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Characterisation of CD8 T cell immune responses directed against cytomegalovirus in people living with human immunodeficiency virus (HIV).

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Résumé

Titre : Caractérisation des réponses immunitaires des lymphocytes T CD8 dirigées contre le cytomégalovirus chez les personnes vivant avec le virus de l'immunodéficience humaine (VIH).

Mots clés : Lymphocytes T CD8 (LT CD8), Infection virale, HIV, CMV, Sénescence

Contexte : Les lymphocytes T CD8 (LT CD8) jouent un rôle crucial dans le contrôle des infections virales. Malgré le traitement antirétroviral (TAR), les personnes vivant avec le VIH (PVV) présentent des dysfonctionnements immunitaires persistants (inflammation chronique, épuisement et sénescence lymphocytaire). La réponse anti-CMV, liée à la sénescence, pourrait contribuer à ces anomalies, notamment chez les PVV âgées.

Objectif : Évaluer l'impact de la co-infection CMV/HIV sur les réponses LT CD8 spécifiques du CMV (CMV-CD8) et évaluer l'impact des réponses CMV-CD8 dans les dysfonctionnements immunitaires associés au VIH, en comparant des PVV sous TAR, des contrôleurs spontanés et des témoins non infectés.

Méthodes : Des PBMC de 40–45 individus/groupe ont été analysées par cytométrie en flux. Les panels incluant des pentamères CMV pour la spécificité, des anticorps dirigés contre CD57, KLRG-1, CD28 pour étudier la sénescence ; PD-1 pour l'épuisement ; CD38/HLA-DR pour l'activation, et d'autres marqueurs associés à l'effet bystander, induit par l'inflammation (CX3CR1 et NKG2D). La sérologie CMV (IgG et avidité) a également été mesurée.

Résultats : Les PVV avaient moins de cellules CMV-CD8 que les témoins. Les CMV-CD8 LT des PVV montraient un phénotype moins sénescent, suggérant un fort renouvellement des CMV-CD8. On observait également une expression accrue de CX3CR1 et NKG2D parmi les cellules CD57+, suggérant une réponse bystander compensatoire.

Conclusions : L'infection chronique par le VIH est associée à des modifications quantitatives et qualitatives des réponses CMV-CD8 LT, suggérant une sénescence lymphocytaire accélérée.

Abstract

Title: Characterisation of CD8 T cell immune responses directed against cytomegalovirus in people living with human immunodeficiency virus (HIV).

Keywords: CD8 T lymphocytes (CD8 LT), Viral infection, HIV, CMV, Senescence

Background: CD8 T lymphocytes (CD8 LT) play a crucial role in the control of viral infections. Despite antiretroviral therapy (ART), people living with HIV (PWH) exhibit persistent immune dysfunction (such as inflammation, exhaustion, and senescence), favored by the persistence of HIV and other factors. The anti-CMV response, linked to senescence, could contribute to these abnormalities, particularly in ageing PWH.

Objective: To assess the impact of the CMV-HIV coinfection on the CMV responses (notably the memory inflation and the senescent profile) and the potential impact of CMV-specific CD8 LT (CMV-CD8) in PWH on the HIV-associated immune dysfunction by comparing PWH on ART, spontaneous controls and uninfected controls.

Methods: PBMC from 40-45 individuals/group were analysed by flow cytometry. The panels included CMV pentamers for the specificity, antibodies targeting CD57/KLRG-1/CD28 for the senescence profile, PD-1 for the exhaustion, CD38/HLA-DR for the activation, and other markers associated with bystander effects induced by chronic stimulation and inflammation (CX3CR1, NKG2D)). CMV serology (IgG and avidity) was also measured.

Results: PWH had fewer CMV-CD8 cells than controls, despite similar IgG titers. CMV-CD8 LT from PWH showed less senescence (CD57+ or CD57+KLRG1+CD28-), suggesting higher turn-over among the CMV-CD8 LT pool. CMV-CD8 LT from PWH also showed increased expression of CX3CR1 and NKG2D among CD57+ cells, suggesting compensatory bystander expansion.

Conclusions: Chronic HIV infection is associated with quantitative and qualitative changes in the CMV-CD8 LT pool, suggesting exacerbated senescence in PWH.

Résumé substantiel

L'infection par le virus de l'immunodéficience humaine (VIH) est désormais considérée comme une maladie chronique dans les pays développés, où en plus des stratégies vaccinales et des stratégies d'éradication du virus, les efforts de recherche se concentrent sur le vieillissement en bonne santé des personnes vivant avec le VIH (PVVIH). L'introduction des traitements antirétroviraux (TAR) a permis d'atteindre une espérance de vie proche de celle de la population générale, bien que l'incidence des comorbidités demeure plus élevée. Les PVVIH, en phase chronique, présentent des signes de vieillissement accéléré, suggérant que les effets de l'infection par le VIH se combine au vieillissement naturel.

Le vieillissement naturel s'accompagne d'altérations physiologiques multi-systémiques, notamment immunitaires (ce processus est désigné sous le terme de « sénescence immunitaire »). Celle-ci se caractérise par une diminution des progéniteurs hématopoïétiques, une perte d'efficacité du thymus et l'installation d'un état inflammatoire chronique, ou inflammaging, contribuant à une susceptibilité accrue aux pathologies liées à l'âge. Généralement asymptomatique chez l'adulte immunocompétent, l'infection à cytomégalovirus (CMV) est associée aux processus de vieillissement immunitaire. Avec l'âge, l'infection à CMV est associée à une susceptibilité accrue aux comorbidités et à une réduction de l'efficacité vaccinale. La réponse de l'hôte contre le CMV, marquée par une inflammation soutenue et le phénomène d'inflation mémorielle (expansion clonale de lymphocytes T (LT) spécifiques), appauvrit le répertoire immunitaire. L'infection par le CMV est ainsi un facteur associé à un vieillissement de moins bonne qualité.

L'impact de la co-infection par le CMV reste à aborder chez les PVV en phase chronique et ce d'autant plus qu'elle est très fréquente chez les PVV. Son rôle est largement discuté lors de la phase aiguë de l'infection à VIH, où la déplétion des LT CD4+ au niveau intestinal pourrait favoriser la réactivation du CMV, amplifiant à son tour l'activation immunitaire systémique et potentialisant la dissémination du VIH, bien qu'un lien causal direct reste à établir. En phase chronique, le rôle du CMV est moins consensuel, reflétant l'hétérogénéité des cohortes de PVV et/ou un rôle plus discret de cette infection dans le contexte de l'infection par le VIH, qui induit de nombreux dysfonctionnements immunitaires. Certaines études montrent néanmoins que le traitement par ganciclovir réduit l'activation immunitaire, suggérant une contribution significative du CMV à ce processus.

De manière surprenante, les conséquences de l'infection à CMV sur le vieillissement immunitaire chez les PVVIH, contrôlant efficacement le VIH, restent peu explorées, malgré sa séroprévalence élevée dans cette population. Notre hypothèse est que la co-infection VIH/CMV crée un contexte propice à la sénescence et à l'épuisement lymphocytaire. Ces phénomènes seraient susceptibles de compromettre le contrôle du CMV, d'accélérer la génération et l'accumulation de LT CD8+ spécifiques du CMV à phénotype sénescent et de favoriser le phénomène de sénescence touchant le compartiment T. Ce phénomène pourrait être d'autant plus critique que les PVV vieillissent progressivement grâce à l'introduction des TAR qui a permis d'augmenter la durée de vie de ces personnes.
Une question clinique majeure consiste à comprendre comment le vieillissement naturel interagit avec un système immunitaire déjà altéré par l'infection à VIH, aggravant potentiellement ces dysfonctionnements et contribuant au fardeau des comorbidités. Même si le vieillissement immunitaire est un phénomène complexe et multifactoriel, l'évaluation de l'impact du CMV sur le vieillissement immunitaire chez les PVV permettra de mieux comprendre son rôle dans le développement du vieillissement accéléré chez les PVV, dans l'incidence accrue de comorbidités et d'envisager de nouvelles stratégies thérapeutiques pour en limiter les effets délétères potentiels.

L'objectif de cette thèse était d'évaluer l'impact à long terme de l'infection VIH sur le compartiment des LT CD8+ spécifiques du CMV(CMV-CD8+). Pour répondre à cette question, nous avons analysé des échantillons cryopréservés provenant de trois groupes d'individus : des PVV sous traitement antirétroviral avec contrôle viral efficace (ART), des contrôleurs spontanés du virus (HIC), et des donneurs non infectés par le VIH (dans le cadre de dons de sang (Etablissements français du sang). Les échantillons des PVV, issus des biobanques des cohortes ANRS-MIE PRIMO et CODEX permettant un accès à l'historique complet des patients. Nous avons sélectionné des échantillons de participants présentant une infection contrôlée depuis plus de cinq ans. La sélection a également ciblé les patients exprimant les allèles HLA de classe I (HLA-A02, A03, B07 ou B35), un prérequis technique indispensable pour l'utilisation des pentamères CMV.

Les cellules mononucléées du sang périphérique (PBMC) de 46 PVVIH sous ART, 44 contrôleurs HIC et 22 donneurs sains non infectés ont été analysées par cytométrie en flux en utilisant des pentamères spécifiques et un large panel de marqueurs associés à la sénescence,

l'épuisement, l'activation et à l'effet bystander (CD57, KLRG-1, PD-1, CD38, HLA-DR, CX3CR1, NKG2D et IL-15R).

Notre investigation s'est articulée autour de trois axes principaux : (1) la quantification de la proportion de CMV-CD8+ ; (2) la caractérisation détaillée de leur phénotype ; et (3) l'évaluation de leur fonction via des tests de survie cellulaire. En parallèle, une sérologie CMV a confirmé une infection ancienne chez tous les participants.

Nos résultats révèlent que les PVVIH, bien que séropositifs pour le CMV avec des titres d'IgG et une avidité comparable aux témoins, présentent un compartiment de CMV-CD8+ profondément altéré. Le premier résultat frappant a été l'observation d'une réduction drastique (environ 8 fois) de la proportion de ces cellules chez les PVVIH (médiane : 1,6% sous ART, 1,0% chez les HIC vs 9,8% chez les témoins), démontrant que l'infection par le VIH module profondément le répertoire immunitaire. Cet effet n'était pas médié par une différence dans la distribution des allèles HLA, écartant un biais génétique.

Ces résultats nous ont tout d'abord semblé contre-intuitif, car on aurait pu présumer que l'inflammation chronique résiduelle, caractéristique chez une fraction non négligeable des PVV, exacerberait le phénomène de l'inflation mémorielle. Cette réduction quantitative s'accompagnait de remaniements phénotypiques complexes. Néanmoins, nous avons observé une proportion plus élevée de LT CD8+ activés parmi les cellules spécifiques du CMV des PVVIH, suggérant une activation résiduelle persistante. Le niveau d'activation global restait toutefois inférieur à celui décrit chez les PVV avec un faible degré de reconstitution immunitaire. Cette activation était marquée par une augmentation généralisée de l'expression de CX3CR1, plus élevée parmi les cellules spécifiques du CMV comparé au pool total de CD8+, et plus élevée chez les PVV que chez les témoins. Cette plus forte expression de CX3CR1 sur les LT CD8+ peut être interprétée comme une signature de l'inflammation chronique constitutive au VIH. Paradoxalement, cette activation s'accompagnait d'une réduction de la fraction CD57+. Puisqu'aucun processus de dédifférenciation des cellules CD57+ vers le phénotype CD57- n'est décrit, cette diminution ne pourrait être liée qu'à un remplacement progressif des fractions CD57+ par des cellules CD57-. Nous avons observé une corrélation inverse entre la proportion de cellules CMV-CD8+ et le pourcentage de cellules CD57+ au sein de ce compartiment confirment que la faible expression de CD57 est intrinsèquement liée à la réduction quantitative globale et suggèrent un défaut de génération ou de maintenance des cellules sénescentes matures.

L'analyse des profils de survie de ces cellules a montré que l'infection VIH chronique altère sévèrement la survie de la fraction progénitrice CD57-. Nous proposons donc un modèle d'interaction où l'infection par le VIH réduit la capacité de survie des fractions CD57- des LT CMV-spécifiques, ce qui réduit considérablement sa capacité à contribuer au renouvellement du pool, l'empêchant de reconstituer le pool sénescent CD57+. Une susceptibilité apoptotique intrinsèque de la fraction CD57+ a également été observée, mais était indépendante du statut VIH. Ce modèle est compatible avec l'observation d'un déplacement de l'équilibre de différenciation (TCM/TEM/TEMRA) vers des phénotypes moins différenciés et un renouvellement accéléré mais inefficace. Fait marquant, cet impact spécifique au compartiment CMV-CD8+ n'a pas été observé dans le pool total de LT CD8+, suggérant une vulnérabilité particulière des LT CD8 spécifiques du CMV, liée à leur état de différenciation avancée et/ou le rôle de stimulation antigénique chronique. Néanmoins, le suivi de ce compartiment pourrait révéler le degré de sénescence active chez chaque patient.

Collectivement, ces données identifient le compartiment CMV-CD8+ comme une cible privilégiée (ou plus aisément accessible à l'analyse) de l'immunopathogenèse du VIH et révèlent un nouveau mécanisme par lequel l'inflammation chronique peut induire un vieillissement immunitaire accéléré, qui n'est paradoxalement pas associé à l'inflation mémorielle des fractions LT CD8 spécifiques du CMV.

En conclusion, ce travail démontre que l'infection VIH chronique remodèle profondément la réponse T CD8+ spécifique du CMV, se traduisant par une déplétion quantitative et une atténuation inattendue du phénotype sénescent. Nous proposons que ce phénomène soit orchestré par un défaut de survie des précurseurs CD57-, reflétant un vieillissement immunitaire accéléré et dysfonctionnel.

Les implications de ces modifications sont doubles et paradoxales : une réduction potentielle des effets délétères de l'inflation mémorielle d'un côté, et un risque possible de mauvais contrôle du CMV de l'autre.

Les mécanismes précis (tels que la fréquence des réactivations CMV ou le rôle de l'environnement cytokinique) restent à élucider.

La principale limite de cette étude réside dans son confinement à l'analyse du sang périphérique, qui ne reflète pas nécessairement la dynamique virale et immunitaire au sein des sites de réservoir et de réplication.

Des recherches futures visant à corréler la réponse immunitaire systémique avec la charge virale CMV dans des tissus plus accessibles (comme le tissu adipeux, site connu de persistance virale et d'inflammation résiduelle) ou dans des échantillons de biopsies accessibles (ganglions lymphatiques, intestins) seront cruciales. Cette approche est essentielle pour comprendre pleinement les interactions complexes entre le VIH et le CMV et pour évaluer avec précision le risque clinique associé à ces modifications immunologiques. Ces études ouvriront la voie au développement de nouvelles stratégies thérapeutiques ciblées.

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List of abbreviations

Ag: Antigen

AIDS: Acquired immunodeficiency syndrome

APC: antigen presenting cell

ART: antiretroviral therapy

CCR5: C-C chemokine receptor 5

CCR7: C-C Chemokine Receptor 7

CD4: CD4+ T cells

CD8: CD8+ T cells

CD57: cluster of differentiation 57

CMV: Cytomegalovirus

CRP: C reactive protein

CTL: Cytotoxic T lymphocytes

CTLA-4 : cytotoxic T lymphocyte antigen 4

CX3CR1: CX3C motif chemokine receptor 1

DAMP: Damage Associated Molecular Pattern

DC: Dendritic cell

DNA: deoxyribonucleic acid

Env: Envelope

EOMES: eomesodermin homologue

GALT: Gut-associated lymphoid tissue

HCMV: Human CMV infection

HIC: HIV Controllers

HIV-1: Human immunodeficiency virus 1

IE: Immediate Early

Ig: Immunoglobulin

IL: Interleukin

IFN: Interferon

IRP: immune risk phenotype

KLRG1: Killer cell lectin-like receptor subfamily G member 1

LAG-3: Lymphocyte activation gene 3

LPS: Lipopolysaccharide

MFI: median fluorescence intensity

MHC: Major histocompatibility complex

NF-κB: Nuclear factor-kappa B

NK cells: Natural killer cells

ORF: Open reading frame

PAMP: Pathogen Associated Molecular Patterns

PBMC: Peripheral blood mononuclear cells

PD1: Programmed cell death protein 1

PWH: People living with HIV

POL: DNA polymerase

PRR: Pattern recognition receptor

RNA: Ribonucleic acid

SAMHD1: SAM domain and HD domain-containing protein 1

SASP: senescence associated secretory phenotype

Tcm: Central memory T cells

TCR: T cell receptor

TE: Effector T lymphocyte

TEM: effector memory T cells

TEMRA: Effector Memory-Expressing CD45RA

Th: T helper cell

TLR: Toll-like receptor

TNF: tumor necrosis factor

4-1BB (CD137 ou TNFRSF9): Tumor Necrosis Factor Receptor Superfamily Member 9

TM: memory T lymphocyte

TN: naive T lymphocyte

TTM: Transitional memory cells

Vpr: Viral protein R

INTRODUCTION

1 CD8 T cell biology

1.1 Generalities

1.1.1 Generation and activation

CD8 T lymphocytes (LT), known to give rise to cytotoxic LT when activated, are key players involved in the immune response against intracellular infections (viruses, bacteria, protozoa) and tumor cells or autoimmune diseases (Zhang & Bevan, 2011).

Production. CD8 LT start their development in the bone marrow and mature in the thymus, where the thymic selection of the LT repertoire eliminates autoreactive clones and produces immunocompetent naive LT (Tough & Sprent, 1994). These mature CD8 LT join the secondary lymphoid organs (lymph nodes, spleen and Peyer's patches) via the bloodstream and migrate continuously until they specifically recognise their cognate antigen (Ag).

Activation. The activation of naïve CD8+ T lymphocytes, which requires three signals, is carried out by specialised antigen-presenting cells (APCs). Among them, conventional dendritic cells (cDCs) are the only ones reported to be capable of inducing priming in vivo. Other APCs, such as monocytes/macrophages and B lymphocytes, can also play a role in antigen presentation (Figure 1):

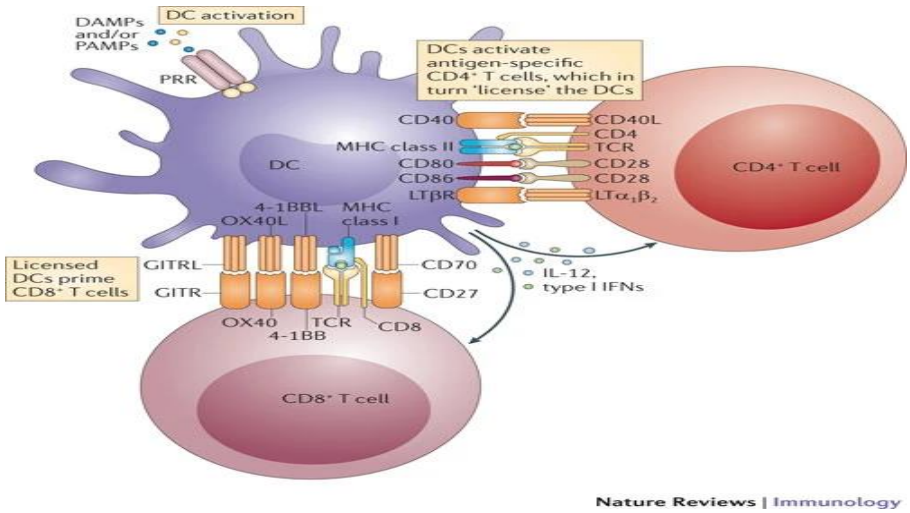


Figure 1: Activation of CD8 LT by DCs

The activation of CD8 LT is dependent on the activation and maturation of DCs. DCs are initially stimulated by pathogen- or damage-associated molecular patterns (PAMPs/DAMPs), which leads to the upregulation of CD40, CD80, CD86 and MHC molecules. This maturation process enables the presentation of peptide Ags to specific CD4 LT, thereby activating them and inducing the expression of CD40L and LTα1β2 licensing factors. DCs that have been 'licensed' in this way acquire the ability to prime naïve CD8 LT, which triggers the upregulation of CD27, 4-1BB, OX40 and GITR receptors on their

surface. The combined stimulation of these receptors by their respective ligands (IL-12 and type I interferons) ultimately results in the robust activation of CD8 LT (Summers deLuca & Gommerman, 2012).

(i) the interaction between the T cell receptor (TCR) and the peptide/MHC class I complex; (ii) the interaction between costimulatory molecules such as CD28 (expressed on the surface of LT CD8) and CD80/CD86 (expressed on mature DCs); (iii) the final signal is induced by proinflammatory cytokines (L-12, IL-18, interferon (IFN)-α, IFN-β and γ-IFN) produced by DCs, NKs, macrophages and activated LTs (Curtsinger et al., 1999; Sigal, 2016; Watts, 2005). CD4 (helper) LT are involved in assisting the optimal activation of CD8 LT by ensuring the full maturation of DCs (Eickhoff et al., 2015).

Differentiation in effector CD8 T cells. CD8 LT activation induces the acquisition of new functions, notably effector functions, including the production of cytotoxic molecules (such as granzymes and perforins, which induce cellular cytotoxicity) and the secretion of cytokines (γ-IFN and TNF-α), which will amplify the immune response (Harari et al., 2009). Effector CD8 LT thus include both lytic and nonlytic factors (**Figure 2**).

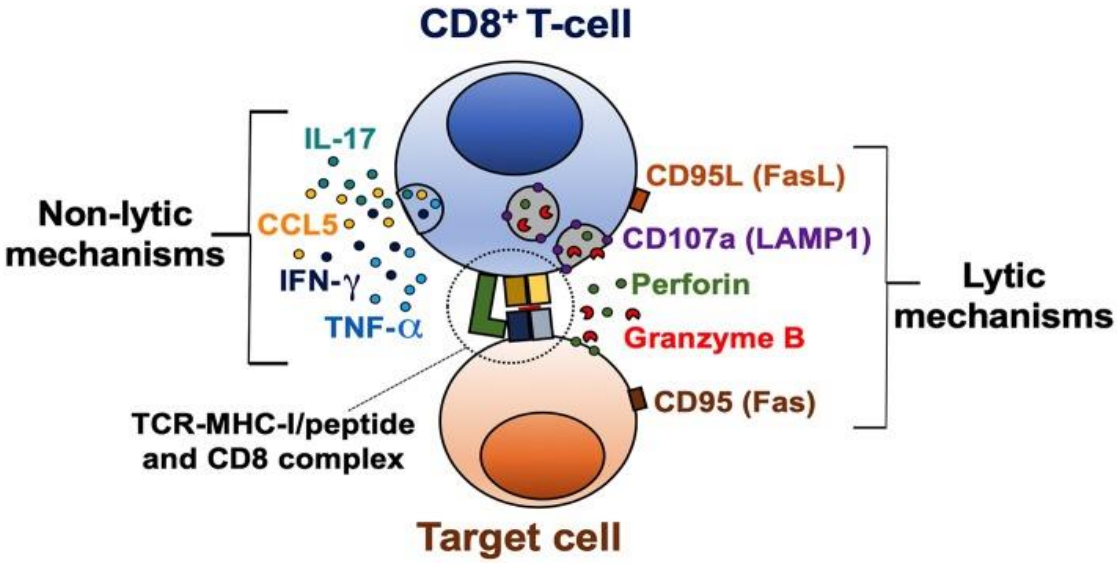


Figure 2 : Lytic and non-lytic effector mechanisms of CD8 LT

CD8 LT exert their cytotoxic functions via two main mechanisms: lytic and non-lytic. Activation is triggered by recognition of the MHC-I/peptide complex and leads to the expression of cytotoxic molecules, such as perforin and granzyme B. Coordinated release of these molecules during degranulation (detectable by CD107a/LAMP-1 on the cell membrane) induces target cell death. Another major lytic pathway involves the interaction between CD95L and CD95 (Fas). In parallel, activated CD8 LT produce various cytokines (e.g. γ-IFN, TNF-α and IL-17) and the chemokine CCL5. These non-lytic mediators have diverse antiviral, inflammatory and regulatory functions. (Perdomo-Celis et al., 2019).

***Differentiation in memory CD8 LT*.** Some activated CD8 LT (5 to 10%) will survive the contraction phase, in which most cells undergo apoptosis, and will differentiate into memory cells, capable of persisting for years. These CD8 LT respond more rapidly and effectively to re-infection by the same pathogen and will allow more rapid viral clearance than during primary infection and therefore greater protection against a pathogen (Kaech & Ahmed, 2001). These memory CD8 LT produce chemokines (CCL5/RANTES) a few minutes after encountering the Ag (Walzer et al., 2003), and cytokines a few hours after encountering the Ag (Veiga-Fernandes et al., 2000) compared with several days for a naive CD8 LT.

Memory CD8 LT persist at a high frequency and are long-lived (Veiga-Fernandes et al., 2000). The maintenance of this immune memory depends on their capacity for survival and for self-renewal by homeostatic proliferation (Cui & Kaech, 2010). The homeostatic proliferation is regulated by homeostatic cytokines such as IL-7 and IL-15 (Kieper et al., 2002). These processes are independent of Ag stimulation, but rechallenges with the specific pathogen will also modulate the size of the Ag-specific LT CD8 compartment.

1.1.2 Diversity in memory CD8 LT

Memory CD8 LT are a heterogeneous and diverse group of cells, which can be divided into several subpopulations based on (i) their expression of different phenotypic markers including chemokine receptor (Masopust et al., 2001; Sallusto et al., 1999), (ii) their effector function (adapted to their location) and (iii) their lifespan.

1.1.2.1 Central and effector memory T cells (TCM and TEM)

The first level of discrimination relied on the expression of CCR7 and CD62L among blood T cells (Hamann et al., 1997; Sallusto et al., 1999) to identify two subsets:

The central memory CD8 LT (TCM) that exhibit the following phenotype: CCR7+ CD62L+ CD27+ CD45RO+. They are present in lymph nodes (LN), show high proliferative capacity and produce IL2. They are dedicated to localised responses in lymphoid tissues, where they contribute to the effector response after a rapid proliferation response.

The effector memory CD8 LT (TEM) that do not express the migration receptors CCR7 and CD62L but are CD27+CD45RO+. They circulate in peripheral blood and non-lymphoid tissues (particularly liver and lung) (Rutishauser et al., 2009). They provide rapid effector activity. In terms of proliferative capacity, TEM show low proliferation after cytokine or antigenic

stimulation, and also a shorter telomere length than TCM (Gett et al., 2003; Wherry et al., 2003). However, in terms of differentiation, TEM are more differentiated and show a more rapid acquisition of effector functions after stimulation than TCM.

Both TCM and TEM contribute to the efficiency of the memory response, providing complementary activities. This classification was then extended to a third memory population described in humans but not mice.

1.1.2.2 Terminal effector memory cells that re-express CD45RA (TEMRA)

These cells are highly cytotoxic and produce proinflammatory cytokines (Hamann et al., 1997; Mahnke et al., 2013). Their proportion increases with age and is usually associated with recurrent/chronic viral and bacterial infection. The CMV infection is a prototypical example of a chronic infection associated with TEMRA induction..

By combining these observations, a strategy using CCR7, CD45RA, CD28, CD27 and CD57 allows assessing the CD8 LT differentiation status (**Table 1**).

Cell subset	Phenotype	Degranulation (CD107a/b expression)	Cytotoxic molecules expression (granzymes and/or perforin)	*In vitro* cytotoxicity	*De novo* synthesis of granzymes and/or perforin*	IFN-γ production*	Main location
Naïve	$CD45RA^+$ $CD45RO^-$ $CCR7^+$ $CD62L^+$ $CD28^+$ $CD27^+$ $CD57^-$	-	-	-	-	-	Secondary lymphoid tissues
Central memory	$CD45RA^-$ $CD45RO^+$ $CCR7^+$ $CD62L^+$ $CD28^+$ $CD27^+$ $CD57^-$	++	++	++*	++	+++	Secondary lymphoid tissues
Effector memory	$CD45RA^-$ $CD45RO^+$ $CCR7^-$ $CD62L^-$ $CD28^{+/-}CD27^{+/-}CD57^{-/+}$	+++	+++	+++	+	+++	Blood and inflamed tissues
Terminal effector	$CD45RA^+$ $CD45RO^-$ $CCR7^-$ $CD62L^-$ $CD28^-$ $CD27^-$ $CD57^+$	+++	+++	+++	+	+++	Blood and inflamed tissues

*After 5 h stimulation.

Table 1: Functions of the different memory T cell subset

Several subpopulations of CD8 LT can be identified by their combined expression of various differentiation markers, including CD45RA, CD45RO, CCR7, CD62L, CD27 and CD28. The main classification groups them into four categories: naïve (TN), central memory (Tcm), effector memory (Tem), and T effector memory RA (Temra. Each population exhibits a unique functional profile, as assessed by parameters such as granzyme and perforin expression, γ-IFN production, degranulation capacity, and in vitro-measured cytotoxicity. (Perdomo-Celis et al., 2019).

1.1.2.3 Other memory subsets

In the following decades, the characterisation of memory LT has evolved with the identification of transitional memory, stem cell memory, tissue-resident memory but also exhausted LT (Muroyama & Wherry, 2021).

Transitional memory T cells (TM)

Whereas TCM (CCR7+) express CD28 and CD27, TEM (CCR7-) do not. However, a fraction of CCR7- CD62L- CD28+ cells was identified and referred to as transitional memory. This subset is more differentiated than TCM but less than TEM. They exhibit an intermediate profile between TCM and TEM (Mahnke et al., 2013).

Stem cell memory T cells (TSCM)

Another degree of characterisation revealed the existence of a small subset of memory LT named memory stem cells CD8 LT (T_{SCM}) (Gattinoni et al., 2011). Their phenotype is close to that of naïve LT, but they differ by the expression of CD95, CD122, CXCR3 and LFA1. These cells exhibit high proliferative capacity, providing them with a long lifetime.

Tissue-resident memory T cells (TRM)

The exploration of tissue-specific immunity also revealed the existence of tissue-resident memory LT (TRM). They are mostly dedicated to the immune defences of peripheral tissues (Jiang et al., 2012), but their functional properties may differ depending on the site. Differences in phenotype, functions, and migration profile depending on the anatomical site are emerging and are still under investigation.

Differentiation profile associated with chronic stimulation: Exhausted T cells (TEX)

Most of these characterisations were performed in healthy individuals or during an acute infection. The process of memory differentiation indeed referred to contexts of Ag elimination. The question was whether this strategy was appropriate in the context of chronic infection. Some groups used the expression of additional chemokine receptors to distinguish CD8 LT involved in chronic immune responses: CXCR3, CXCR5 and CX3CR1 (Böttcher et al., 2015). Another approach was to identify these cells using PD-1. The characterisation of the CD8 LT during chronic infection led to the identification of the specific upregulation of PD-1 on these cells. These cells do not allow pathogen elimination but provide containment (or delay) of the infection. Interestingly, PD-1 is also associated with exhaustion in LT and as such

associated with dysfunctions. The characterisation of these exhausted cells will be addressed more precisely below.

As a whole, the outcome of CD8 LT activation appears more diverse than originally thought. A new model of CD8+ T cell differentiation following antigenic stimulation has been formulated **(**Figure 3), although it is not yet fully consensual. This model proposes that the outcome of the CD8+ response is not linear but rather diverges early into several distinct lineages:

-Memory cells (Tmem) (capable of self-renewal and secondary response),

-Terminal CD45RA+ effector cells (Temra) (highly cytotoxic but with a limited lifespan),

-Exhausted cells (Tex) (functionally impaired but providing partial pathogen control in chronic contexts).

The main points of debate surrounding this model concern:

- Plasticity: To what extent these lineages are fixed and irreversible.

- Initial decisional factors: The relative importance of early signals (TCR signal strength, inflammation, microenvironment) in determining cell fate.

- Population heterogeneity: The existence of subsets within the Tex population (precursors vs. terminally exhausted).

- Classification of Temra: Their origin and their relationship with exhaustion.

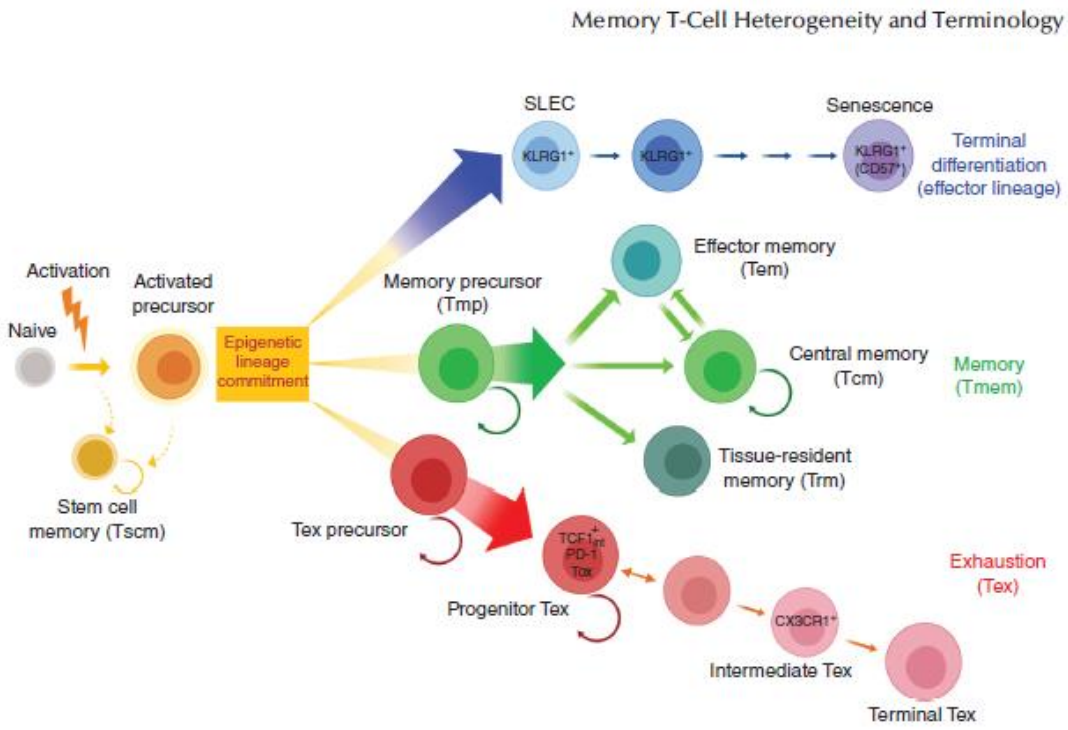


Figure 3: CD8 T cell differentiation

Upon antigen encounter, activated naïve $CD8^+$ T cells can commit to one of three major lineage trajectories, each with distinct functional outcomes:

- (Green pathway and cells) T memory (Tmem) cell lineage: This pathway leads to the generation of long-lived, self-renewing memory subsets (e.g., Tcm, Tem) that provide durable protection and rapid recall capacity.

- (Blue pathway and cells) Terminal effector (Teff) cell lineage: This pathway results in short-lived effector cells (SLECs), characterised by high expression of cytotoxic molecules and KLRG1, which undergo apoptosis after the peak of the immune response. This population includes terminally differentiated cells that re-express CD45RA (Temra).

- (Red pathway and cells) T exhausted (Tex) cell lineage: This pathway occurs under conditions of persistent antigen, as in chronic infection or cancer. It leads to a state of dysfunction, characterised by progressive loss of effector functions and sustained expression of inhibitory receptors (e.g., PD-1). The Tex population is heterogeneous, containing progenitor (Texprog) and terminally exhausted (Texterm) subsets.

Arrows indicate the proposed differentiation routes and potential relationships between subsets. The model highlights the divergence of cell fate early after activation (Muroyama & Wherry, 2021).

These three lineages exhibit distinct phenotypic, functional, and transcriptional characteristics that dictate their role in the immune response. The Tmem lineage is distinguished by its strong proliferative capacity and IL-2 production, its oxidative metabolic profile, and its expression of

homing markers such as CD62L and CCR7. In contrast, terminal effectors (Teff/Temra) exhibit high cytotoxicity but limited proliferative capacity and are often associated with senescence markers such as CD57. Finally, the Tex lineage is defined by the progressive expression of multiple inhibitory receptors (PD-1, LAG-3, TIM-3), a loss of effector functions, and a blockade in its differentiation, orchestrated by the transcription factor TOX. It is important to note that the Tex lineage itself is heterogeneous, comprising a progenitor subset (Tex prog) that retains some capacity to proliferate and respond to immunotherapy, and a terminally differentiated subset (Tex term) that is irreversibly dysfunctional (Koh et al., 2023).

Although this model defines exhaustion and senescence as differentiation stage per se, exhaustion and senescence are also described as immune dysfunctions, differently impacting the memory/effector T cell subsets. For simplicity, these two phenomena will described below, independently of the effector/memory stages.

1.2 T cell exhaustion

1.2.1 General definition

Chronic immune responses, in a tumor environment and in the presence of a high antigenic load, are classically associated with a phenomenon of exhaustion. Exhaustion is characterised by a progressive and hierarchical loss of cellular functional capacity, beginning with a reduction in IL-2 secretion, proliferation potential and cytokine polyfunctionality. This is followed by loss of TNF-α production and cytotoxic activity. At a severe stage of exhaustion, -γ-IFN secretion is finally compromised (Fuller & Zajac, 2003) (**Figure 4**).

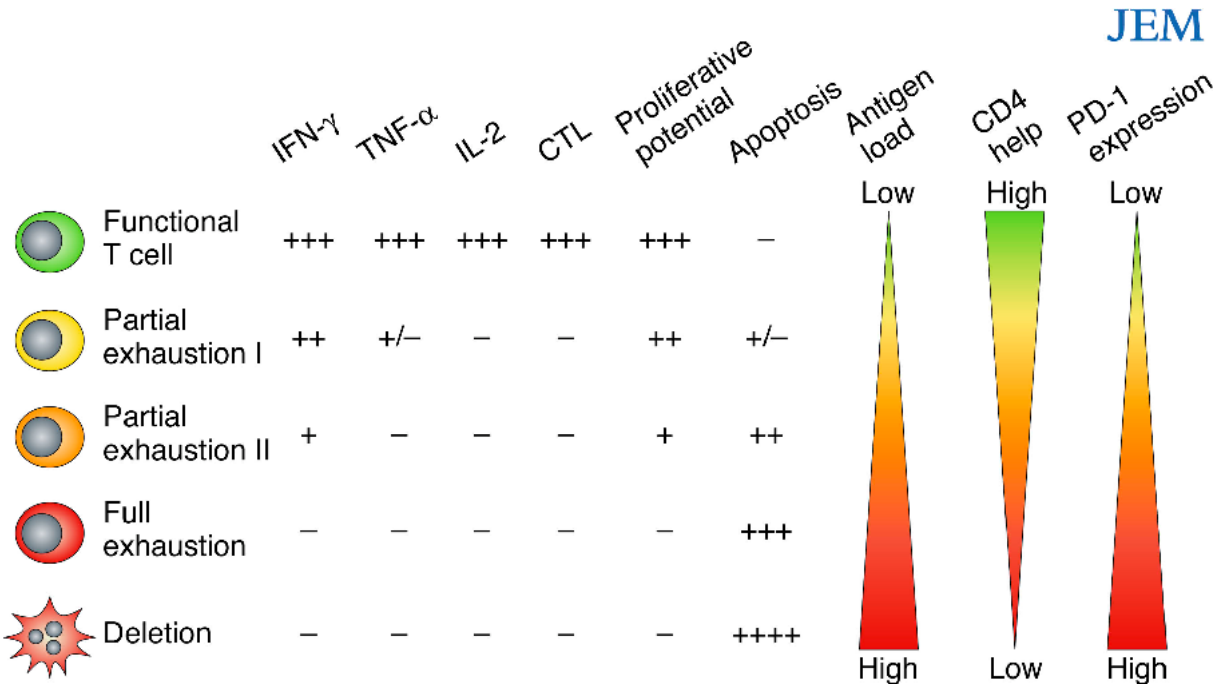


Figure 4 : The spectrum of T cell exhaustion

Virus-specific CD8 LT possess multiple functions including production of γ-IFN, TNF-α, and IL-2, cytotoxicity, Ag-driven proliferation, and resistance to apoptosis. During chronic infections, functions can be exhausted. Exhaustion represents a spectrum from mild (Partial exhaustion I: little IL-2 and poor TNF-α and cytotoxicity) to moderate (Partial exhaustion II: modestly defective γ-IFN, cytotoxicity, and little IL-2 or TNF-α) to severe (Full exhaustion: lack of γ-IFN, TNF-α, IL-2, and cytotoxicity). Finally, physical deletion (apoptosis) of LT occurs. Proliferative potential decreases concomitantly with the loss of other functions while apoptosis increases. Ag load and CD4 help strongly influence exhaustion; as Ag load increases and/or CD4 help decreases, virus-specific LT become more exhausted (G. J. Freeman et al., 2006).

The phenomenon (although not called exhaustion at the time) was described for the first time in mice during chronic viral infection by LCMV with the Clone 13 strain (LCMV-C), with evidence of the loss of effector functions of virus-specific CD8 LT (Barber et al., 2006; Zajac et al., 1998). Importantly, the identification of various stages of exhaustion also allowed the discovery of a reversible functional alteration mediated by PD-1 in LCMV infection that rapidly encouraged the study of this pathway. Since then, it has been observed in human during chronic infections, such as HIV, HBV, HCV or in the context of tumors (Wherry & Kurachi, 2015).

1.2.2 Expression of inhibitory receptors

Exhaustion is associated with the sustained expression of several inhibitory receptors or immune checkpoint proteins (ICPs), which inhibit functional and proliferative responses, such as PD1, T cell immunoglobulin domain and mucin domain protein 3 (TIM3), lymphocyte activation gene 3 (LAG3), T cell immunoreceptor with Ig and ITIM domains (TIGIT) and CTLA4 (**Figure 5**). The inhibitory receptor that is the most characterised is PD-1.

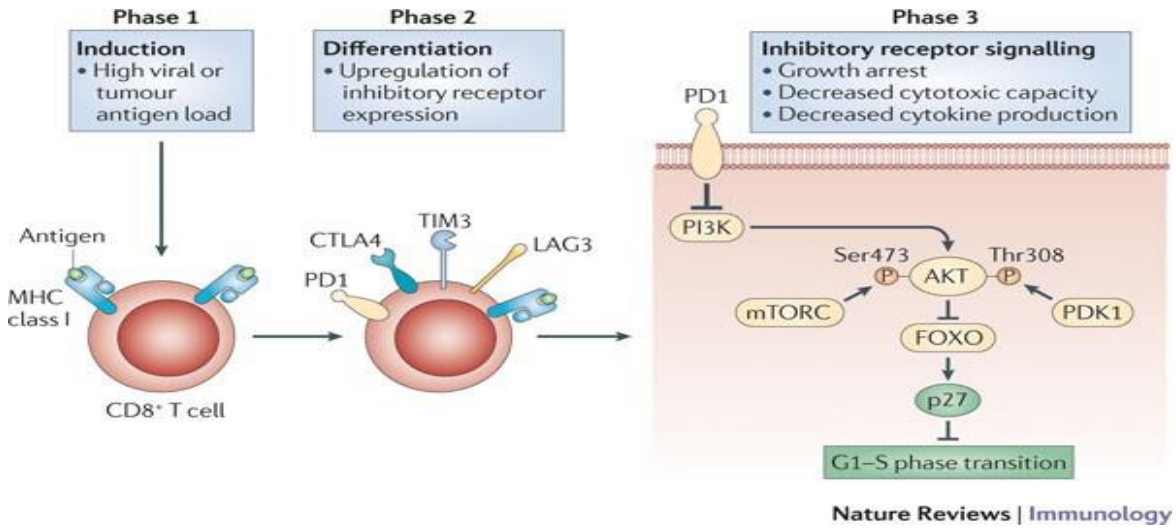


Figure 5 : The exhaustion pathway

T cell exhaustion occurs in response to persistent antigenic exposure, as observed in chronic viral infections or cancers. Following activation, T lymphocytes differentiate into effector cells and progressively express inhibitory receptors (PD1, CTLA4, TIM3, LAG3). Signaling through these receptors induces proliferative arrest and loss of effector functions, including cytokine production and cytotoxic activity. Mechanistically, inhibition of the AKT pathway—via suppression of phosphoinositide 3-kinase (PI3K), which is required for AKT phosphorylation leads to the removal of inhibition on FOXO transcription factors. These then activate the transcription of p27/KIP1, a cyclin-dependent kinase inhibitor, thereby blocking the G1/S transition in the cell cycle. Abbreviations: mTORC, mTOR complex; PDK1, 3-phosphoinositide-dependent protein kinase 1(Akbar & Henson, 2011).

