.

362. Berry N, Jaffar S, Schim van der Loeff M, Ariyoshi K, Harding E, N'Gom PT, et al. Low level viremia and high CD4% predict normal survival in a cohort of HIV type-2-infected villagers. AIDS Res Hum Retroviruses. 2002;18(16):1167-73.

363. Wong JK, Yukl SA. Tissue reservoirs of HIV. Curr Opin HIV AIDS. 2016;11(4):362-70.

364. So-Armah K, Benjamin LA, Bloomfield GS, Feinstein MJ, Hsue P, Njuguna B, et al. HIV and cardiovascular disease. Lancet HIV. 2020;7(4):e279-e93.

365. Jaffar S, Van der Loeff MS, Eugen-Olsen J, Vincent T, Sarje-Njie R, Ngom P, et al. Immunological predictors of survival in HIV type 2-infected rural villagers in Guinea-Bissau. AIDS Res Hum Retroviruses. 2005;21(6):560-4.

366. Zuliani-Alvarez L, Govasli ML, Rasaiyaah J, Monit C, Perry SO, Sumner RP, et al. Evasion of cGAS and TRIM5 defines pandemic HIV. Nat Microbiol. 2022;7(11):1762-76.

367. Jallow S, Alabi A, Sarge-Njie R, Peterson K, Whittle H, Corrah T, et al. Virological Response to Highly Active Antiretroviral Therapy in Patients Infected with Human Immunodeficiency Virus Type 2 (HIV-2) and in Patients Dually Infected with HIV-1 and HIV-2 in The Gambia and Emergence of Drug-Resistant Variants. Journal of Clinical Microbiology. 2009;47(7):2200-8.

368. Collins DR, Urbach JM, Racenet ZJ, Arshad U, Power KA, Newman RM, et al. Functional impairment of HIV-specific CD8+ T cells precedes aborted spontaneous control of viremia. Immunity. 2021;54(10):2372-84.e7.

369. Rocha C, Duarte J, Borrego P, Calado R, Marcelino JM, Tendeiro R, et al. Potency of HIV-2-specific antibodies increase in direct association with loss of memory B cells. AIDS. 2017;31(17):2431-3.

370. Marcelino JM, Nilsson C, Barroso H, Gomes P, Borrego P, Maltez F, et al. Envelope-specific antibody response in HIV-2 infection: C2V3C3-specific IgG response is associated with disease progression. Aids. 2008;22(17):2257-65.

371. Klein F, Diskin R, Scheid Johannes F, Gaebler C, Mouquet H, Georgiev IS, et al. Somatic Mutations of the Immunoglobulin Framework Are Generally Required for Broad and Potent HIV-1 Neutralization. Cell. 2013;153(1):126-38.

372. Wagar LE, Salahudeen A, Constantz CM, Wendel BS, Lyons MM, Mallajosyula V, et al. Modeling human adaptive immune responses with tonsil organoids. Nature Medicine. 2021;27(1):125-35.
373. Kaw S, Ananth S, Tsopoulidis N, Morath K, Coban BM, Hohenberger R, et al. HIV-1 infection of CD4 T cells impairs antigen-specific B cell function. The EMBO Journal. 2020;39(24):e105594.
374. Caetano DG, Ribeiro-Alves M, Hottz ED, Vilela LM, Cardoso SW, Hoagland B, et al. Increased biomarkers of cardiovascular risk in HIV-1 viremic controllers and low persistent inflammation in elite controllers and art-suppressed individuals. Sci Rep. 2022;12(1):6569.
375. Hassan AS, Hare J, Gounder K, Nazziwa J, Karlson S, Olsson L, et al. A Stronger Innate Immune Response During Hyperacute Human Immunodeficiency Virus Type 1 (HIV-1) Infection Is Associated With Acute Retroviral Syndrome. Clin Infect Dis. 2021;73(5):832-41.
376. Zhang W, Ambikan AT, Sperk M, van Domselaar R, Nowak P, Noyan K, et al. Transcriptomics and Targeted Proteomics Analysis to Gain Insights Into the Immune-control Mechanisms of HIV-1 Infected Elite Controllers. EBioMedicine. 2018;27:40-50.
377. Filbin MR, Mehta A, Schneider AM, Kays KR, Guess JR, Gentili M, et al. Longitudinal proteomic analysis of severe COVID-19 reveals survival-associated signatures, tissue-specific cell death, and cell-cell interactions. Cell Rep Med. 2021;2(5):100287.
378. Uhlén M, Karlsson MJ, Hober A, Svensson A-S, Scheffel J, Kotol D, et al. The human secretome. Science Signaling. 2019;12(609):eaaz0274.
379. The proteins actively secreted to human blood - Human Protein Atlas [Available from: https://www.proteinatlas.org/humanproteome/blood+protein/secreted+to+blood [Accessed February 8, 2023].
380. Malmstrom E, Kilsgard O, Hauri S, Smeds E, Herwald H, Malmstrom L, et al. Large-scale inference of protein tissue origin in gram-positive sepsis plasma using quantitative targeted proteomics. Nat Commun. 2016;7:10261.
381. Yusa T, Tateda K, Ohara A, Miyazaki S. New possible biomarkers for diagnosis of infections and diagnostic distinction between bacterial and viral infections in children. Journal of Infection and Chemotherapy. 2017;23(2):96-100.