1.2.3 Molecular mechanisms governing T cell exhaustion and inhibitory receptorexpression

The molecular mechanisms governing T cell exhaustion and inhibitory receptor expression involve a complex network of transcriptional factors, epigenetic modifications, metabolic disturbances, and microenvironmental signals triggered by persistent antigenic stimulation (Wherry & Kurachi, 2015; Z. Zeng et al., 2020) (**Figure 6**). Exhaustion results from profound transcriptional reprogramming, distinct from conventional memory or effector states (Paley et al., 2012) . Chronic TCR signaling is the central driver of this process, causing a NFAT/AP-1 imbalance favoring NFAT, directly leading to the expression of inhibitory receptors PD-1, LAG-3, and TIM-3 (Youngblood et al., 2011) . In the absence of the transcription factor AP-1, NFAT activates a negative regulatory program in CD4+ and CD8+ T cells, inducing low cellular reactivity.

The T-box factors T-bet and Eomes exhibit opposing dynamics during exhaustion, with a progressive decrease in T-bet and an increase in Eomes correlating with worsening dysfunction (Kao et al., 2011; Paley et al., 2012) . The transcription factor IRF4, induced by chronic TCR activation, significantly contributes to the establishment of the exhausted state during chronic infections (Chennupati & Held, 2017; Man et al., 2017) by promoting inhibitory receptor expression and limiting the development of memory-like cells.

TCF-1 proves essential for maintaining exhausted progenitors (Man et al., 2017) and contributes to the development of stem-like properties in tumor-infiltrating lymphocytes (TILs). It mediates the transition from the T-bet to the Eomes transcription factor in precursor exhausted T cells by promoting Eomes expression, which in turn promotes exhaustion through B7S1 induction. TOX emerges as a master regulator induced by NFAT/calcineurin, with its absence completely abolishing exhausted cell development (O. Khan et al., 2019) . The NR4A factor, co-regulated with TOX, actively participates in this transcriptional programming (Seo et al., 2019) and attenuates the antitumor effect of T cells.

Other molecular actors include BATF (downstream of PD-1), which alters cell proliferation and cytokine secretion, and Blimp-1, which promotes the transition to an exhausted phenotype during chronic infections. Metabolically, mTOR upregulates the expression of inhibitory

receptors via HIF-1α and c-Myc. In the tumor microenvironment (TME), TGF-β and IL-10 cytokines inhibit the secretion of IFN-γ and granzyme B (Z. Zeng et al., 2020).

Exhausted cell heterogeneity encompasses a differentiation spectrum ranging from TCF-1+ progenitors to terminally differentiated cells, characterised by progressive loss of T-bet and gain of Eomes (Beltra et al., 2020) . Stable epigenetic modifications, including persistent demethylation of the PDCD1 locus, lock in the exhaustion program and limit reversibility following immunotherapy (Pauken et al., 2016; Sen et al., 2016) . These mechanisms explain the persistence of the exhausted state even after antigen clearance and represent promising therapeutic targets.

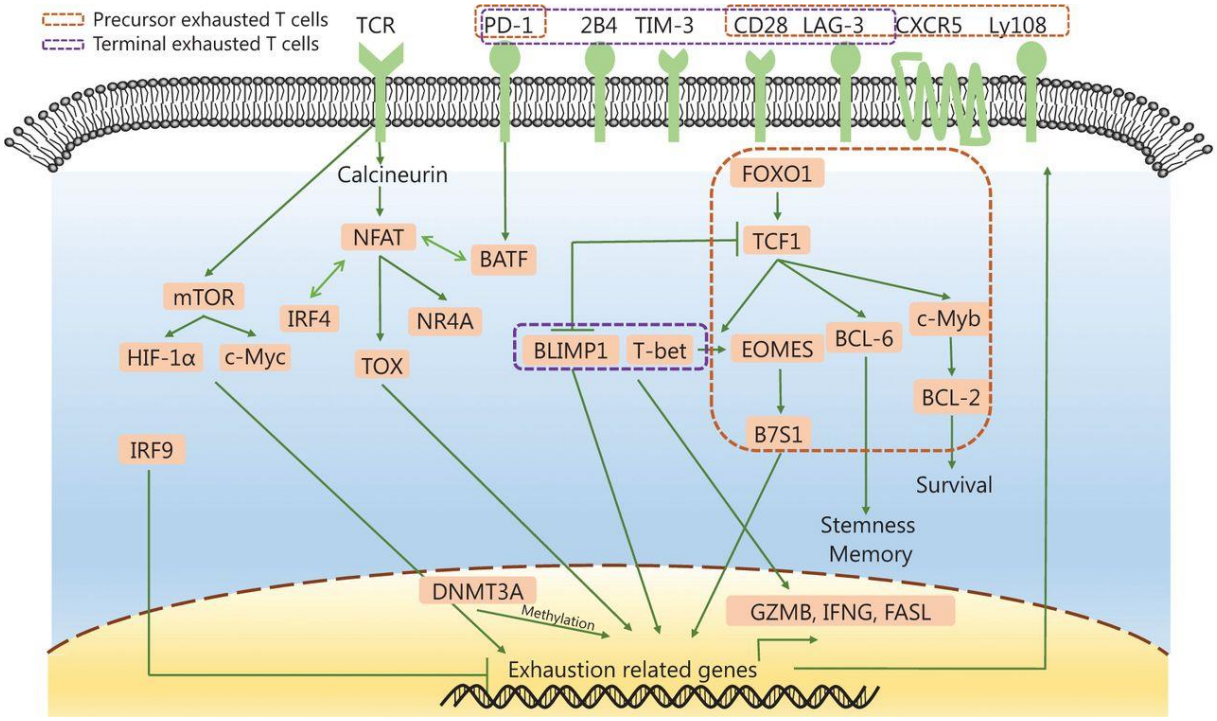


Figure 6: Mechanisms driving T cell exhaustion

Schematic of the intracellular signaling cascade whereby chronic T cell receptor (TCR) activation triggers transcriptional (NFAT, TOX, NR4A, TCF1) and epigenetic (DNMT3A) reprogramming, resulting in an exhausted phenotype characterised by checkpoint inhibitor expression, loss of effector function, and antigen-dependent persistence (Z. Zeng et al., 2020).

1.3 Senescence of CD8 LT

1.3.1 General definition

1.3.1.1 Replicative senescence as initially described by Hayflick

Replicative senescence is characterised by a progressive loss of proliferative capacity and telomere shortening. Historically, senescence is a cellular phenomenon that was described in 1961 by Hayflick as the cessation of fibroblast growth after approximately 50 passages in culture (Hayflick & Moorhead, 1961; Hayflick, 1965)**.** Later it was shown that this "replicative limit" or "Hayflick limit" was caused by somatic erosion of telomeres: the size of telomeres was progressively reduced during successive divisions (telomere-dependent senescence) (Harley et al., 1990). In prolonged in vitro culture, CD8 LT cells progressively lose expression of CD28 (Effros et al., 1994), a costimulatory marker, together with telomere shortening and an inability to proliferate, all characteristic features of senescence. Senescence is caused by the activation of the DNA damage response and can be triggered by a series of stresses: telomere defects, damaged DNA, oxidative stress, oncogenic activity, metabolic defects (Campisi & d'Adda di Fagagna, 2007).

Senescent cells promote chronic inflammation, contribute to immunosenescence, and are involved in the development of certain cancers (via oncogene-induced senescence) as well as degenerative diseases (Kirkland & Tchkonia, 2014).

1.3.1.2 Senescence: a mosaic of phenotypic and functional features

The functional characterisation of senescent cells remains complex due to the diversity of induction pathways and the heterogeneity of their phenotypic features (**Figure 7**).

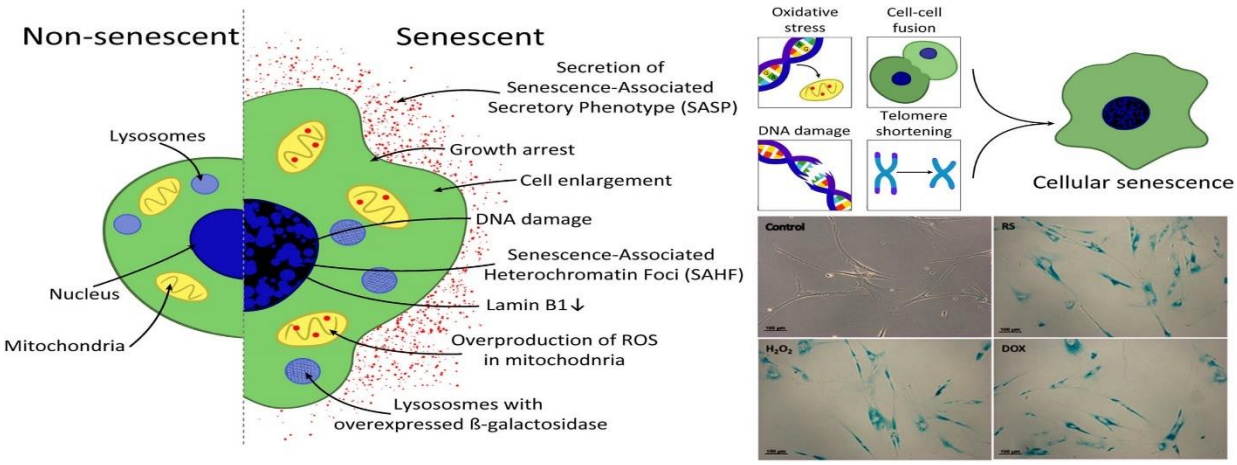


Figure 7 : Schematic representation of the most basic changes between senescent and non-senescent cells

Examples of naturally occurring or stress-induced cellular senescence and their effect on β-gal expression. Cellular senescence is driven by diverse genetic and molecular perturbations, including oxidative stress, DNA damage, or telomere attrition. Senescent cells are marked by irreversible cell cycle arrest, upregulation of cyclin-dependent kinase inhibitors (e.g., p16, p21), and significant alterations in cellular morphology and protein expression profiles. This state can be self-sustained through autocrine signaling or propagated to adjacent tumor cells via paracrine mechanisms, mediated by the senescence-associated secretory phenotype (SASP). The SASP releases a repertoire of cytokines, chemokines, and proteases that remodel the tissue microenvironment, facilitating senescence dissemination or fostering immunosuppressive niche (Henschke et al., 2024).

Their main characteristics include the irreversible proliferation arrest mediated by p16/p21, the morphological changes (increase in size, flattening, vacuolisation), the chromatin reorganisation, including senescence-associated heterochromatin foci that contribute to the silencing of proliferation-promoting genes and the remodeling of the lysosomal compartment marked by senescence-associated β-Gal activity (Calcinotto et al., 2019; Dimri et al., 1995; Narita et al., 2003). On the other hand, senescent cells secrete extracellular matrix remodeling molecules and pro-inflammatory cytokines (Coppé et al., 2008). This senescence-associated secretory phenotype (SASP) is involved in modulating the tumor microenvironment. Senescent cells also exhibit resistance to apoptosis favoring their persistence. We will focus on these last two key properties: SASP and resistance to apoptosis.

1.3.2 Senescence applied to T cells

1.3.2.1 T Cell Senescence: Definition

T cell senescence is an irreversible state of cellular aging acquired by T lymphocytes following repeated antigen-driven divisions or prolonged exposure to inflammatory and genotoxic

stress. This process results in the permanent loss of proliferative capacity and effector functions, while the cells remain metabolically active and persist in the organism, often adopting a pro-inflammatory phenotype.

1.3.2.2 Phenotypic changes

Senescent LT highly express CD57 (N. Khan et al., 2002), a marker associated with the history of excessive proliferation and the loss of the cytokine receptors CD122 (IL-2Rβ) and CD127 (IL-7Rα) (Cicin-Sain et al., 2012; Mojumdar et al., 2011). Overexpression of CD57 and KLRG1 is also considered a marker of senescence (Brenchley et al., 2003d; Henson & Akbar, 2009), as well as the gradual loss of CD27 or CD28 expression (terminal differentiation stage).

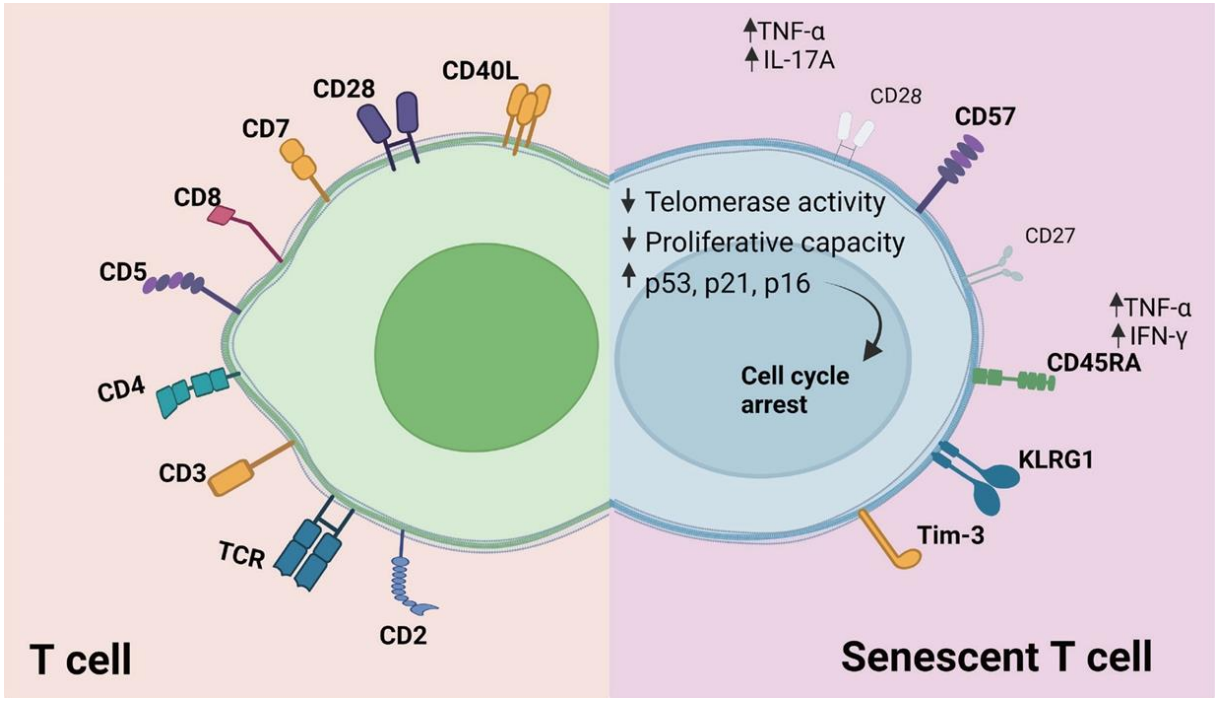


Figure 8 : The comparison of T cell and senescent T cell surface markers

In contrast to normal T cells, senescent T cells demonstrate elevated expression levels of cluster of differentiation 45 isoform RA (CD45RA), CD57, Tim-3, and KLRG1, while displaying reduced levels of CD27 and CD28. TCR, T-cell receptor; TNF-α, tumor necrosis factor-α; IL-17A, interleukin-17A; γ-IFN, interferon-γ (Nga et al., 2024).

The combination of these changes, loss of CD28, defective response to IL-2/IL-7, and irreversible telomere erosion (**Figure 8**), creates a favourable ground for replicative senescence of $CD8^+$ T cells (Effros et al., 1994). This process, further exacerbated by the inability of telomerase to restore telomere length, confines these cells to a dysfunctional state,

contributing to inflammation and potentially participating in the development of comorbidities such as cardiovascular diseases, obesity, and diabetes (Nga et al., 2024).

Table 2 summarises the key surface markers and functional features associated with CD8 T cell senescence, in comparison with exhaustion

CD8 LT type	Surface markers	Other characteristics
Exhausted	PD-1^{+}	• Impaired cytokine expression— γ-IFN, TNF-α, IL-2
Senescent	CD28^{-} CD57^{+} KLRG1^{+} CD27^{-}	• Shortened telomeres • Cell cycle arrest • Altered sensitivity to apoptosis • DNA damage • Defective mitochondrial function • Higher p53

Table 2: Markers used to define exhaustion and senescence

Exhausted T cells are primarily characterised by sustained expression of inhibitory receptors such as PD-1 and by a progressive loss of cytokine production (IFN-γ, TNF-α, IL-2). In contrast, senescent T cells display a specific set of surface markers (CD28^{-}, CD57^{+}, KLRG1^{+}, CD27^{-}) and exhibit the classical features of replicative senescence, including telomere shortening, cell cycle arrest, resistance to apoptosis, DNA damage, mitochondrial dysfunction, and increased expression of p53 (Donovan et al., 2021) .

1.3.2.3 The senescence-associated secretory phenotype (SASP)

Senescent cells activate a complex inflammatory program characterised by the release of bioactive molecules that modulate both intercellular communication and the microenvironment. Among these components, cytokines (IL-6, IL-8), chemokines (CCL2 and CXCL1) , growth factors (TGF-β, GM-CSF) and proteases (MMP1, MMP3, MMP9) act in synergy. IL-6 and IL-8 amplify inflammation, while chemokines recruit immune cells, favoring an environment conducive to fibrosis or tissue destruction (Acosta et al., 2008; Coppé et al., 2008; López-Otín et al., 2023) (**Figure 9**). These molecules can be divided into three groups

according to their mode of action. The first group includes mediators that bind to cell receptors (cytokines, chemokines, growth factors) and trigger signaling cascades involving NF-κB or JAK/STAT to regulate inflammation or proliferation. The second group includes enzymes (MMPs, tissue plasminogen activator) that act directly by cleaving membrane proteins, degrading signaling molecules and remodeling the matrix, to facilitate tumor dissemination for example.

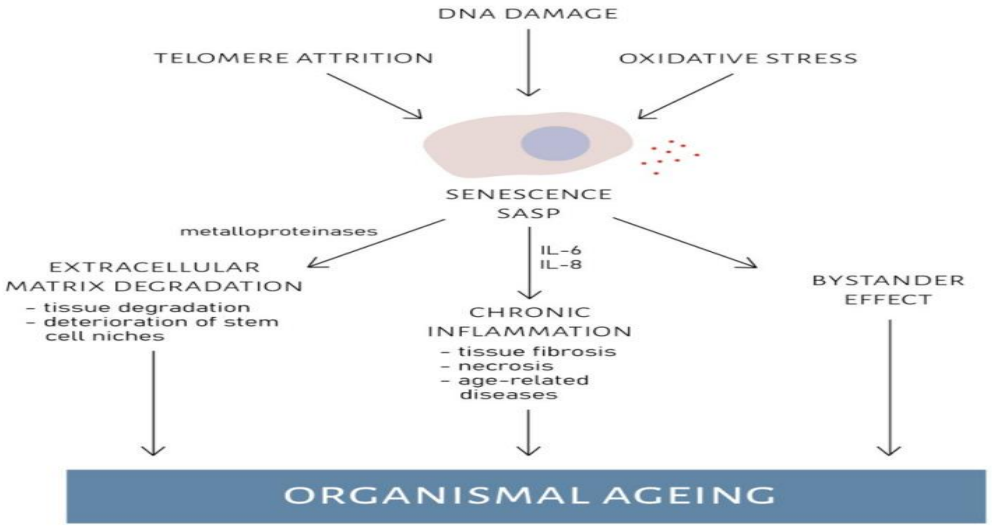


Figure 9 : Effects of SASP on organismal ageing

Telomere attrition, DNA damage or oxidative stress can cause the cell to enter senescence. Senescent cells can develop SASP. Substances that are excreted during SASP can have detrimental effects on extracellular structures and neighboring cells. Metalloproteinases cause extracellular matrix degradation leading to deterioration of tissues and stem cell niches. Inflammation factors (e.g., IL-6, IL-8) might cause chronic inflammation, that can lead to tissue fibrosis, necrosis and age-related diseases. Finally, SASP has a bystander effect on neighboring cells, causing them to enter senescence. All these effects propagate organismal ageing (Sławińska & Krupa, 2021).

The third group includes inhibitors (TIMP, PAI-1) that regulate these processes by slowing down protease activity, although this system becomes less effective with age. This duality is explained by its transcriptional regulation, orchestrated mainly by NF-κB and C/EBPβ, and amplified by recently discovered mechanisms such as the cGAS/STING pathway. The latter, activated by cytosolic DNA sensing during cellular stresses, stimulates the production of interferons and cytokines, reinforcing SASP (Glück et al., 2017; Rodier & Campisi, 2011; Yang et al., 2017).

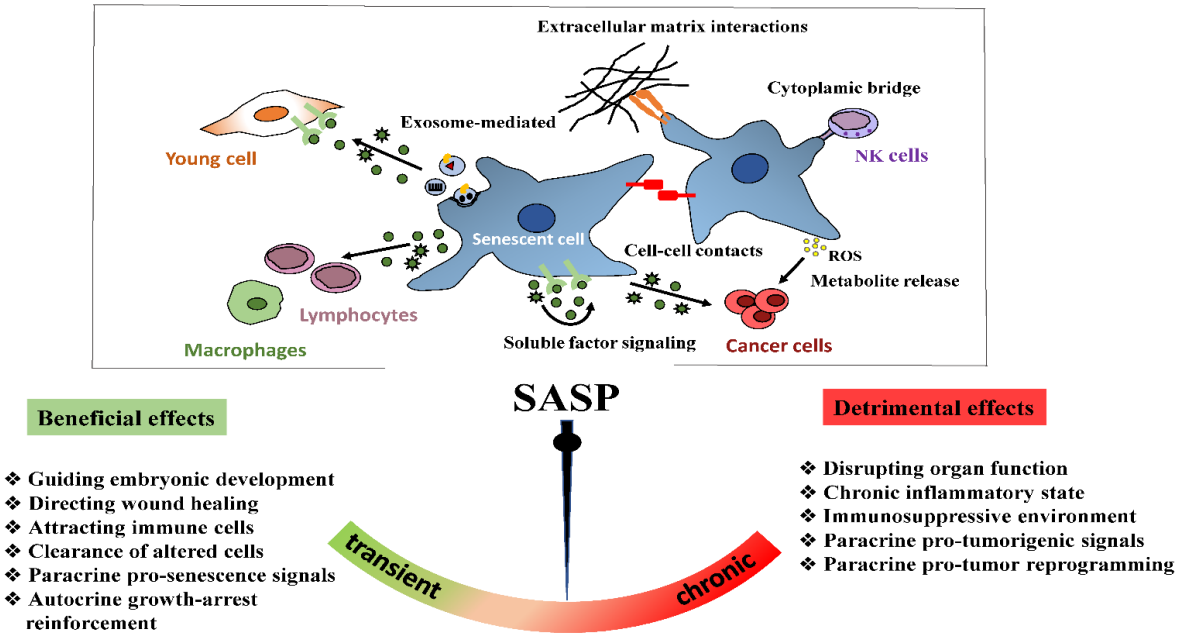


Figure 10 The beneficial or detrimental effects of SASP

Senescent cells dynamically shape their microenvironment through diverse communication mechanisms: secretion of soluble factors (cytokines, proteases), release of extracellular vesicles (exosomes), direct cell-cell contacts, cytoplasmic bridges, extracellular matrix remodeling and metabolite signaling. While a transient SASP promotes wound healing, immune surveillance (e.g., via NK cell recruitment), and tumor suppression, its chronic activation drives pathological outcomes such as fibrosis, cancer progression, and age-related degenerative diseases. This duality underscores the SASP as a context-dependent switch, where timing and persistence dictate its impact on tissue health (Roger et al., 2021).

Responsible for the ambivalent effects of senescent cells, the SASP recruits immune cells to eliminate them and acts as a tumor suppressor via the recruitment of innate and adaptive immune cells during tumor initiation (Xue et al., 2007). It also plays a key role in tissue repair following injury (Demaria et al., 2014). However, prolonged exposure to SASP components such as IL-6, CXCL8 or CXCL1 promotes senescence via an autocrine mechanism (Kuilman & Peeper, 2009), while diffusion of these molecules induces paracrine senescence in neighboring cells (Acosta et al., 2013). This phenomenon partly explains the chronic inflammation and age-related dysfunctions associated with the pathological accumulation of senescent cells. The SASP thus may produce opposite effects (**Figure 10**).

1.3.2.4 Resistance to apoptosis

Apoptosis, a stress-induced programmed cell death mechanism, acts as a brake on tumorigenesis. However, senescent cells can escape this process through pro-survival

mechanisms. For example, the transcription factor FOXO4, which is overexpressed at the transcriptional and protein levels, neutralizes p53 by sequestering it, thereby inhibiting apoptosis (Baar et al., 2017). In addition, anti-apoptotic proteins of the BCL-2 family (Bcl-2, Bcl-XL, Mcl-1) are upregulated, blocking caspase activation (Yosef et al., 2016). Finally, epigenetic inactivation of the pro-apoptotic gene Bax via trimethylation of histone H4 at lysine 20 (H4K20me3) contributes to this resistance (Sanders et al., 2013). These adaptations would explain the persistence of senescent cells in vitro and their progressive accumulation during ageing, contributing to tissue degradation and age-related pathologies.

1.3.2.5 The duality of senescence

As previously discussed, cellular senescence represents a fundamental physiological programme, characterised by an irreversible cell cycle arrest and a distinctive secretory activity, known as the SASP(Coppé et al., 2008) . Beyond its well-documented deleterious effects, senescent cells retain important antiviral functions, notably through the secretion of type I and III interferons, which contribute to host defence(Moiseeva et al., 2006) . As shown in (**Figure 11)**, the conceptual distinction between acute and chronic senescence is now crucial to understand its dual effects – beneficial in the short term but detrimental over the long term (Campisi, 2013; van Deursen, 2014).

Acute senescence, induced by transient sub-lethal stress (such as DNA damage, transient oncogenic activation, or radiation exposure), is a protective mechanism. It rapidly triggers a local inflammatory response and the expression of activating ligands on the cell surface (such as ULBP2, recognised by the NKG2D receptor), enabling efficient immune clearance by NK cells and macrophages (Krizhanovsky et al., 2008; Xu et al., 2015). This process, limited to a few days or weeks, promotes tissue regeneration and acts as an essential brake on tumour development (Muñoz-Espín & Serrano, 2014) .

In contrast, chronic senescence develops gradually over months or years with ageing or in persistent pathological contexts (obesity, atherosclerosis, chronic toxic exposures). Immunosenescence (the age-associated decline in immune function) reduces the capacity of the immune system to efficiently eliminate senescent cells, leading to their accumulation (Laphanuwat et al., 2023; Ovadya et al., 2018). These persistent senescent cells continuously release a complex SASP, rich in pro-inflammatory cytokines (IL-6, IL-8, TNF-α), chemokines, proteases (such as matrix metalloproteinases), and growth factors (Coppé et al., 2010). The chronic SASP maintains a inflammaging (Franceschi & Campisi, 2014), impairs stem cell

function, promotes fibrosis, and disrupts tissue homeostasis. Chronic senescence thereby actively contributes to the development and progression of a broad spectrum of senopathies, including cancer (via a permissive tumour microenvironment), neurodegenerative diseases, metabolic disorders, atherosclerosis, osteoarthritis, and organ failure (Childs et al., 2016; He & Sharpless, 2017).

This understanding has paved the way for senotherapies, strategies aimed either at selectively eliminating senescent cells (senolytics) or at modulating their deleterious phenotype by inhibiting SASP production (senomorphics). Some senolytic agents, such as the dasatinib–quercetin combination or fisetin, have shown beneficial effects in preclinical models and are currently under exploratory clinical investigation (Amaya-Montoya et al., 2020; Kirkland & Tchkonia, 2017).

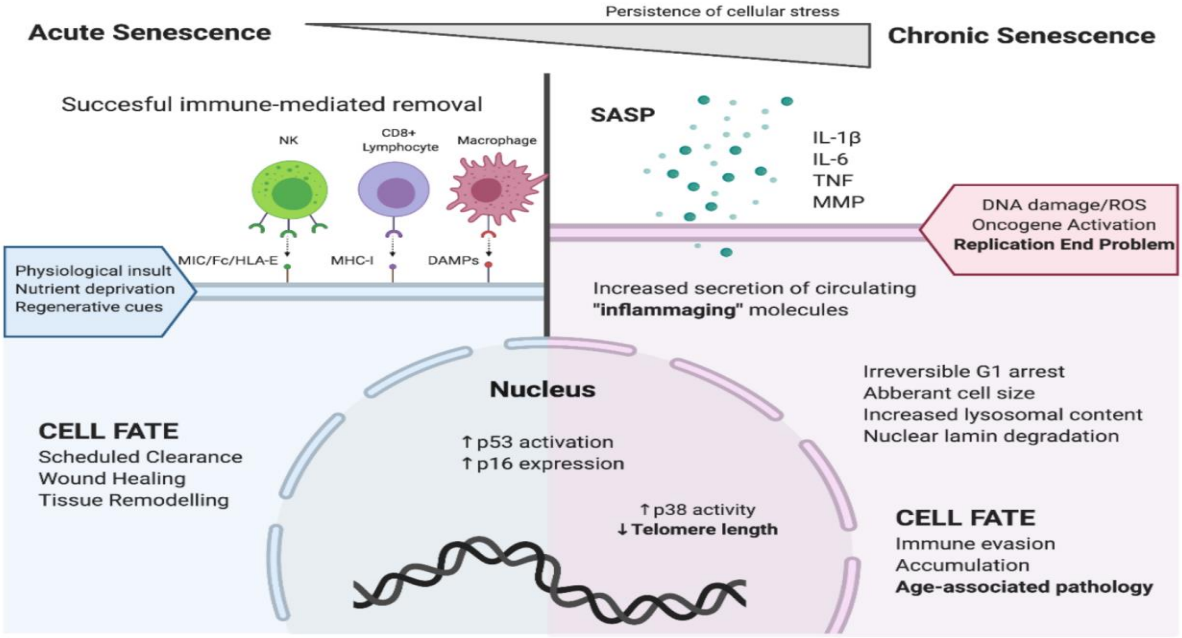


Figure 11 : Two types of cellular senescence

Acute senescence (left): Senescent cells are rapidly identified and eliminated by the immune system thanks to coordinated detection mechanisms, allowing them to be replaced by functional cells.

Chronic senescence (right): Senescent cells accumulate progressively, releasing SASP. This leads to persistent inflammation, both local and systemic, which exacerbates tissue senescence and promotes the development of age-related pathologies (J. Heath & D. Grant, 2020).

1.4 T cell senescence : a fraction of the global phenomenon of immunosenescence

Immunosenescence is a complex process of immune aging distinct from replicative cellular senescence, characterised by a progressive and multifactorial decline in immune system functions related to age. This phenomenon leads to increased vulnerability to infections and significantly contributes to the development of degenerative pathologies, including neurodegenerative disorders, cancers, cardiovascular diseases, and autoimmune conditions (Stahl & Brown, 2015).

Immunological alterations include decreased functionality of hematopoietic stem cells, pronounced thymic involution, reduction in circulating naïve T lymphocytes, and expansion of CD28- T lymphocytes with limited proliferative potential. Concurrently, there is an increase in the production of pro-inflammatory cytokines (IL-6, TNFα) and a decrease in the CD4/CD8 ratio (Czesnikiewicz-Guzik et al., 2008; Deng et al., 2004).

Phenotypically, this condition manifests as a reduction in CD8+ effector LT, loss of CD28 expression, telomere shortening, and reduced telomerase activity, associated with overexpression of CD57 and KLRG1 markers. These alterations lead to a decrease in naïve CD8+ LT, inversion of the CD4/CD8 ratio, generalised T lymphocyte activation, reduced lymphocyte proliferation, and progressive thymic involution.

Functionally, immunosenescence results in significant limitations in both innate and adaptive immune responses, reduced vaccine efficacy, increased susceptibility to autoimmune manifestations, and the development of a chronic low-grade inflammatory state (inflammaging) contributing to geriatric frailty syndrome. The underlying mechanisms, although not fully elucidated, involve multiple factors including cellular senescence affecting all immune compartments and correspond to a "multi-hit" model where physiological aging and associated comorbidities interact.

This progressive immune dysregulation typically emerges around age 70 and represents a major determinant of age-related morbidity and mortality. It is particularly influenced by chronic infections and comorbidities that shape immune repertoires. CMV infection powerfully modulates immune repertoires, with anti-CMV cellular responses representing up to 10% of the circulating immune repertoire, a proportion that increases with age. This clonal

expansion significantly contributes to the acceleration of immunosenescence and the deficit in vaccine responses (Solana et al., 2012).

1.5 Conclusions

Although dysfunctional, both senescent and exhausted cells exhibit functional activity. There is ongoing debate on whether these cells should be considered dysfunctional or adapted to the antigenic contexts. Interestingly, different chronic viral infections led to different outcomes, as exemplified by the context of HIV and CMV infections that we will introduce in this manuscript. Additionally, limited information is available on the combined impact of senescence and exhaustion.

2 The Human immunodeficiency virus (HIV) infection

2.1 Epidemiology

Since its discovery more than 40 years ago (1983), 84 million people have been infected by HIV, leading to the deaths of over 40 million individuals. The incidence of infection has fallen over time but remains high. In 2023, the estimates indicate that there are over 39.9 million people living with HIV (PWH) with 1.3 million new infections and 630,000 HIV-related deaths reported (ONUSIDA, 2023) (**Figure 12A**). This is 60% less than the peak in 1995, when 3.3 million people were infected. In its latest national bulletin, titled 'Surveillance of HIV and bacterial STIs in France in 2023', Santé publique France estimates that there will be 3,650 new HIV infections in France in 2023. The annual number of new infections fell between 2012 and 2021, primarily due to a decline among French-born men who have sex with men (MSM), thanks to intensified screening and pre-exposure prophylaxis. However, this downward trend has since levelled off and does not apply to all populations, particularly those most at risk of HIV transmission (Santé publique France, 2024).

Since the first tritherapies were introduced in 1996 and allowed for effective infection control, the prevalence of PWH has increased as a result of longer survival times. Compared to its highest level in 2005 (the black year of the pandemic with 2.5 million deaths), the number of deaths has decreased by 48% in 2018 (ONUSIDA, 2024). UNAIDS launched an ambitious program called “90-90-90: Treatment for All”. The goal was to ensure that, by 2020, 90% of PWH knew their serostatus, 90% were receiving antiretroviral treatment, and 90% were showing viral suppression. Data from 2018 showed that 23 million PWH had access to treatment, including 82% of pregnant women, which prevented mother-to-child transmission. However, the 90-90-90 targets were not globally achieved in 2020 due to persistent regional disparities and chronic underfunding of HIV programs (ONUSIDA, 2020a).

The estimated number of deaths worldwide in 2016 is one million. More than half of the deaths cannot be directly attributed to the virus's infection. The primary causes of death are no longer those associated with the stage of acquired immunodeficiency syndrome (AIDS) but rather with comorbidities (cardiovascular, neurodegenerative, …) (**Figure 12B**). Importantly, the population of PWH is now naturally ageing thanks to the introduction of antiretroviral treatment (ART), and the incidence of comorbidities may highly depend on the setting of an ageing HIV-infected population (Morlat et al., 2014; C. J. Smith, Ryom, et al., 2014). It is

estimated that 84% of PWH could have at least one age-related non-transmissible comorbidity associated with age by 2030, compared with 28% in 2010, including 28% with 3 or more associated comorbidities (Smit et al., 2015). This worrying increase in age and subsequently increase in comorbidities may require appropriate clinical follow-up and further investigations regarding the ageing processes in PWH.

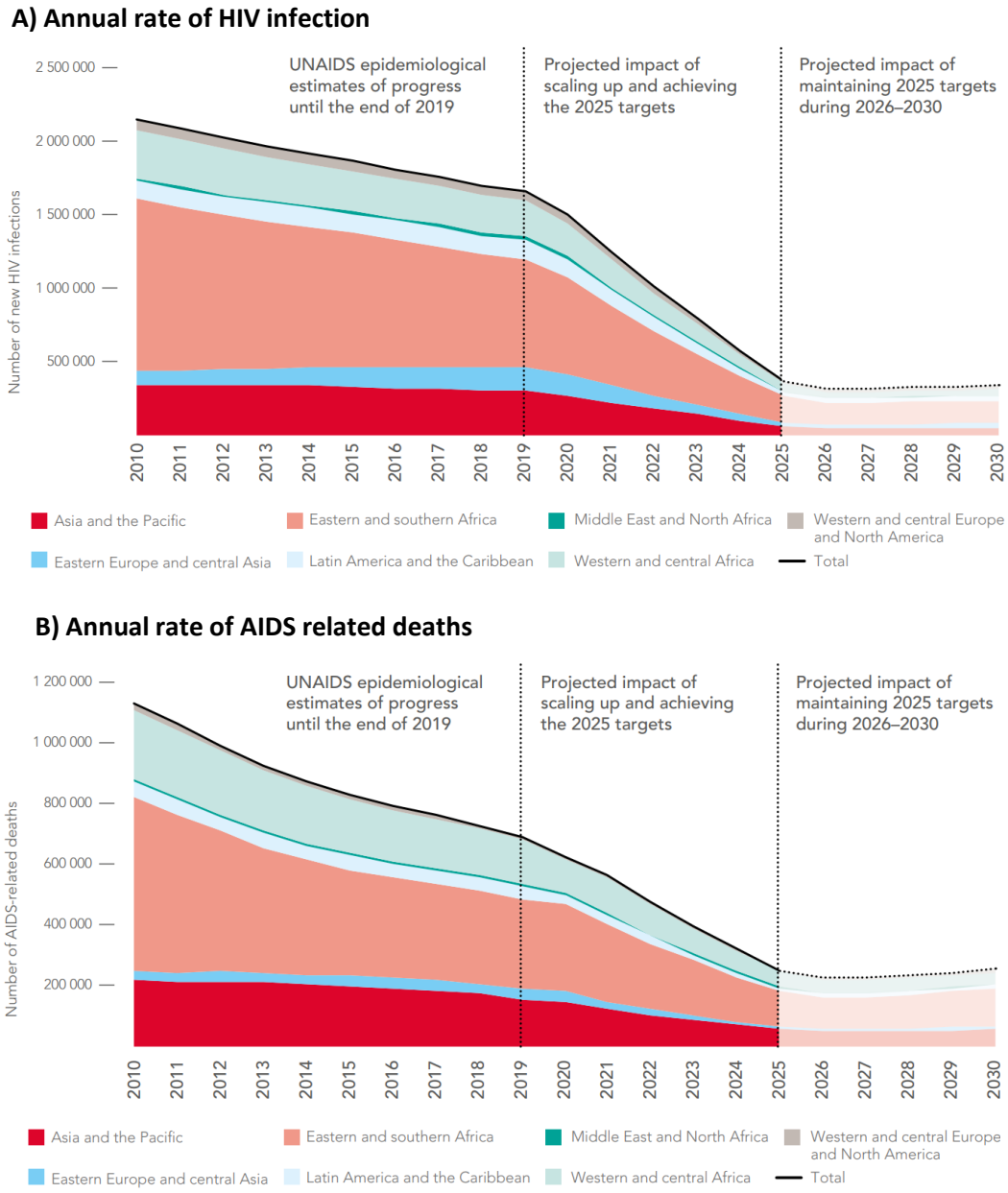


Figure 12 : Worldwide prevalence of novel HIV infection and AIDS related death from 2010 to 2025 and projected prevalence for 2025-2030

Epidemiological models predict that achieving the 95-95-95 targets by 2025 would result in a 90% decrease in new HIV infections by 2030, in line with the Sustainable Development Goals. Equivalent reductions in AIDS-related mortality are projected (ONUSIDA, 2020b).

2.2 Virology

2.2.1 HIV is a retrovirus

The International Committee on Taxonomy of Viruses classifies the human immunodeficiency virus (HIV) in the Retroviridae family and the Lentivirus genus. It was first discovered and characterised in 1983 by Luc Montagné's team (Barré-Sinoussi et al., 1983). HIV is an RNA retrovirus that infects $CD4^+$ cells and integrates its viral DNA into the target cell's genome. The virus contains the enzyme reverse transcriptase, responsible for converting viral RNA into DNA. Its genome is 9.2 kb in size and contains several genes which produce structural proteins (Gag, Pol and Env) and accessory viral proteins (regulators) such as Tat, Rev, Nef, Vif, Vpr and Vpu. These proteins play roles in viral recognition by the host cell's membrane or intracellular, as well as in activating the immune response (**Figure 13**).

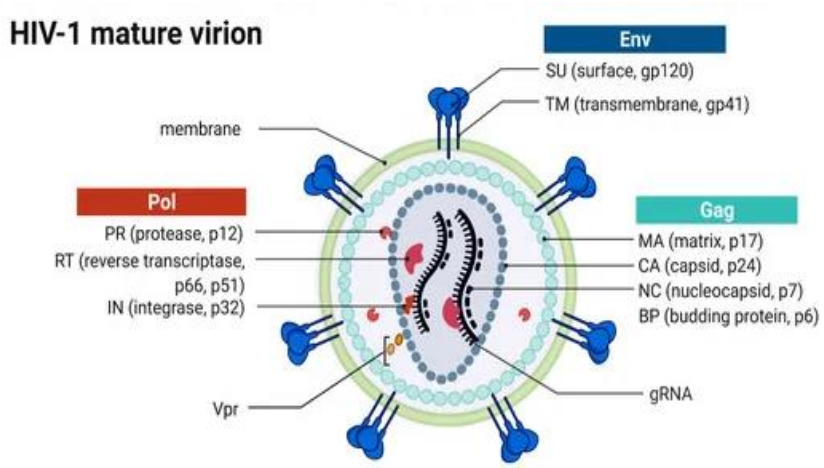


Figure 13 : The HIV-1 virion structure and genome

The mature enveloped virion has a spherical shape and is enveloped by a lipid bilayer membrane derived from the host cell containing 7–35 envelope glycoproteins trimers, encoded by the gene env. The inner layer of the membrane anchors the Gag-derived matrix proteins (MA) and also harbors Vpr and PR. The capsid (CA) is found within the center of the virion and contains the two copies of genomic RNA, the reverse transcriptase (RT), the integrase (IN) encoded by Pol. The gRNA is stabilised by the nucleocapsid (NC) proteins. The two regulatory genes rev and tat and three accessory genes vif, vpr, and vpu, are not presented in the scheme (van Heuvel et al., 2022).

HIV viruses exhibit a high degree of genetic diversity, which has important implications for transmission, pathogenesis, and response to therapy. They are classified, based on their phylogeny, into distinct groups, clades, or subtypes within the M group of HIV-1**(** **Figure 14****)**, the most prevalent type worldwide (Désiré et al., 2018).

In addition to HIV-1, there is HIV-2, which is less widespread, largely confined to West Africa, and genetically distinct, generally associated with slower disease progression. In this thesis, we will focus primarily on HIV-1, given its global prevalence and clinical significance.

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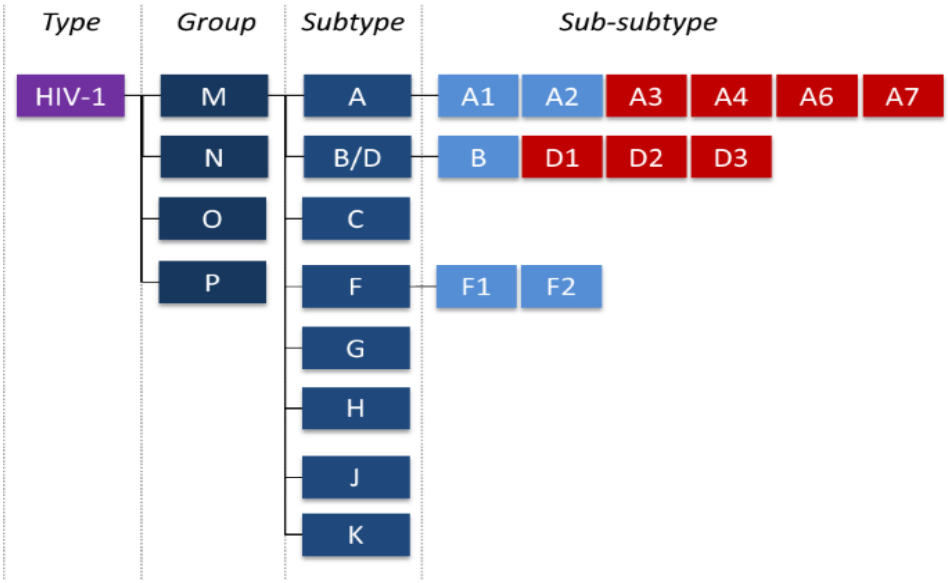


Figure 14 : HIV-1 updated classification

HIV-1 remains classified into groups, subtypes and sub-subtypes, with changes from the previous classification colored in red. The groups were originally named M for main, O for outlier, and N for Non-M-Non-O (DeLeys90, Charneau94, Simon98). Phylogenetic analysis of envelope sequences revealed the existence of multiple phylogenetic clusters (or clades), named as subtypes (Désiré et al., 2018).

2.2.2 Cellular targets of HIV

The main targets of HIV virus are activated CD4 T, monocytes/macrophages, and dendritic cells that express both the CD4 receptor and CXCR4 or CCR5 co-receptors (Coleman & Wu, 2009; Klatzmann et al., 1984; Popov et al., 2005). The virus enters the cell using the gp120 and gp41 proteins, encoded by Env. Then, the cellular machinery of the host is hijacked in order to produce new viral particles, which will infect other cells from one cell to another.

2.2.3 Viral cycle and HIV persistence in latent form

The HIV-1 replication cycle can be summarised in different steps: 1) binding, 2) fusion, 3) reverse transcription, 4) integration, 5) replication, 6) assembly and 7) budding. The knowledge of the cell cycle provides important information for the outcome of the infection:

- The crucial role of reverse transcription: an important feature of the HIV biology is its significant genetic variability, especially in the env gene, that is mostly driven by the infidelity of the reverse transcriptase. This variability also contributes to the persistence of the virus.
- The identification of the whole sequence allowed to identify antiviral drug targets for different stages of the cycle (**Figure 15**).
- Finally, the virus is not always in a replicative form. The HIV virus may persist in latent form. The incapacity of ART to eliminate the virus reflects their inability to target the virus in its latent form. The HIV cure program includes the "shock and kill" strategy aiming at reactivating the virus hiding in infected cells by using latency-reversing agents. These cells can then be targeted by ART, contributing to the viral eradication and/or reduction of viral load.

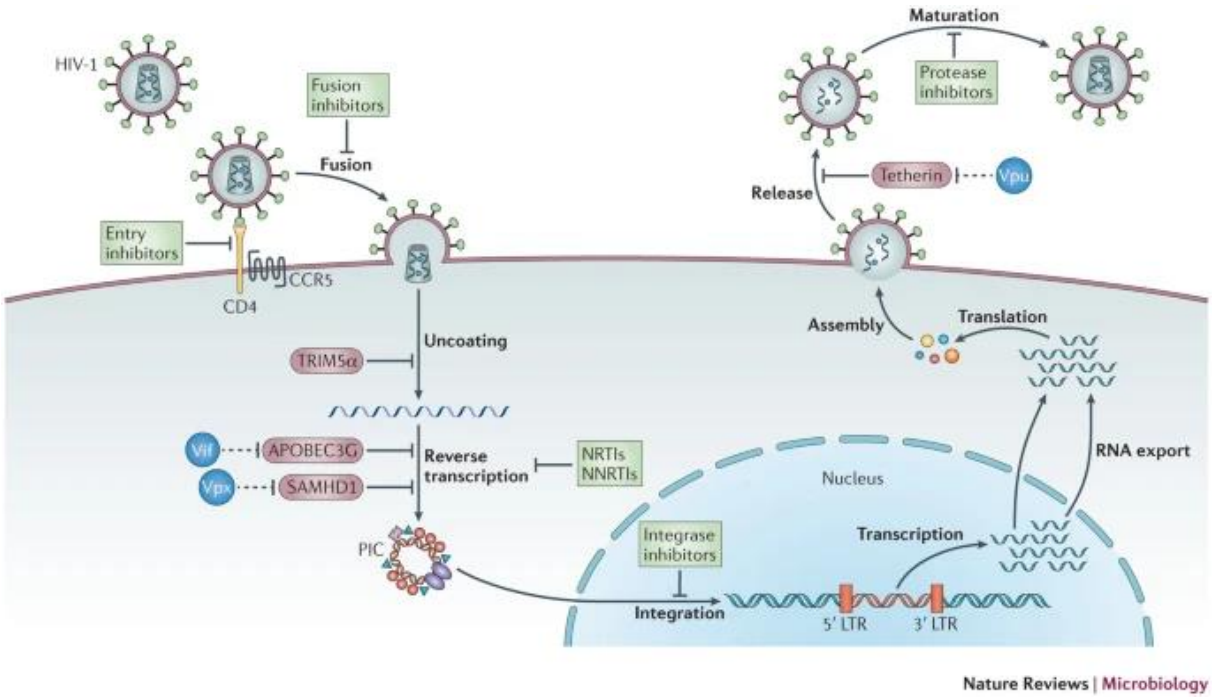


Figure 15 : Schematic overview of the HIV-1 replication cycle

The major families of antiretroviral drugs (green), and the step of the life cycle they block, are indicated. The virus fuses with the host cell membrane and releases the contents of its capsid into the

cytoplasm. Reverse transcription produces proviral DNA from the viral RNA, which is transported to the nucleus in the form of a pre-integration complex. transported to the nucleus in the form of a pre-integration complex. The cellular machinery transcription and translation of the viral genome. The virions self-assemble and are released by budding on the surface of the infected cell. Maturation is completed by the action of proteases (Barré-Sinoussi et al., 2013).

2.3 Pathophysiology

2.3.1 The primary phase of HIV infection

The initial infection phase corresponds to the weeks following the infectious encounter. The acute HIV infection refers to the first month, whereas the primary infection refers to the six-month period after infection. This period is characterised by a very high HIV viral load and corresponds to important damage to the immune system: the depletion of CD4 LT seen in the blood is also occurring in intestinal mucosa and lymph nodes (M. S. Cohen et al., 2011). In the intestinal mucosa, HIV rapidly depletes a large population of activated memory CD4+ CCR5+ LT (Shale et al., 2013). The myeloid cell compartment is also disrupted during the initial stages of infection (Rodrigues et al., 2017). Although an immune response is mounted early on, it fails to clear the virus.

2.3.2 Progression of HIV infection

The pathophysiology, evolution and outcome of HIV infection drastically differs depending on the introduction of ART or not.

Evolution in the absence of ART treatment

The chronic phase, known as "asymptomatic," typically lasts for several years with a gradual decline in CD4 LT cell numbers. This decline is attributed to direct infection of target cells causing cell lysis but also to the apoptosis of uninfected CD4 LT due to chronic activation. This major cause of lymphopenia is mediated by the induction of death proteins such as TRAIL and Fas-Ligand expressed on the surface of CD4 LT under the influence of IFN-alpha (Février et al., 2011).

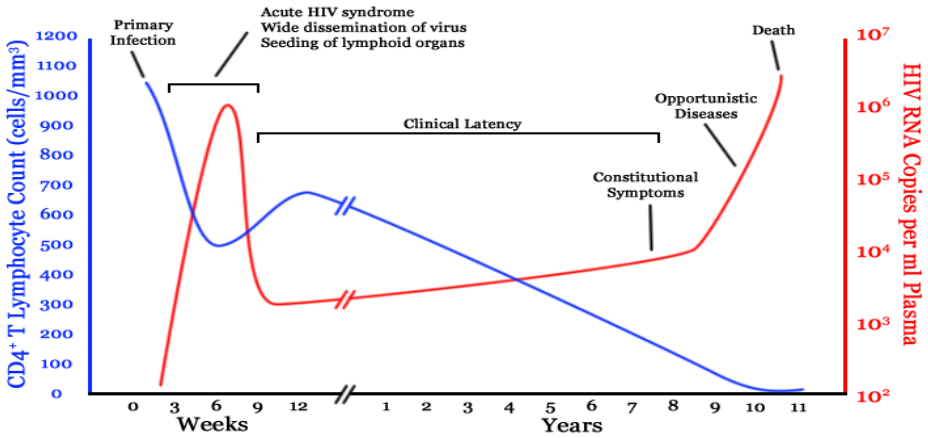


Figure 16 : HIV infection and disease progression in the absence of treatment

There are two main indicators of the disease progression: the viral load and the CD4 count. The viral load (HIV RNA) reaches 10^6 copies/mL during the acute phase of the infection, and then falls to lower titers due to the development of the anti-HIV immune responses. In the absence of ART, the immune response becomes less efficient, and the viral load increases. The CD4 count reflects the degree of immunodeficiency and is predictive of near-term risk of opportunistic diseases and mortality(Situma, 2013).

The destruction of infected CD4 LT by HIV-specific cytotoxic CD8 LT helps control viral replication during the initial infection and contributes to CD4 LT depletion. However, the immune response progressively loses the ability to control the virus. AIDS is defined as having a CD4 LT count of less than 200 cells/µl. This significantly increases the risk of developing opportunistic infections and certain cancers, such as Kaposi's sarcoma (Battistini Garcia et al., 2025). To note, a rare fraction of PWH (<0.5%) are able to control spontaneously the virus: the HIV controller.

The crucial role of ART treatment

The introduction of ART changed the outcome of the infection (**Figure 17**). The effect of ART is significant during the 3 phases of the infection (the primary phase, the asymptomatic phase and the end stage). The WHO recommends treating anyone infected with HIV as soon as possible after diagnosis, with the main aim of preventing progression to AIDS.

The ART is usually composed of three drugs, targeting essential functions for the viral cycle, that are combined to prevent resistance. The tritherapies usually target the reverse transcriptase and the integrase or protease. When efficient, the ART inhibits viral replication (leading to an viremia below the detection limit (50 copies/mL)), restores the CD4 LT pool

(greater than 500 cells/mm3), reduces immune deficiency and opportunistic infections, as well as the activation of the immune system and its deleterious consequences, such as cardiovascular, metabolic, bone and neurological risks (Sandler et al., 2011) and lymphocyte exhaustion (Maartens et al., 2014; Trautmann et al., 2006).

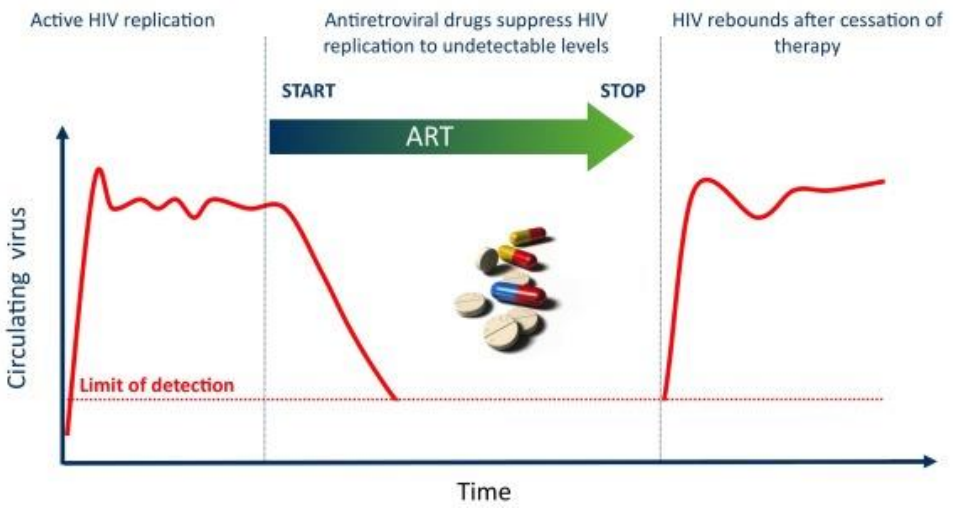


Figure 17 : Clinical definition of the HIV reservoir

Without treatment, HIV infection is characterised by intense viral replication, which can be quantified in the plasma of people living with the virus. ART suppresses this replication to undetectable levels (less than 50 copies/mL) according to standardised methods. However, when ART is interrupted, viral replication resumes, revealing the persistence of HIV in cellular and anatomical reservoirs that can reactivate full-blown infection (Kulpa & Chomont, 2015).

ART is also a highly effective strategy for preventing HIV transmission. However, ART cannot eradicate viruses that persist in a latent state in cellular or anatomical reservoirs that are more difficult to access (Trono et al., 2010). These reservoirs represent a barrier to curing the infection; consequently, the interruption of ART leads to viral rebounds in most cases. According to a meta-analysis of 24 prospective studies (n = 382 participants), 4% of PWH maintain an undetectable viral load (less than 50 copies/mL) 84 days after stopping ART. This outcome was more prevalent among individuals who initiated ART early (6% vs. 1% for late ART initiation) (Gunst et al., 2025).

2.4 HIV infection as a chronic disease with residual immune defects

Thanks to the introduction of ART, HIV infection is no longer a fatal disease and is now a chronic illness associated with mainly non-infectious complications (Deeks, Tracy, et al., 2013). People living with HIV experience survival rates approaching those of individuals not infected

with HIV (Helleberg et al., 2012; McManus et al., 2012; Sabin, 2013; Sabin & Reiss, 2017; Trickey et al., 2023) but still exhibit lower quality of life (Marcus et al., 2020). In PWH receiving very long courses of treatment, it has been observed that these patients present complications 'indirectly' linked to HIV, with morbidity and even mortality no longer due to opportunistic infections or cancers classified as AIDS-related, but rather to early atherosclerosis, coronary risk, accelerated neurocognitive changes, cancers not classified as AIDS-related, etc... (Morlat et al., 2014). At the level of the immune system, some immune dysfunctions persist.

Heterogeneity in CD4 T cell restoration

In most patients, ART effectively inhibits viral replication and restores CD4 LT numbers in the peripheral blood. However, this recovery is incomplete in the intestinal mucosa, where structural and functional changes persist despite treatment (Brenchley et al., 2004; Kök et al., 2015). These alterations are particularly pronounced when treatment is initiated late. At least 30% of successfully treated patients who achieve complete viral suppression experience limited improvement in CD4 LT counts (virological responders) and remain at increased risk of long-term clinical complications (Kaufmann et al., 2005; Battegay et al., 2006; Tuboi et al., 2010).

Low-grade immune activation

Chronic controlled HIV infection is commonly associated with residual chronic inflammation (Macal et al., 2008; Hatano et al., 2013). The nature of the low-grade inflammation is still difficult to comprehend among PWH. These difficulties reflect (i) the absence of consensual characterisation of inflammation as exemplified by the abundancy of soluble markers of inflammation providing a large spectrum of inflammatory profiles, (ii) the probable heterogeneity in inflammatory status among PWH (including confounding factors (age, sex, BMI, substance consumption, …) and (iii) the difficulty to define the appropriate control group for comparison (Novelli et al., 2020). The residual inflammation is typically linked to various mechanisms, including HIV persistence, sustained damage to the intestinal mucosal barrier (disrupting the balance between CD4+ T lymphocytes and regulatory T cells (Treg) or T helper 17 cells (Th17), ART toxicity, co-infections, and metabolic as well as immunological alterations (**Figure 18**).

The consequences of this low-grade inflammation are uneasy to evaluate but is commonly associated with the incidence of comorbidities such as cardiovascular diseases, neurocognitive decline, … The inflammation may also contribute to the development of immune dysfunctions (Teer et al., 2025).

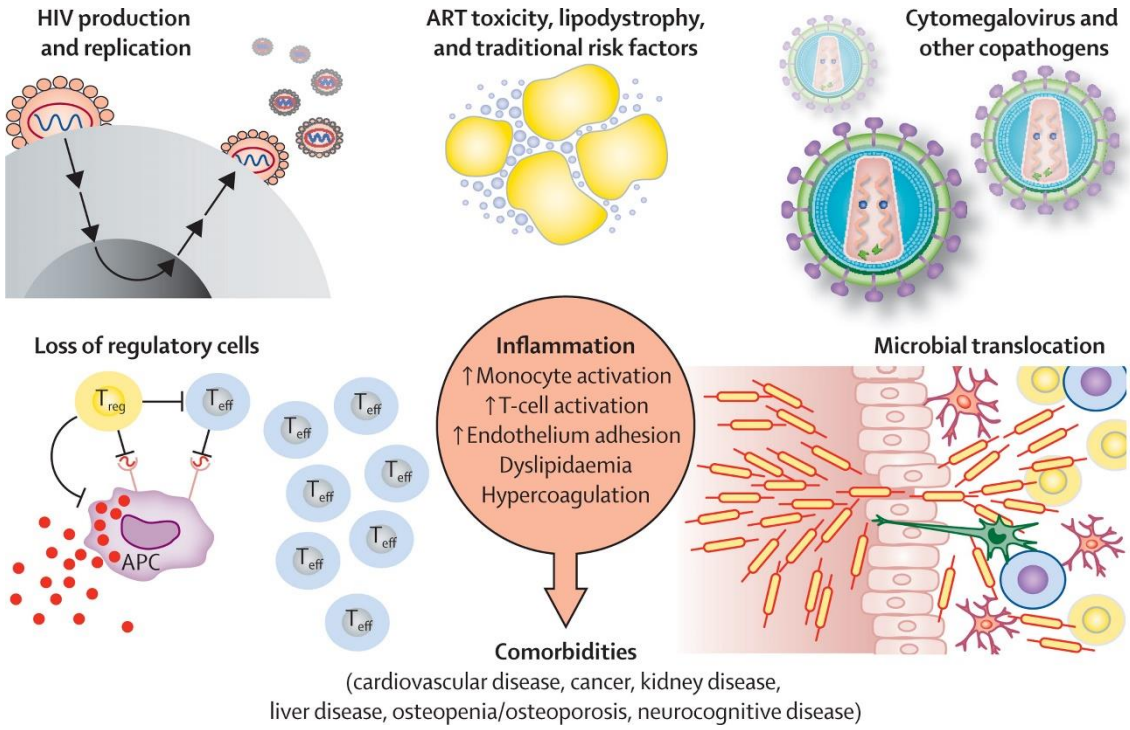


Figure 18 : Causes and consequences of HIV-associated inflammation

In the chronic phase of the HIV infection, multiple factors may contribute to persistent inflammation: low but constant HIV production, metabolic inflammation related to ART toxicities, high levels of other pathogens, persistent immune defects impacting CD4 LT or other immune cells, persistent damage to the gut mucosa, leading to microbial translocation (Deeks, Lewin, et al., 2013).

Accelerated ageing in PWH

The accelerated immunosenescence observed in PWH, even when on effective ART, corresponds to premature ageing of the immune system which persists despite viral suppression. The notion of accelerated ageing in PWH initially relied on the similar changes impacting the immune system in PWH and aged individuals: lower CD4/CD8 ratio, lower naïve/memory ratio, enrichment in senescent LT, low-grade chronic inflammation, exhaustion of hematopoietic progenitors (Appay & Kelleher, 2016). It was also substantiated by the higher incidence of comorbidities as described in normal ageing (Pathai et al., 2014). According to

the work of Chauvin and Sauce (2022), this complex immunosenescence is essentially based on three interdependent mechanisms: (i) chronic immune activation is maintained through persistent inflammation and high levels of cytokines (IL-6 and TNF-α). This inflammation is fueled in particular by microbial translocation. (ii) LT develop a senescent phenotype characterised by the loss of CD28 and the expression of the CD57 molecule (CD28-CD57^{+}), resulting from excessive and repeated stimulation by viral Ags. (iii) HIV induces a thymic deficiency that compromises the production of new naive LT, thereby limiting the renewal of the immune system's repertoire (Chauvin & Sauce, 2022). However, these observations did not meet consensus until the development of new approaches studying patterns of methylation (Gross et al., 2016; Navas et al., 2025) to predict an epigenetic age. Based on these strategies, a 5-year increase in biological age is described in PWH compared to uninfected individuals, with an epigenetic imprinting developing during the primary phase of infection.

These aspects are all the more crucial as the population of PWH naturally ages. The combined effects of natural and HIV-induced ageing are still probably not at the extreme, as only less than 10% are more than 70 years old (Marty et al., 2022). Projection estimates than more than 20% of PWH will be more than 70 years old by 2030. The factors involved in this accelerated ageing are not fully understood and partly overlap those described as involved in chronic low-grade inflammation. Among them, the role of the CMV coinfection may be particularly crucial. Indeed, the majority of PWH are co-infected with CMV and CMV is strongly associated with senescence and senescent associated comorbidities.

2.5 Immune dysfunctions associated with HIV infection

2.5.1 Defects impacting the innate immune responses

2.5.1.1 The activation of the innate immunity as the first line of defense

The activation of the innate immune system by HIV induces a cascade of cytokines and chemokines during primary infection, which precedes the peak of the viral load. The innate immune response is triggered by the coordination of a number of cells which recognise the virus non-specifically, via universal pattern-recognition receptors (PRRs) located on the cell surface or in the cytoplasm (such as TLRs, RLRs and DNA-sensors). This recognition leads to the production of a cascade of multiple pro- or anti-inflammatory cytokines.

2.5.1.2 The production of cytokines

The cytokine storm during the primary phase of HIV infection

Activation of the innate immune system triggers a cytokine storm that occurs prior to the peak viral load (**Figure 19**). A sequential elevation in plasma levels of multiple inflammatory mediators is observed: a rapid and transient increase in IFNα and IL-15, a sharp rise in IP-10, a rapid and sustained increase in TNFα and MCP-1, a more delayed elevation in IL-6, IL-8, IL-18, and IFNγ, and finally a late increase in IL-10. These cytokines collectively contribute to controlling viral replication, either directly or indirectly through the activation of other effector mechanisms. However, this excessive cytokine response also contributes to early immunopathology during infection.

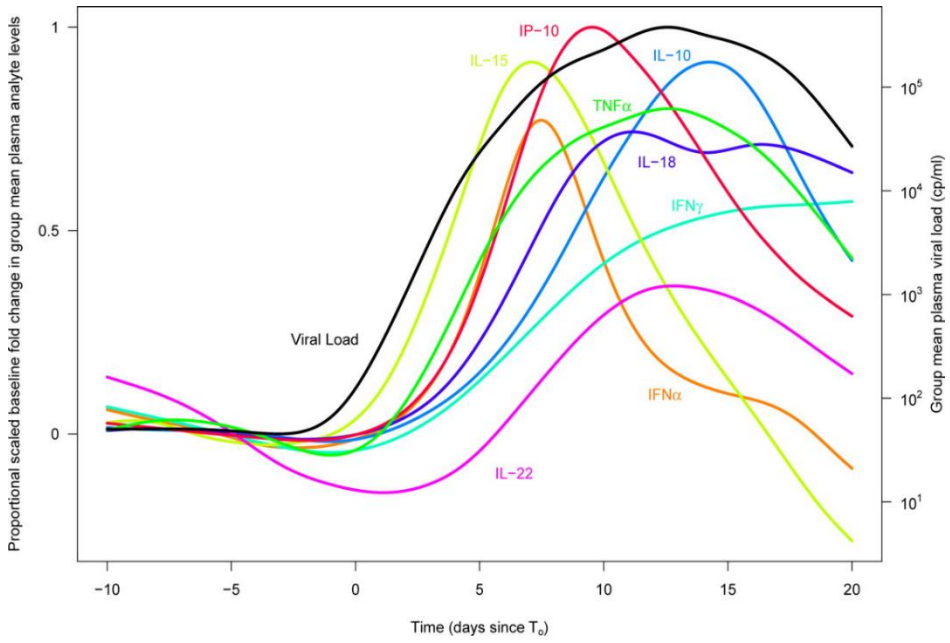


Figure 19 : The cytokine storm during the acute HIV-1 infection

The proportional change relative to baseline in plasma levels of selected analytes (see key) and the viral load over time in the entire group of HIV-infected subjects are shown. The peak heights for different analytes are scaled according to the percentage of HIV-infected subjects studied who exhibited an elevation in plasma levels of the analyte concerned. Time is plotted in days relative to T_0. cp, copies (Stacey et al., 2009).

These cytokines represent a mean of inducing an intrinsic antiviral response, by stimulating restriction factors and other IFN-induced genes, but also to mobilize adaptive immunity by helping to activate and polarize LT. On the other hand, they allow the recruitment and activation of CD4 LT, the main targets of HIV, allowing HIV to spread to the rest of the body. They also contribute to the hyperactivation and exhaustion of the immune system.

Low-grade inflammation during the chronic stage

Chronic inflammation in HIV-infected patients involves both systemic and neuroinflammatory markers, even under effective ART. Among systemic markers, we find CRP, LPS, IL-6, TNF-α, sCD14, sCD163, and D-dimers, whose elevation is correlated with an increased risk of cardiovascular and hepatic pathologies (**Figure 20**). Notably, fasting triglyceride levels and CRP, the latter produced by the liver under the influence of IL-6 are significantly associated with cardiovascular diseases in people living with HIV. Furthermore, increased LPS concentrations, secondary to microbial translocation resulting from impaired intestinal barrier function, activate the immune system; this is reflected particularly by elevated sCD14 and sCD163, which contribute to the pathogenesis of cardiovascular and hepatic complications (Poesen et al., 2015; Shive et al., 2015).

On the neuroinflammatory side, mediators such as TNF-α, IL-1β, IL-6, sCD14, LPS, and increased expression of CD38/HLA-DR on the surface of T lymphocytes are directly involved in HIV-associated neurocognitive disorders (HAND). These factors compromise the integrity of the blood-brain barrier and facilitate the recruitment of inflammatory cells within the central nervous system (CNS), leading to cognitive deficits whose severity ranges from mild impairment to HIV-associated dementia. Thus, all these markers reflect the dual impact systemic and neurological of chronic inflammation in HIV infection, with both peripheral and central consequences (J. A. Smith et al., 2012).

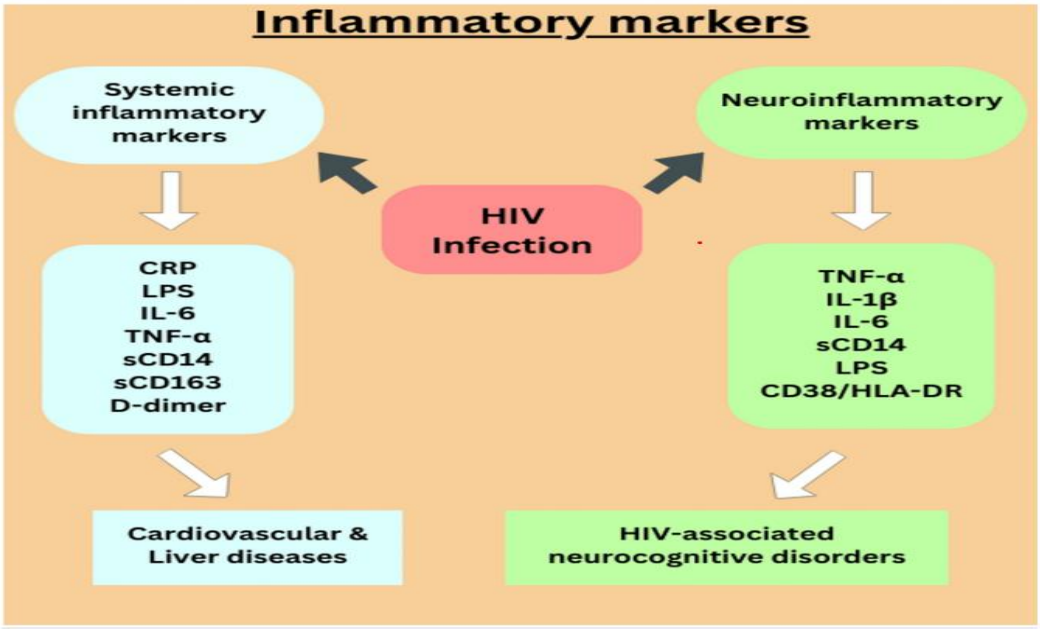


Figure 20: Inflammatory markers drive HIV-1 disease progression:

Systemic inflammation, mediated by C-reactive protein (CRP), lipopolysaccharide (LPS), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), soluble CD14 (sCD14), soluble CD163 (sCD163), and D-dimer, contributes to end-organ damage. Concurrently, neuroinflammation characterised by elevated TNF-α, interleukin-1 beta (IL-1β), IL-6, sCD14, LPS, and CD38/HLA-DR expression on T cells is linked to HIV-associated neurocognitive disorders (HAND) (Gnanaskandan & Srikanth, 2024).

The existence of low-grade inflammation is still debated because of (i) its multifactorial origins, (ii) the lack of consensual markers, (iii) the heterogeneity of biological profile of inflammations (Psomas et al., 2016; Younas et al., 2019) and (iv) the existence of a proportion of PWH with no inflammatory signature.

Example of two cytokines

Among the multitude of cytokines produced during HIV infection, we will focus on IFN I (which have a dual role) and IL-15 (a cytokine that plays a role on memory T cell homeostasis as a survival factor and an inducer of bystander activation).

- Type I IFN family

The type I IFN (IFN-I) is a family of multigenic cytokines with 13 IFNα subtypes, a single IFNβ and several as yet poorly defined monogenic products (IFNε, IFNτ, IFNκ, IFNω, IFNδ, IFNζ).

These cytokines are well known for their involvement in antiviral defense. IFN-I exhibit a dual role in the context of HIV-1 infection, depending on the phase of the infection.

During the primary phase of HIV infection: IFN-I (in particular IFN-α and -β) are heavily secreted, with a peak in production around day 6, concomitant with a major pro-inflammatory cytokine cascade and preceding the peak in plasma viral load (Stacey et al., 2009). They contribute to an effective anti-viral response that interferes with viral replication (Yan & Chen, 2012). In acute SIV infection in rhesus macaques, IFNAR blockade early in SIV infection results in increased viral load, a decrease in CD4 LT and more rapid progression to AIDS. In contrast, injection of IFN-α in the early phase of infection protects monkeys from systemic infection (Kader et al., 2013; Sandler et al., 2014).

In the chronic phase of HIV infection: The production of IFN-I is no longer detectable in plasma for a few weeks to a few months after infection (Kamga et al., 2005), but remains detectable at the tissue level (Nascimbeni et al., 2009). However, it is again detectable in plasma in a disparate way during the late phases of HIV (Hardy et al., 2013; von Sydow et al., 1991). The production of IFN-I is thus highly specific to HIV infection. In the context of a classical controlled viral infection, IFN-I levels return to baseline. In contrast, during the chronic phase of HIV, IFN-I continue to be secreted at low levels, as evidenced by continuous transcription of interferon-stimulated genes (ISGs), a set of genes that encode proteins mediating antiviral responses. The low-noise production of IFN-I correlates with immune activation and induces various defects in immune responses. In mice, chronic injection of IFN-I alone is capable of inhibiting CD8 antiviral responses through the accumulation of immunosuppressive monocytes (Ly6Chi) (Taleb et al., 2017; Teijaro et al., 2013). The persistence of IFN-I (at low doses) is also implicated in CD4 LT depletion. The main pathways involved are the TRAIL-DR5-mediated apoptosis pathway (Herbeuval, Hardy, et al., 2005; Herbeuval, Grivel, et al., 2005; Herbeuval, Boasso, et al., 2005) and the Fas-FasL pathway (Kaser et al., 1999). In addition, IFN-I induces the expression of immunoregulatory molecules such as indoleamine-2,3-dioxygenase, which may contribute to the alteration of mucosal barriers in the digestive system through the imbalance of the Th17/Treg ratio (Favre et al., 2009; Malleret et al., 2008), thereby maintaining immune activation. IFN-I are also associated with the development of an exhaustion profile (Sumida et al., 2022).

Finally, IFN-I play a dual role. They exert crucial protective role in triggering the anti-HIV immune response through their antiviral activity, but they also play a deleterious role through their involvement in immune dysfunctions (Nganou-Makamdop & Douek, 2019).

- Interleukin 15

Identified in 1994 (Grabstein et al., 1994), interleukin-15 (IL-15) is a potent pleiotropic hematopoietic cytokine produced by a variety of cells. IL-15 is an immunostimulatory cytokine: it modulates the immune system, particularly the homeostasis of memory NK (natural killer), NK-T, γδ LT and memory CD8 (and CD4) LT. Among CD8 LT, IL-15 exerts a primordial role in the survival of memory CD8 LT (Jabri & Abadie, 2015) but also contribute to the bystander activation of CD8 LT, as demonstrated in patients infected with hepatitis A virus (Kim et al., 2018). Importantly, IL-15 acts on these immune cells independently of the presence of Ag. Under normal homeostatic conditions (i.e. without lymphopenia), plasma IL-15 levels are either undetectable (by standard assays) or very low (Jabri & Abadie, 2015).

Previous studies have suggested that IL-15 may also play an important role in HIV-1 infection, enhancing immune function and stimulating the expansion of HIV-specific cytotoxic T lymphocytes (Chehimi et al., 1997; Kanai et al., 1996). During the initial phase of untreated HIV infection, levels of IL-15 are elevated and progressively decrease after ART initiation (Swaminathan et al., 2016). The plasma IL-15 levels correlate negatively with CD4 LT counts and positively with plasma viral RNA levels (Swaminathan et al., 2016). In turn, high plasma levels of IL-15 are associated with down-regulation of their cellular receptors (Tarkowski et al., 2012). Ex vivo studies indicate that IL-15 stimulation improves cellular responses against HIV-1 in vitro and ex vivo without expanding the size of the HIV-1 reservoir (Gunst et al., 2023). However, IL-15 also enhances the survival of CCR5+ LT, thus facilitating HIV infection in CCR5+ CD4 LT (Li et al., 2023). IL-15 treatment during acute SIV infection increases viral set point and accelerates disease progression despite the induction of stronger SIV-specific CD8 LT responses (Mueller et al., 2008). During chronic infection, the role of IL-15 is less described. No significant change in viral load was observed when comparing chronically infected animals with/out IL-15 (Mueller et al., 2005).

2.5.1.3 Key innate cellular players in HIV

- **NK cells**

NK cells are also involved in innate anti-infectious and anti-tumor immunity, via cytotoxic and immunoregulatory cytotoxic functions and secrete cytokines that direct the adaptive immune response. This lymphocyte population represents approximately 10% of blood mononuclear cells. NK cells are characterised by two levels of expression of CD56: CD56+ fraction or CD56 low CD16+ fraction (Lanier et al., 1986).

- The CD56lowCD16high fraction exerts cytotoxic functions by recognition and engagement of their natural inhibitory receptors (Killer Immunoglobulin-like receptor (KIR)) with MHC class I molecules expressed on the surface of target cells. This interaction inhibits the activation signal of the cytolytic program. In the absence of recognition, the NKs' natural cytotoxicity program will be set in motion and the target cells will be lysed. Besides inhibitory receptors, activating receptors such as NKG2D, NKp44 or NKp30, in interaction with the expression of their ligands such as MICA, MICAB, ULPBP1-4, or NCR-L also contribute to the elimination of the target infected cells. This cytotoxicity can be exerted either by direct natural cytotoxicity via the release of perforin (creating pores in the target cell's membrane) and granzymes (triggering apoptosis once they enter the cell) or by cellular cytotoxicity mediated by antibody-dependent cellular cytotoxicity(ADCC) antibodies, interacting with the engagement of CD16/FcγRIII (Gasser & Raulet, 2006; Gooneratne et al., 2014).

- The second CD56high immunoregulatory NK population, <5% in circulating blood, but predominant in lymphoid organs, exerts cytotoxic functions via the production of pro- and anti-inflammatory cytokines (TNF-α, γ-IFN, IL-10 and IL-13) (Cooper et al., 2001).

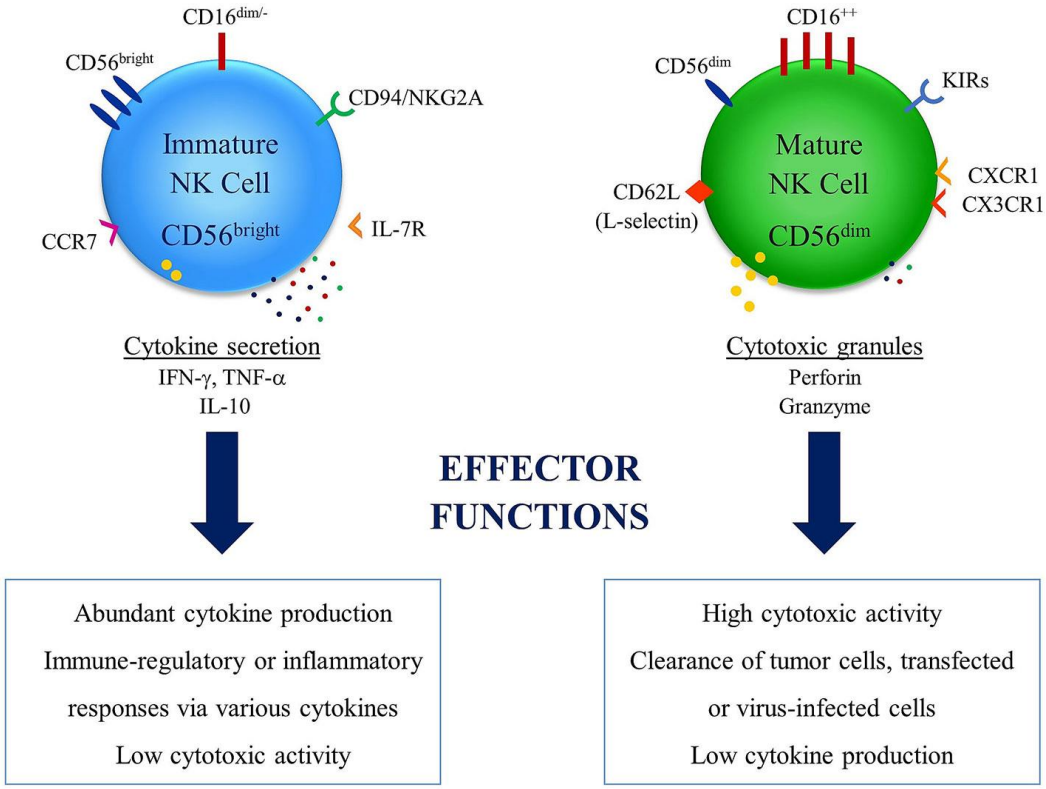


Figure 21 : Schema of human natural killer (NK)-cell subsets

Differences in effector functions between CD56^{dim} NK cells and CD56^{bright} NK cells (Kucuksezer et al., 2021).

In the acute phase of HIV infection, the number of cytotoxic NK cells increases, as do their production of γ-IFN and their cytotoxic degranulation capacity, which contribute to the initial control of viral replication (Alter et al., 2005; Fogli et al., 2008). It has also been shown that NKs have greater cytotoxic functions in HICs and long-term non-progressors than in progressors, with greater production of γ-IFN after stimulation (Vieillard et al., 2010).

In the chronic phase, alterations in NKs have been described, although some discrepancies exist, presumably due to the lack of control of confounding factors such as age, gender, and CMV serostatus (Scully & Alter, 2016).

In addition, HIV-1 can modulate the NK response via the accessory protein Vpr, which decreases the expression of the co-receptor for NKG2D and NTB-A, necessary for degranulation (Richard et al., 2010; Shah et al., 2010). HIV and more specifically viral proteins such as Nef, Tat, Vpu, also reduces the expression of HLA class I molecules on infected cells. The process impairs Ag presentation to HIV-specific CD8 LT (Andrieu et al., 2001) but favors

NK cell cytotoxicity. The Nef protein does not down-regulate the expression of HLA-C and HLA-E (Specht et al., 2008) which prevents the activation of NK cells.

- Monocytes/Macrophages

Macrophages are involved in anti-HIV immunity, playing an important role in tissues by phagocytosing free virions and debris from infected cells and maintaining inflammation. They are either resident macrophages or tissue-infiltrating monocytes that transform into macrophages. These resident macrophages can be differentiated according to M1 phenotype (sensitive to pro-inflammatory stimuli or LPS) or M2 phenotype (responding to IL-4, IL-10).

As macrophages are one of the two main targets of HIV, they constitute reservoirs where HIV persists (they do not die as a result of viral replication, unlike lymphocytes), preventing eradication of the virus by ART (Ganor et al., 2019).

Monocytes are generally not easily infected by HIV, except for the CD16+ subpopulation (intermediate), which strongly expresses CCR5 and has a less efficient APOBEC3G restriction factor (Ellery et al., 2007). As a result, these CD16+ monocytes are more easily infected than others (Lambotte et al., 2000).

During HIV infection, especially during the uncontrolled phases of viremia, there is an increase in CD16+ and CD16++ (non-classical) monocytes (Campbell et al., 2014), which is associated with significant production of pro-inflammatory cytokines such as TNF (Dutertre et al., 2012), IL-1 (Guo et al., 2014) or CXCL10 (SIMMONS et al., 2013).

- Neutrophils

Finally, neutrophils are also involved in the fight against HIV, playing an anti-infectious role, but also causing tissue damage. They recognise the virus via TLR-7 and TLR-8 receptors, generating reactive oxygen species (ROS)and Neutrophil Extracellular Traps to eliminate the virus (Saitoh et al., 2012). HIV infection is associated with neutropenia, reduced phagocytosis capacity of neutrophils and impaired antimicrobial function (Hensley-McBain & Klatt, 2018). Other neutrophil dysfunctions have also been described in PWH, such as increased expression of CD11b and production of oxygen radicals, highlighting a state of basal hyperactivation linked to cytokine production, such as IL-18 (Campillo-Gimenez et al., 2014).

ART treatments do not restore the functions of neutrophils, which present an immature phenotype (Lemaitre et al., 2020). Importantly, neutrophils may exert a regulatory function

on the LT response. Expression of PD-L1 by neutrophils correlates with PD1 and CD57 expressed by CD8 and CD4 LT (Bowers et al., 2014).

2.5.2 Defects impacting the adaptive immunity

2.5.2.1 Crucial role of CD8 LT in the context of HIV infection

The role of CD8 LT in antiviral control is a very important factor in the pathophysiology of HIV infection, with a different impact during acute and chronic phases.

Acute phase of infection

The activation of CD8 LT and their cytolytic action against infected cells appear early in the acute phase (Musey et al., 1997). The innate response and inflammation amplify the expansion of the CD8 LT response specific to HIV already activated (Decrion et al., 2005). This specific CD8 LT response does not eradicate the virus, but it is correlated with the viral load at the end of the acute phase (Borrow et al., 1994; Koup et al., 1994). The cytotoxic CD8 response must constantly adapt to viral variants, as the immune response induces the selection of mutants that resist cytotoxic CD8 LT and escape antibodies (Couillin et al., 1994). The CD8 LT response has been shown to have a protective role in simian models, with CD8 LT depletion strongly increasing virus replication (Gaufin et al., 2010).

The quality of the CD8 LT response is not limited to its cytotoxic function and also depends on its polyfunctionality, which is assessed by its production of IL-2, TNF-α, γ-IFN, CD107a and MIP-1β and its capacity to secrete perforin and granzyme (**Figure 22**). The HIV-specific CD8 LT response is associated with long-term control of viral replication (Betts et al., 2006). A protective role for the CD8 LT response was associated with viremia control in elite controllers compared with progressive patients, and is associated with certain class I alleles such as HLA-B27, HLA-B57 (Rinaldo et al., 1995; Migueles et al., 2000; Hersperger et al., 2010). Similarly, the anti-gag specific CD8 LT response is more important in controllers (Sáez-Cirión et al., 2009). In addition, high TCR avidity and Ag sensitivity are factors involved in the efficiency of CD8 LT response (Price et al., 2005; Almeida et al., 2009).

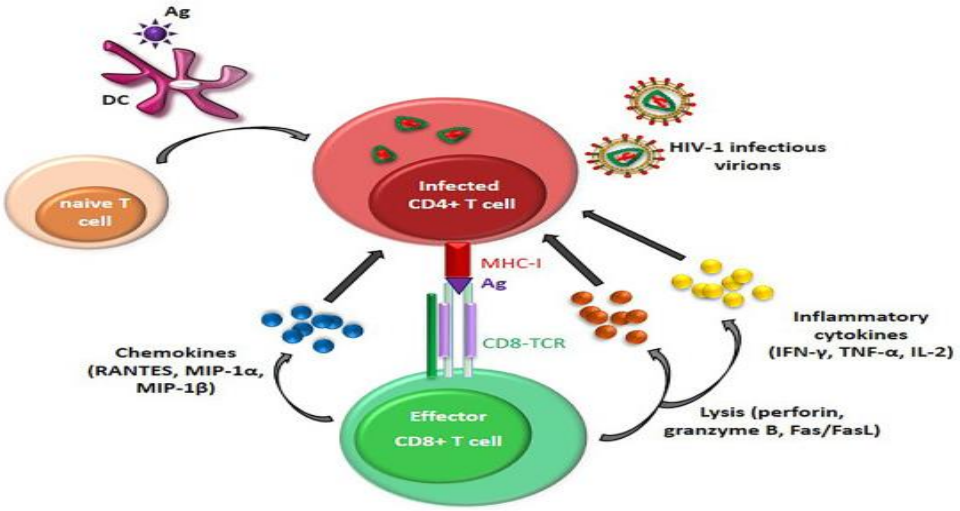


Figure 22 : Cytotoxic, HIV-inhibitory and pro-inflammatory roles of CD8 LT during HIV-1 infection

The Ag-specific lysis of target cells requires recognition of a peptide of viral origin presented by an HLA I molecule. The CD8 LT activation induces the production of cytolytic molecules such as perforin and granzyme and the secretion of pro-inflammatory cytokines such as IL-12, IL-18, IFN-I (IFN-α and β) and type II IFN (γ-IFN) or chemokines MIP-1α, MIP-1β and RANTES (Gonzalo-Gil et al., 2017).

Chronic phase of infection

CD8 LT activation during primary infection persists into the chronic phase, with 20% of circulating HIV-1-specific CD8 LT in untreated chronic subjects (Betts et al., 2001). Even under treatment, these CD8 LT functions associated with cell activation persist (Lécuroux et al., 2009; Lc et al., 2008; Paiardini & Müller-Trutwin, 2013). However, prolonged activation of HIV-specific CD8 LT has a long-term adverse effect on their function and leads to progressive functional deterioration and exhaustion. It includes the reduction in their cytotoxic and proliferative capacity in response to stimulation and higher sensitivity to apoptosis. These defects are related to the expression of inhibitory markers such as PD-1 and negative costimulatory molecules (CTLA-4) (Brenchley et al., 2003e; Dagarag et al., 2003; Day et al., 2006) and immune senescence (Sauce et al., 2011). In addition, HIV, via its viral protein Nef, escapes the cytotoxicity of CD8 LT by reducing the expression of MHC I, which is involved in the recognition of the infected cell (K. L. Collins et al., 1998). Importantly, HIV controllers exhibit a more functional CD8 LT compartment (D. R. Collins et al., 2020).

2.5.2.2 CD8 T cell dysfunction associated with chronic HIV infection

Chronic HIV infection is characterised by defects in CD8 LT, such as exhaustion and senescence. These phenotypes are associated with defective regulation of the transcription factors T-bet and EOMES, which are essential for the differentiation of effector cells (Buggert et al., 2014; Simonetta et al., 2014).

CD8 LT exhaustion in HIV infection

In the context of HIV, the persistence of the virus providing continuous antigenic stimulation, the chronic immune activation and inflammation lead to exhaustion. As previously mentioned, CD8 LT exhaustion is characterised by a progressive loss of functional capacity, such as cytotoxicity and cytokine production, and an inability to control viral replication. In the advanced phase of exhaustion, other transcriptional and metabolic disturbances are observed, such as a reduction in glycolysis and mitochondrial defects (Bengsch et al., 2016). Furthermore, it has been shown that PD-1 signaling itself promotes lipolysis and fatty acid oxidation while inhibiting glycolysis, thereby contributing to the limitation of the cells' functional capacity (Patsoukis et al., 2015).

PD-1 expression on HIV-specific LT

Several teams have demonstrated over-expression of PD-1 on HIV-specific CD8 LT: the expression of PD-1 on HIV-specific CD8 LT was greater than that on CMV-specific CD8 LT (Day et al., 2006; Trautmann et al., 2006). Similarly, in PWH, PD-1 expression on CD8 and CD4 LT correlated positively with plasma viral load and altered CD8 LT functionality, and inversely with CD4 LT numbers (a marker of HIV progression) (Day et al., 2006; Petrovas et al., 2007; Trautmann et al., 2006). However, initiation of ART reduced the expression of PD-1 on HIV-specific CD8 LT. In spontaneous controllers, expression of PD-1 on HIV-specific CD8 LT was lower than in progressive patients (Zhang et al., 2007).

Petrovas et al showed that PD-1 expression on HIV-specific CD8 LT made them more susceptible to apoptosis. This susceptibility was mediated by higher levels of Fas receptor and lower levels of Bcl-2, compared with LT expressing low levels of PD-1 (Petrovas et al., 2007).

In vivo studies in animal models have shown that SIV-infected rhesus monkeys have SIV-specific CD8 LT expressing PD-1, and that PD-1 expression was higher in lymphoid tissue and

the intestine than in circulating blood. Blocking PD-1 restores SIV-specific CD8 LT proliferation (Petrovas et al., 2007; Velu et al., 2007). PD-1 blockade also improved polyfunctionality, TNF, IL-2 and IFN secretion and HIV-specific CD8 LT expansion (Velu et al., 2008).

PD-1 expression was progressively reduced in HIV-specific CD8 LT whose epitopes had undergone mutational escape, highlighting the role of TCR stimulation (Petrovas et al., 2007).

On the other hand, other inhibitory receptors expressed independently or in combination, such as TIM3, LAG3, TIGIT and 2B4, are involved in the exhaustion of CD8 LT (Blackburn et al., 2009; Chew et al., 2016; Day et al., 2006; Jin et al., 2010; Jones et al., 2008). For example, Chew et al. showed that the co-expression of PD1 and TIGIT correlated with the progression of SIV infection in macaques (Chew et al., 2016).

We focus this review of literature on HIV specific CD8 LT but the expression of PD-1 on HIV-specific CD4 LT has also been correlated with viral replication, disease progression and CD4 LT cell depletion (D'Souza et al., 2007).

Other teams have confirmed that this PD-1 overexpression is not confined to HIV-specific LT but also affects total CD8 LT populations. This contributes to the systemic immunosuppression observed in chronic HIV infection (Day et al., 2006; Petrovas et al., 2006; Zhang et al., 2007).

CD8 LT senescence in HIV infection

It is not yet clear whether HIV-1 infection is responsible for T-cell senescence, but PWH in a chronic phase present a long-term risk of complications unrelated to AIDS, which can affect their life expectancy and quality of life, such as cardiovascular and metabolic diseases, and cancer. They also exhibit characteristics of immune ageing such as an overrepresentation of terminally differentiated memory LT effector CD28-, a low ratio of naive/memory LT, of CD4/CD8 and a reduced repertoire (Kalayjian et al., 2003). It has been shown that the expression of the senescence marker CD57 on CD8 in PWH, whether viremic, on treatment or spontaneous *controllers* was higher than in HIV-uninfected individuals (Ladell et al., 2008; Palmer et al., 2016; Simonetta et al., 2014).

Victor Appay et al have reported an increase in CD57+ cells in chronic PWH (Appay et al., 2007), and it has been shown that these senescence characteristics are more marked in PWH who are unable to restore CD4 counts under treatment (Robbins et al. 2009). Additionally, the senescence and exhaustion of CD8 LT is more marked in progressors (ART) than in

spontaneous controls (Quigley et al., 2010). Simonetta et al. demonstrated that there is functional heterogeneity within CD57+ cells (Simonetta et al., 2014), while another study reported a lower CD57+ fraction within effector CD8 LT in controller patients (Henriquez et al., 2019).

2.5.2.3 CD4 LT response

Effector CD4 LT play an important auxiliary role in the adaptive immune response: they are called “helper”. During HIV infection, CD4 LT function is important in the pathogenesis of the virus. CD4 LT are a prime target for viral entry, and their depletion over time is then reflected in the circulating blood and will constitute the principal characteristic of the severity of HIV pathogenesis (Rowland-Jones, 2003).

The inflammation induced by the entry of the virus encourages the recruitment of CD4 LT target cells to the site of infection. The virus then spreads rapidly to the secondary lymphoid organs, particularly the GALT (Gut-associated lymphoid tissue), where memory CD4 LT are present in large numbers (Rowland-Jones, 2003; Mattapallil et al., 2005).

In vivo studies have shown that HIV-specific CD4 LT are preferential targets of infection, irrespective of the stage of infection (Douek et al., 2002). This explains the inefficiency of the anti-HIV response in primary infection and the progressive death of HIV-specific CD4 LT, because each time they are antigenically stimulated, these LT are again targets of infection.

In controller patients, HIV-specific CD4 LT contribute to better control of the infection, but in patients who progress, there is a quantitative and qualitative defect in CD4 LT from the time of primary infection (Lissina et al., 2016), particularly in the TCM CD4 LT. In simian models, it has been shown that in the periphery and in lymphoid organs, TCM CD4 LT are the most impaired (Mattapallil et al., 2005), and this impairment persists under treatment (Elrefaei et al., 2004).

Defects in the CD4 LT response are characterised by a loss of cytokine (IL-2) production (Younes et al., 2003) and a loss of proliferative capacity in response to viral proteins (Lichterfeld et al., 2004), but y-IFN production persists. All of these dysfunctions may explain the failure of an effective early immune response in so-called progressive subjects. However, it has been shown that strong activation of CD4 LT, in parallel with viral load, is linked to inflammation (Catalfamo et al., 2011).

2.6 *Clinical consequences of chronic HIV infection*

PWH have a higher risk of developing non-AIDS-related pathologies than uninfected people of the same age (Freiberg et al., 2013), with morbidity and mortality related to cardiovascular disease, liver disease, kidney disease, bone disease, neurological disease, *osteoporosis* and cancers (not classifiable as AIDS) (Morlat et al., 2014; Pereyra et al., 2012). Numerous other comorbidities have been observed (Allavena et al., 2018): sarcopenia, onset of myopathies and muscle fatigue. There are also various cardiovascular manifestations such as atherosclerosis, arterial hypertension, heart failure and myocardial infarction. The combined impact of inflammation, immune cell dysfunction and coagulation contribute to these defects.

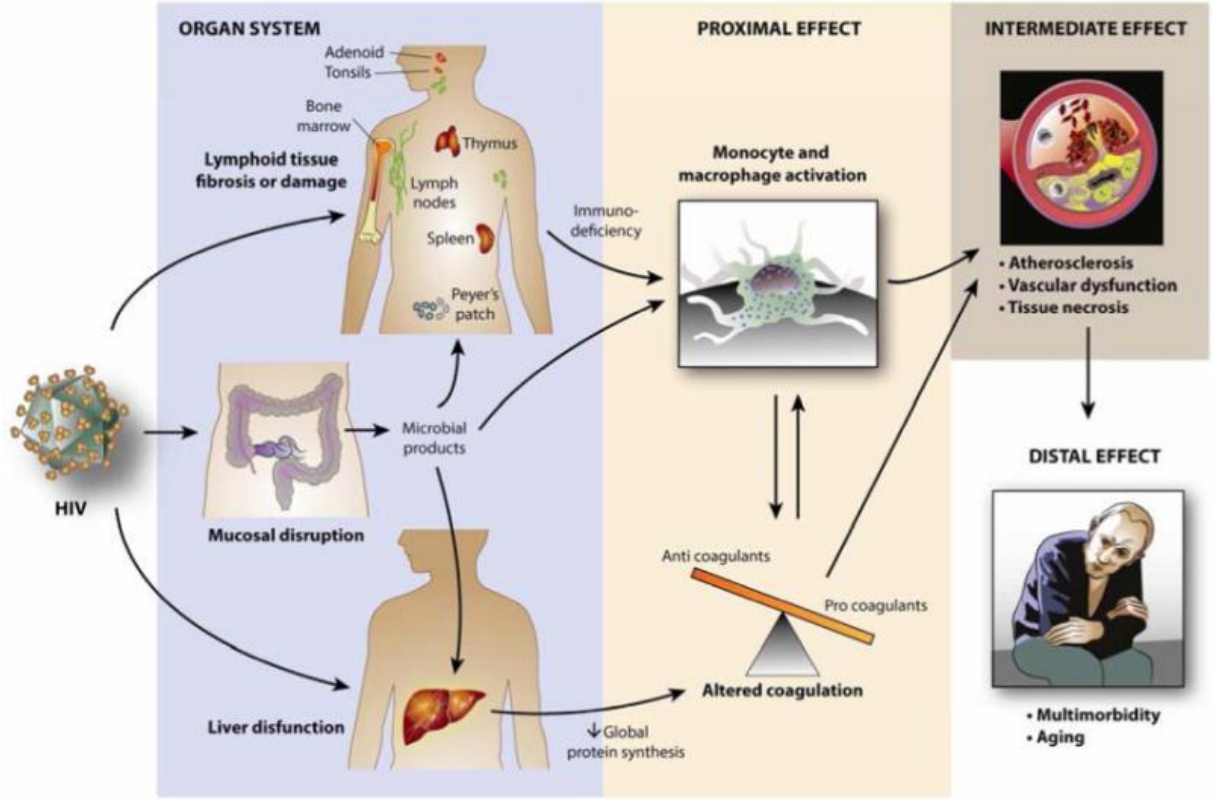


Figure 23 : Pathogenesis of inflammation-associated disease in HIV-infected adults

HIV infection damages lymphoid and gut mucosal tissues, causing progressive immunodeficiency, increased pathogen levels (including HIV), inflammation, and microbial translocation. HIV and its treatment also impair liver function. These combined effects trigger chronic monocyte/macrophage activation and hypercoagulation. This leads directly to vascular injury, end-organ damage, and multi-morbidity, potentially manifesting later as geriatric syndromes (Deeks, Tracy, et al., 2013).

3 The human cytomegalovirus (HCMV)

3.1 *Epidemiology*

3.1.1 A strictly human virus that is highly ubiquitous

Cytomegalovirus (CMV) comes from the Greek roots “cyto” and “megalo” meaning big cell. CMV is an endemic virus present throughout the world. HCMV was first isolated and cultivated in 1956 (Craig et al., 1957). Its prevalence varies considerably worldwide according to several factors, including age and socio-economic status. The prevalence differs depending on the geography. It is estimated at 50% in industrialised countries and up to 100% of the population in developing countries (Cannon et al., 2010). Zuhair M and al, conducted a systematic survey of the published literature to describe the global seroprevalence of HCMV IgG antibodies. They estimated a global CMV seroprevalence of 83% (95%UI: 78-88) in the general population, 86% (95%UI: 83-89) in women of childbearing age, 86% (95%UI: 82-89) in donors of blood or organs. For each of these three groups, the highest seroprevalence was seen in the Eastern Mediterranean region 90% (95%UI: 85-94) and the lowest in European region 66% (95%UI: 56-74) (**Figure 24**) (Zuhair et al., 2019). There are also differences in prevalence between individual’s groups. Fowler K and al performed a survey on HCMV prevalence showing that CMV seroprevalence correlated with race, ethnicity, socioeconomic status and education level. As a whole, seroprevalence increases with age and is generally lower in developed than in developing countries (K. Fowler et al., 2022).

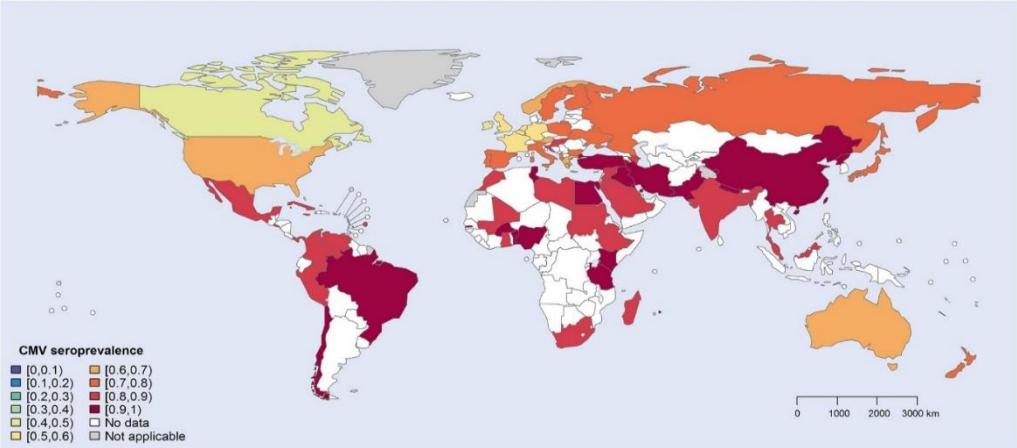


Figure 24 : Worldwide seroprevalence of HCMV

This figure illustrates the geographical variation in exposure to CMV. In countries with a high seroprevalence rate, such as those in Africa and Asia, most congenital infections result from maternal reactivation. In contrast, in Europe and North America, primary infections during pregnancy are more prevalent, posing an increased risk to the fetus (30–40%) (Zuhair et al., 2019).

3.1.2 Transmission of CMV

HCMV is an enveloped virus and therefore fragile. In infected people, the virus is excreted in all bodily secretions: saliva, blood, urine, semen, tears, breast milk and vaginal secretions. There are two peaks in the incidence of CMV infections: (i) In infancy (0 and 2 years), contamination occurs through vertical transmission from mother to fetus (during childbirth and breastfeeding) (K. B. Fowler & Boppana, 2018) as well as horizontal transmission between children (Hutto et al., 1986) and (ii) in adulthood through saliva or sexual contact .

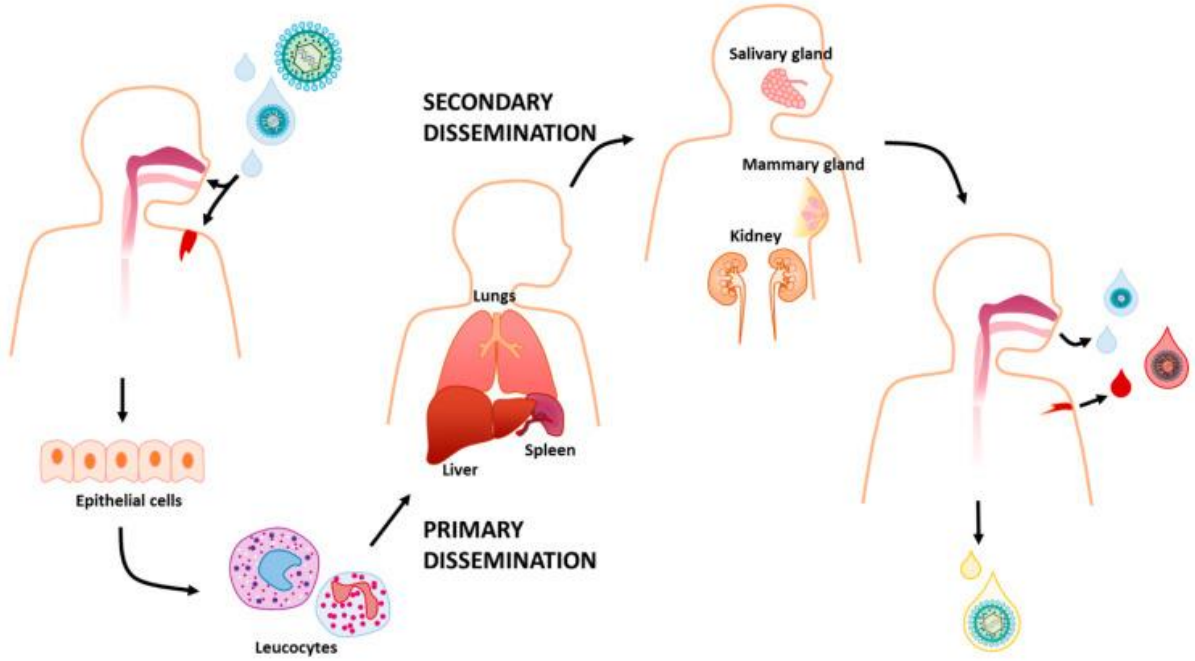


Figure 25 : How HCMV travels through the body

HCMV is transmitted via direct contact with infected bodily fluids (saliva, urine, cervicovaginal secretions, breast milk, semen, blood, tears). Initial infection typically occurs through the upper gastrointestinal or respiratory tract epithelium, where viral replication begins. Following epithelial infection, HCMV disseminates systemically via infected leukocytes (monocytes, dendritic cells) during primary viral dissemination, seeding organs like the lungs, liver, and spleen. Subsequently, secondary viral dissemination establishes infection in secretory organs (salivary glands, mammary tissue, kidneys), enabling sustained viral shedding into bodily fluids and perpetuating transmission cycles (Gugliesi et al., 2020).

3.2 Virology

3.2.1 CMV is a herpesvirus

HCMV is a virus belonging to the Herpesviridae family (herpesvirus type 5 HHV-5) and the ß-herpesvirinae subfamily (Boeckh & Geballe, 2011). HCMV is a linear double-stranded DNA virus with an icosahedral capsid which is surrounded by a lipid bilayer harboring viral glycoproteins (**Figure 26**).

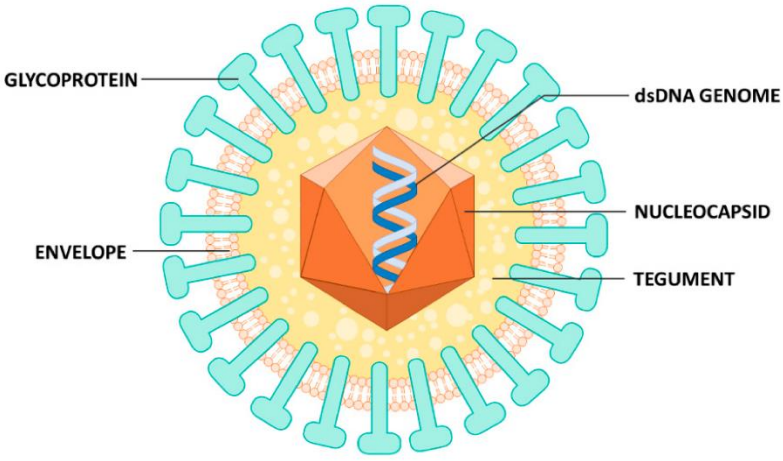


Figure 26 : Human CMV virion structure and structural components

The genome is packaged in an icosahedral capsid surrounded by a lipid envelope. Viral phosphoproteins (eg, pp65 Ag) are found in the tegument, a space between the nucleocapsid and the envelope (Gugliesi et al., 2020).

HCMV genome: It has the largest genome of any human herpesvirus, at around 235,000 base pairs, with large GC-rich repeat regions (Karamitros et al., 2018). The DNA genome is formed by 2 covalently linked genome segments (L, long; S, short), each consisting of a central unique region (U_L, unique long; U_S, unique short). These unique regions are flanked by inverted repeats (IR) either at the ends (TR, terminal repeat) or internally at the intersection of the long and short segments (IR_L, internal repeat long; IR_S, internal repeat short). It displays more than 200 open reading frames encoding 165 genes and various types of RNA, including mRNAs, long non-coding RNAs (lncRNAs), circular RNAs and microRNAs, which play an essential role in viral replication and interaction with the host (J. Zeng et al., 2023; Tsingine, 2020). During CMV replication, a single long DNA chain is synthesised. This chain contains multiple repeated gene

sequences known as concatemers. Each concatemer is cleaved into multiple gene sequences known as monomers, forming the genetic material for each virion (El Chaer et al., 2016).

HCMV membrane proteins and glycoproteins: The HCMV genome encodes around 54 membrane proteins, of which 25 are glycoproteins integrated into the virion envelope (Varnum et al., 2004). However, the exact list of proteins essential for viral entry remains incomplete. Indeed, most HCMV mutants have been classified as 'essential' or 'non-essential' for replication without any specific assessment of their role in viral entry (Dunn et al., 2003; Long et al., 2021). Glycoproteins include:

-gB (UL55): a viral fusogen required for entry into all cell types (Vanarsdall & Johnson, 2012). It is highly expressed and is also a major immunodominant target after natural infection, making it a promising vaccine candidate (Schleiss, 2018).

-gH/gL forms a complex with UL128, UL130 and UL131 (gH/gL/UL128-131), which is crucial for infecting epithelial and endothelial cells, as well as macrophage-monocytes (Adler et al., 2006).

- gM and gN are also involved in viral assembly and entry (Masse et al., 1999).

- In addition, the tegumentary phosphoprotein pp65 (pUL83) is a major Ag that is clinically detectable and plays a central role in immunomodulation and evasion of host immune defenses.

3.2.2 Cellular tropism

Humans are the only known reservoir of HCMV. It has a broad cellular tropism that contributes significantly to its pathogenesis. It includes

- **Epithelial cells**: Epithelial cell infection contributes to host-to-host transmission and is widespread in the gastrointestinal tract, particularly in HIV/AIDS patients (Sun et al., 2022).

- **Endothelial cells**: Infection of endothelial cells facilitates systemic propagation in the host, with infection rates influenced by specific viral strains and their genetic make-up (Sinzger et al., 2008).

- **Hematopoietic cells**: Macrophages and dendritic cells are also targets, their sensitivity varying according to the different strains of CMV (Sinzger et al., 2008). Myeloid cells,

particularly CD34+ progenitors, are the most frequent site of latency (M. S. Smith et al., 2004; Sissons et al., 2002).

- Fibroblasts and smooth muscle cells are also predominant targets of viral replication.

3.2.3 Viral cycle

Primary CMV infection involves a replication phase in the mucosal epithelium, from which the virus spreads to endothelial cells, monocytes, fibroblasts and CD34+ progenitors in the bone marrow.

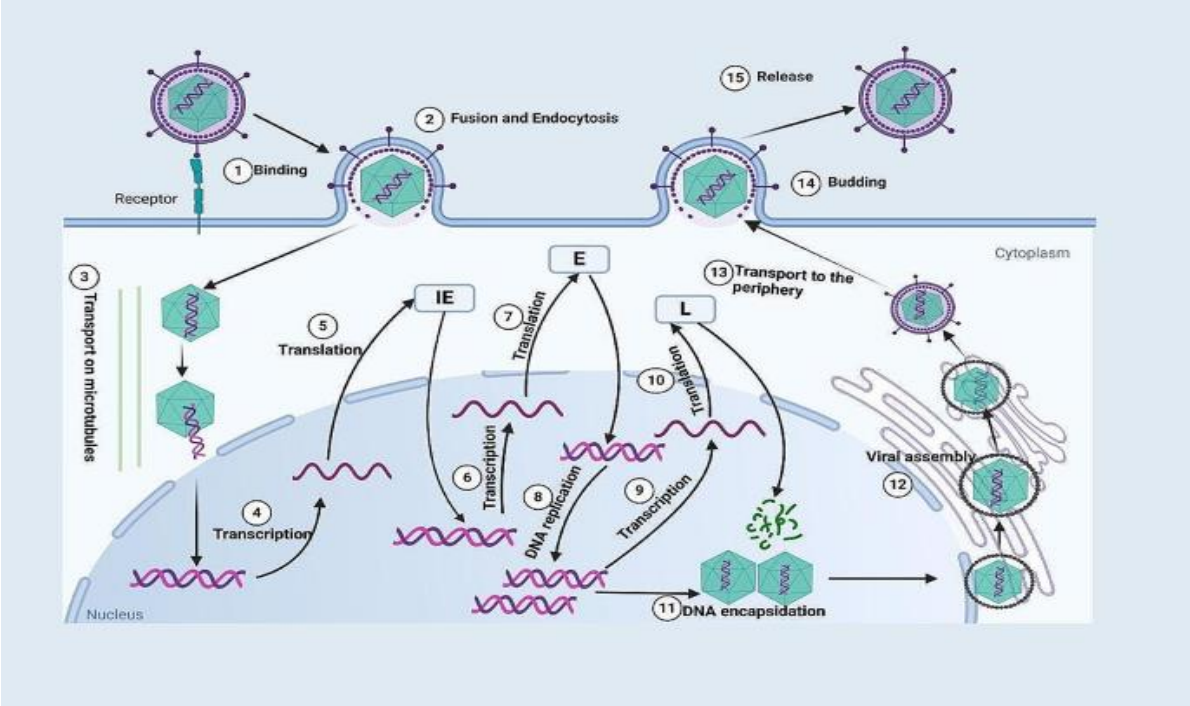


Figure 27 : The life cycle of HCMV

HCMV glycoproteins bind to specific cellular receptors (1), triggering endocytosis and fusion with the plasma membrane (2). The nucleocapsid is transported to the nucleus via microtubules (3), where the linear viral DNA is released. (4 and 5) Immediate-early (IE) viral genes are transcribed and translated. (6 and 7) Early (E) viral genes are subsequently transcribed and translated. (8) Viral genome replication occurs within the nucleus. (9 and 10) Late (L) proteins, primarily capsid proteins, are expressed and assembled into new nucleocapsids (11). (12) Viral membrane proteins and glycoproteins assemble within the endoplasmic reticulum and the Golgi apparatus to form mature virions. (14, 15) Mature virus particles are released into the extracellular space by budding (Li et al., 2024).

The CMV replication cycle comprises 3 phases: a very early /immediate early phase, an early phase and a late phase. The immediate early phase occurs within the first two hours, with synthesis of activator proteins IE1 and IE2. It is followed by an early phase occurring within the first 24 hours, with synthesis of enzymatic proteins such as viral DNA polymerase, tegument proteins (pp65, pp52) and the third so-called late phase with synthesis of the

protein components of the capsid and envelope glycoproteins (gB, gH). Replication of the CMV viral DNA separates the early and late phases (Stinski, 1978).

3.2.4 Latency and reactivation

HMCV (like all viruses in the Herpesviridae family) persists in its host, either by maintaining a chronic, low-noise infection or by entering the latency phase and not being eliminated from the infected organism after a primary infection. This latency is the ability of CMV to persist by synthesizing a small quantity of viral proteins with persistence of the virus genome in non-integrated episomal form, unlike the lytic phase, or active replication of the virus (production of viral particles).

This viral latency forms a reservoir mainly in endothelial cells, haematopoietic progenitor cells (HPCs) of the bone marrow, monocytes (Sinclair & Sissons, 1996), and dendritic cells (Reeves et al., 2005). Current models of HCMV infection and dissemination in the host highlight a crucial role for myeloid cells and haematopoietic stem and progenitor cells (HSPCs) in ensuring viral persistence. Epithelial cells of the host are infected by contact with HCMV-containing bodily fluids. HCMV then replicates and spreads to monocytes in the peripheral blood by an unknown mechanism and establish a quiescent infection. The exact contribution of monocytes, macrophages and hematopoietic progenitor cells (HPC) to the latency and persistence of CMV is still debated (**Figure 28**).

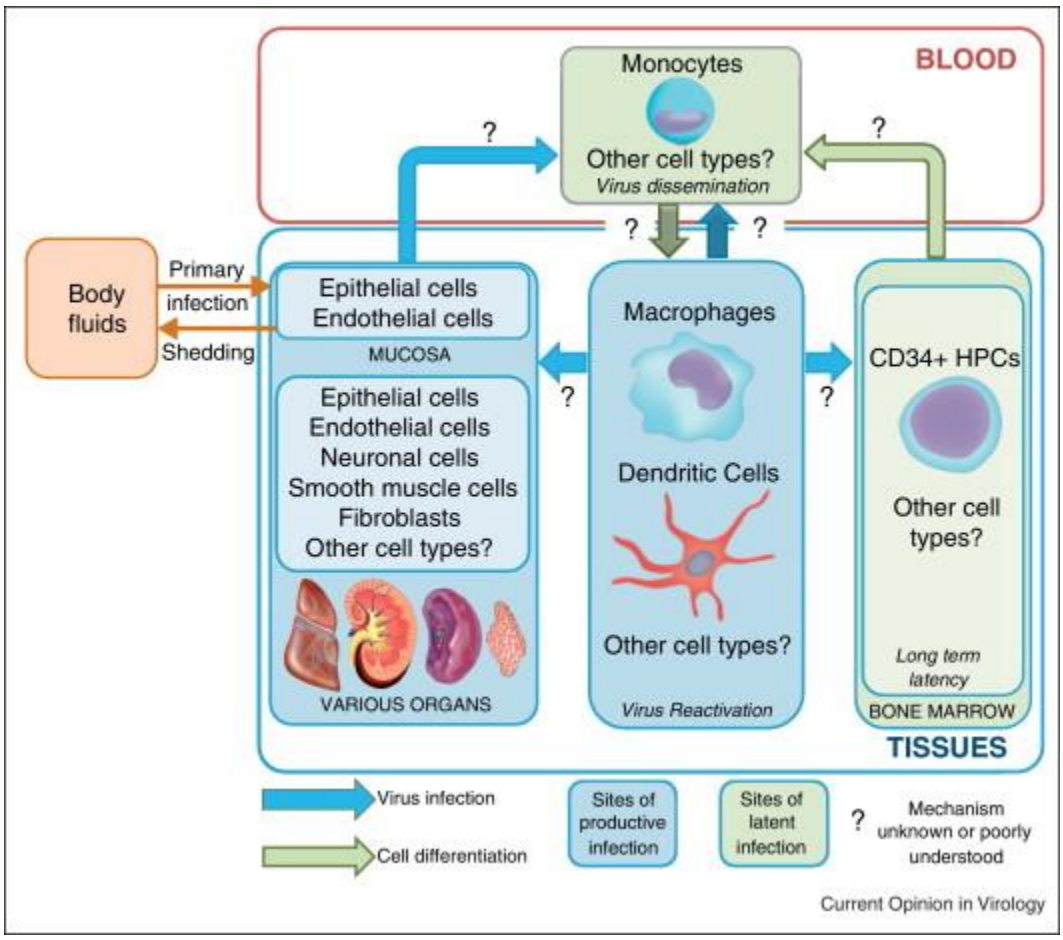


Figure 28: Systems-level model of CMV infection, latency, and persistence in the host

Primary CMV infection occurs when mucosal epithelial cells are exposed to infectious body fluids such as saliva, urine, or semen. The virus disseminates through the body via latent infection of circulating monocytes, and potentially other cell types. Interactions between monocytes and endothelial cells at blood vessel walls likely facilitate monocyte infection. Upon entering tissues and differentiating into macrophages or dendritic cells, these monocytes support viral reactivation, producing infectious particles that infect multiple tissues, including mucosal sites (enabling viral shedding and transmission) and bone marrow $CD34^+$ hematopoietic progenitor cells, which serve as long-term latency reservoirs. Extracellular stimuli, such as cytokines or allogeneic signals, can induce latently infected progenitor cells to differentiate into monocytes, triggering stochastic events of viral dissemination and reseeding that maintain long-term viral persistence. Many components and mechanisms of this process remain poorly understood due to the complex, dynamic nature of CMV latency and persistence (Landais & Nelson, 2013).

Some studies suggest that the primary infection of monocytes promotes their migration into host organ tissue and their survival and differentiation into long-lived, viral replication-permissive macrophages. These macrophages could become sites of persistent infection in the host tissue (Chan et al., 2012). The possibility also exists that HCMV-induced macrophages could migrate into the bone marrow and infect CD34+ myeloid progenitor cells, which are believed to be sites of HCMV latency.

The mechanisms involved in CMV latency depend on:

- **Epigenetic changes**: Suppression of the major IE promoter is a pre-requisite for latency and is driven by repressive epigenetic changes during latent infection (E. Poole et al., 2023).

- **The expression of various viral proteins** such as UL138 (Petrucelli et al., 2009) or Luna. **LUNA** plays a key role in efficient latency-associated viral gene expression and carriage of viral genome during latent carriage and interacts with transcription factors such as GATA-2, which are essential for maintaining an open chromatin configuration around these genes (E. Poole et al., 2023).

3.3 *Pathophysiology*

The severity and the clinical manifestations of the CMV infection greatly vary depending on the immune status of the individuals. Schematically, three groups of individuals are identified: the immunocompetent individuals, the neonates (and newborns) presenting a congenital infection and the immunodeficient patients (**Figure 29**).

3.3.1 CMV Infection in immunocompetent patients

In immunocompetent subjects, primary and reactivated CMV infection is usually asymptomatic. Primary infection may give rise to a mononucleosis syndrome, as well as a flu-like syndrome, diarrhea and non-specific constitutional symptoms, in some cases resulting in severe gastrointestinal bleeding (Wang et al., 2022; N. Kumar et al., 2023).

Other rare clinical manifestations may reflect the impact of CMV on the gastrointestinal tract, the central nervous system and the liver, leading to conditions such as ileitis, hepatitis and even pancreatitis (N. Kumar et al., 2023; Schattner, 2024).

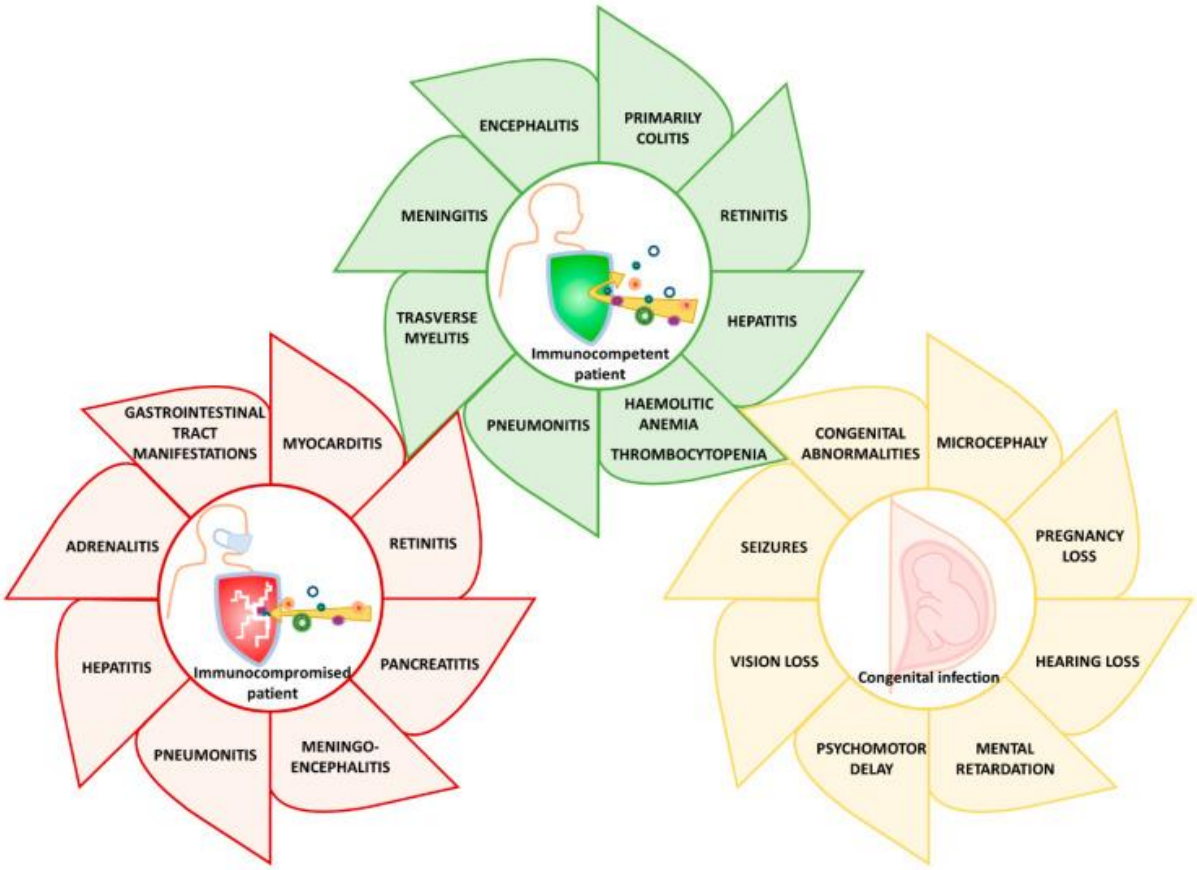


Figure 29 : HCMV clinical manifestations depending on the immune status

Different outcomes in immunocompetent (severe infections), immunocompromised (AIDS, transplant recipients) patients and congenital infection (Gugliesi et al., 2020).

3.3.2 Congenital CMV infection

The consequences of congenital CMV infection are highly deleterious as it causes neurodevelopmental disorders or hearing loss in up to 25% of cases. The congenital infection is linked to an increase in morbidity and mortality (Luck & Sharland, 2009).

3.3.3 CMV infection of immunocompromised patients

There are different types of immunosuppression considered in the context of CMV: (i) iatrogenic immunosuppression linked to transplantation-related treatment, cancer chemotherapy and other immunosuppressive treatments, and (ii) immunosuppression linked to infection, such as HIV, which is associated with severe CD4 lymphopenia.

3.3.3.1 Iatrogenic immunosuppression

Patients with diseases such as multiple myeloma and those receiving treatments such as steroids or rituximab are at increased risk (Ilyas et al., 2022; Tay et al., 2024). Patients

submitted to allogeneic or autologous hematopoietic stem cell transplantation, intensive care unit are also at risk patients (Styczynski, 2018).

3.3.3.2 Cytomegalovirus infection in patients with HIV infection

CMV infection poses significant risks for PWH, particularly those with advanced immunosuppression. CMV infections are one of the criteria used to define AIDS and is a cofactor enhancing progression of AIDS (Griffiths, 2006). CMV infections with severe clinical manifestations occur when the CD4 LT count is less than 200/mm3. Clinically, the most frequent manifestations are CMV retinitis (affecting 10 to 15% of patients with a CD4 count of less than 50/mm3) (Ferreira Tátá et al., 2021) and digestive tract infections (affecting 10% of patients with a CD4 count of less than 100/mm3). Other disorders, such as neurological disorders: encephalitis, myoradiculitis, pancytopenia and pneumonitis are also present.

With the therapeutic progress of ART, severe CMV infections are less frequent in PWH. A recent study observed that CMV viremia at the initiation of the ART therapy is associated with advanced infection. Persistent viremia after ART initiation is associated with a higher risk of morbidity and mortality. Other studies have shown that CMV can exacerbate HIV progression, potentially increasing morbidity and mortality rates in co-infected individuals (Nikita Sherwani, et al., 2018).

3.4 Treatment for CMV

The drugs currently used to treat CMVH infections, including ganciclovir (GCV), its prodrug valganciclovir (VGCV), cidofovir (CDV) and foscavir (FOS), have major drawbacks that limit their clinical use. These molecules, which are mainly inhibitors of viral DNA polymerase UL54, have low oral bioavailability and are associated with dose-related toxicities such as bone marrow suppression, nephrotoxicity, hepatotoxicity, and increasing viral resistance (Sahu et al., 2015; Sun et al., 2018). This situation highlights the urgent need to develop new approaches that potentially combine several mechanisms of action and incorporate immunomodulatory strategies (Hostetler, 2010). New treatment approaches are emerging to address these challenges. Competitive inhibitors of viral kinase UL97, maribavir, and letermovir, which target the HCMV terminase complex, represent interesting alternatives with a better safety profile (Piret & Boivin, 2019; Prichard, 2009). They inhibit the release of the viral capsids, i.e. a different mechanism than the action of conventional DNA polymerase inhibitors, meaning that it may provide an alternative to resistant strains (Chou & Watanabe,

2024; Piret & Boivin, 2024). The different anti-herpes viral agents available as licensed treatments are presented in **Table 3** (C. L. Poole & James, 2018).

Drug	Antiviral Activity	Mechanism of Action	Approved Clinical Indications
Acyclovir (valacyclovir)	All herpesviruses	Nucleoside analogue–polymerase inhibitor	Treatment and suppression of HSV and VZV infections
Penciclovir (famciclovir)	HSV VZV	Nucleoside analogue–polymerase inhibitor	Treatment of zoster and treatment and suppression of genital HSV Penciclovir topical for HSV labialis
Ganciclovir (valganciclovir)	All herpesviruses	Nucleoside analogue–polymerase inhibitor	Treatment and suppression of CMV infections
Foscarnet	All herpesviruses	Pyrophosphate analogue–polymerase inhibitor	Treatment of acyclovir- or ganciclovir-resistant HSV, VZV, and CMV infections
Cidofovir	All herpesviruses	Nucleotide analogue–polymerase inhibitor	Treatment of acyclovir-, ganciclovir, and foscarnet-resistant HSV and CMV infections
Letermovir	CMV	Terminase complex inhibitor; inhibits cleavage of CMV genome units and viral particle packaging	Suppression of CMV infection posttransplantation

Table 3 : List of Approved Antiherpesviral Drugs

HSV= herpes simplex virus; VZV = varicella zoster virus (C. L. Poole & James, 2018).

Although there is no vaccine against HCMV on the market, some candidate vaccines are in the clinical development stage (Chen et al., 2019). In the absence of an effective preventive approach, however, research is considering resorting to other novel solutions, such as immunotherapy, which may serve as an alternative to traditional antiviral therapy, the shortcomings of which are becoming increasingly apparent.

3.5 The immune response to CMV

A complex multilevel cellular response is required to inhibit CMV dissemination. The innate immune responses are the first line of defense against CMV and allow the host to rapidly mount antiviral measures. It involves a major role of IFN-I produced by DCs and NK cells in particular. Adaptive immunity (particularly CD8 and CD4 LT, and to a lesser extent B cells) are subsequently involved (**Figure 30**).

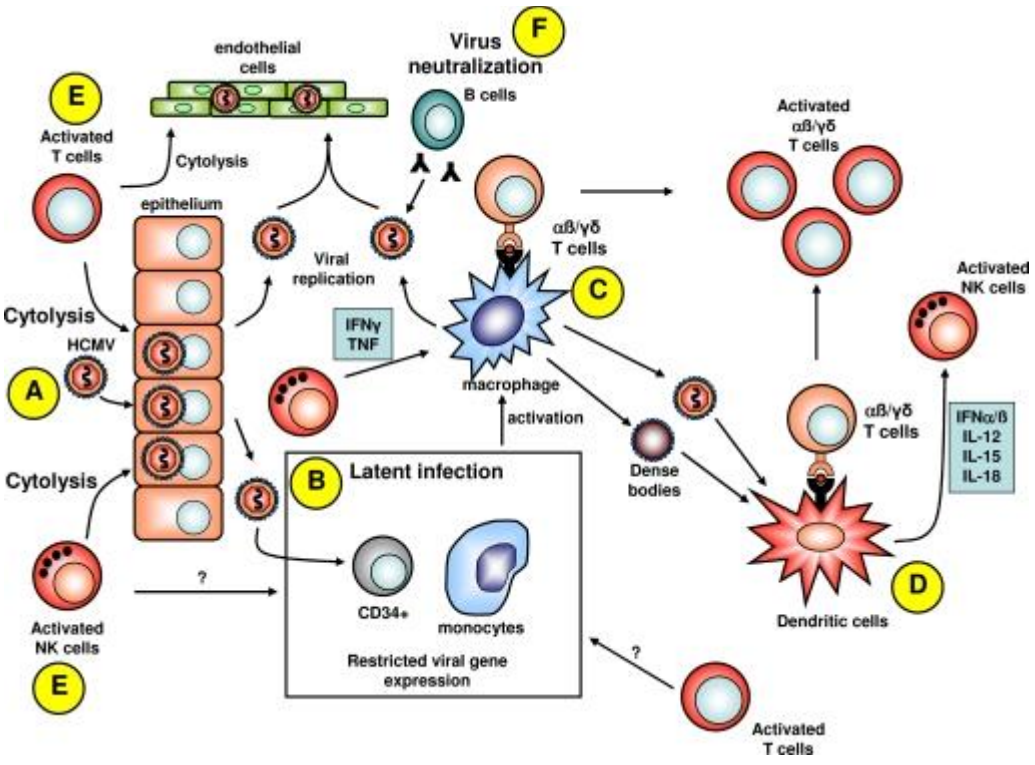


Figure 30 : Innate and adaptive CMV specific immune response

infection in healthy individuals typically begins with viral replication in the mucosal epithelium (A). The virus subsequently disseminates to myeloid-lineage monocytic cells, including monocytes and CD34+ progenitors, where it establishes latent infection (B). In these latently infected cells, restricted viral gene expression limits recognition by effector immune cells. Differentiation of infected monocytes into macrophages can trigger productive infection (C).

Virus particles or virus-associated dense bodies are processed by professional antigen-presenting cells (e.g., dendritic cells, DCs), which activate antigen-specific T cells (D). TLR-activated DCs also secrete cytokines and chemokines that stimulate innate immunity, including NK cells (D). Virus-infected macrophages can additionally present antigens directly to T cells (C). Activated T cells (CD8+, CD4+, and/or γδ) and NK cells eliminate infected cells through cytolysis or inhibit viral replication via cytokine secretion (e.g., IFN-γ, TNF) (E). B cells, activated by antigen-presenting cells, provide another adaptive immune arm by controlling extracellular virus through antibody-mediated neutralisation (F)(Crough & Khanna, 2009).

3.5.1 The innate immunity: the first line of defense against CMV

3.5.1.1 NK cells: The early contributor to the antiviral activity

NK lymphocytes play a crucial role in the control of CMV by various mechanisms. The HCMV infection is characterised by expansion of NK lymphocytes. They exert cytotoxic functions either by direct (perforin and granzymes secretion) or indirect lysis (by ADCC) of infected cells (Costa-Garcia et al., 2015; Parikh et al., 2015). They produce chemokines and pro-inflammatory cytokines such as TNF-α and γ-IFN, which activate macrophages and reinforce the antiviral immune response. Cytokines such as IL-15, IL-18 and IL-12 released by APCs promote the activation and survival of LT (Coudert et al., 2010). A deficiency in NK cells is linked to severe HCMV infections (Mace & Orange, 2019), confirming their crucial role in the anti-CMV response.

The activation of NK cells is modulated by the ligation of inhibitory (leukocyte Ig-like receptors (LIR), lectins CD94/NKG2A and NKG2B) and activating receptors (lectins CD94/NKG2C, E and H - NCR - NKG2), as previously described. A defining characteristic of the NK cells in the context of CMV is their elevated expression levels of the activating CD94/NKG2C+ receptor for HLA-E and absence of expression of its inhibitory homologue CD94/NKG2A+ (Gumá et al., 2006; Asenjo et al., 2024).

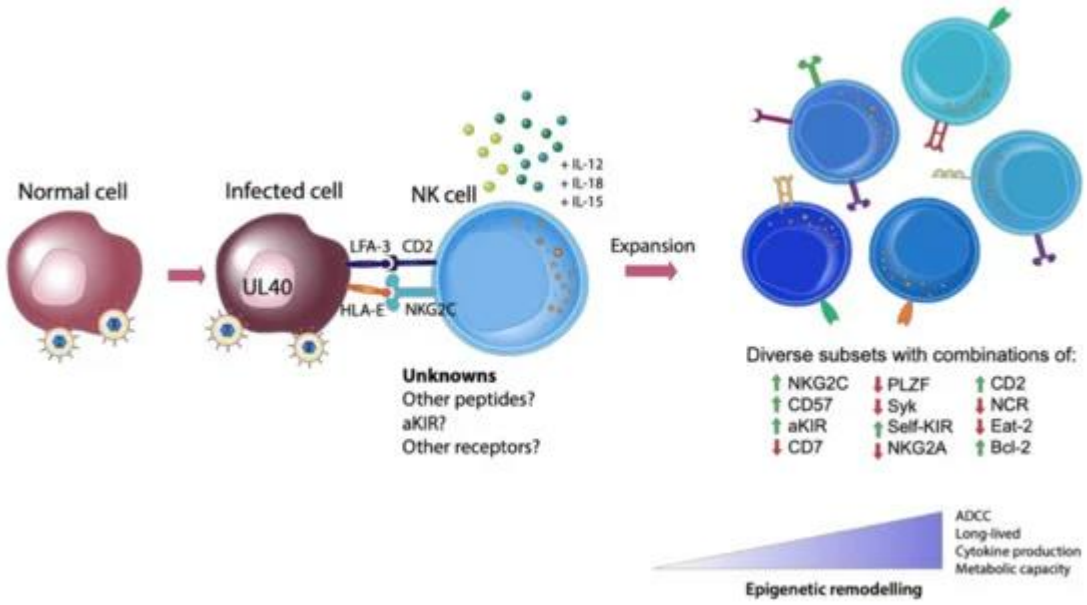


Figure 31 : Expansion of NK cells

The expansion of adaptive NK cells relies on three essential signals: (a) recognition of viral or self-peptides by specific receptors (notably HLA-E and NKG2C); (b) a co-stimulatory signal; and (c) pro-inflammatory cytokines. Interacting with an infected cell in this way induces the formation of diverse sub-populations of NK cells (Barnes et al., 2020).

CD94 /NKG2C+ NK phenotype is correlated with protection from HCMV disease in pregnant women (primary infection or reactivation) (Bayard et al., 2016) and in hematopoietic stem cell transplant patients (Foley et al., 2012). HCMV also induces an amplification of CD57+NKG2C+ NK cells in a specific manner, as EBV infection does not result in an amplification of the proportion of NK cells presenting this NKG2C+ CD57+ phenotype (Hendricks et al., 2014).

Importantly there is a specific interplay between NK cells and CMV. HCMV-encoded genes (US2, US3, US6, and US11) efficiently down-regulate MHC class I molecules, which may favor NK activation. In parallel, HCMV encodes multiple genes to suppress NK cell recognition. The UL16 gene product sequesters activating ligands (MICB, ULBP1, and ULBP2) in the endoplasmic reticulum. The HCMV gpUL40 stimulates expression of the nonclassical MHC class I molecule HLA-E, which binds the NK cell inhibitory receptor CD94/NKG2ALIR-1 (or LILRB1). Conversely, the CMV UL18 protein is an MHC class I homolog. Regardless this complex degree of interaction, NK cells play a critical role in the cytotoxicity of CMV-infected cells, in particular through the recognition of the UL40 Ag coupled to HLA-E by the quasi-specific CMV receptor NKG2C (**Figure 31**).

3.5.1.2 γδ LT: First-line of defense in tissues

The gamma delta LT (LTγδ) are unconventional LT expressing a TCR composed of a γ and δ chain. They are a minority population of LT in the peripheral blood, representing 1 to 10% of circulating LT (Gully et al., 2021). This small proportion is explained by their predominant location in epithelial tissues and mucous membranes, where they constitute up to 50% of LT and play an essential role in immune surveillance (Zheng & Yang, 2014). Unlike conventional LTαβ, which recognise Ags presented by MHC molecules, LTγδ recognise Ags independently of the MHC. In particular, they recognise stress molecules such as MIC-A, MIC-B or CD1d expressed on the surface of APC or infected cells (Willcox et al., 2020). LTγδ can respond to a wide variety of stimuli, whether peptide, lipid or phospho-antigenic in origin, giving them a unique ability to detect and respond rapidly to multiple danger signals. They are polarised in different effectors producing different cytokines (γδ1, γδ2 and γδ3 LT) (Gaballa et al., 2021).

Primary infection with HCMV is characterised by a rapid response of LTγδ activated by pro-inflammatory cytokines such as IL-12, IFNα/β and IL-18. They exhibit cytotoxic activity (production of perforin and granzymes) and produce other pro-inflammatory cytokines, particularly γ-IFN and TNF-α, which inhibit viral replication and activate other immune cells (Halary et al., 2005). This oligoclonal expansion of TCR Vδ T is a feature of HMCV, but not of other viral infections such as those caused by HSV, VZV, EBV, and influenza (Déchanet et al., 1999; Tuengel et al., 2021).

It has also been shown that HCMV induces long-term expansion of effector/memory Vδ2− γδLT (exhibiting an effector/memory TEMRA phenotype) in the peripheral blood of both immunocompetent and immunosuppressed patients (Pitard et al., 2008).

Recent studies have highlighted that not all γδ T lymphocytes respond to CMV in the same way. While Vγ9Vδ2 T cells dominate the peripheral blood under normal conditions, **non-Vγ9Vδ2 γδ T lymphocytes** (including $V\delta1^+$ and $V\delta3^+$ subsets) expand specifically in response to CMV infection. These cells exhibit adaptive-like features, including clonal expansion and long-term persistence, suggesting they play a key role in controlling viral replication and maintaining immune surveillance (Kaminski et al., 2020) (**Figure 32**).

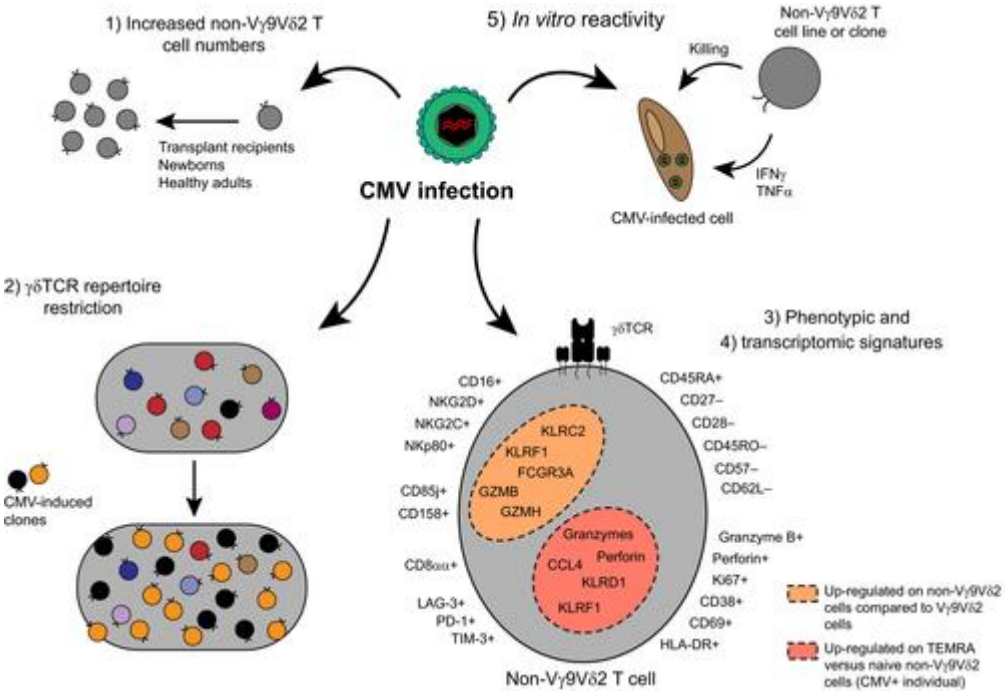


Figure 32 : Non-Vγ9Vδ2 T cell involvement in the anti-CMV immune response

Several clinical observations demonstrate the ability of **non-Vγ9Vδ2 gamma delta T lymphocytes** (non-Vγ9Vδ2 LT) to respond to CMV infection in vivo. After infection, these cells proliferate in the peripheral blood of various groups, including recipients of solid organ or hematopoietic cell transplants, as well as healthy donors. Their expansion is clonal and accompanied by restriction of the γδTCR receptor repertoire. CMV-induced non-Vγ9Vδ2 LT exhibit a unique molecular profile characterised by the expression of TEMRA markers, activation and cytotoxicity molecules, immune checkpoint inhibitors, and NK-like receptors. In vitro studies confirm their functionality by demonstrating their ability to produce pro-inflammatory cytokines and eliminate CMV-infected cells (Kaminski et al., 2020).

3.5.1.3 Other key players in the initiation of the anti-CMV innate responses

- DCs are specialised APCs that recognise CMV (CMV DNA or proteins) using PRRs such as Toll-like receptors (TLR-2, 3, 4, 7 and 9) and DC-SIGN molecules (DC-specific ICAM grabbing nonintegrin). Following this recognition, mature DCs present CMV Ags via MHC class I or II molecules to CD8 and CD4 LT for activation (Gredmark-Russ & Söderberg-Nauclér, 2012; Wujcicka et al., 2015).

Activated plasmacytoid DCs (pDCs) produce cytokines (IFN-I) and chemokines that recruit macrophages and NK cells to the site of infection (Yun et al., 2021). Monocyte-derived DCs (MoDCs) act by producing soluble antiviral factors that are not dependent on interferon signaling (Kasmapour et al., 2017).

- **Monocytes/macrophages** also express PRRs such as TLR (TLR-2, 4, 7 and 9) that recognise CMV Ags. They produce pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and IFN-α/β, and chemokines (CCL2, CCL3) that limit viral replication. However, CMV can establish latent infection in monocytes and replication in macrophages. Additionally, CMV significantly alters the function of monocytes/ macrophages, by decreasing their capacity for Ag presentation and phagocytosis while increasing pro-inflammatory cytokine production. CMV also alters monocyte migration to sites of infection while promoting trans-endothelial migration, thus aiding viral dissemination. This could lead to immune evasion and chronic inflammation (Planchon et al., 2024).

3.5.2 Adaptive immune responses: Control of CMV

3.5.2.1 CD8 LT response leading to CMV the memory inflation

CD8 LT are considered the main players in the immune response against CMV. They recognise viral Ags displayed on MHC class I molecules by infected cells (e.g., epithelial cells, macrophages) or via cross-presentation by DCs (Pavić et al., 1993; Busche et al., 2013). Once activated, CD8 LT induce the death of infected cells through the production of large quantities secretion of cytokines, in particular TNF-α and γ-IFN (antiviral effects) and also through the production and release of cytotoxic granules (perforin and granzymes) (Einsele et al., 2002).

The critical role of CD8 LT in the control of CMV infection has long been demonstrated both in experimental mouse models and in humans by several teams (Reddehase et al., 1987; Riddell et al., 1992). Riddell et al, on the one hand, and Elinsele et al, on the other, reporte that anti-CMV LT clone transfer in immunosuppressed patients allowed the reconstitution of a specific T cell response and reduced the viral load to undetectable levels (Riddell et al., 1992; Einsele et al., 2002). Quinn et al also have validated that passive transfer of MCMV-specific CD8 LT into murine Rag-/- mice (that are completely devoid of LT) protected these mice against CMV infection (Quinn et al., 2015). Peggs et al. have shown that in humans, adoptive transfer of CMV-specific CD8 LT can control not only the acute phases of infection, but also the chronic phases of CMV reactivation (Walter et al., 1995; Peggs et al., 2003).

The anti-HCMV CD8 LT response is strongly influenced by CMV-specific peptides presented by different HLA class I allotypes, in particular immunodominant peptides derivatives of 65 kDa phosphoprotein (pp65) and 55 kDa protein immediate early proteins (IE1). Studies have

shown that CD8 T responses to HCMV peptides pp65 and IE1 presented by HLA-A* 02:01 and HLA-B* 07:02 are associated with stronger responses, whereas HLA-C allotypes HLA-C, A2402 and HLA-B3501 show weaker CD8 T responses to the same peptides (Giest et al., 2010; Hyun et al., 2017).

Memory differentiation profile

The CMV-specific CD8 LT response is associated with a TEMRA phenotype. During the acute phase of CMV infection, CD8 LT play a critical role in controlling viral replication. These CMV-specific cells can be identified in the blood of infected individuals approximately 20 days after primary infection (Gamadia et al., 2003). During this acute phase, CD8 LT display an activated phenotype characterised by the expression of CD45RO, CD27 and CD28 in human cells. During the chronic phase of CMV infection, CD8 LT primarily differentiate into two subpopulations with distinct phenotypes and functions.

- The first one, TCM, displays a $CD45RA^{-}$ $CCR7^{+}$ $CD27^{+}$ phenotype in humans. This population is maintained at low levels mainly due to persistent viral Ags that promote terminal differentiation into effector cells. Despite their rarity, TCM retain the capacity for proliferation and self-renewal, an essential feature of long-term immune memory reservoirs (Appay et al., 2002; Holtappels et al., 2023).

- The second subpopulation, TEMRA, characterised by a $CD45RA^{+}$ $CCR7^{-}$ $CD27^{-}$ phenotype. These cells are highly functional with high cytotoxic activity (perforin and granzyme secretion), high proliferative capacity (stimulated by CMV antigenic reactivations) and sustained production of pro-inflammatory cytokines such as γ-IFN and TNF-α (Kuijpers et al., 2003; Gamadia et al., 2004; Wiesel et al., 2009). TEM, characterised by the $CD45RA^{-}$ $CCR7^{-}$ phenotype, are rare in chronic CMV as the virus promotes accelerated differentiation towards terminal cells (Appay et al., 2002). This dichotomy shows that CMV modulates the immune response, prioritizing immediate cytotoxicity (TEMRA) over long-term memory maintenance (TCM).

Memory inflation: a phenomenon unique to the CMV CD8 LT response

The anti-CMV immune response is characterised by massive clonal expansion of CD8 LT. In healthy CMV-seropositive individuals, CMV-specific CD8 LT represent on average 10% of the memory T cell repertoire and can reach > 40% in certain elderly individuals (Sylwester et al., 2005). This importance in the repertoire is striking as compared with other viral infections

such as EBV, for which less than 1-2% of virus-specific CD8 LT are detected (Hislop et al., 2007) or influenza, for which less than <0.5-1% are detected (Kedzierska et al., 2008). This phenomenon is known as "memory inflation" and is a marker of chronic CMV infection (**Figure 33**). Unlike classical memory LT which progressively decline after acute infection, inflationary CD8 LT increase in number over time, even in the absence of active viral reactivation.

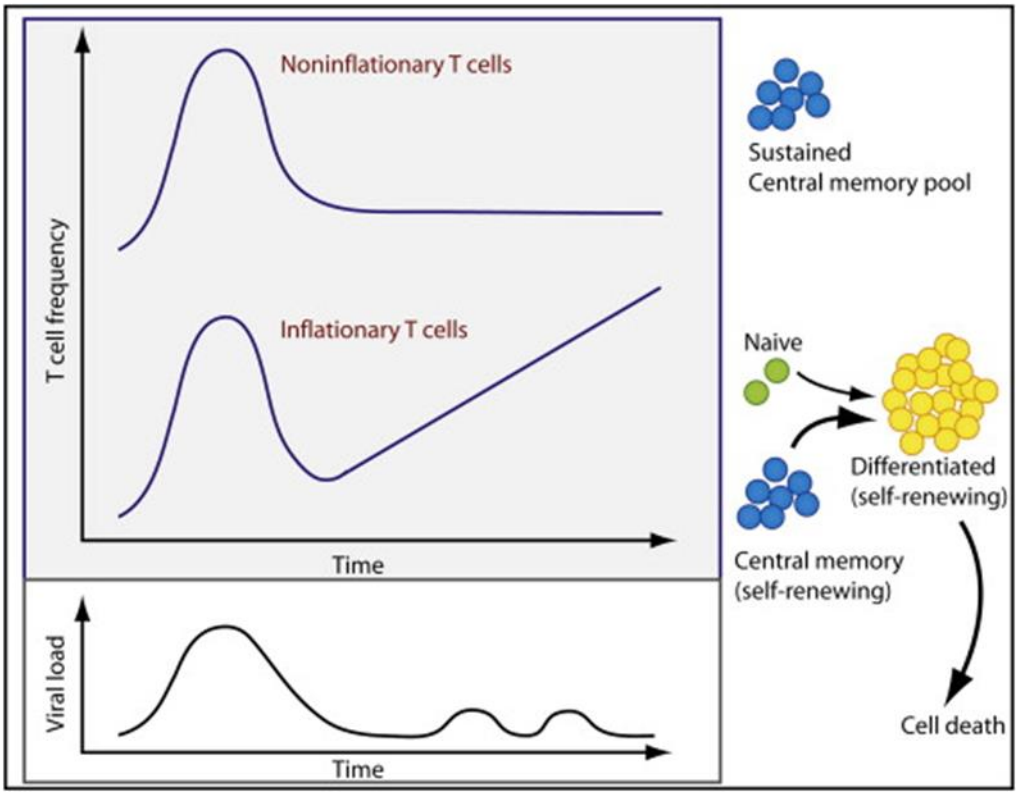


Figure 33 : Mechanisms of T cell memory after CMV infection

CMV infection induces two types of T cell memory response. On the right, in the case of the conventional memory response (non-inflationary), the central memory pool (blue) is maintained at a low level, mainly localised in lymphoid tissues. The inflationary memory response, on the other hand, shows a particular dynamic. In this inflationary response, large populations of differentiated cells are observed in the blood and peripheral tissues (yellow), which are continually renewed by three main sources: differentiated cells capable of self-renewal, reservoirs of central memory cells and naive precursors (green). This process compensates for natural cell death, maintaining stable pools of effector cells. Viral reactivations of CMV over time specifically stimulate this inflationary response (Klenerman & Dunbar, 2008).

In humans, CMV-specific CD8 LT predominantly adopt a highly differentiated phenotype ($CD45RA^{+}CD27^{-}CD28^{-}CCR7^{-}$), corresponding to the TEMRA profile. Up to 70-90% of these cells display this phenotype in some responses, reflecting chronic Ag exposure and terminal differentiation as described in persistent infections (Appay et al., 2002). In mice, CMV-specific

CD8 LT display a TEM phenotype (CD62L$^-$ KLRG1$^+$) characterised by progressive loss of CD127, characteristic of terminal differentiation induced by intermittent antigenic stimulation. Although this phenotype is phenotypically distinct from human TEMRA (CD45RA$^+$), it shares its functional features: persistent cytotoxicity and accumulation over time (inflationary memory) (Karrer et al., 2003; Snyder et al., 2008a). In contrast, CD8 T cells specific for antigens derived from acute and self-limiting infections (such as influenza) retain a central memory phenotype (CD62L$^+$, CCR7$^+$) characteristic of a less differentiated long-term memory capable of lymphoid recirculation as described in transient immune responses (Sallusto et al., 1999; Wherry et al., 2003).

Phenotype of CD8 LT involved in the CMV response

The CD8 LT responses directed against CMV thus include naïve, effector, memory and terminally differentiated CD8 LT (**Figure 34**).

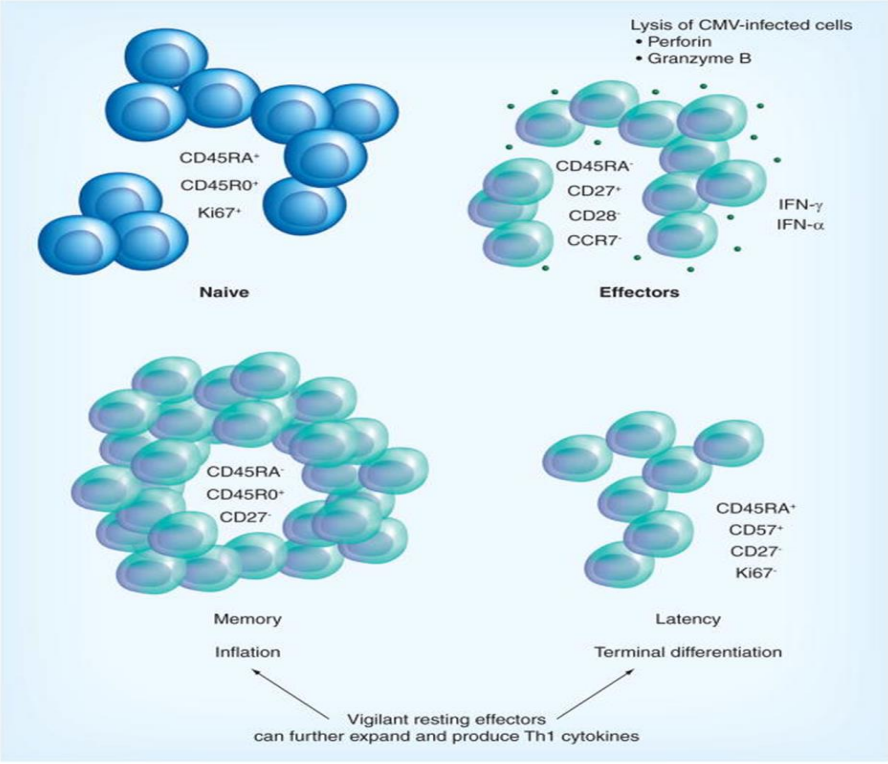


Figure 34 : Phenotypes of CMV-specific CD8 LT

CMV-specific CD8 LT display distinct phenotypic characteristics depending on the stage of infection (primary infection, memory and latency). The effector cells are markedly cytolytic and produce cytokines, notably of the Th1 type, a signature that persists in the early and late stages of infection. Unlike other viral infections, the memory pool does not regress once the virus has been eliminated, but continues to swell, eventually dominating the memory T cell compartment. Despite displaying a

terminally differentiated phenotype during the latent phase, these cells retain a remarkable capacity to develop rapidly and to produce Th1 cytokines as resting effectors, an essential property for controlling viral reactivation. However, this vigorous response is accompanied by a sustained production of γ-IFN, which could contribute to maintaining a pro-inflammatory state, potentially involved in the pathogenic effects of CMV (La Rosa & Diamond, 2012).

Phenotype of CMV-specific TEMRA cells

The inflationary LT correspond predominantly to TEMRA profile. They express CD45RA but are negative for the costimulatory molecules CD27 and CD28 and for the lymphoid guidance molecule CCR7 (Appay et al., 2002; Klenerman & Oxenius, 2016a). This phenotype is accompanied by high expression of CD57 and KLRG1 (senescence markers) (Henson et al., 2009). Their low expression of the guidance molecules CCR7 and CD62L favors their retention in peripheral tissues rather than their migration to lymphoid organs. Finally, the reduced expression of CD127 (IL-7Rα) and IL-15Rβ receptors limits their dependence on survival cytokines. This reduced expression is considered as an adaptive mechanism linked to their long-term persistence in the absence of chronic antigenic stimulation (Nikolich-Žugich, 2018). In the context of the CMV infection, inflationary CD8 LT are also characterised by the expression of the CX3CR1 receptor (**Figure 35**). These cells are mainly found in the blood and spleen. Their maintenance is linked to the ability of CX3CR1 to interact with its ligand, fractalkine (CX3CL1), on endothelial cells, an essential mechanism for their homing to tissue niches and survival via induction of BCL-XL (BCL2L1), an anti-apoptotic protein that inhibits caspase activation(Böttcher et al., 2015). Morris et al showed that these **CX3CR1+CD57+CD8 LT** populations are generated and amplified by IL-15, a key cytokine in LT homeostasis and survival. IL-15 acts in synergy with CX3CR1 signaling to promote clonal expansion and persistence of inflamed cells, in particular by activating pathways such as STAT5 that regulate BCL-XL expression. This cooperation explains their resistance to apoptosis and their progressive accumulation in response to persistent antigenic stimuli (Morris et al., 2020b). Conversely, when confronted with non-inflationary epitopes, CD8 LT lose CX3CR1, depriving them of both fractalkine interactions and IL-15 dependent pro-survival signals. Deprived of these mechanisms, these cells undergo accelerated apoptosis, in contrast to the inflationary populations supported by CX3CR1 and IL-15 (Hertoghs et al., 2010). The combination of these factors - chemokine receptor interaction, BCL-XL activation and cytokine signaling - forms a molecular network in which CX3CR1 and IL-15 act in concert to maintain a pool of inflationary memory LT. Interestingly, a recent article provide a different observation and shows that

CX3CR1^{+} LT exhibit a selective survival disadvantage without stimulation (Pokharel et al., 2024).

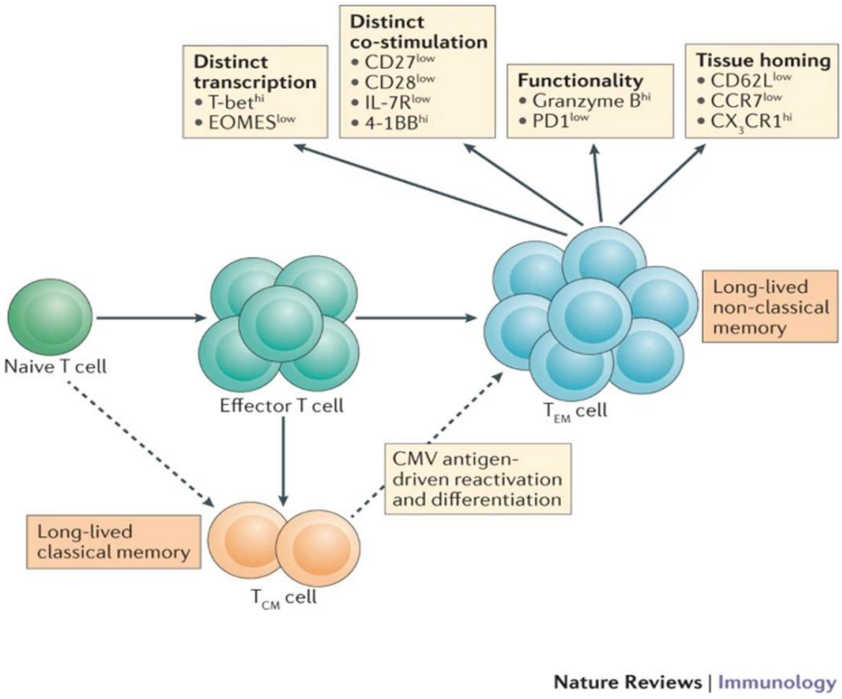


Figure 35 : Inflation of anti-CMV memory CD8 LT over time

Classical memory CD8 LT involves marked population expansion and acquisition of effector capacity, followed by contraction and maintenance of long-term Ag-independent memory pools with a T_{CM} cell phenotype. CMV infection and some low-level persistent infections and vaccines seem to support the recruitment of memory LT to an T_{EM} cell pool that is maintained at high levels, has an effector memory phenotype and maintains effector function (Klenerman & Oxenius, 2016b).

3.5.2.2 Factors modulating the CMV CD8 T cell response

The mechanisms underlying the expansion of CD8+ memory T cells in HCMV infection are not yet fully understood. Nevertheless, this clonal expansion appears to depend on synergistic factors operating within a dynamic equilibrium (**Figure 36**).

Before examining the factors that contribute to CD8+ T cell memory inflation, it is important to introduce the concept of immunodominance, a central principle that structures the hierarchy of T cell responses and governs the clonal selection observed during CMV infection.

-Immunodominance : The concepts of immunodominance and TCR repertoire restriction are fundamental features of T cell responses against CMV. Immunodominance is defined as the hierarchical targeting of the immune response towards a limited set of epitopes, driven by

inter-epitope competition (where dominant epitopes actively suppress subdominant responses) and by T cell receptor affinity for the presented antigen (Yewdell & Bennink, 1999). This leads to a restricted TCR repertoire, characterised by oligoclonal expansion of T cells with limited diversity specific to these dominant epitopes (Akram & Inman, 2012).

In CMV infection, these phenomena are pronounced. In HLA-B44:03 carriers, CD8+ T cells targeting the NW8 epitope (from IE-2) can constitute up to 17% of circulating CD8+ T cells, illustrating extreme immunodominance(Attaf et al., 2018). Similarly, epitopes derived from the matrix protein pp65 (e.g., NLVPMVATV for HLA-A02:01) and IE1 (e.g., TPRVTGGGAM) are major immunodominant targets in different HLA contexts (Giest et al., 2012; Hyun et al., 2017) . Clinical studies show that the protective efficacy of CMV-specific CD8+ T cells varies depending on the HLA/peptide combination; for instance, HLA-A2402/pp65 (341–349) and HLA-B3501/pp65 (123–131) T cells confer protection at lower frequencies than HLA-A0101/pp50 (245–253) or HLA-A0201/pp65 (495–503) T cells, indicating HLA- and peptide-dependent differences in immune response efficiency (Giest et al., 2010).

This immunodominance is accompanied by severe TCR repertoire restriction, with preferential V and J segment usage and recurrent public TCR clones shared across individuals, suggesting convergent selection of optimal TCR sequences. Recent identification of recurring CDR3 motifs in these public TCRs supports this hypothesis(May et al., 2024).

Underlying mechanisms include epitope competition (immunodomination) and HLA-mediated differences in antigen presentation efficiency (Balasubramanian et al., 2025) . These phenomena have dual consequences: extreme targeting can limit immune diversity and promote viral escape via antigenic mutation, although restricted targeting may also represent a host strategy to mitigate this risk. Simultaneously, these features provide opportunities for targeted cellular immunotherapies exploiting high-affinity public TCRs, such as adoptive T cell transfer to control CMV reactivation in immunocompromised patients (Ritmahan et al., 2020; Song et al., 2017).

Clinical data confirm that immunodominance influences therapeutic outcomes: CMVpp65-specific CD8+ T cells restricted by HLA-B07:02 and HLA-A02:01 consistently elicit protective responses, whereas certain HLA-B*35–restricted responses, although immunodominant, fail to lyse infected cells or control CMV in adoptive therapy, indicating that immunodominance does not always correlate with protective efficacy (Hasan et al., 2022).

Factors fueling memory CD8 T cell inflation

Memory inflation is driven by a combination of viral and host factors that sustain controlled proliferation of effector CD8 T cells over time:

-**Antigen persistence and repeated stimulation**: Sporadic reactivation of latent CMV in non-hematopoietic cells (fibroblasts, endothelial cells) results in chronic antigen presentation via the constitutive proteasome. High-affinity CD8 T cells preferentially recognise these epitopes and dominate the inflationary response (Klenerman & Oxenius, 2016b).

-**Initial viral load and tissue distribution**: A high viral inoculum during acute infection correlates with more latent genomes and larger inflationary T cell pools. Tissue reservoirs such as spleen and lungs play key roles in maintaining memory inflation (Holtappels et al., 2020; Redeker et al., 2014; C. J. Smith, Turula, et al., 2014). The route of infection also affects tissue distribution and local immune responses (Oduro et al., 2016).

-**Inflationary T cell renewal**: Central memory T cells (TCM) and CX3CR1^{-} peripheral memory T cells contribute to replenishing the inflationary pool, preventing exhaustion. Naïve T cells play a limited role during latency in adult hosts (Gordon et al., 2018; Welten et al., 2019).

-**Cytokines and co-stimulatory signals**: IL-15 is critical for the survival of inflationary CD8 T cells in peripheral tissues, supporting anti-apoptotic pathways. IL-2 signaling via CD25 (IL-2Ra subunit) is essential for the proliferation of TCM-derived effector cells. Co-stimulatory molecules such as CD27, 4-1BB, and OX40, often mediated by CD4 T cell help, enhance expansion and long-term persistence of inflationary T cells (Humphreys et al., 2007, 2010; Kim et al., 2015; Morris et al., 2020a; Welten et al., 2013).

-**CD4 T helper cells**: For certain immunodominant epitopes, CD4 T cells are required to optimise CD8 T cell responses through IL-2 secretion and APC maturation (Snyder et al., 2008b).

Factors limiting CD8 LT inflation

Memory T cell inflation is constrained by host-derived regulatory mechanisms that prevent excessive or pathological immune responses. Regulatory T cells are key, as their depletion has

been shown to enhance inflationary CD8 T cell responses during latent MCMV infection. Anti-inflammatory cytokines such as IL-10 further limit the accumulation of virus-specific CD8 T cells. Moreover, the expression of inhibitory receptors such as KLRG1 helps to balance effector differentiation with long-term maintenance (Welten et al., 2019).

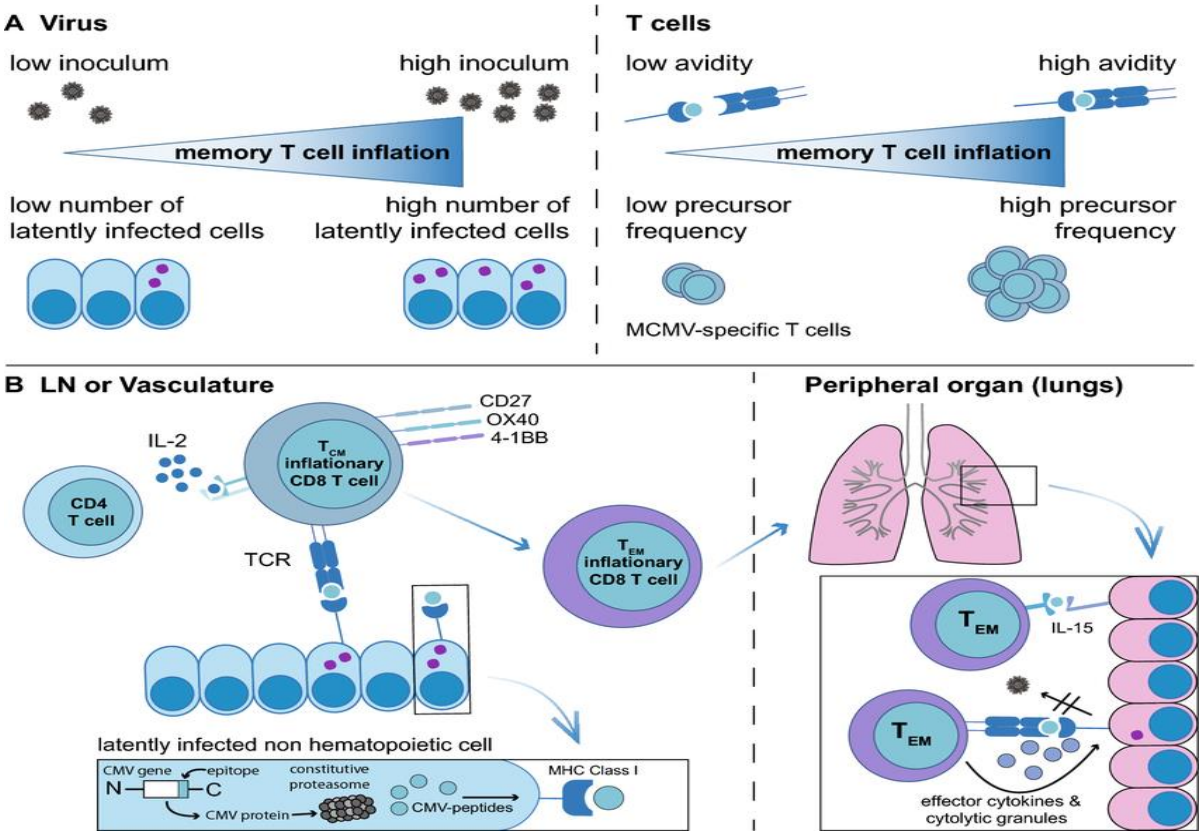


Figure 36 : Factors involved in memory T cell inflation

Factors influencing memory T cell inflation. (a) The extent of memory inflation is shaped by the initial viral inoculum and the number of latently infected cells. From the host perspective, a higher precursor frequency of MCMV-specific T cells and strong TCR avidity favour a more pronounced inflationary response. (b) Mechanistically, in latently infected non-haematopoietic cells, viral proteins are processed by the constitutive proteasome, generating peptides that are presented during viral reactivation events. Proliferation-competent TCM cells with inflationary specificity recognise these antigens in lymph nodes or vascular niches, leading to their expansion and differentiation into effector memory cells that subsequently migrate to peripheral tissues, where they control viral reactivation. In addition, IL-15 signals in peripheral sites support the survival of inflationary T cells. Other critical factors include CD4 T cell help, IL-2, and co-stimulatory pathways mediated through CD27, OX40, and 4-1BB (Welten et al., 2019).

In conclusion, CD8 T cell memory inflation in response to CMV results from sustained antigenic stimulation by dendritic cells, favored by immunodominant epitopes, a high initial viral load, the recruitment of naïve and TCM, as well as the presence of homeostatic cytokines such as IL-15. It is regulated by brakes such as KLRG1, regulatory T cells, and IL-10, which modulate CD8 T cell inflation and help maintain immune balance.

3.5.2.3 Replicative senescence as a feature of CD8 T responses to CMV

A key feature of CD8 T responses directed against CMV is the induction of replicative senescence. CMV infection promotes the accumulation of terminally differentiated T cells (CD27-CD28-, $CD57^+$, largely corresponding to the TEMRA phenotype), displaying reduced proliferative capacity and expressing markers associated with senescence, which limits their functional efficacy (Hoji et al., 2007). These features are particularly marked in persistent CMV infection, where chronic antigenic stimulation favours the massive adoption of a terminal phenotype by specific CD8 LTs.

Unlike other chronic viral infections (such as HIV or HCV), CMV-specific CD8 LTs retain their cytotoxic function despite their terminal state. This combination of replicative senescence and functional maintenance could reflect an adaptation favouring prolonged viral control (Appay et al., 2000).

An additional aspect of CMV-induced replicative senescence of CD8 LTs is the acquisition of a SASP. These senescent cells can release pro-inflammatory cytokines such as IL-6, TNF-α and various chemokines, contributing to inflammaging (Campisi, 2013; Solana et al., 2012). The amplification of this SASP is promoted by antigenic persistence and by the activation of signalling pathways such as cGAS-STING/NF-κB, which detect viral DNA and stimulate the production of pro-inflammatory mediators (Hopfner & Hornung, 2020). Although most of the data comes from experimental models or in vitro analyses, these mechanisms offer a plausible explanation for the observed link between CMV seropositivity and certain age-related comorbidities (Simanek et al., 2011).

Expression of other modulating markers

In contrast to the exhausted CD8 LT observed in models of chronic infection such as LCMV in mice or in humans during HIV, HBV and HCV infection (associated with functional exhaustion) (Barber et al., 2006; Day et al., 2006; Heim et al., 2019; Sekyere et al., 2015), CMV-specific CD8 LT retain robust effector function, and do not express exhaustion markers such as PD-1 and

TIM-3. This duality is further enhanced by the co-stimulatory receptor 2B4 (CD244), which specifically enhances γ-IFN production, thereby optimizing antiviral control without inducing exhaustion (Snyder et al., 2008b). However, recent data suggest substantial heterogeneity in the expression of PD-1 among CMV-specific CD8 LT (Welten et al., 2019).

In the latent phase, CMV-specific CD8 LT weakly express the inhibitory receptors classically associated with exhaustion (PD-1, TIM-3, LAG-3 and CD160) (Hertoghs et al., 2010; Snyder et al., 2008b). However, they have high expression of other regulatory receptors such as KLRG1 and CD85j (ILT2 an inhibitory receptor of the LILR family specific to humans) (van den Berg et al., 2019). Still, the high expression of CD85j in these TEMRAs does not reflect immune dysfunction because these cells retain a robust effector function. CD85j primarily functions as a modulator of activation rather than a classical inhibitory receptor, helping to balance antiviral responsiveness and homeostatic control.

Conclusion

CMV-specific CD8 T cells represent a relevant model for investigating the mechanisms of T cell senescence. This effector memory subset displays unique adaptive properties, enabling it to respond to viral evolution while retaining cytotoxic function despite chronic infection. Such functional specialisation may also reflect features of the SASP, contributing to low-grade inflammation and immune dysfunction associated with cellular aging.

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3.5.2.4 CD4 T cell compartment

CD4 LT also play an early role in the anti-HCMV immune response (Rentenaar et al., 2000). In mice, CD4 LT depletion induces CMV viral persistence in the salivary glands and delays resolution of infection (Jonjić et al., 1989). The CD4 LT response is a key element of the anti-CMV defense and its assessment is particularly important in patients at risk of CMV reactivation (immunocompromised). They recognise HCMV viral Ags presented by APCs via MHC II and co-stimulatory molecules (CD28 and ICOS) and then differentiate into effector CD4 LT that produce Th1-type cytokines such as TNFα and γ-IFN. These cytokines, particularly γ-IFN and IL-2, potentiate the cytotoxic CD8 LT response, NK cells and the maintenance of a humoral response (Jonjić et al., 1989).

The HCMV-specific CD4 LT are detectable early in the primary anti-CMV response, as early as 7 days after detection of HCMV viral load in the blood of transplant patients (Rentenaar et al.,

2000). Sylwester et al. showed that approximately 4% of the total CD4 LT population recognises HCMV peptides and that approximately 9% of memory CD4 LT are specifically directed against HCMV. They also observed that more than 50% of HCMV seropositive individuals develop CD4 LT responses directed against viral proteins such as UL55 (gB), UL83 (pp65), UL86 (major capsid protein - MCP), UL99 (pp28) and UL122 (IE86) (Sylwester et al., 2005); these proteins are involved in different phases of the viral cycle.

Phenotypically, during the acute phase of infection, CMV-specific CD4 LT have a $CD45RO^+$ $CCR7^-$ phenotype (TEM) for immediate control of the virus in the tissues and a fraction exhibit a $CD45RO^+$ $CCR7^+$ phenotype (TCM) for systemic surveillance and rapid secondary response (Crough & Khanna, 2009). In the chronic phase of infection, CD4 LT adopt a $CD45RA^-$ $CD27^-$ or $CD44^+$ $CD62L^-$ memory effector phenotype (Rentenaar et al., 2000). This CD4 TEM population plays a key role in the long-term control of CMV by providing a rapid response and immune surveillance against viral reactivation. A high proportion of $CD28^-$ GrB^+ CD4 LT has been identified in the peripheral blood of CMV-seropositive individuals (van Leeuwen et al., 2004). This subpopulation mainly secretes TNF-α and γ-IFN and may also have cytotoxic activity (via Granzyme B, Perforin and Fas/FasL), allowing lysis of CMV-infected cells in vitro (van Leeuwen et al., 2004; Casazza et al., 2006).

3.5.2.5 Humoral response

Although cellular immunity (CD8, CD4 LT and NK cells) is essential for controlling replication and eliminating infected cells, the humoral response plays a complementary role by preventing the spread of the virus and reducing the risk of reactivation.

Britt et al. showed that 40 to 70% of anti-HCMV IgG antibodies specifically target gB (Britt et al., 1990). Other viral glycoproteins such as gH/gL or tegument proteins (pp65, pp150) can also be targeted by antibodies, but to a lesser extent (Landini & La Placa, 1991). Subsequent studies have shown that the most potent anti-HCMV neutralizing antibodies (in terms of neutralizing efficacy) target protein complexes essential for viral entry into host cells, in particular the pentameric gH/gL/UL128-UL130-UL131A complex, with a small fraction directed against the gH/gL and gB (less than 10%) (Fouts et al., 2012; Vanarsdall & Johnson, 2012). Although these neutralizing antibodies represent only a small proportion of total IgG,

their ability to block viral attachment and entry makes them important players in the protective response.

In mouse models, B cell deficiency does not affect the immune response during the acute phase of CMV infection. However, anti-CMV antibodies become more important in the chronic phase and play a crucial role in preventing reactivation and limiting the long-term spread of the virus (Waisman et al., 2008).

Anti-CMV IgG plays a protective role in preventing congenital transmission. Several studies, have demonstrated that pregnant women who were immunised prior to pregnancy (i.e., pre-pregnancy infection) and developed mature anti-CMV IgG with high affinity/avidity transmit the infection to the fetus less frequently and with less severe outcomes compared to non-immunised women (K. B. Fowler et al., 1992; Nigro et al., 2005; Palav et al., 2025).

Conclusion

A study of monozygotic twins discordant for CMV (one infected and one uninfected) found that 58% of the immune parameters analysed, including key populations such as γδ LT, CD8 LT and neutrophils, were significantly altered in CMV+ individuals (Brodin et al., 2015). In particular, these changes included clonal expansion of CD8 LT (differentiation into effector memory cells), chronic neutrophil activation (pro-inflammatory phenotype) and altered γδ LT homeostasis, highlighting the central role of CMV in modulating adaptive immunity.

This observation is complemented by a quantitative multiparametric study carried out on 1,000 genotyped individuals (SNP analysis), which shows that CMV infection reduces non-conventional LT populations (NKT and MAIT, associated with mucosa) while increasing conventional LT (CD8 and CD4 TEMRA LT subpopulations) (Patin et al., 2018). Furthermore, this study shows that the natural variation in the parameters of innate immune cells (e.g. monocytes, NK cells) is mainly determined by genetic factors, in contrast to conventional LT, whose variability is more influenced by environmental factors such as CMV.

3.6 *Clinical consequences of the HCMV infection*

Although associations have been observed, the causal role of CMV infection in the development of pathologies such as immunosenescence, chronic inflammation or cardiovascular disease has not been clearly established.

3.6.1 *CMV-related* comorbidities

A meta-analysis has shown that HCMV is associated with elevated inflammatory markers (IL-6, CRP) and an increased risk of cardiovascular disease (Wang et al., 2017), but these associations could reflect confounding factors (age, co-infections) rather than a direct causal relationship. Similarly, studies on mortality show conflicting results: some associate CMV with increased mortality, while others find no significant association. These discrepancies suggest that the impact of CMV depends on complex interactions with immune ageing and age-related comorbidities rather than a unidirectional effect.

In the absence of controlled prospective studies, it remains difficult to disentangle the precise mechanisms by which CMV influences these processes. Future studies will need to clarify whether these associations reflect direct causality or epiphenomena associated with other risk factors .

3.6.2 CMV infection in elderly

3.6.2.1 Age-dependent impact

CMV infection exerts a complex and multifaceted influence on vaccine responses, with effects that vary depending on the vaccine type, age, and immune status of the individual.

In older adults, CMV can reduce the effectiveness of several vaccines, particularly those requiring robust T-cell immunity. This reduction is primarily explained by the virus-induced acceleration of the immunosenescence process (Pawelec et al., 2012).

Specific studies demonstrate that: For the **zoster vaccine**, CMV seropositivity is associated with reduced humoral and cellular immune responses (Weinberg et al., 2025). For the **yellow fever vaccine (YF17D)**, a decrease in the frequency of specific CD8+ cells has been observed in older seropositive males (Santos-Peral et al., 2025). Notably, for **mRNA COVID-19 vaccines**, one study found no negative impact of CMV seropositivity on anti-RBD IgG antibody levels,

viral variant neutralisation capacity, or the production of durable CD4+ and CD8+ memory T lymphocytes (Breznik et al., 2022).

In young adults (20-30 years), the picture is different. Latent CMV infection is associated with an **enhanced immune response** to the influenza vaccine, characterised by higher antibody levels and more robust T-cell responses than in non-infected individuals of the same age (Furman et al., 2015).

However, this apparent immune advantage in young adults may prove to be a double-edged sword with age. The chronic activation and clonal expansion of CMV-specific T lymphocytes, while beneficial in the short term for fighting pathogens, progressively contributes to immunosenescence. This phenomenon is characterised by a limited T-cell repertoire and persistent systemic inflammation, creating fertile ground for the development of age-related diseases such as cardiovascular disorders (atherosclerosis), neurodegenerative conditions (dementia), and metabolic diseases (type 2 diabetes).

Furthermore, the dominance of CMV-specific T clones in the immune repertoire, coupled with increased reactivity to the virus with age, could contribute to reduced vaccine efficacy in older adults, although this association requires further investigation (Furman et al., 2015) .

Thus, CMV illustrates the paradox of increased immune stimulation that is beneficial in young adults but potentially detrimental in the long term due to its effects on immune senescence.

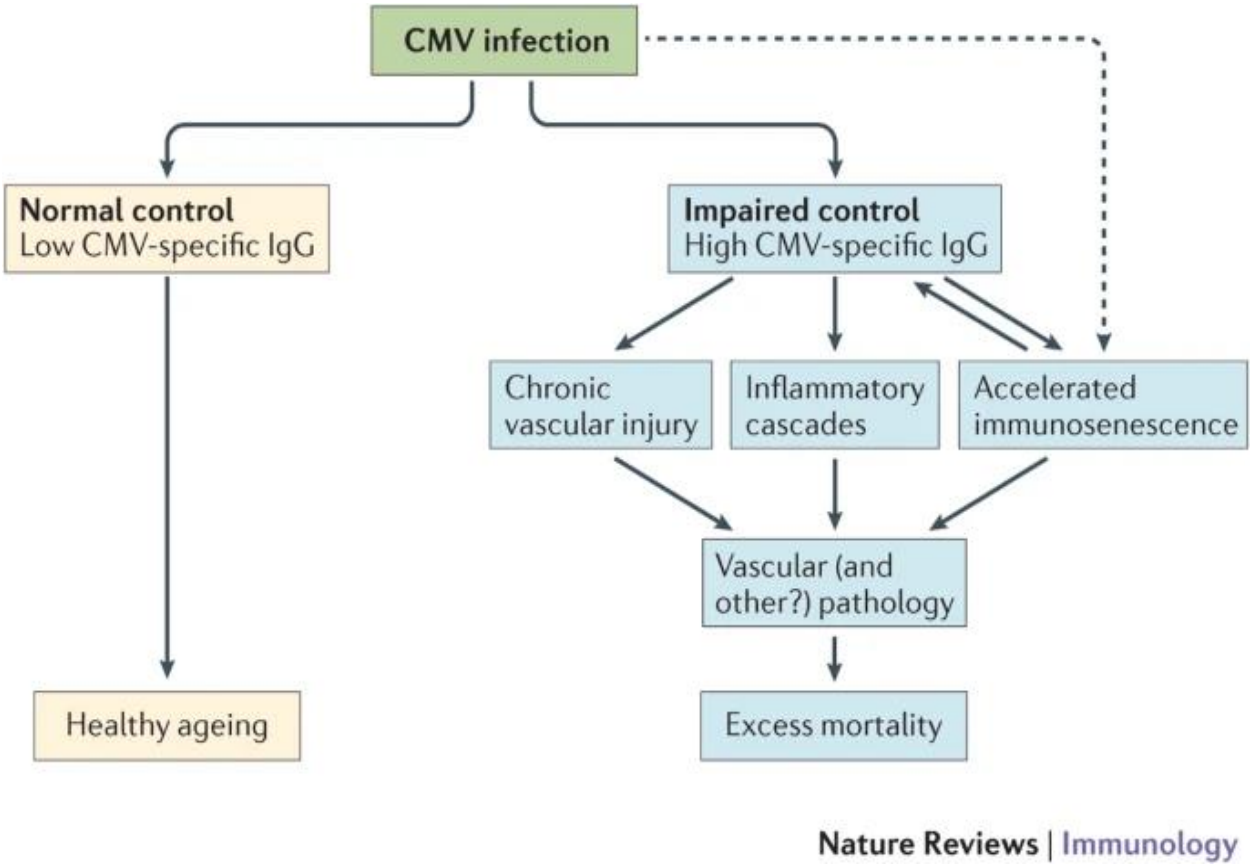


Figure 37 : CMV infection, memory inflation and consequences for ageing.

CMV infection, particularly when associated with high levels of CMV-specific IgG, has been correlated with adverse outcomes such as cardiovascular disease and increased mortality in some studies, although these associations are not systematic. In healthy elderly individuals, CMV is generally well controlled, as evidenced by low levels of specific IgG, suggesting an adaptive immune response. The mechanisms behind the loss of control of viral replication and therefore high IgG levels are not yet well understood (Klenerman & Oxenius, 2016b).

The robust associations between high frequencies of CMV-specific LT, markers of replicative senescence (such as loss of CD28, expression of CD57 or accumulation of β-galactosidase) and advancing age suggest that anti-CMV immunity may act as a key driver of chronic immune senescence. This phenomenon may be involved in the pathogenesis of age-related comorbidities (such as cardiovascular disease, cancer …)(Campisi et al., 2011; Solana et al., 2012) (**Figure 38**).

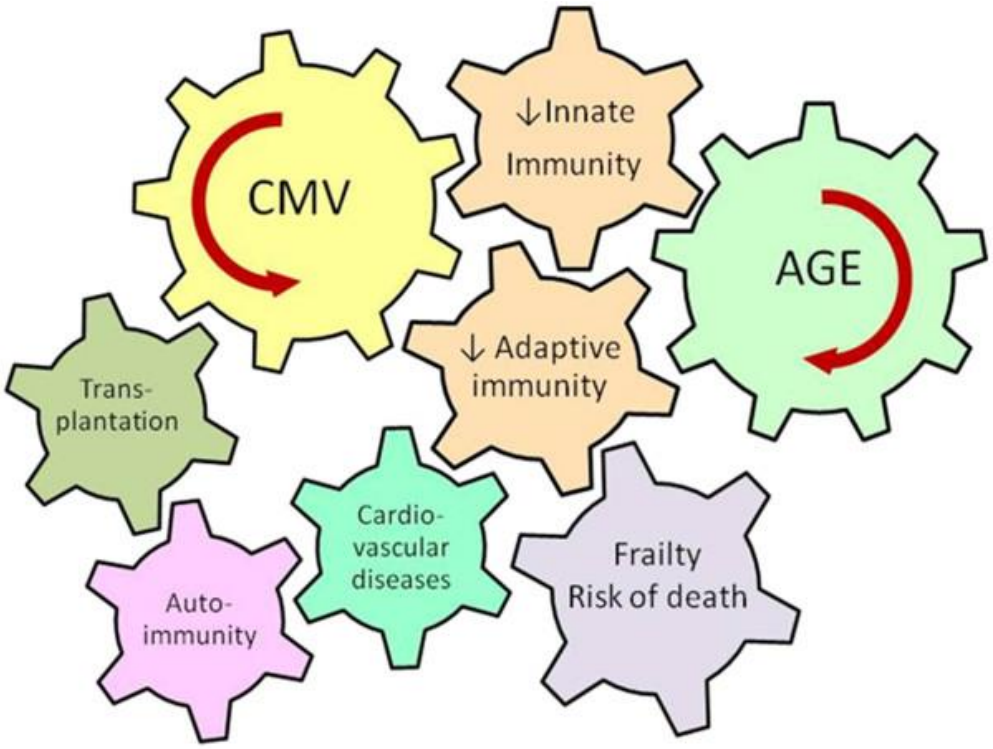


Figure 38 : Age and CMV infection are major driving forces contributing to the deterioration of innate and adaptive immunity

Age-related weakening of adaptive immunity (immunosenescence) and dysregulation of innate immunity, linked to inflammation, contribute critically to the progression of various age-related pathologies. In the elderly, these interconnected processes increase vulnerability to frailty and mortality (Solana et al., 2012).

3.6.2.2 Immune risk profile (IRP)

The concept of the immune risk profile (IRP) emerged from the Swedish OCTO and NONA cohort studies conducted between 1994 and 1995 (Pawelec et al., 2001). The aim was to identify fraction of elderly more susceptible to comorbidities and mortality. In the population of nonagenarians studied, 15% of individuals had IRP. This profile was associated with twice the risk of mortality over a 4-year period compared with those without IRP (Ferguson et al., 1995), highlighting its predictive role in frailty and decline in the very old. Underlying mechanisms include chronic inflammation (inflamm-aging), reduced T-cell diversity and altered responses to pathogens or vaccines. IRP highlights the heterogeneity of ageing and enables the identification of high-risk subgroups for targeted prevention strategies, such as the treatment of latent infections or therapies that modulate inflammation. The IRP was defined by three key immunological markers: an expansion of CD8 LT accompanied by an inversion of the CD4/CD8 ratio (below 1, reflecting a decrease in CD4 LT and an increase in

CD8 LT), a low proliferative capacity of LT after concanavalin A stimulation (a mitogen), and B cell lymphopenia.

The OCTO study, re-examined by Olsson et al, showed that the IRP initially defined without taking into account CMV serological status was systematically associated with this virus. In fact, 100% of individuals classified as having an IRP were CMV seropositive. This finding highlights the central role of chronic CMV infection in the immune abnormalities characteristic of IRP, such as the inversion of the CD4/CD8 ratio and the proliferation of virus-specific T lymphocytes. It challenges the original definition of IRP and suggests that CMV may be a key factor in the accelerated ageing of the immune system in the very elderly (Olsson et al., 2001).

The IRP is also observed in younger populations or those suffering from chronic pathologies, revealing accelerated immune ageing independent of chronological age. In middle-aged adults or asymptomatic individuals, a 'premature' IRP has been described, characterised by immunological changes similar to those observed in the elderly. These profiles include a decrease in naive LT and an expansion of differentiated memory LT, markers classically associated with immune senescence (Strindhall et al., 2013; Wikby et al., 1998).

Chronic inflammatory diseases such as rheumatoid arthritis and chronic obstructive pulmonary disease have immunological mechanisms that overlap with those of IRP. These conditions are associated with chronic lymphocyte activation, oxidative stress and cellular senescence, mimicking changes observed in physiological immune ageing (Franceschi et al., 2018; MacNee, 2016). The context of chronic HIV infection will be specifically considered in the next chapter, as PWH are susceptible to accelerated ageing and present features of ageing. Low CD4/CD8 ratio persists in almost 30% of individuals, even under effective ART treatment. This imbalance, combined with residual immune activation and an increased risk of non-AIDS-related co-morbidities (cardiovascular diseases, cancers) illustrates accelerated immune senescence ($CD28^-$ and $CD57^+ CD28^-$) despite virological control (Serrano-Villar et al., 2014).

4 The HIV-HCMV coinfection

4.1 Epidemiology

4.1.1 CMV seroprevalence in PWH

The prevalence of the HIV/CMV co-infection varies according to population, mode of HIV acquisition and geographical context. In developed countries, MSM living with HIV have a CMV seroprevalence of almost 100%, due to the high risk of sexual transmission of this virus (Kryger et al., 1984; Shepp et al., 1996). In contrast, the prevalence of anti-CMV antibodies among HIV-infected haemophiliacs in the United States was 57% (Jackson et al., 1988) and 67% among injecting drug users (Shepp et al., 1996). These differences reflect the different modes of transmission of CMV (not only sexual, but also by blood or blood products), which explains the differences between risk groups.

In developing countries, where vertical transmission of CMV occurs early and preventive measures are limited, CMV seroprevalence among PWH often exceeds 90%. A study conducted in rural Lesotho found seroprevalence of 100% among PWH (Rabenau et al., 2010). This trend is confirmed in sub-Saharan Africa, where more than 90% of PWH are seropositive for CMV due to adverse socio-economic conditions and early exposure to the virus (Grønborg et al., 2017)

4.1.2 Simultaneous coinfection or HIV infection on preexisting CMV infection

In China, a retrospective study of 1,552 patients reported a prevalence of active co-infection of 3.53% prior to ART initiation (Li et al., 2022). In Europe, a German cohort showed that 83.7% of HIV+ patients had anti-CMV IgG, indicating widespread prior exposure (Lichtner et al., 2012).

4.2 Virological Interactions

4.2.1 Interactions in the gut

4.2.1.1 HIV induces massive CD4 LT depletion in the gut favoring CMV reactivation

In the acute phase of HIV infection, the virus rapidly targets the GALT, causing massive depletion of local CD4 LT and disruption of the intestinal epithelial barrier. This direct damage to the mucosa facilitates the translocation of microbial products (such as LPS) into the systemic circulation, activating a chronic immune response and exacerbating inflammation.

4.2.1.2 CMV reactivation contributes to HIV dissemination, loss of gut integrity, mucosal translocation and inflammation

CMV reactivation, which is common in patients with advanced immunodeficiency (CD4 < 50 cells/mm³), further aggravates intestinal damage. CMV actively infects intestinal epithelial cells, as demonstrated in studies of rectosigmoid biopsies, where its replication leads to disruption of tight junctions between cells, reducing the integrity of the intestinal barrier(Maidji et al., 2017) (**Figure 39**). This disruption not only facilitates microbial translocation, but also increases the exposure of local immune cells to HIV, thereby increasing the viral reservoir. In addition, HIV/CMV co-infection stimulates the production of pro-inflammatory cytokines (such as TNFα and IL-6) and activates molecular pathways such as NF-κB that promote both replication of both viruses and chronic inflammation.

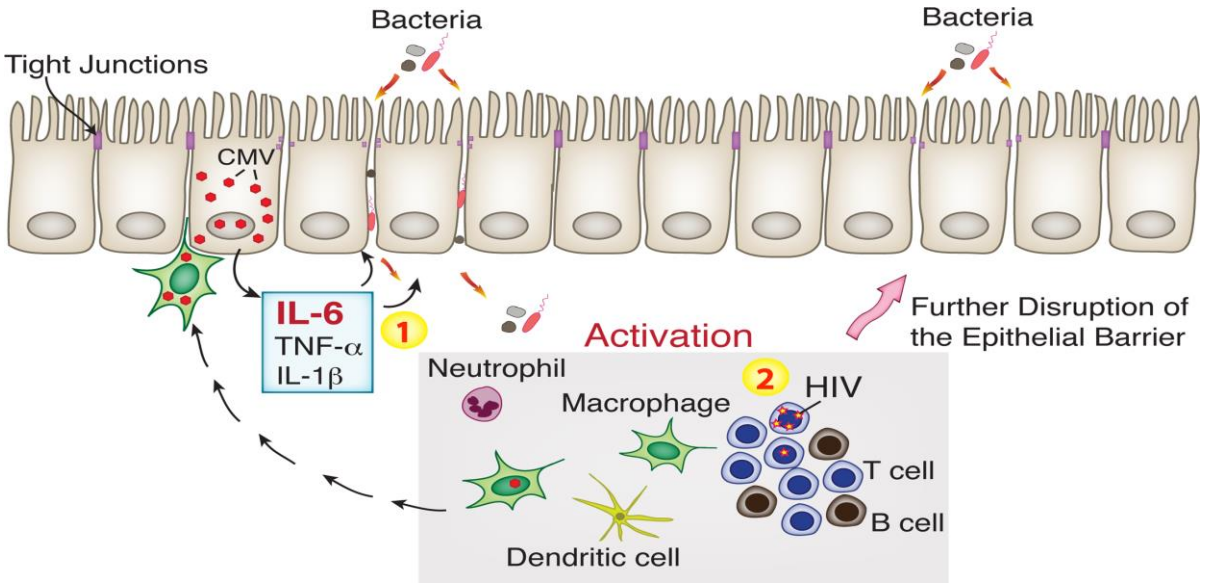


Figure 39 : Reactivation of intestinal CMV

Reactivation of CMV in the intestine induces local bacterial translocation, causing inflammation. Intestinal macrophages harboring reactivated CMV transmit the virus to intestinal epithelial cells. These cells secrete pro-inflammatory cytokines (IL-6, and possibly TNF-α and IL-1β), causing the tight junctions between the epithelial cells to break down. These alterations increase the permeability of the intestinal barrier, facilitating the passage of bacteria and microbial components (LPS) into the bloodstream. This permeability in turn triggers an influx of immune cells, which promotes inflammation. In addition, cytokines produced by CMV-infected cells promote reactivation of the latent HIV provirus, stimulating its replication. The resulting immune activation increases the destruction of the intestinal barrier, perpetuating a vicious cycle of inflammation, particularly in patients on ART (Maidji et al., 2017).

4.2.2 Interactions in the bone marrow

Studies reveal a complex interaction between HCMV and HIV in CD34⁺ HPC. These cells, which are essential for the production of blood cells, act as a latent reservoir for both viruses. HIV can persist latently in CD34⁺ cells, evading ART (Carter et al., 2010; McNamara et al., 2012), while CMV also establishes a quiescent infection in CD34⁺ cells without immediate active replication (Cheung et al., 2017). Bidirectional interactions are described in CD34⁺ progenitors (**Figure 40**).

- When previously CMV-infected CD34⁺ cells are exposed to HIV, HIV infection is significantly amplified. This is thought to be due to upregulation of HIV co-receptors (CCR5, CXCR4) and alteration of antiviral defense mechanisms, such as suppression of restriction proteins, making cells more vulnerable to HIV.
- Conversely, HIV promotes the maintenance of CMV latency in CD34⁺ cells through modulation of cell signaling pathways (e.g. NF-κB) or disruption of local immune responses, creating an environment conducive to the persistence of both viruses.

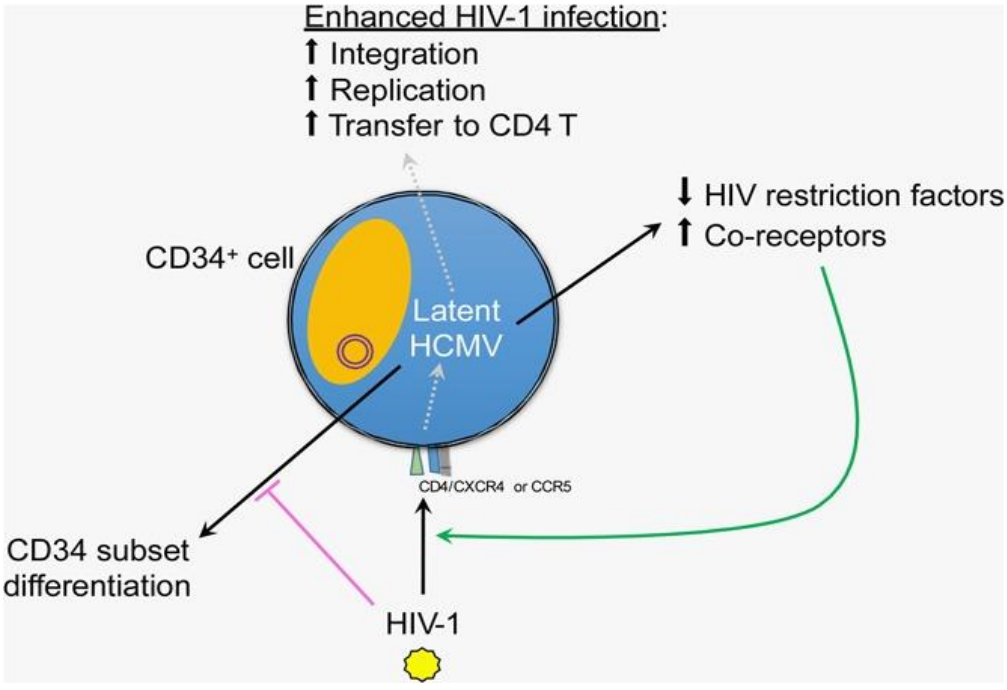


Figure 40 : Establishment of HCMV latency reprograms CD34+ cells to facilitate HIV-1 infection

HCMV latency reprograms CD34+ cells, which favors HIV infection. This mechanism involves increased expression of HIV entry co-receptors (CCR5 /CXCR4) and suppression of antiviral restriction factors in these cells, constituting a pro-viral cellular environment conducive to HIV replication (Cheung et al., 2017).

4.2.3 Molecular interaction between the two viruses

It has been described that HCMV can amplify HIV infection in vitro, by several mechanisms (**Figure 41**).

- CMV transactivator proteins derived from its IE gene region directly activate latent HIV proviral DNA in co-infected cells, thereby promoting viral replication (Davis et al., 1987; Johnson et al., 2018; Skolnik et al., 1988).

- CMV induces the release of cytokines (such as TNF-α) by surrounding immune cells, triggering indirect activation of the HIV provirus.

- Exposure to CMV Ags can reactivate latent HIV in CMV-specific memory CD4 LT by direct antigenic stimulation (Peterson et al., 1992).

- CMV may alter the tropism of HIV. The generation of viral pseudotypes (HIV enveloped in CMV glycoproteins) allows the virus to infect cells that express CMV receptors (Margalith et al., 1995). The CMV US28 gene plays a key role by encoding a chemokine receptor that facilitates HIV entry into CD4 LT independently of CCR5. In addition, CMV can activate CD4 expression in normally CD4-negative cells, broadening the spectrum of HIV target cells.

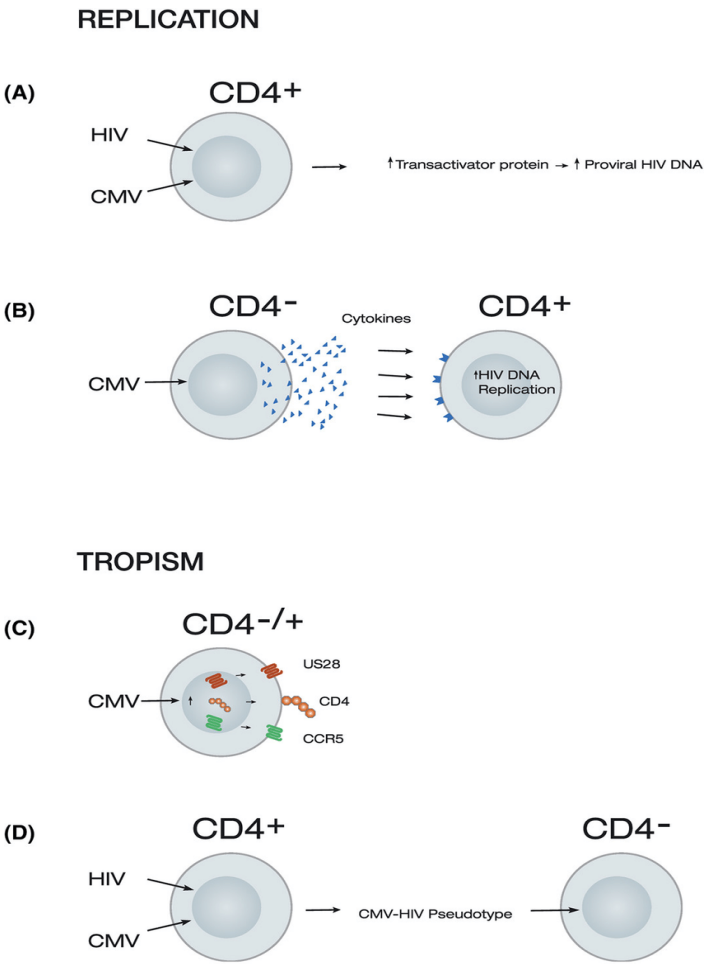


Figure 41 : Potential mechanisms by which CMV might affect HIV replication and tropism

CMV could promote HIV replication and extend its tropism via: (A) direct viral transactivation, (B) cytokine-dependent activation, (C) regulation of viral receptors, and (D) generation of viral pseudotypes (Grønborg et al., 2017).

- Finally, CMV expresses Fc receptors on its surface, which facilitate the infection of cells by HIV-antibody complexes. This occurs when non-neutralizing antibodies bind to HIV, enabling viral entry through Fc receptor-mediated mechanisms (Pleskoff et al., 1997).

These synergistic interactions between CMV and HIV may explain the deterioration of viral replication and contribute to the persistence of HIV reservoirs, highlighting the importance of controlling CMV co-infection in the management of HIV (Emery, 2015).

4.3 Pathophysiology

4.3.1 CMV coinfection in PWH with immunosuppression /AIDS

4.3.1.1 Still a reality in developing country

Co-infection with HIV and CMV was a major concern when ART was not available and PWH were progressing towards AIDS. The CMV infection is indeed a major clinical challenge in immunocompromised individuals. Before the ART era, CMV infection was one of the most common opportunistic infections in HIV patients, with about 40% developing severe clinical manifestations such as retinitis or colitis (Steininger, 2007). In co-infected patients with immunosuppression, the HIV-CMV co-infection worsens the pathogenesis and co-infected patients develop AIDS more rapidly, probably due to increased HIV replication and chronic inflammation induced by CMV (**Figure 42**).

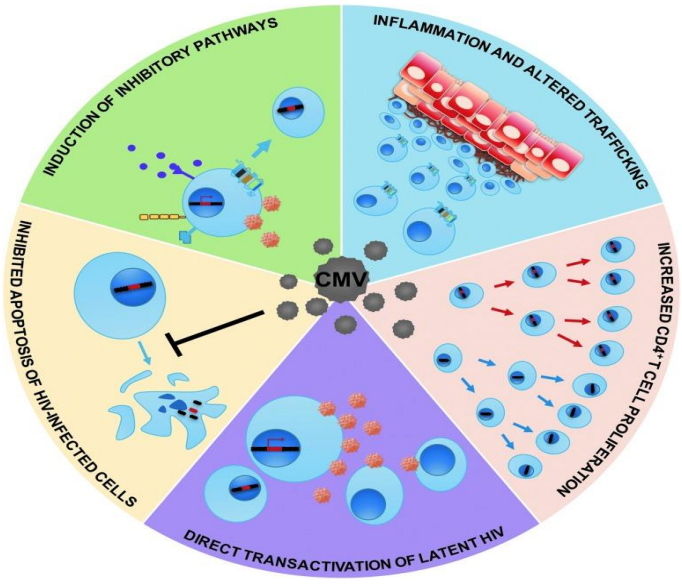


Figure 42 : Proposed mechanisms for CMV-facilitated HIV persistence

CMV may promote HIV persistence through several synergistic pathways. CMV-induced inflammation, inhibitory signals, stimulation of cell proliferation and inhibition of apoptosis could increase the pool of cells harboring latent HIV. At the same time, CMV could directly transactivate latent HIV, leading to increased viral dissemination. In this representation: CD4 LT (blue circles) contain or do not contain integrated HIV; inhibitory cytokines appear as purple dots; HIV viral particles are represented by orange circles; CMV by grey circles; and inflammatory epithelial cells by squared patterns (Christensen-Quick et al., 2017).

Since the introduction of ART, the incidence of CMV disease has fallen by more than 95%, making these complications rare in patients who have achieved sustained virologic control (Dioverti & Razonable, 2016; Jabs et al., 2007; Schwarcz et al., 2013). However, CMV seroprevalence remains high in elderly and low-income populations with limited access to screening and ART (Dioverti & Razonable, 2016). Socio-economic disparities exacerbate underdiagnosis, with up to 20% of cases in sub-Saharan Africa identified only at autopsy (Afessa et al., 1992; d'Arminio Monforte et al., 1997; Zhao et al., 2020), revealing a disseminated disease that remains undetected throughout life.

4.3.1.2 Current management of CMV in PWH

The management of CMV in PWH is based on an integrated strategy combining optimised ART and monthly virologic monitoring of CMV to allow targeted use of antivirals. ART prevents the majority of CMV reactivations by restoring CD4 counts above 100 cells/µL (Jabs et al., 2015). For IgG+ CMV patients with CD4 < 100 cells/µL, CMV viremia > 10,000 IU/mL (or > 5,000 IU/mL if CD4 < 50 cells/µL) defines a threshold associated with increased risk of complications (Zhao et al., 2020). If positive, a fundus examination is performed to identify early lesions in retina. Preemptive treatment with VGCV or foscarnet is initiated in cases of high (>5,000 IU/mL), ascending viremia or with risk factors (immunosuppression, active co-infection), as demonstrated in intensive care studies (Mayaphi et al., 2014; Mizushima et al., 2015).

Studies have shown that preemptive strategies using VGCV in patients on effective ART are not justified due to the low incidence of CMV disease in this population. A study by Wohl et al. confirmed the lack of any significant benefit from such preemptive treatment in patients with access to ART, despite the presence of CMV viremia or low CD4 LT counts (Wohl et al., 2009). These data reinforce the importance of rapid initiation of ART as the cornerstone of prevention, coupled with targeted anti-CMV treatment in the event of proven disease. The overall prognosis has improved considerably, with an estimated 3-year survival rate of 64% in France between 2001 and 2003 (Grabar et al., 2008), reflecting the combined impact of therapeutic advances and multidisciplinary management.

4.3.2 CMV/HIV coinfection in maternofetal context

Co-infection with HCMV may increase the risk of vertical transmission of HIV-1 in utero. The placenta, which normally provides a partial barrier to HIV becomes more permeable in the presence of HCMV. Studies show that placental cells pre-infected with HCMV exhibit a 2- to 5-fold increase in HIV-1 replication, with increased viral load and viral RNA expression (Johnson et al., 2018). This mechanism suggests that HCMV facilitates HIV transmission to the fetus by altering placental integrity and creating a pro-inflammatory microenvironment. In HIV-positive pregnant women, CMV screening and control could reduce the risk of neonatal HIV transmission. In infants born to mothers co-infected with HIV and CMV, co-infection leads to early immunodeficiency characterised by accelerated decline in CD4 counts, higher HIV viral load and a doubling of infant mortality, often due to hepatic or pulmonary complications (Slyker et al., 2009).

4.3.3 CMV coinfection in ART-treated PWH

4.3.3.1 Impact on immune reconstitution following ART introduction

CMV co-infection significantly impairs immune reconstitution in HIV patients on ART, particularly in the elderly. It exacerbates age-related thymic decline and reduces the production of naive CD4 LT (CD45RA+/CCR7+), which are essential for a diversified immune response. At the same time, CMV induces the clonal expansion of senescent CD8 LT ($CD28^{-}/CD57^{+}$), monopolizing immunological space and reducing the prevalence of polyfunctional LT. This limits the diversity of the antiviral repertoire. This dual process contributes to the incomplete immune reconstitution observed in many HIV-infected individuals despite sustained viral suppression under ART (M. L. Freeman et al., 2016; J. J. Heath et al., 2018; J. Heath & D. Grant, 2020).

4.3.3.2 Impact on the progression of the HIV infection

CMV seroprevalence is particularly high among PWH who are receiving ART and appears to be more prevalent in those with symptomatic HIV. This suggests a link between immunosuppression and CMV persistence (Rabenau et al., 2010). CMV infection influences HIV progression. In co-infected patients, CMV induces immune activation that can result in elevated production of pro-inflammatory cytokines (IL-6 and TNF-α,) and systemic markers

(CRP) (Adland et al., 2015). This inflammation contributes to accelerated immunosenescence. Clinically, CMV is associated with more rapid progression of HIV disease. A study conducted in Zimbabwe showed that each log10 increase in maternal CMV viral load increased the risk of infant mortality by 1.6 times, independently of HIV viral load (Evans et al., 2024).

4.3.3.3 Contribution to chronic inflammation

In well-controlled ART-treated PWH, CMV seropositivity is associated with a significant increase in markers of microbial translocation (LPS) and epithelial damage (I-FABP), highlighting the key role of CMV in the persistence of immune activation (Ramendra et al., 2020). This residual inflammation may contribute to long-term comorbidities such as cardiovascular disease or insulin resistance, and maintains an environment conducive to HIV replication. Although ART partially restores immunity and reduces the risk of serious opportunistic CMV disease (such as retinitis), asymptomatic reactivation of CMV is frequent. This reactivation contributes to the maintenance of a chronic inflammatory state, which is identified as a factor that increases the risk of comorbidities in patients living with HIV. CMV is considered a key factor in the immunological phenotype of premature ageing (inflammaging), which is characteristic of this population (Forte et al., 2020).

In conclusion, CMV acts as a major brake on immune recovery in PWH by maintaining an inflammatory environment and promoting premature lymphocyte senescence.

4.4 Immune dysfunctions associated with the CMV/HIV coinfection

4.4.1 *The notion of accelerated ageing*

HCMV appears to exacerbate premature immune senescence in the context of HIV and subsequently contribute to the accelerated onset of age-related comorbidities (cardiovascular pathologies, cognitive decline). In PWH, work by Lichtner et al, highlights that CMV co-infection is associated with a 40-60% increased risk of developing non-AIDS-related pathologies, such as cardiovascular, renal or liver disease, and a 30% increase in all-cause mortality (Lichtner et al., 2015). These effects persist despite virological control of HIV, suggesting that CMV acts through independent mechanisms such as immune senescence and prolonged release of pro-inflammatory cytokines, mimicking the effects of physiological ageing.

Barrett et al. describe this HIV/CMV interaction as a "perfect storm" where clonal expansion of CMV-specific CD8 LT accelerates replicative senescence. These cells, characterised by the loss of CD28 and expression of CD57, accumulate in inflammatory tissues, fueling a pro-senescent microenvironment via the SASP (Barrett et al., 2012). Heath et al. confirmed that CMV-targeted CD8 LT in PWH are near-senescent, reflecting premature immune ageing (J. J. Heath et al., 2018). Veel et al. documented an expansion of TEMRA CD8 LT and a reduction in naïve cells among co-infected patients, a profile typical of accelerated ageing (Veel et al., 2018). This phenomenon of (clonal) competition monopolizes immunological space, reducing T repertoire diversity and compromising responses to novel pathogens (Effros, 2016).

The study by Mueller MC et al**.** highlighted that CMV could be a dominant factor driving abnormal differentiation of CD8 LT subsets in these patients, surpassing even the impact of treated HIV (Mueller et al., 2021). These disruptions would not only promote the persistence of senescent cells in tissues but also propagate senescence via the SASP. The latter induces an inflammatory and pro-senescent microenvironment, affecting neighboring cells. These peripheral CD8 LT, recruited into these inflammatory tissues, perpetuate a vicious cycle, their excessive activation leads to clonal expansion, functional exhaustion, and accelerated telomere shortening.

People living with HIV on ART showed greater response of CMV-specific CD8 LT compared to the general population (as assessed by γ-IFN detection following stimulation), as well as better NK responses (as assessed by the percentage of $NKG2C^{+}$ $CD57^{+}$ NK cells) and antibody responses (J. Heath et al., 2016). This age-independent effect reflects chronic antigenic stimulation by CMV, exacerbated by HIV infection. Barrett et al illustrate this phenomenon by showing that PWH co-infected with CMV have a lower CD4/CD8 ratio, even after adjusting for age and HIV viral load (Barrett et al., 2016a). This immunological imbalance is a marker of premature ageing and increased risk of cardiovascular and metabolic comorbidities. HIV infection is associated with an increase in the absolute number of CD8 LT (Barrett et al., 2016b), a memory/senescent phenotype ($CD28^{-}CD57^{+}$) similar to that seen in elderly CMV-infected individuals (Blackman & Woodland, 2011). This expansion occurs despite effective HIV suppression and partial CD4 LT recovery (Barrett et al., 2016b).

Smith et al showed that even asymptomatic CMV replication in the early stages of HIV infection is associated with a lower CD4/CD8 ratio on ART, regardless of baseline viral load or

CD4 LT count (D. M. Smith et al., 2016). This observation highlights that the deleterious effects of CMV on immune balance begin early and persist despite HIV suppression (Effros, 2016).

CMV directs the repertoire of CD8 LT towards a senescent phenotype ($CD28^-$ $CD57^+$), characterised by loss of cytotoxic function and increased production of pro-inflammatory mediators. These cells, which persist on long-term ART, maintain residual immune activation (Naeger et al., 2010). The CD4/CD8 ratio, a clinical marker of immune competence, is often inverted (<1.0) in CMV co-infected patients. This low ratio may signal a disproportionate expansion of CD8 LT and may increase the risk of serious clinical events (cardiovascular pathologies, neurodegeneration) (Serrano-Villar et al., 2014). In HIV-infected children co-infected with CMV, these abnormalities (reduced CD4/CD8 ratio, terminally differentiated CD8 LT) are observed from the earliest stages, regardless of the detectable viral load (Lu et al., 2015).

4.4.2 Low grade inflammation and activation

In young adults starting ART, active CMV infection exacerbates CD8 LT activation and delays CD4 LT reconstitution, demonstrating the early impact of CMV on immunity (Ariyanto et al., 2018). Barrett et al, reinforce this observation by demonstrating that CMV-seronegative PWH show improved immune resilience, with normalised CD4:CD8 ratios and a reduction in senescent LT (Barrett et al., 2014).

The trial by Hunt et al. demonstrated that valganciclovir, an antiviral agent targeting CMV, significantly reduced immune activation in HIV-positive patients on antiretroviral therapy with incomplete immune reconstitution. In this randomised, double-blind, placebo-controlled study, 30 patients with persistent CD4 counts < 350 cells/mm^3 received 900 mg/day of valganciclovir or a placebo for 8 weeks. Valganciclovir suppressed CMV replication (undetectable DNA in biological fluids) and reduced the proportion of activated CD8+ T lymphocytes (CD38+/HLA-DR+ markers) by 4%, representing a relative reduction of approximately 20% compared to placebo. No impact on HIV viral load or CD4 count was observed (Hunt et al., 2011) . These results indicate that asymptomatic CMV replication significantly contributes to persistent immune activation in this population, suggesting that CMV control could improve the immunological health of co-infected patients.

These findings were expanded upon by Beck-Engeser et al., who reported an approximate one-quartile decrease in innate immune markers (sCD14, sCD163) during VGCV treatment

(*Beck-Engeser G et al., 2O11*). Clinically, this reduction in systemic inflammation corresponds to a modeled 22% decrease in cardiovascular risk and a ~50% reduction in type 2 diabetes risk (Brown et al., 2010; Tenorio et al., 2014).

CMV co-infection is also a key factor in chronic immune activation and systemic inflammation in PWH even when on effective ART (Schnittman & Hunt, 2021). This interaction worsens the immunological profile and contributes to non-AIDS comorbidities, blurring the boundary between natural ageing and viral pathology. CMV wages a silent war by amplifying the immunosenescence mechanisms common to ageing and HIV infection through residual immune activation and persistent inflammation (Effros, 2016).

CMV induces persistent antigenic stimulation leading to clonal expansion of activated CD8 LT ($CD38^+$ $HLA\text{-}DR^+$), reflecting a state of constant immune alert (Hunt et al., 2011). This clonal expansion is directly linked to an increase in systemic inflammatory markers (sTNF-RII, IP-10, IL-6) in ART-treated PWH, highlighting the link between residual CMV viral load and chronic inflammation (M. L. Freeman et al., 2016). These cells, often CMV-specific, become more active in the immune system, differentiate into senescent CD8 LT ($CD28^-$ $CD57^+$), a phenotype associated with premature immunosenescence (M. L. Freeman et al., 2016). Ballegaard et al show that while CMV-specific LT are clearly associated with immunosenescence, they do not directly correlate with systemic markers of inflammation (e.g. IL-6, CRP), suggesting different mechanisms for these two phenomena (Ballegaard et al., 2018). In parallel, residual CMV replication correlates with an increase in inflammatory mediators such as IL-1β, IL-6, IP-10 (Hunt et al., 2011) and CRP (M. L. Freeman et al., 2016). This chronic inflammation is exacerbated by the synergistic interaction between HIV and CMV, which disrupts immune homeostasis and promotes a persistent pro-inflammatory environment (M. L. Freeman et al., 2016). For example, higher production of γ-IFN by CMV-specific CD8 LT is associated with increased carotid intima-media thickness, an early marker of atherosclerosis in HIV patients (Hsue et al., 2006). High anti-CMV IgG titers are also associated with an increased risk of coronary lesions (Parrinello et al., 2012), highlighting the key role of the anti-CMV immune response in atherogenesis.

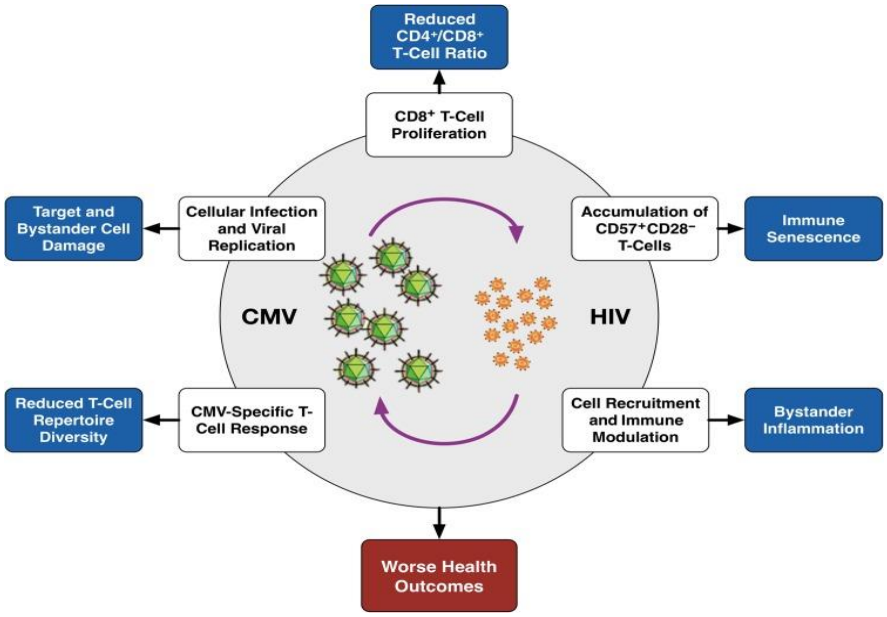


Figure 43 : The model highlights a vicious circle in which HIV and CMV reinforce each other's effects

HIV infection leads to the depletion and dysfunction of CD4 LT, rendering them unable to provide optimal support to CD8 LT. This immune failure promotes accelerated replication of CMV. Together, these viral infections exacerbate chronic inflammation, accelerate immune senescence and stimulate excessive CD8 LT proliferation. The association of this CD8 LT expansion with CD4 LT loss (lowering of the CD4/CD8 ratio) correlates with severe clinical complications in HIV-CMV co-infection. At the same time, CMV can directly damage endothelial cells and other tissues, exacerbating organ damage (Gianella & Letendre, 2016).

4.5 Clinical consequences

The efficacy of ART has transformed the prognosis of HIV infection, but it also reveals a paradox, patients are ageing prematurely, with an increased burden of non-AIDS related comorbidities. These conditions occur up to 15 years earlier in HIV-infected patients than in the general population (Serrano-Villar et al., 2014).

The clinical implications go beyond cardiovascular disease. CMV contributes to the frailty syndrome (sarcopenia, chronic fatigue, functional decline) in ageing PWH (Tavenier et al., 2019). This frailty is exacerbated by inflammaging, a persistent systemic inflammation that acts as a molecular bridge between HIV infection and accelerated ageing (Effros, 2016). Inflammatory markers such as fibrinogen, CRP and IL-6 are independent predictors of mortality in these patients (Kuller et al., 2008; Tien et al., 2010). In a clinical trial with valganciclovir, the reduction in CMV replication led to a significant decrease in sTNF-RII, confirming the central role of CMV in chronic inflammation (Schnittman & Hunt, 2021).

Neurocognitive decline is of particular concern. Letendre et al, showed that high levels of anti-CMV IgG are associated with reduced neurocognitive performance in patients on suppressive ART, even in the absence of active viral replication in the CSF (Letendre et al., 2018). This association suggests that CMV-induced chronic inflammation, via mechanisms such as the SASP, may impair brain function.

The synergy between HIV and CMV is not limited to neurological damage. Gianella et al showed that CMV replication in semen correlates with higher HIV DNA reservoirs in peripheral blood mononuclear cells (PBMC) . For each doubling of time on ART, viral DNA decay slowed by 0.14 log10 in patients with detectable CMV (Gianella et al., 2014). This phenomenon can be explained by CMV activation of pro-inflammatory pathways (NF-κB) that promote HIV latency.

Lichtner et al, found in the ICONA cohort that CMV seropositivity predicts an increased risk of serious non-AIDS events, independent of virological control (Lichtner et al., 2015).

5 Objectives of the study

As the population of PWH is naturally ageing, it is now important to consider how the PWH are ageing. Considering the well-documented impact of HCMV on ageing, we wanted to evaluate the impact of the HIV infection on CMV-specific CD8 LT. We hypothesise that the senescence associated with CMV-specific CD8 LT may differ in HIV/CMV coinfected PWH compared to HIV-/CMV+ individuals.

The aim of this project is to assess whether there are quantitative and qualitative modifications in CMV-specific CD8 LT responses in the context of chronic HIV. We examined the evolution of CMV-specific CD8 LT proportion (using pentamer staining) and phenotype (senescence, exhaustion, and activation) during HIV infection in various contexts of viral control. We analysed PBMC from spontaneous controllers (HIC) and from PWH with ART-induced control (ART). We benefited from samples collected from two cohorts of the ANRS-MIE: the ANRS-MIE CODEX cohort (that has enrolled PWH showing spontaneous control (i.e. viral control in the absence of ART), and the ANRS PRIMO cohort (that has enrolled PWH as soon as they were diagnosed for HIV infection). We compared these two populations with each other and with a control group of HIV-negative patients. We also aimed to evaluate associations between impairments in the anti-CMV responses and standard parameters evaluating the severity of HIV infection (nadir CD4, cumulative HIV viral load, residual inflammation and CMV avidity testing).

In the course of our work, we asked ourselves the following questions:

- In PWH coinfected with CMV, is there a difference in the quantity (proportion) or quality (senescence, exhaustion and activation) of the CD8 LT response directed against CMV compared with HIV-uninfected subjects?

- If so, are these differences in profiles specific to the CMV-specific compartment and/or linked to the chronic inflammation?

- Are there differences in these profiles between PWH under different control profiles (under ART treatment or spontaneous HIC control)?

We hypothesise different profiles of senescence, exhaustion, and activation in CMV-coinfected PWH patients compared to HIV-/CMV+ individuals.

MATERIELS AND METHODS

1 Study population

The study was conducted using frozen peripheral blood mononuclear cells (PBMC) isolated from two cohorts of PWH and a control group of HIV-negative individuals.

1.1 Three groups of participants

1.1.1 Group of patients on long-term controlled ART (n=40)

This group consisted of patients from the ANRS CO6 PRIMO cohort who had been monitored since the primary infection (i.e. the date of diagnosis) and treated with antiretrovirals. Samples were selected at timepoint where PWH had sustained control of viral replication for at least 5 years.

Among the PRIMO cohort, the inclusion criteria for the study were:

- Diagnosis of HIV infection more than 5 years ago;

- ART treatment started more than 4 years ago;

- CD4 LT count greater than 500 cells/mm^3 at last examination;

- Undetectable plasma viral load (HIV RNA <40 copies/mL) for more than 2 years, with tolerance to isolated blips not exceeding 400 copies/mL (according to standard tests).

- Patients co-infected with hepatitis B/D or C viruses were excluded.

1.1.2 Natural Control of HIV-1 group (n=40)

These patients, capable of spontaneously controlling the infection without treatment, were identified at the Bicêtre hospital in 2005 (Lambotte et al., 2005). HIC patients are characterised by an anti-HIV response that is both quantitatively and qualitatively superior to that of patients on long-term ART. Their HIV-specific CD8 LT are not only more numerous, but also have enhanced ex vivo suppressive capacities. These cells display remarkable polyfunctionality, simultaneously secreting multiple cytokines (TNF-α, γ-IFN, MIP-1β, IL-2) and

expressing the cytotoxicity marker CD107a, while showing high avidity for peptide-HLA complexes, an indicator of optimal Ag recognition (Sáez-Cirión et al., 2007).

Patients showing this profile of “spontaneous control” were enrolled in the ANRS CO21 CODEX cohort (currently comprising around 300 patients).

Among the CODEX cohort, the inclusion criteria for the study were:

- Documented HIV-1 infection for more than 5 years;

- Last five consecutive viral loads below 400 copies/mL;

- Complete absence of ART treatment during the control period.

- Patients co-infected with hepatitis B/D or C viruses were excluded.

Samples were collected from the biobank and we also had access to historical clinical, biological and virological data.

1.1.3 Seronegative Healthy Donor Control Group (n=40)

HIV-uninfected participants were recruited through the Établissement Français du Sang (EFS). No history of HIV infection or viral hepatitis was reported in this group. Samples were selected to provide equivalent age and sex profile than the PWH groups

1.2 Patient selection based on HLA

1.2.1 Generalities on HLA

The originality of the study is to evaluate the CD8 LT response using pentamer staining which require to identify the HLA allele of the participants and select HLA available in all groups. The detection of LT specific for a given peptide is based on the use of pentamers, molecular complexes formed by biotinylated HLA class I molecules coupled to avidin-phycoerythrin. These pentamers allow the detection of Ag-specific LT by direct labelling of their TCR with a complex of 5 MHC units bound to a specific peptide and therefore dependent on the individual's HLA status. This technique, first described in the context of CMV by Altman et al, makes it possible to identify within a polyclonal lymphocyte population those cells that specifically recognise a given Ag via their TCR receptor (Altman et al., 1996).

1.2.2 Complement-dependent cytotoxicity (CDC) or microlymphocytotoxicity test

The conventional and most widely used method for determining HLA antibodies is the cell-based complement-dependent cytotoxicity (CDC) test, introduced by Terasaki and McClelland in the early 1960s (Terasaki & Mcclelland, 1964). In this test, the patient's serum is exposed to a panel of lymphocytes from donors with known HLA typing, in the presence of complement. The presence of anti-HLA antibodies directed against Ags present on these lymphocytes activates complement, resulting in cell lysis. This lysis is visualised under the microscope after staining: the damaged lymphocytes appear stained in contrast to the intact cells.

Results are quantified by the percentage of cell lysis, with a positivity threshold of 20%. To distinguish between antibodies directed against HLA class I molecules (expressed on T and B lymphocytes) and those directed against class II molecules (present on B lymphocytes and APC), B and T lymphocytes are separated prior to testing. Thus, lysis of LT indicates anti-class I antibodies, whereas lysis of B lymphocytes indicates anti-class II antibodies.

Although this technique has since been superseded by more sensitive methods (e.g. Luminex tests), it remains a cornerstone in the history of clinical immunology, particularly for pre-transplant screening or the study of alloimmune responses.

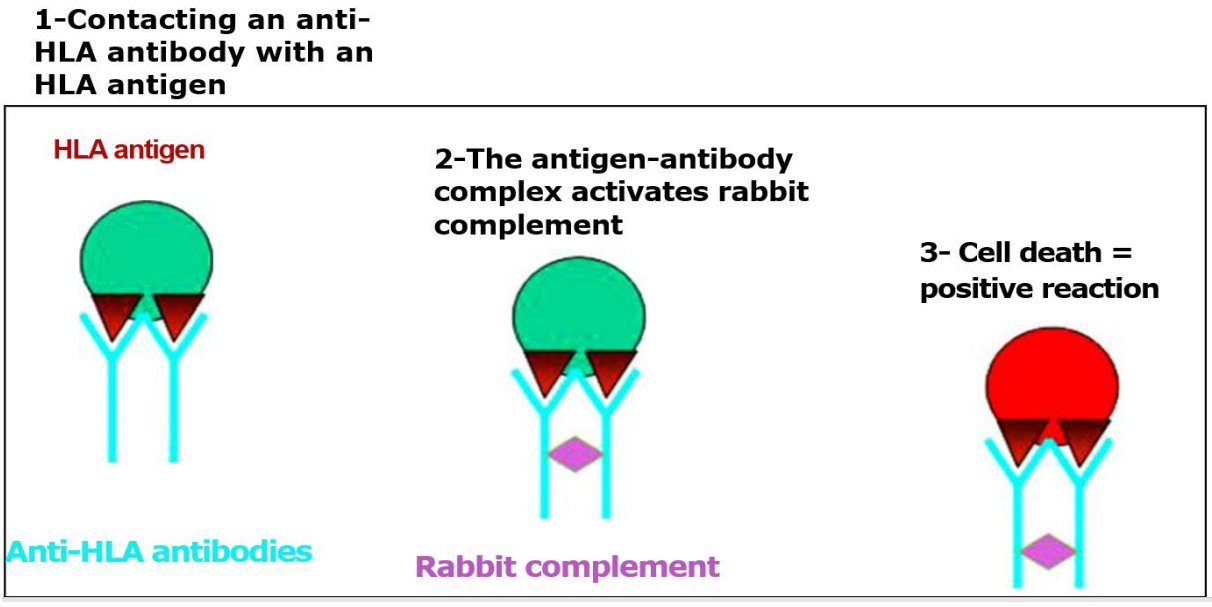


Figure 44 : Principle of a positive microlymphocytotoxicity reaction

Anti-HLA antibodies bind to HLA Ags on lymphocytes, which triggers the activation of the complement system. This leads to the formation of membrane pores and cell lysis. Lymphocyte death can be detected by the penetration of exclusion dyes, which confirms a positive reaction (Moalic, 2008).

The HLA typing was performed using lambda monoclonal trays (ONeLambda, Thermo-Fisher) that provide a plate with monoclonal antibodies and complement that are premixed in each well. To note, for the participants from the ANRS-MIE cohorts, additional HLA genotyping was performed.

1.2.3 Patients selected for the expression of HLA compatible with identification of CMV-specific cells

PWH were selected according to strict criteria previously described: they had to be in a controlled chronic phase of infection (duration of infection ≥ 5 years, undetectable viral load on treatment or spontaneous control) and matched to the control group for age, sex. To perform the CMV-specific pentamer staining, we also had to select patients based on the HLA status. We used the pentamer structure commercially available from ProImmune LTD, which define different sequences for immunodominant peptides of CMV in the following HLA: A*0201 (NLPMVATV, HCMV pp65), A*0301 (KLGGALQAK, HCMV IE1), A*2402 (QYDPVAALF, HCMV pp65), B*0702 (TPRVTGGGAM, HCMV pp65), B*3501 (IPSINVHHY, HCMV pp65). As a prerequisite, we evaluated the relative proportion of these alleles in the patient population to identify the alleles that were shared among the three groups and were compatible for CMV-pentamer detection.

2 Methods

2.1 Flow cytometry

PBMCs PWH with ART-induced control (ART), controllers HIC , and healthy uninfected donors were analysed by flow cytometry. CMV-specific pentamers were used to determine the percentage of CMV-specific pentamer-positive cells, alongside a broad panel of markers associated with distinct phenotypic profiles. Senescence was assessed by the expression of CD57, or CD57 in combination with KLRG1 and loss of CD28 ($CD57^+$ or $CD57^+$ $KLRG1^+$ $CD28^-$), whereas exhaustion was evaluated using PD-1 and 4-1BB. TCR-mediated activation was assessed via CD38 and HLA-DR, and non-TCR-mediated activation, corresponding to a bystander effect, was examined through CX3CR1, NKG2D and IL-15R.

Four panels were employed: the **CMV-specific CD8 T-cell panel**, comprising the CD8 T-cell panel and the CD8 T-cell bystander effect panel, the **NK panel** for NK cell analysis, and the **CD4 panel** for CD4 T-cell analysis. Analyses were performed on a **FACS Fortessa** configured for 14 parameters.

The antibody combinations used are presented in **Table 4.**

Antibody	Fluorochrome	Supplier	Clone	Cat number	Panel(s)
PD-1	BV421	Biolegend	EH-12-2H7	329920	CD8, CD8 Bystander Effect, NK, and CD4
CD28	BV510	Biolegend	CD28.2	302936	CD8, CD8 Bystander Effect, NK, and CD4
KLRG1	BV605	BD Bioscience	Z7-205rMab	568891	CD8 and CD4
CD215 (ILR15α)	BV605	BD Bioscience	IL-15Ra	747702	CD8 Bystander Effect
CD8a	BV650	Biolegend	RPA-T8	301042	CD8 and CD8 Bystander Effect
CD45RO	BV711	BD Bioscience	UCHL-1	563722	CD8, CD8 Bystander Effect, NK, and CD4

CD3	BV786	BD Bioscience	SP34-2	563918	CD8, CD8 Bystander Effect, NK, and CD4
CD56	PEVio770	Miltenyi	AF-12 7H3	130-113-308	NK
CD38	FITC	BD Bioscience		555459	CD8 and CD4
NKG2D	VioBrightB515	Biolegend	1D11	320820	CD8 Bystander Effect and NK
CMV-pp65 Pentamer	PE				CD8, CD8 Bystander
CD27	PE	Miltenyi	REA499	130-113-640	NK
CD57	PE-CF594	BD Bioscience	NK-1	562488	CD8, CD8 Bystander Effect, NK, and CD4
CX3CR1	PerCP-Cy5,5	Biolegend	ZA9-1	341614	CD8
41BB	PE-Cy5.5	BD Bioscience	4B4-1	551137	CD8 Bystander Effect
CD4	PerCP-Cy5,5	Biolegend	SK3	344608	CD4
CD27	PE-Cy7	BD Bioscience	M-T271	560609	CD8, CD8 Bystander Effect, and CD4
CCR7	APC	BD Bioscience	2-L1A	566762	CD8, CD8 Bystander Effect, and CD4
NKG2A	APC	Biolegend	S19004C	375108	NK
HLA-DR	APC-R700	BD Bioscience	G46-6	565127	CD8 and CD4
CD16	AF680/700	BD Bioscience	3G8	560713	NK

Table 4: Flow cytometry antibody panels for CD8, CD4, and NK cell analysis

RESULTS

1 HLA study prior to the study of CMV specific responses

1.1 Description of HLA-A allele frequencies in the PRIMO and CODEX cohorts

Among the PWH enrolled in the PRIMO cohort for which samples were available (n =708), the most common alleles were HLA-A*02 (21%), A*03 (11%), A*01 (10%), A*19 (8%), A*24 (7%), A*11 (5%) A*09 (4%), A*10 (3%) and A*28 (3%) (**Figure 45**). Only HLA-A alleles with a frequency greater than or equal to 3% were shown.

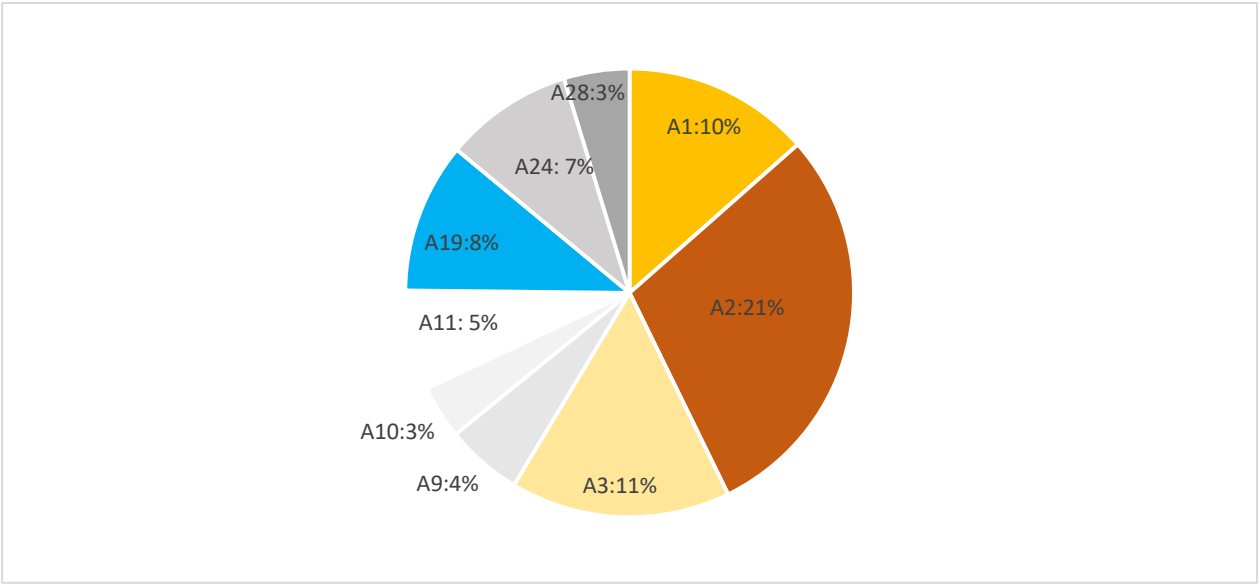


Figure 45: Distribution of HLA-A allele among PWH in the PRIMO cohort

Based on the data already collected in the cohort, we performed an analysis of the HLA-A allele distribution among PWH enrolled in the PRIMO cohort and for which frozen PBMC were available (n=708).

In the CODEX cohort (spontaneous controls HIC, n =359), the most common alleles were HLA-A*02 (17%), A*19 (15%), A*03 (12%), A*01 (7%), A*28 (7%), A*11 (6%), A*30 (5%) and A*10 (4%) (**Figure 46**).

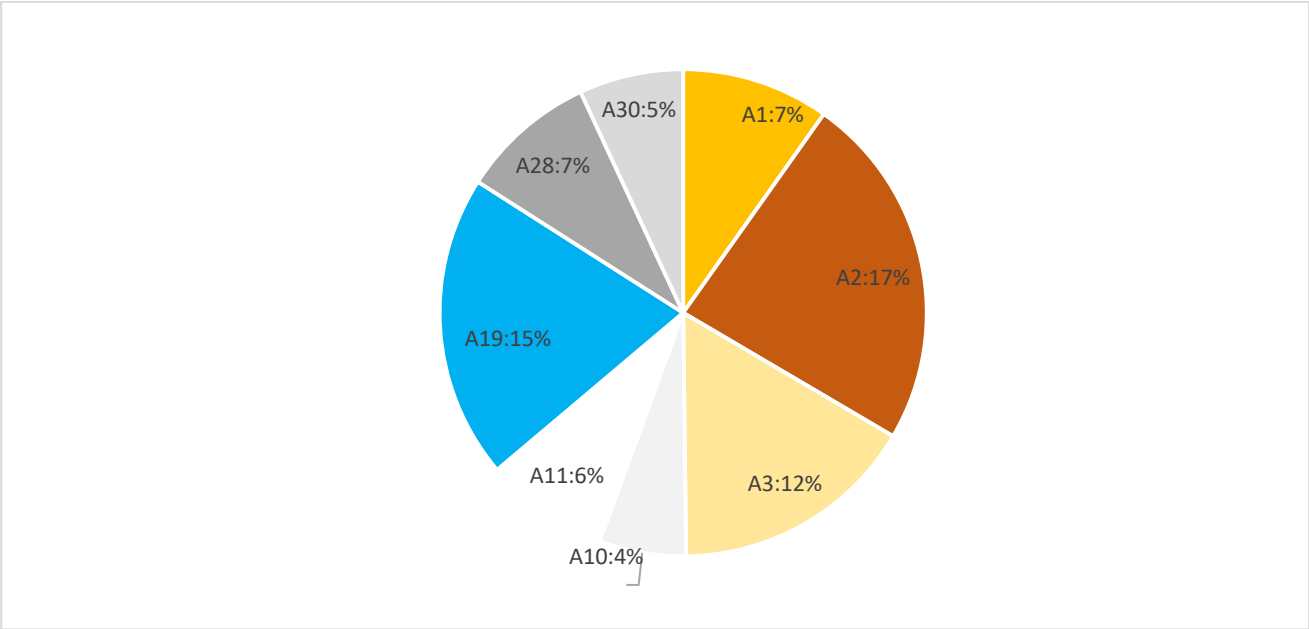


Figure 46 : Distribution of HLA-A allele among PWH in the CODEX cohort

Based on the data already collected in the cohort, we performed an analysis of the HLA-A allele distribution among PWH enrolled in the CODEX cohort and for which frozen PBMC were available (n =359).

Based on these distributions, only patients carrying the **HLA-A*02 and/or HLA-A*03** alleles were included in the final study, as these were the only two alleles significantly represented in both the PRIMO and CODEX groups.

1.2 Description of HLA-B allele frequencies in the PRIMO and CODEX cohorts

We performed the same analyses for the HLA-B alleles. Only HLA-B alleles with a frequency greater than or equal to 3% were included in the analysis.

In the PRIMO cohort, the most common alleles were HLA-B*07 (9.5%), B*35 (8,7%), B*44 (8,1%), B*08 (5,9%), B*18 (5,4%), B*12 (4,9%), B*14 (3,6%), B*51(3,6%) and B*40 (3,3%).

In the CODEX cohort the most common alleles were B*57 (11,4%), B*27 (7,1%), B*17 (7%), B*44 (5,8%), B*14 (5,6,%), B*07 (5,4%), B*15 (4,2%), B*08 (3,8%), B*40 (3,6%), B*51 (3,6%), B*70 (3,5%), B*35 (3,3%) and B*12(3,3%).

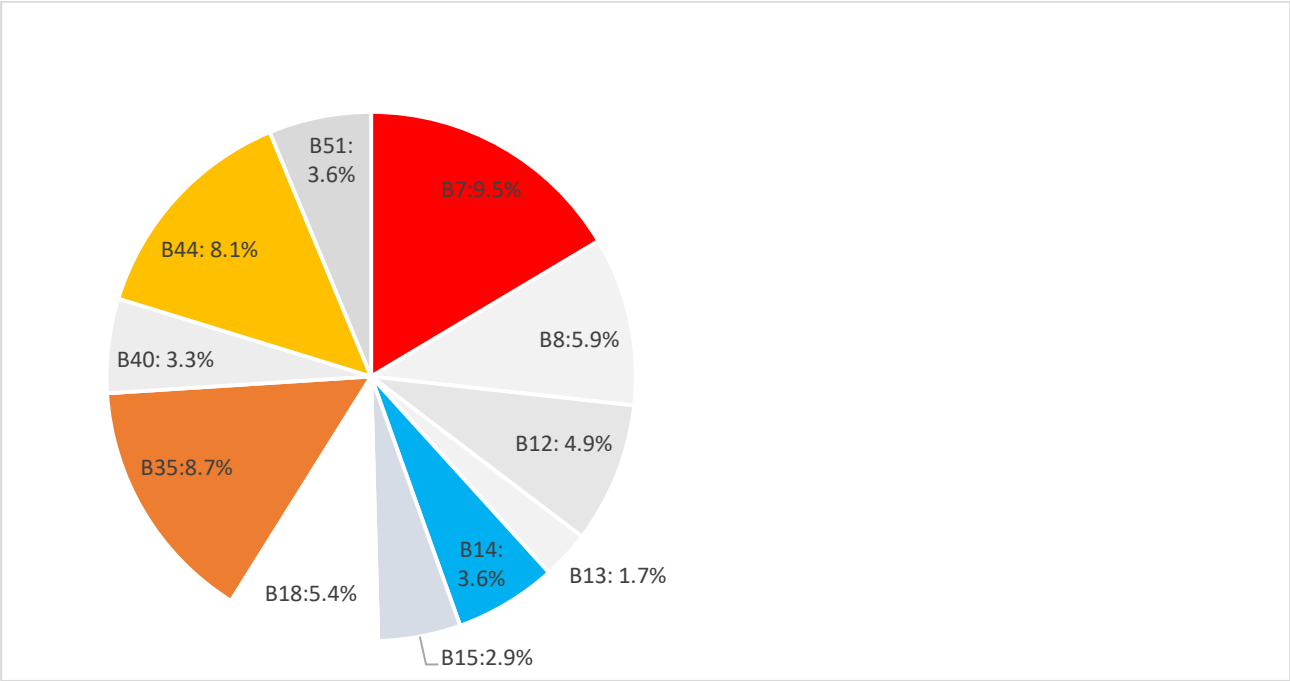


Figure 47 : Selection of patients with HLA-B*07 et HLA-B*35 in the PRIMO cohort (n=708)

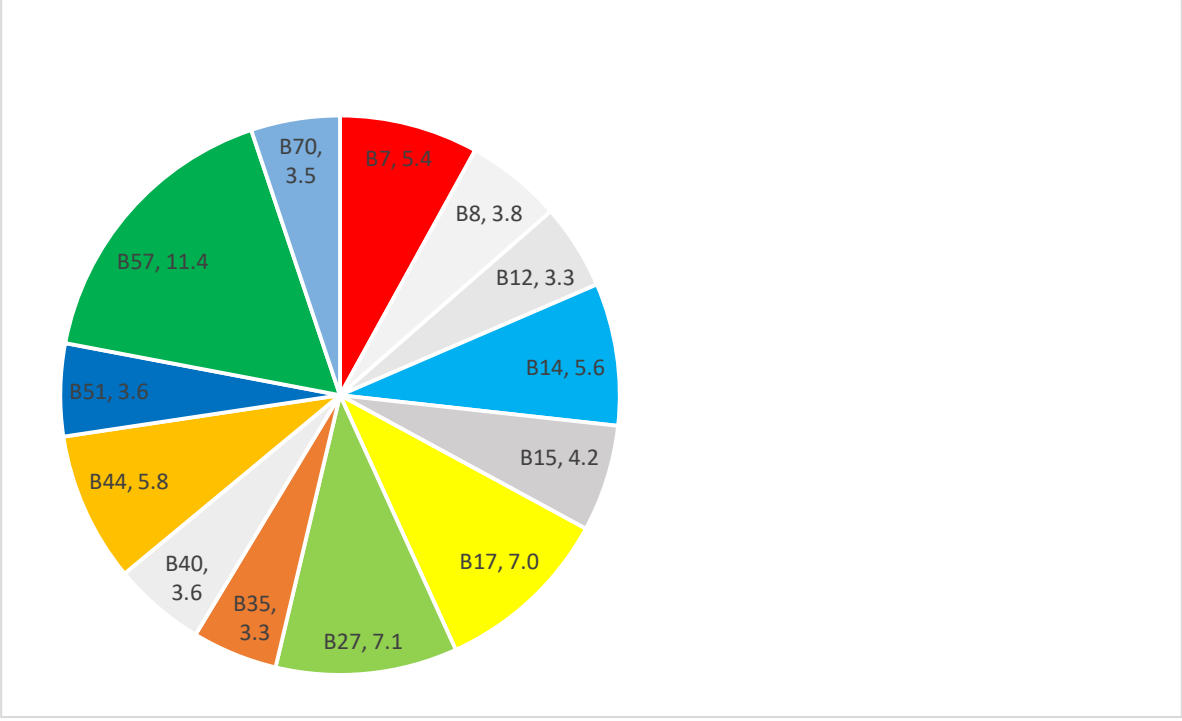


Figure 48 : Distribution of HLA-B allele among PWH in the CODEX cohort (n =359)

To allow a reliable comparison between the two cohorts according to HLA status, only patients carrying **HLA-B*07 and HLA-B*35** alleles were included in the final study, as these were the only two alleles significantly represented in both groups. Although HLA-B*40 and HLA-B*44

were also detected in both cohorts, they could not be analysed due to the lack of specific peptide pentamers for CMV-specific CD8 LT labelling.

Despite the initial heterogeneity in the distribution of HLA-B alleles between the PRIMO and CODEX cohorts, a methodological adjustment made it possible to compare the groups.

1.3 Comments on HLA alleles in HIV and CMV control

HLA class I molecules play a central role in controlling viral replication particularly through their ability to present viral Ags to CD8 LT. Each individual can express up to six different alleles of these molecules, two for each of the three HLA-A, HLA-B and HLA-C loci, providing a diversity essential for recognition of a wide range of viral epitopes.

In the context of HIV infection, the HLA allele is of importance

- HIV partially evades this immune response via its Nef protein, which selectively reduces the expression of HLA-A and HLA-B - which are more sensitive due to structural differences - while sparing HLA-C and HLA-E, thereby exposing infected cells to NK cell cytotoxicity (G. B. Cohen et al., 1999). This viral regulation highlights the delicate balance between immune evasion and susceptibility to innate mechanisms.

- Homozygosity at HLA class I loci, by limiting the diversity of CD8 LT responses, correlates with rapid disease progression, highlighting the benefit of a diverse allelic repertoire (Carrington & O'Brien, 2003).

- Among HICs, a high frequency of specific (protective) alleles such as HLA-B*27:01, HLA-B*57:01, HLA-B*58:01, HLA-A*25:01, HLA-B*13, HLA-B*14:02, HLA-B*52:02 has been observed, in contrast to alleles such as HLA-B*35 or HLA-B*07, which are associated with accelerated progression to AIDS (Carrington & O'Brien, 2003; Pereyra et al., 2010).

Among the protective alleles, HLA-B*57 and HLA-B*27 stand out for their affinity for conserved viral epitopes favoring robust memory LT responses and durable suppression of viral replication (Altfeld et al., 2003; Fellay et al., 2007). Nearly 67% of HICs carry at least one of these alleles, compared with 37% of non-HICs, suggesting a major genetic advantage (Emu et al., 2008). Synergistic interactions between HLA class I and NK cell KIR receptors, such as the HLA-B*57/KIR3DL1*001 association, enhance viral control by combining adaptive and innate mechanisms (Boulet et al., 2008; Martin et al., 2007).

In the context of HCMV infection

The CD8 LT response to HCMV is modulated by specific viral peptides presented by different HLA class I allotypes. Key immunodominant targets include peptides derived from pp65 (e.g. NLVPMVATV) and IE1 (e.g. TPRVTGGGAM) (Giest et al., 2010; Hyun et al., 2017).

The HLA-A*02:01 and HLA-B*07:02 allotypes are associated with robust CD8 LT responses, particularly in HLA-A*02:01 carriers, where almost half of individuals demonstrate strong reactivity against pp65. HLA-A*01:01 is associated with significant responses in certain cohorts (15–20% of carriers), but does not achieve systematic immunodominance status (Giest et al., 2010). Conversely, HLA-C allotypes (excluding HLA-C*08:01) and HLA-B*35:01 are associated with weak or absent CD8 LT responses and fail to reach the critical threshold for protection (Hyun et al., 2017). For HLA-A*03:01, responses are variable, although this allotype has IE1-derived epitopes, such as VLEETSVML, they are recognised less intensely and are highly dependent on the host's genetic context.

2 Perturbation of the CMV-specific CD8 T cells compartment in people living with HIV, suggesting ongoing senescent process in PWH

The result section is presented in the form of an article that is still under preparation.

Background: With HIV now a chronic disease in developed countries, many efforts are being focused on ensuring that people living with HIV (PWH) age well. Indeed, the introduction of antiretroviral treatment (ART) has made it possible to achieve a lifespan similar to that of uninfected people, although the incidence of comorbidities remains higher. CMV infection is included in the immune risk phenotype defined to identify people at higher risk of morbidity and mortality among the elderly (>85 years). Co-infection with HIV and CMV constitutes a context of exhaustion and senescence which may alter the control of CMV and/or the dynamics of the accumulation of CMV-specific senescent cells.

Materials and methods: Frozen peripheral blood mononuclear cells (PBMC) from 40 to 45 individuals per group were analysed by flow cytometry using pentamer labelling and markers associated with senescence, exhaustion, activation and differentiation profiles (CD57, KLRG-1, PD-1, CD38, HLA-DR, CX3CR1, NKGD2 and IL-15R). We also analysed the CD4 T and NK cell compartments. CMV serology and IgG avidity were determined on plasma samples.

Results: PWH had a significantly lower percentage of CMV-CD8 cells than uninfected donors (1.6% on ART and 1.0% in HIC vs 9.8% in HD, $p<0.001$), although anti-CMV IgG titers and avidity did not differ. Interestingly, PWHs showed an attenuated senescent profile among CMV-CD8, with reduced expression of CD57+KLRG1+CD28- and an increase in inflammaging markers such as CX3CR1. This apparent contradiction was explained by the greatly reduced survival of CMV-specific CD57- cells in PWH ($p<0.01$), limiting their differentiation into senescent CD57+ cells. In addition, our data suggest an increased contribution of bystander mechanisms to maintain the CMV-CD8 cell compartment (higher fraction of NKG2D+ and IL-15R+ among CD57+ cells), suggesting a profound remodeling of the CMV-specific compartment under the effect of chronic HIV infection.

Conclusions: We observed quantitative and qualitative changes in the CMV-CD8 compartment, in chronically infected PWH. The reduction in their proportion is at least partially linked to a defect in the survival of CD57- cells, limiting the replenishment of the CD57+ CMV-CD8 compartment. The attenuated senescent profile, and the altered differentiation (with more MCTs and fewer senescent TEMRAs) also fit with an early depletion of the CMV-CD8 cell pool, and may reflect accelerated ageing in PWH.

This manuscript is based on the first set of data obtained and analyzed during the course of the thesis. The final manuscript may provide a different set of results, as only a fraction of samples were treated.

Perturbation of the CMV-specific CD8 T cell compartment in people living with HIV, suggesting ongoing senescent process in PWH

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Introduction

Because HIV is now a chronic disease in developed countries, many efforts are focusing on the well ageing of people living with HIV (PWH). Indeed, the introduction of ART allowed reaching similar lifespan compared to uninfected individuals although the incidence of comorbidities remains higher (Croxford S, et al. 2017; Marcus JL, et al. 2020, Trickey et al. 2023). In the general population, ageing is associated with defects affecting various organs including the immune system (Oh SJ. Et al, 2019), leading to a higher incidence of comorbidities (cardiovascular, neurodegenerative, …). The immune senescence includes a reduction of immune progenitors, a pro-inflammatory profile called inflammageing, … (Liu Z et al, 2023). The cytomegalovirus (CMV) infection may greatly contribute to the occurrence of age-related immune dysfunctions (Muller L. et al, 2024) by the specificities of the host response to the virus including the development of a pro-inflammatory immune response and a phenomenon of memory inflation limiting the T cell repertoire diversity (Vasto, S., et al., 2007). The CMV infection is included in the immune risk phenotype defined to identify people with a higher risk of morbidity and mortality among the elderly (>85 years) (Strindhall J. et al, 2007: Pawelec G. et al, 2009). The immune system of PWH exhibits some persistent defects despite viral control. It includes T cell exhaustion, myeloid dysfunction, and accelerated ageing, with high heterogeneity among PWH. HIV controllers who represent an extreme profile of control, i.e. PWH capable of controlling the virus spontaneously without ART (Lambotte O. et al, 2005) also present some degree of T cell exhaustion and myeloid dysfunction, albeit at lower levels. On the other hand, PWH treated in the late stage present the most marked immune defects. Some studies also mention the link between the severity of the acute/primary phase and the long-term inflammatory signature (Novelli et al., 2020). In clinic, a concern is the impact of natural ageing on the already defective immune system of PWH that may aggravate the defects of the immune system and contribute to the higher incidence of comorbidities. The impact of

CMV infection in PWH is well documented, as the prevalence of CMV/HIV coinfection is high (Robain M. et al, 1998). During the primary phase, the CD4 depletion in the gut may allow CMV reactivation that in turn increases the pool of activated T cells, i.e. the pool of cells targeted by the virus, thus favoring viral dissemination (Gianella S, et al 2015). To note, the direct demonstration of this close link between HIV infection and CMV reactivation is still missing. During the chronic phase, the impact of CMV coinfection is less consensual, which is expected regarding the heterogeneity of immune profile among PWH. The introduction of ganciclovir leads to a reduction of the fraction of activated T cells, suggesting that a fraction of T cell activation is partly driven by immune responses directed against CMV (Hunt PW, et al. 2011). However, the consequences of the CMV infection on the immune ageing of PWH have not been discussed which is surprising as the prevalence of CMV seropositivity is higher in PWH compared to the general population. In this article, we focused on three aspects of the CMV response that are highly associated with immune senescence. (i) The proportion of CMV specific CD8 T cells, to evaluate the impact of HIV infection on memory inflation; (ii) The phenotype of CMV specific and nonspecific CD8 T cells, to evaluate the respective senescent profile of these cells (using either CD57+ or CD57+KLRG1+CD28- definition); (iii) Some functional assays to evaluate their survival capacity and their cytotoxic potentials. Additionally, we analysed the impact of HIV infection on CD4 T cells and NK cells, two subsets also involved in the response directed against CMV. We studied PWH showing efficient viral control either by ART treatment or by spontaneous control, monitored in two French cohorts of PWH: the ANRS-MIE C06 PRIMO cohort and the ANRS-MIE C021 CODEX cohort. These cohorts enroll respectively PWH from the diagnosis and are then monitored regularly (PRIMO cohort) and PWH who control the virus spontaneously for more than 5 years. This rare fraction of PWH (0.5%) are called HIV controllers.

The majority of PWH are coinfected by CMV. The impact of such coinfection has not been clearly defined although bidirectional interferences have been hypothesised. HIV infection may contribute to CMV reactivation and disruption of anti-CMV responses, as a consequence of CD4 T cell depletion, notably in the gut mucosa. Secondarily, CMV reactivation increases the proportion of activated T cells that are more prone to HIV infection. The use of ganciclovir reduces the proportion of activated T cells in PWH, confirming the close link between the two infections. These mechanisms may mostly occur during the primary phase of the HIV infection, but the interferences in the chronic phases of HIV infection remains to be further evaluated. At long-term, one may discuss the potential deleterious effect of these two infections driving respectively T cell exhaustion and T cell senescence. As PWH are progressively ageing, one may fear exacerbated induction of senescence among CMV specific CD8 T cells in PWH. In noninfected individuals, long-term CMV infection translates in memory inflation, i.e. overrepresentation of CMV specific T cells among the memory compartment. CMV infection also constitutes a deleterious factor in aged individuals, being associated with the immune risk profile. On the other hand, CMV specific CD8 T cells exhibit a terminally differentiated profile, ensuring long-term control of CMV. The coinfection of HIV and CMV constitutes a setting of exhaustion and senescence that may alter the control of CMV and/or the dynamic of CMV-specific senescent cell accumulation.

Material and methods

Ethics statement

All participants provided written, informed consent to participate. The study was approved by the local independent ethics committee (CPP Ile-de-France VII, Paris, France; approval reference: 05–22 for ANRS-MIE CO21 CODEX and CPP Ile-de-France III, n. 115 for ANRS-MIE C06 PRIMO). Samples from UI were collected from the blood agency EFS (Etablissement Français du Sang) at Saint-Louis and La Pitié-Salpêtrière Hospitals (Paris, France).

Cohort participants

Samples from PWH are collected from two French national cohorts supported by the ANRS-MIE. The Codex cohort, standing for "cohort for the extreme", is the French national and multicentric cohort recruiting participants showing natural control of the infection without introduction of ART for at least 5 years since infection. The cohort recruitment started in 2011 and is still ongoing and ensures yearly monitoring. The inclusion criteria are the following: known HIV infection for more than five years, five most recent consecutive plasma HIV RNA determinations below 400 copies/ml [testing performed at a median interval of seven months (IQR) without ART (Lambotte O, et al. 2005). The Primo cohort is another French national and multicentric cohort recruiting participants as soon as the diagnosis is established (median of 28 days), before the initiation of ART. The Primo cohort ensures longitudinal monitored (every year during the first three years, at year 5 and then every 5 years). The cohort's recruitment started in 2011 and is still ongoing (Novelli et al., 2020).The latter cohort allows study of long term PWH with exhaustive characterisation of their clinical and biological parameters collected as soon as diagnosis. The time of introduction of ART is monitored as well as the ART combination. Samples from both the chronic phase and the primary/acute phase were collected

from different participants. HIV RNA detection assays with a detection limit of 40 copies/ml were performed on all samples from the Primo cohort.

In both cohorts, we selected samples from participants showing more than 5 years of controlled infection, which is the criteria for the enrollment in the Codex cohort although the exact date of infection is unknown and is based on the first date of diagnosis. Samples at similar period of infection were selected in the primo cohort in which the date of infection is estimated based on the Fiebig classification. As CMV-pentamer technology is not available for all HLA, we further selected participants expressing either/or HLA-A*02, HLA-A*03, HLA-B*07 and HLA-B*35. The HLA genotype is available for the participants enrolled in the two cohorts (Hopital Saint Louis). We tested blood donors using xx assay.

CMV serology

CMV-IgG were measured with LIAISON XL (LXL, DiaSorin®, Saluggia, Italy) completed with CMV-IgG avidity performed on VIDAS (bioMérieux®, Craponne, France) CMV-IgG avidity. CMV-IgM were not tested as all samples were positive for CMV-IgG and showed high avidity.

Flow cytometry

Cryopreserved peripheral blood mononuclear cells (PBMCs) were thawed and numerated before staining. The PBMCs were isolated from EDTA-anticoagulated blood using Ficoll density gradient centrifugation. Cells were cryopreserved in fetal bovine serum supplemented with 10% dimethyl sulfoxide. The following fluorescent labeled MHC class I pentamers (Proimmune LTD, Oxford, UK) were used: A*02:01- NLVPMVATV (specific for the HCMV pp65), A*03:01- KLGGAlQAK (specific for the HCMV -IE1), B*07:02 - TPRVTGGGAM

(specific for the HCMV pp65) or B*35:01 – IPSINVHHY (specific for the HCMV pp65), in combination with the Pro5 fluorotag secondary reagent. The staining protocol was as followed: 2 million cells were first incubated with one specific pentamer (when participants showed multiple HLA compatible with CMV pentamer staining, each pentamer was tested individually). Samples were washed and incubated with human Fc block for 20 min at room temperature before incubation with labeled antibodies against the following surface markers: CD3 BV786, CD8 BV650, PD1 BV421, CD28 BV510, CD45RO BV711, HLA-DR AR700, CCR7 APC, CD38 FITC, DE57 PECF594, CD57 PECy7, mixed with brilliant violet buffer. Additional combinations for further characterising the CD8 T cell, CD4 T cell and NK cell compartment included CD215 (IL15Rα), NKG2D (FITC), 4.1BB (PerCP-Cy5.5), CD56 (PEVio770), CD16 (AF700), CD4 (PerCP Cy5.5)

After fixation with PFA, samples were acquired on the FACS BD Fortessa and analysed using the FlowJo software.

Functional assay for cytokine production, cytotoxic potential and survival assay

The capacity to secrete cytokines and exhibit a cytotoxic potential was evaluated on thawed PBMCs unstimulated or stimulated for 4 hours with PMA (100 ng/mL) and ionomycin (500 ng/mL) in the presence of brefeldin A (1 μg/mL) in RPMI medium, supplemented with 5% SVF. Cells were then stained for intracellular markers: IL-2, TNF, IFN-γ, CD107a after extracellular staining for CD3, CD8, CD57, KLRG1, CMV-specific pentamer.

The survival assay was performed by in vitro incubation of 2 10^6 PBMC for 24h in RPMI medium supplemented with 5% SVF at a concentration of 0.5 10^6/mL. At 24h, equal quantities of beads were added to the samples, the samples were then stained for extracellular markers and the acquisition of beads and cell suspension allowed the quantification of each cell subsets by cytometry (BD count, Becton-Dickinson) to determine the survival capacity.

Statistical analyses

Quantitative and qualitative data are reported as medians [IQR] and numbers (%), respectively. Statistical analyses were performed with GraphPad Prism software (version 10, San Diego, USA). Intergroup comparisons (one-way ANOVA test on GraphPad), followed by multiple comparison assays were performed with nonparametric, unpaired Kruskal–Wallis tests for continuous data and chi-square or Fisher's exact test for categorical data. Statistics for the comparison for paired values among each participant was performed using the Mann-Whitney test.

RESULTS

Patients' characteristics

Ninety persons living with HIV (PWH) for more than 10 years in median including ART-treated patients (n=46) and spontaneous controller (n=44) and 20 uninfected (UI) participants were included in this study. We selected participants seropositive for CMV (approximately 80% of the participants enrolled in the cohorts) and exhibiting either HLA-A2, HLA-A3, HLA-B07 or HLA-B35 alleles, as those alleles were compatible with CMV-specific CD8 T cell detection. The three groups were equivalent in the frequency of HLA-A*02, HLA-A*03 expression but differed for HLA-B*07 and HLA-B*35. The high proportions of HLA-A*02 and A*03 alleles in the French cohort of PWH studied were expected based on the high proportion of caucasian individuals in the French cohorts. The HLA-B*07 allele was highly represented in UI (80%) whereas the HLA-B*35 allele was less represented in the group of spontaneous controller, as previously described (Juarez-Molina et al., 2014). The three groups were equivalent for age, sex and the two groups of PWH were equivalent for estimated infection duration and infection parameters (viral load, and CD4 count).

Reduced proportion and reduced senescent profile of CMV-specific CD8 T cells in PWH

We compared the proportion of CMV-specific CD8 T cells using pentamer staining for the immunodominant epitope of CMV in the context of the four alleles studied (**Figure 1A**). The proportion of CMV-specific CD8 T cells was drastically lower in PWH than in UI (1.6 [0.6-3.8] in ART, 1.0 [0.3-2.6] in HIC compared to 9.8 [3.6-15.8]) (**Figure 1B**). No difference was detected between ART and HIC PWH (p=0.439). We next evaluated the impact of the different HLA alleles by different secondary analyses (**supplemental Figure 1)**. We studied the percentage of CMV specific CD8 T cells in PWH expressing more than one of these alleles. Among the 15 UI participants having more than one allele, the percentage was equivalent

regardless of the HLA allele considered (**Sup Fig 1A**). When considering participants showing two or more alleles compatible with CMV pentamer staining, we evaluated the proportion of CMV-specific CD8 T cells provided by two alleles (**Sup Fig 1B**), confirming the high proportion of CMV-specific CD8 T cells in UI participants, representing 25.6 % [15.0-32.4] of total CD8 T cells compared to 5.1 [1.9-11.7] and 4.2 [1.6-12.9] respectively among ART and HIC. Additionally, we also observed the lower percentage of CMV specific CD8 T cells among PWH when considering respectively either the HLA-A*02, HLA-A*03, HLA-B*07 or the HLA-B*35 alleles bearing individuals (**Sup Fig 1C**).

The quantitative change in the CMV-specific CD8 T cell pool was also associated with some qualitative changes. The percentage of activated CD8 T cells (as assessed by the percentage of HLA-DR+ CD38+ cells) was low in all groups. When comparing the activation profile between CMV-specific and nonspecific CD8 T cells, UI showed lower activation among CMV-specific cells compared to nonspecific CD8 T cells. Such difference was not detected in PWH. Focusing on the impact of HIV infection on the CMV-specific compartment, we observed higher proportion of activated CMV-specific CD8 T cells from PWH compared to uninfected participants, which may indicate some higher degree of activation of the CMV-specific CD8 T cells in PWH. Additionally, no difference was detected in the activation profile of total T cells between the two groups. We also studied the percentage of CX3CR1+ CD8 T cells, a chemokine receptor associated with memory T cell migration (Panigrahi S, et al 2020; Bottcher et al., 2015), activation and/or differentiation in chronic context (Gerlach et al,2016). In the context of CMV infection, CX3CR1+ CD8 T cells are also associated with inflammageing, they are named inflammescent, as reflection of their senescent and pro-inflammatory profile (Morris 2020). In contrast to HLA-DR and CD38 whose expression were limited to a small fraction of CD8 T cells, CX3CR1 was largely expressed, especially on CMV-specific CD8 T cells. We observed a higher proportion of CX3CR1+ cells among CMV-specific CD8 T cells in PWH compared to UI (**Figure 1C**). The changes in CX3CR1+ percentage was observed both in

CMV-specific and global CD8 T cell fractions. We also evaluated the senescent phenotype of CMV-specific CD8 T cells, a standard phenotype commonly associated with CMV-specific T cells (Ouyang et al., 2003) , by assessing the percentage of CD57+KLRG1+CD28- CD8 T cells. As expected, the proportion of senescent cells was higher among CMV-specific CD8 T cells compared to total CD8 T cells, in both PWH and noninfected participants. Interestingly, HIV infection differently impacted the senescent profile of CMV-specific and total CD8 T cells: we observed a lower proportion of senescent CMV-specific CD8 T in PWH compared to noninfected participants, whereas the senescent profile was higher among total CD8 T cells. Similar observations were observed when phenotyping senescent T cells only based on CD57 expression (**Sup Fig. 2A**). Although the same trend was observed when distinguishing ART, HIC and UI participants, statistical difference was only detected when the two groups of PWH were pooled (**Sup Fig. 2A**). We also observed the lower percentage of CD57+ cells among CMV-specific CD8 T cells among PWH when considering respectively either the HLA-A*02, HLA-B*07 or the HLA-B*35 alleles bearing individuals, but not the HLA- A*03 (**Sup Fig 1C**). Lastly, we evaluated the exhaustion profile of CMV-specific CD8 T cells in the context of HIV. Interestingly, we observed a higher percentage of PD-1+ cells among CMV-specific CD8 T cells compared to global T cells, regardless of the infectious status. However, we could not detect a change in the percentage of PD-1+ cells among CMV-specific or not CD8 T cells between PWH and noninfected participants. Collectively, we thus observed an impact of chronic HIV infection on the CMV-specific CD8 T cells compartment, unexpectedly combining lower percentage of CMV-specific T cells with lower percentage of cells exhibiting a senescent profile in PWH compared to noninfected participants.

The decrease in the expression of senescent marker is driven by intrinsic changes among TEM and TEMRA

As the senescent profile is also highly related to the memory differentiation profile, we performed similar analyses taking into account the memory differentiation profile. As expected, the memory differentiation profile observed among CMV-specific CD8 T cells was highly skewed towards effector memory (TEM) and terminally differentiated effector memory cells (TEMRA) at the expense of central memory subsets (TCM) (**Sup Fig. 3**). No difference in the distribution of CMV-specific memory subsets was detected between PWH and UI except for the percentage of TCM, that was higher in PWH than UI participants. When addressing the percentage of CD57+KLRG1+CD28- cells detected among TEM and TEMRA CMV-specific CD8 T cells in PWH versus noninfected participants (**Figure 2**), we confirmed the lower proportion of CD57+KLRG1+CD28- cells among CMV-specific fraction in PWH compared to UI.

The higher percentage of CX3CR1 observed among total CMV-specific CD8 T cells was also observed in TEMRA and TEM. The percentage of PD-1+ cells was higher among TEM compared to TEMRA, as previously described (Henriquez et al) but no difference was associated with CMV specificity or HIV status. These analyses demonstrate that in PWH, CMV-specific CD8 T cells exhibited a less senescent profile, although the terminal differentiation profile was preserved. Collectively, the context of chronic HIV infection was associated with slight shift in the differentiation profile of the CMV-specific CD8 T cells pool towards less differentiated profile, including higher proportion of TCM, and lower CD57 expression among TEM and TCM.

Lower survival capacity of the CD57- fraction of CMV-specific CD8 T cell

The combined observation of low CMV-specific percentage and low CD57 percentage among them was rather counterintuitive as one may presume that the lower percentage of CMV-

specific CD8 T cells was related to exacerbated senescence leading to cell death and quantitative reduction of this fraction. We first investigated whether there was an association between these two phenomena (**Figure 3**). Indeed, a strong association was observed (r 0.619) suggesting these two phenomena were related. We formulated two hypotheses based on the dynamic of the CD57 compartment that is mostly reflecting ongoing replenishment of the senescent pool (Snyder C.M., et al. 2008; Baumann NS, et al 2018): (a) higher susceptibility to apoptosis of the CD57+ fraction, leading to lower proportion of CMV-specific CD8 T cells and change in the CD57+/CD57- ratio and/or (b) direct impact of the chronic HIV infection on the CD57- fraction, that may not contribute efficiently to the constitution of the CD57+ compartment. Accordingly, we evaluate the survival capacity of the CD57+ and CD57- fraction among CMV-specific or total CD8 T cells (**Figure 4**) by in vitro incubation for 24h without stimulation. Regarding the total CD8 T cell fraction, the CD57- fraction was mostly preserved after 24h incubation, the percentage of surviving cells reaching 80 to 110% of the initial number of cells seeded. As expected, the survival capacity of the CD57+ fraction was significantly reduced (ranging from 40% to 60%) compared to CD57-. Additionally, the median of survival was higher in HIC compared to ART or UI. Interestingly, a different picture emerged when focusing on the CMV-specific CD8 T cell response: the survival capacity of the CD57+ fraction was low, as previously described, but the main impact was observed on the CD57- fraction: whereas CMV-specific CD8 T cells from UI exhibited survival capacity equivalent to the total fraction, we observed a drastically impaired survival of the CMV-specific CD57- CD8 T cells in PWH. This analysis provides a potential rationale to explain the lower percentage of CMV-specific CD8 T cells due to a reduced survival capacity of the CD57- fraction from which the CD57+ fraction originated.

Relative contribution of bystander activation and CMV-specific activation to the dynamic of the CMV-specific CD8 T cell compartment

The memory inflation of CMV-specific clones is usually explained by chronic CMV antigen presentation (resulting from CMV reactivation events) leading to reactivation and re-expansion of TCM. However, bystander activation by cytokines such as IL-15 may also contribute to the maintenance of this inflammatory pool. As CMV detection in the blood is difficult to perform with sufficient sensitivity, we indirectly evaluated the stage of CMV infection, by measuring anti-CMV IgG titers and avidity (**Figure 5A**). Anti-CMV IgM titers were also not measured because the too transient production of IgM does not allow to conclude on potential reactivation. We could thus confirm that the CMV status was equivalent in all groups: equivalent levels of IgG titers (although a statistical difference was observed between ART and UI) and high avidity of anti-CMV IgG, suggesting preexisting CMV infection. None of these tools allowed us to evaluate the occurrence of more frequent episodes of CMV reactivation and/or different threshold of CMV viral load in the context of chronic HIV infection as a reflect of altered immune control. However, we evaluated the expression of markers on immune cells commonly associated with chronic stimulation (such as TCR downregulation (Bronstein-Sitton, N et al, 2003) or bystander activation by cytokines, some markers specifically associated with IL-15 driven bystander activation (such as NKG2D (Lee H., et al. 2025; Correia MP, et al. 2011)). The MFI for the CMV-specific pentamer staining was lower in PWH compared to UI, suggesting TCR regulation due to chronic stimulation (**Figure 5B**). Additionally, we observed higher percentage of NKG2D+ cells among CMV-specific CD57+ cells in PWH compared to UI (**Figure 5C**). The increase of NKG2D+ cells is in accordance with the increase in the percentage of CX3CR1+ cells among CD57+ cells in PWH, although the higher expression of CX3CR1+ cells was not specific to CMV-specific CD8 T cells (**Figure 2**). We analysed the expression of IL-15R to confirm the sensitivity of CMV-specific CD8 T cells to IL-15 and observed high percentage of IL-15R+ cells among CD57+ T cells, the percentage being higher

among CMV-specific compared to total CD8 T cells in UI (with a similar trend for PWH). Lastly, we evaluated whether the NK cell subsets exhibited any difference among PWH and UI (**Sup Figure 4**). The proportions of CD56high CD16low NK cells was low in all groups at the expense of the CD56dim fraction that was predominant, in accordance with the literature on the impact of CMV on the NK cell subsets (Campos C et al, 2014). A difference in the relative proportion of the CD56high CD16low fraction was observed in ART compared to HIC but PWH did not show any difference compared to UI. In contrast, the percentage of CD56dimCD16low NK cells expressing CD57+ was high in all groups and significantly higher among PWH compared to UI. The analysis of the CD4 T cell compartment revealed the expected changes in the memory/naïve distribution related to the HIV infection: reduction in the percentage of naïve CD4 T cells and increase in the TEMRA fraction in PWH. We observed an increase in the proportion of CD57+ cells among CD4 T cells in PWH compared to UI participants.

Discussion

The impact of the CMV infection differs depending on the clinical status of the individuals. Mostly innocuous in healthy adult individuals, its impacts are more severe in the elderly or in immunodeficient individuals. In the elderly, the CMV infection and notably its consequences on the CD8 T cell compartment are associated with higher susceptibility to comorbidities and lower efficacy of vaccination. The aim of this work was to evaluate the long-term impact of HIV infection on the CMV CD8 T cell compartment because (i) the vast majority of PWH are also coinfected with CMV and (ii) HIV and CMV may synergize to exacerbate the extent of immune defects, and contribute to accelerated ageing**.**

The first striking result was the observation of the reduction of CMV-specific CD8 T cell percentage among CD8 T cells in PWH. This observation clearly shows that HIV infection impacts the T cell repertoire and modulates the frequency of CD8 T cells directed against other viruses. This difference was counterintuitive, as one may presume that the low chronic inflammation and the putative CMV reactivation during the primary phase would exacerbate memory inflation, that is a common characteristic of the CMV infection. This was not the case in both ART-treated and spontaneous controllers who showed approximately 8 fold reduction in the proportion of CMV-specific CD8 T cells. This effect was not mediated by difference in the distribution of HLA alleles as no overt overexpression of T cells specific for one peptide/MHC complex was observed (sup Fig. 1). This observation differs from the work of Naeger et al (Naeger PlosOne 2010), describing higher proportion of cells responding to CMV peptide stimulation. The difference in methods to identify the CMV-specific fraction may contribute to these discrepancies. However, it is interesting to note the difference in ages between the two studies that may partly explain the differences. The more exacerbated impact on the CMV-specific compartment in the elderly may reflect the silent process of degrading the immune responses when CMV and HIV are coexisting, even in latent forms.

This quantitative change was also associated with phenotypic changes. It is usually described that the HIV infection is associated with exhaustion and the CMV infection is associated with senescence. We observed a more nuanced picture in this study. PD-1 expression is equally highly expressed in CMV-specific CD8 T cells both among UI and PWH. When considering CD8 T cell as a whole, the expression of PD-1 was even higher in CMV specific than nonspecific CD8 T cells. However, this effect was mostly related to the differentiation status. The expression of PD-1 was higher among TEM than TEMRA regardless of the specificity and the CMV status. It thus indicates that the TEM fraction is highly impacted by both senescence and exhaustion in UI, whereas TEMRA are mostly affected by senescence. One may presume that the exhaustion profile may prevent appropriate differentiation. Among PWH who all showed high degree of T cell reconstitution (the median of CD4 count was 633 for ART and 736 for HIC), the percentage of activated CD8 T cells (as assessed by the percentage of HLA-DR+ CD38+ cells) was low, suggesting low chronic immune activation. Nevertheless, we observed higher proportion of activated cells among CMV-specific CD8 T cells from PWH compared to uninfected participants, suggesting at some degree, residual activation of the CMV specific CD8 T cell compartment. The level of activation was obviously lower than what was described in a pioneer study demonstrating the activation of the T cell compartment in PWH with low degree of reconstitution, and the positive impact of valganciclovir to reduce CD8 T cell activation (Hunt et al 2011). This observation was even more striking when considering the proportion of CX3CR1+ cells that was higher among CMV-specific compared to total CD8 T cells in both PWH and UI participants, but also higher in PWH compared to UI, in both CMV-specific and total fractions. The role of CX3CR1 expression on T cells is complex as multiple processes are associated with CX3CR1 expression: migration capacity (Panigrahi S, et al 2020; Bottcher et al., 2015), response to chronic stimulation (Gerlach et al,2016) and/or inflammageing (Morris et al, 2020). As the expression is higher in both CMV-specific and total CD8 T cells, we interpretate this increase as a reflect of chronic inflammation that defines

chronic HIV infection. Similarly, the reduction of the fraction of CD57+ cells among CMV-specific T cells in PWH was counterintuitive, as one may presume that the low chronic inflammation would exacerbate senescence. There is no dedifferentiation of CD57+ cells towards the CD57- fraction, implying that this reduction mostly reflects the loss of the CD57+ fraction and/or a preferential emergence of the CD57- fraction from progenitors. We first assessed the inverse association between the percentage of CMV-specific CD8 T cells and the percentage of CD57+ cells among CMV-specific CD8 T cells, thus confirming that the lower expression of CD57 was related to the reduction in CMV-specific CD8 T cell percentages. As memory inflation is maintained by continuous production of T cells (Snyder C.M., et al. 2008), we evaluated whether the reduced percentage of CMV-specific CD8 T cells may reflect either an impact of chronic HIV infection on the CD57+ fraction or on the progenitor CD57- fraction. As shown in Figure 4, the survival of CMV-specific CD8 T cells was limited due to the intrinsic susceptibility to apoptosis of the CD57+ fraction, regardless of the HIV status. However, the chronic HIV infection drastically reduced the survival capacity of the CD57- CMV-specific fraction. (b) direct impact of the chronic HIV infection on the CD57- fraction, that mat not contribute efficiently to the constitution of the CD57+ compartment. This hypothesis also fits with the change in the proportion of TCM among the CMV-specific CD8 T cells fraction. Altogether, these results suggest that chronic HIV infection increases the renewal rate of the CMV-specific compartment, leading to the shift in the balance between TCM/TEM/TEMRA towards less differentiated fraction, impacting the progenitor fraction thus precluding replenishment of the CMV-specific pool. Importantly, the impact of chronic HIV infection on CD57 expression on CD8 T cell was not observed when considering CD8 T cells. One may speculate that it reflects a specific impact of chronic HIV infection on the CMV-specific compartment or it may essentially concern the most differentiated fractions, that are enriched in the CMV-specific compartment. In this latter option, the monitoring of the CMV compartment may reveal the degree of active senescence ongoing in each patient.

Altogether, this work demonstrates an unexpected impact of the chronic HIV infection on the CMV-specific compartment that leads to the reduction of the CMV-specific compartment due to exacerbated ageing, leading to apoptosis of the CMV-specific fraction. The mechanisms that may underly this defect are uneasy to study: it may rely on CMV reactivation (transient or prolonged), higher production of CMV proteins due to a less controlled latency or bystander stimulation by cytokines. The blood samples studied did not allow us to evaluate differences in CMV viral load. However, reactivation or latency may mostly occur in tissues, such as the gut or the bone marrow. It would be interesting to address this question in a model allowing to combine studies on local CD8 T cell response and local viral load.

Groups		ART	HIC	UI	
Nber		46	44	20	
Age	(year)	52.5 [49-59]	55.5 [47-60]	51,6 [49-57]	ns (HIC/ART)
Sex	F/M	20 F/26M	19 F/25M	10F/10M	ns (HIC/ART)
HLA	A*02	30 (65%)	26 (59%)	14 (70%)	
	A*03	28 (61%)	24 (55%)	4 (20%)	
	B*07	19 (41%)	15 (34,1%)	16 (80%)	
	B*35	15 (33%)	6 (13,6%)	6 (30%)	
Infection duration	(year)	10 [6,4-15]	11 [8 -15]	na	ns (HIC/ART)
CD4 count		633 [459-814]	736 [528 -1060]	na	ns (HIC/ART)

Table 1: Clinical and demographic characteristics of the study participants.

Abbreviations. ART: ART-treated participants; HIC: HIV controllers; UI: uninfected individuals.

Data are presented as the median [interquartile range] or number (percentage).

P values were calculated using multiple comparison assay using Kruskal-Wallis or Mann-Whitney tests for continuous data and chi-square or Fisher's test for categorical data.

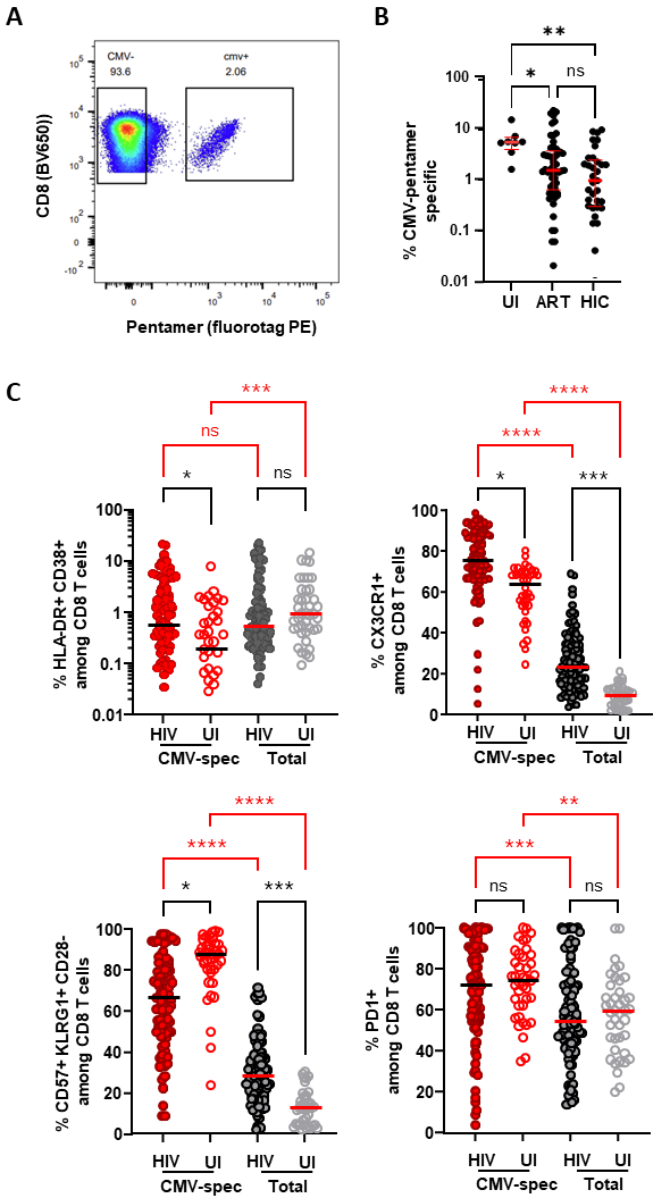
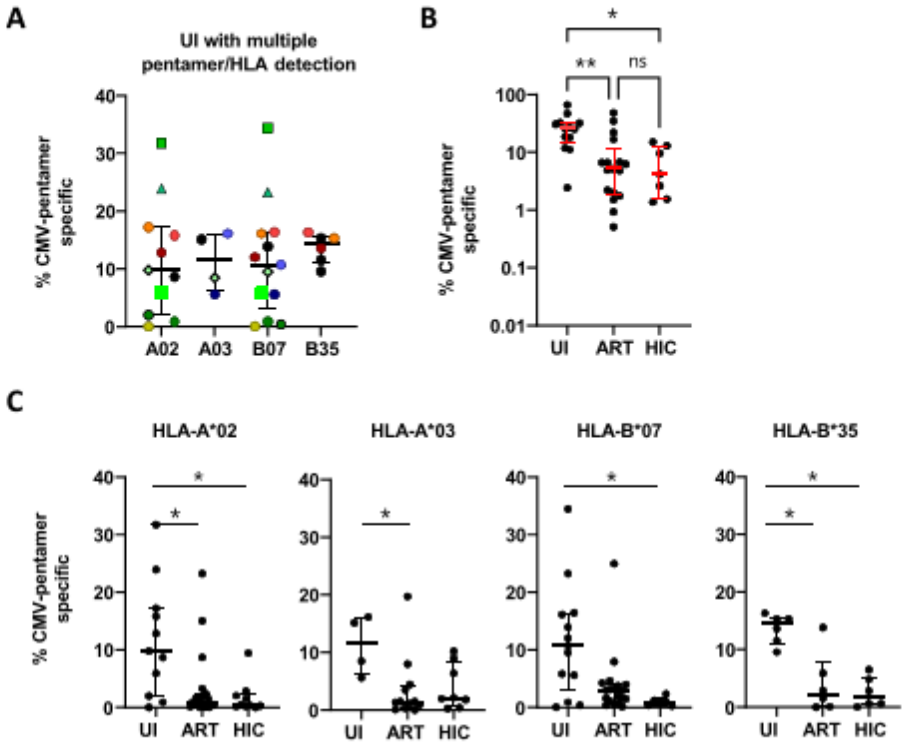


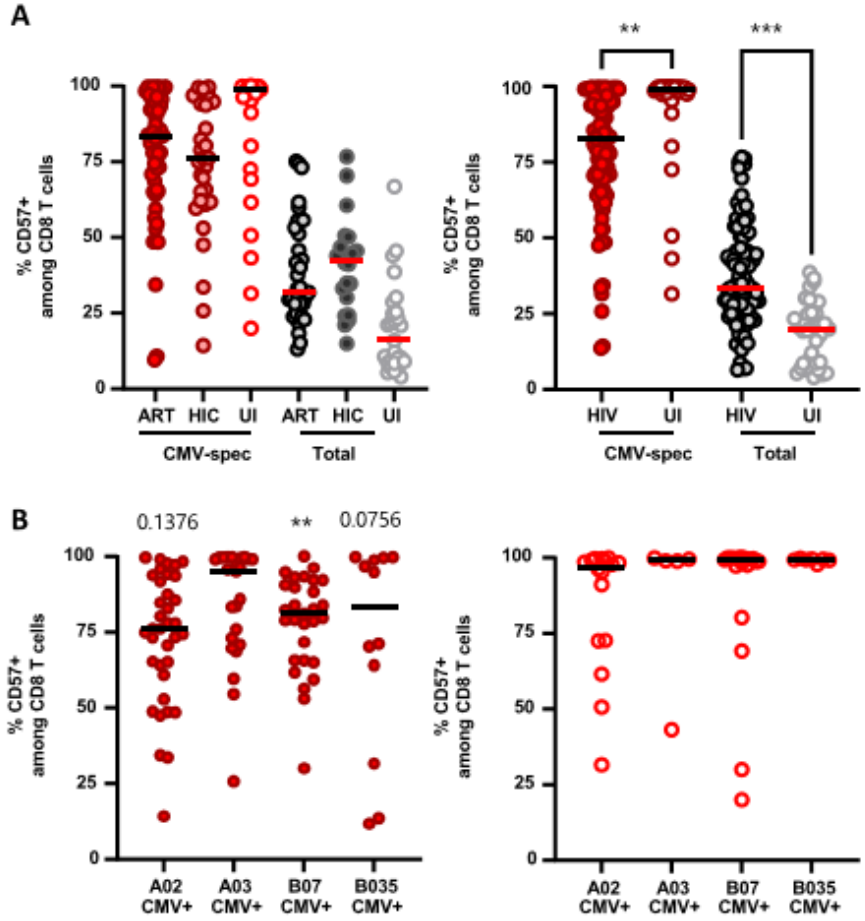
Figure 1. Perturbation of the CMV specific CD8 T compartment in the blood of PLWH

(A) Percentage of CD8 T cells stained by CMV specific pentamer. On the left, dop plot showing representative CMV-specific pentamer staining (sample from the UI group). On the right, graphs showing the percentage of CMV specific CD8 T cells in the different groups. For the participants exhibiting 2 to 3 relevant alleles, all values are shown. Analyses showing results for 39, 54 and 44 HLA/pentamer detection (respectively based on 20 UI, 46 and 40 HIC participants). (B) Phenotypic profile of CMV-specific compared total CD8 T cells in PLWH and UI. Graphs showing the percentage of HLA-DR+CD38+ fraction, CX3CR1+ fraction, CD57+KLRG1+CD28- fraction and PD1+ fraction. Statistics for the comparison between PLWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was performed using assay relevant for paired and nonparametric data, i.e. the Mann-Whitney test. P value (indicated in red): ** <0.01, *** <0.001, ****<0.0001.

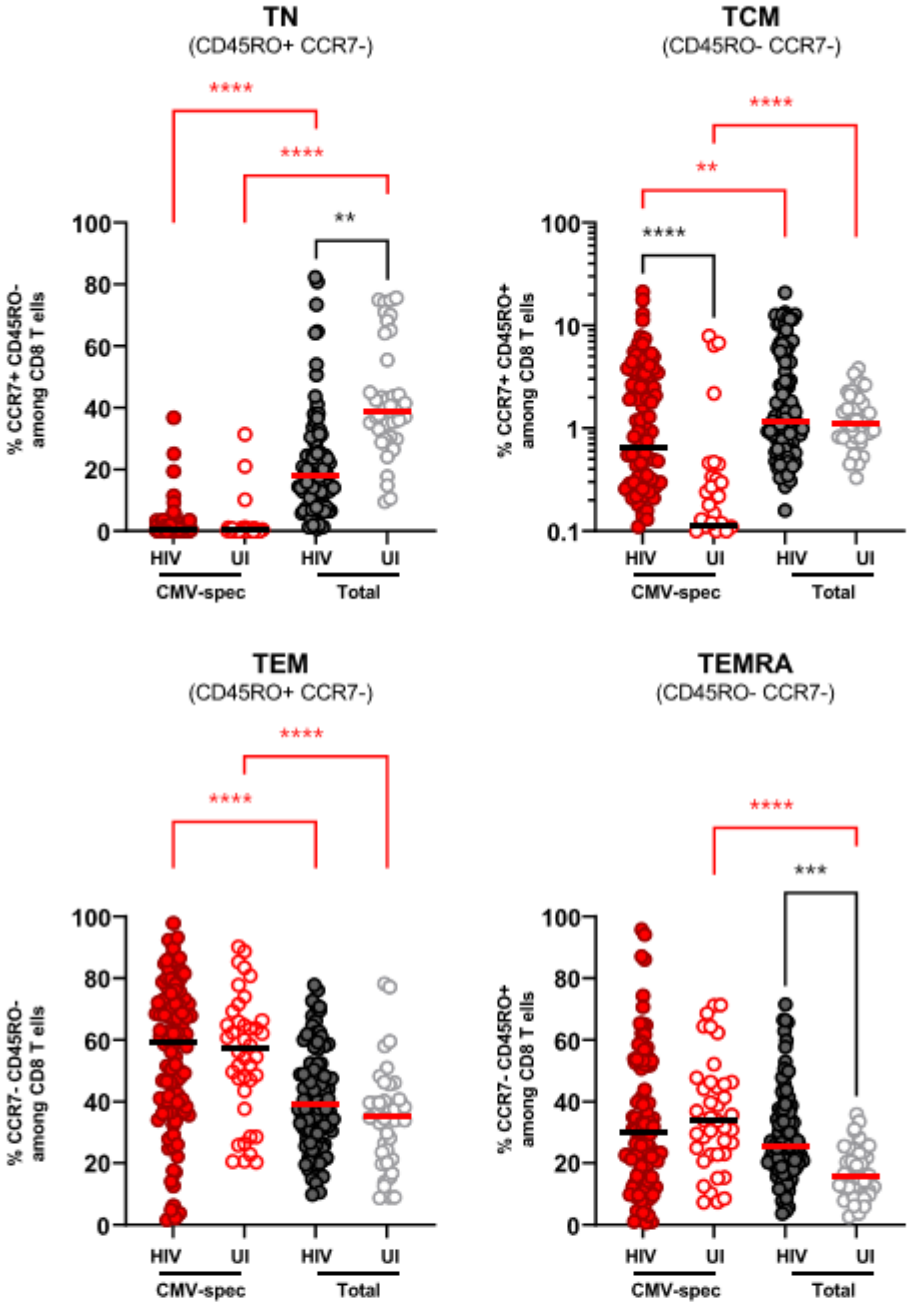


Supplemental Figure 1. Additional data on the impact of the HLA allele on CMV specific percentage

(A) Analysis of the percentage of CMV-specific CD8 T cells detected as a function of the HLA allele among the UI participants. Fifteen out of the 20 participants expressed more than one relevant HLA alleles. We analyzed whether the percentage of CMV-specific differed depending on the HLA allele. Each participant is shown with a different color. The median value and interquartile are shown. (B) Analyses of the percentage of CMV-specific CD8 T cells when cumulating values obtained for 2 alleles. Percentages calculated as the sum of the percentage obtained for 2 alleles, or as 2 times the mean value for samples from participants with 3 alleles. (C) Analysis of the percentage of CMV-specific CD8 T cells in the context of HLA-A*02, HLA-A*03, HLA-B*07 or HLA-B*35. The median values and interquartile are shown. Statistics for the comparison between PLWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001.



Supplemental Figure 2. Analyses of the senescent profile, based on CD57 expression
(A) Graphs showing the percentage of CD57+ cells among CMV-specific and total CD8 T cells on the same samples as shown in Figure 1B. On the left, analyses distinguishing the three groups: ART, HIC and UI. On the right, analyses comparing PLWH and UI. Statistics for the comparison between PLWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value: ** <0.01, ***<0.001. (B) Analyses of the percentage of CD57+ cells among CMV-specific CD8 T cells as a function of the HLA among PLWH (left graph) or UI (right graph). The median values are shown. Statistics for the comparison between the percentage CMV-specific CD8 T cells among participants exhibiting different HLA was performed using the same assay.



Supplemental Figure 3. Differentiation profile of CMV-specific CD8 T cells
Graphs showing the relative percentage of naïve, central memory, effector memory and terminally differentiated memory CD8 T cells among CMV-specific and total CD8 T cells, PLWHIV and UI. Statistics for the comparison between PLWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was performed using assay relevant for paired and nonparametric data, i.e. the Mann-Whitney test. P value (indicated in red): ** <0.01, *** <0.001, ****<0.0001.

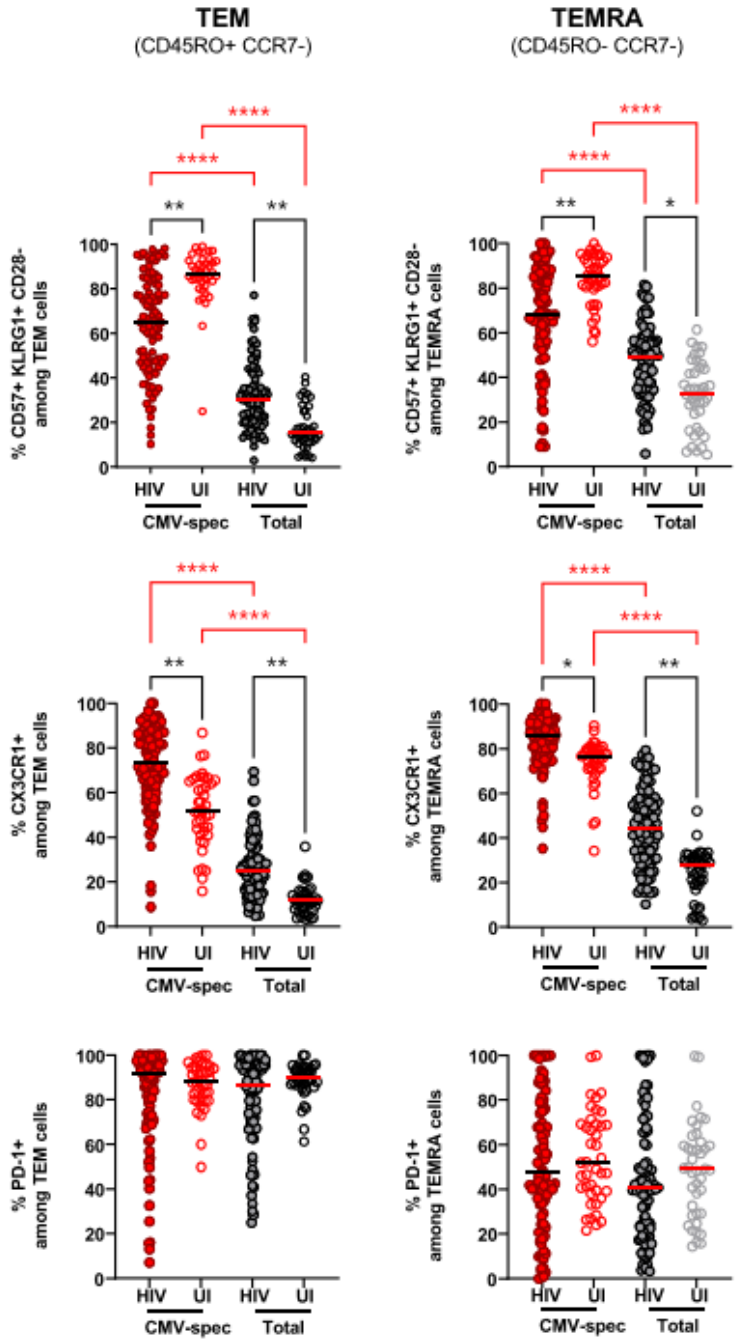


Figure 2. Phenotypic analysis of the CMV-specific CD8 T compartment in the blood of PLWH, integrating the memory differentiation profile

Graphs showing the percentage of CD57+KLRG1+CD28- fraction, CX3CR1+ fraction and PD1+ fraction among TEM (left column) and TEMRA (right column) CD8 T cells, in CMV specific and total CD8 T cells from PLWH and UI. Statistics for the comparison between PLWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was performed using assay relevant for paired and nonparametric data, i.e. the Mann-Whitney test. P value (indicated in red): ** <0.01, *** <0.001, ****<0.0001.

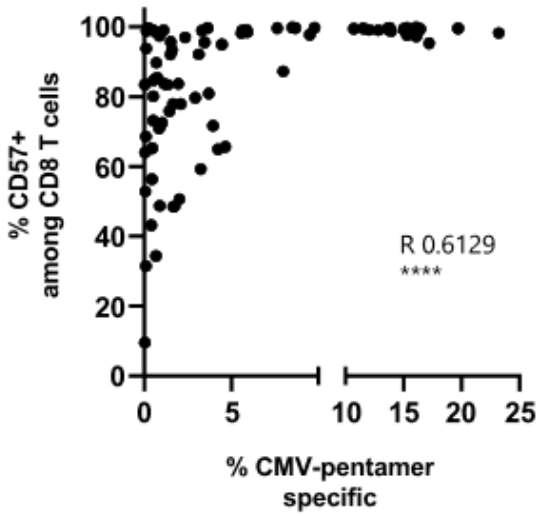


Figure 3. Association between the percentage of CMV-specific CD8 T cells and the percentage of CD57+ cells.

Graphs showing the percentage of CMV-specific CD8 T cells as a function of the percentage of CD57+ cells. Correlation assay using nonparametric Spearman correlation, based on 78 XY pairs.

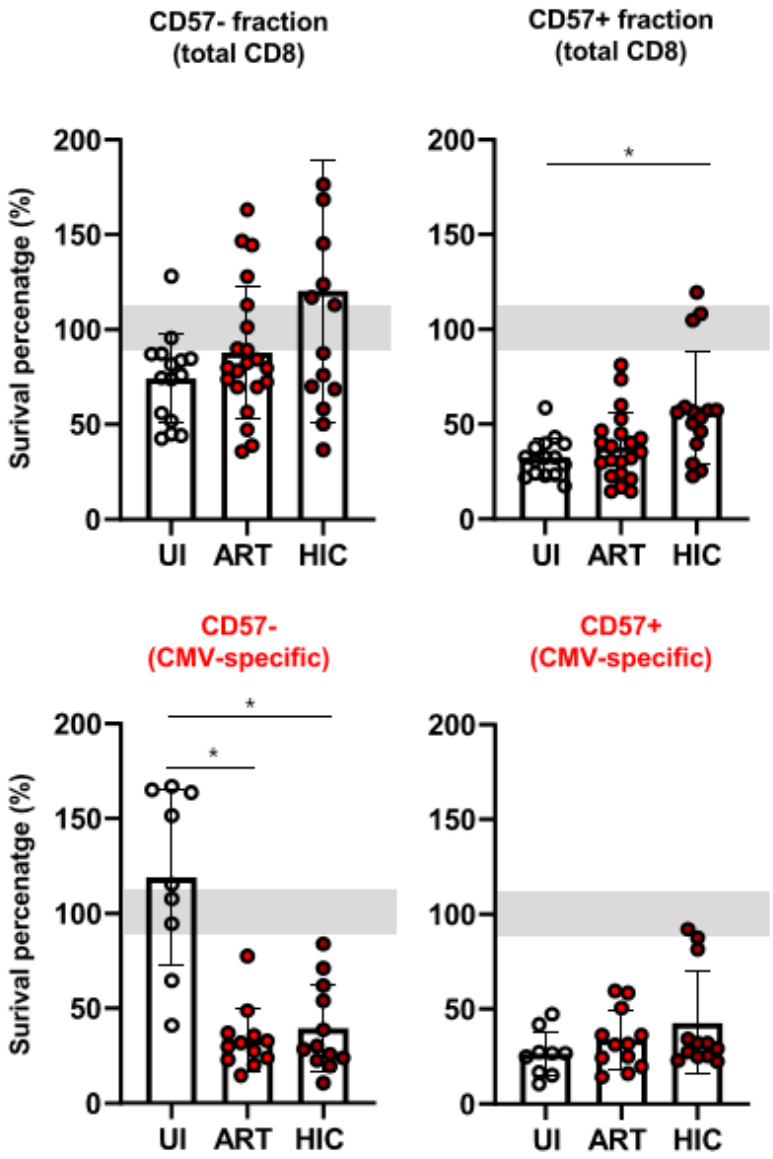


Figure 4. Chronic HIV infection impacts the survival capacity of CD57- CMV specific CD8 T cells

Graphs showing the survival capacity of total CD8 and CMV specific CD8 T cell fractions after 24h incubation of PMBC without stimulation. Percentage of survival was based on the numeration of the cell at day 0 and 24 hours later. Culture assay were generated in triplicates. The median and interquartile are shown. Statistics for the comparison between PLWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001.

(Data generated by Souhila Boucif (M2 student))

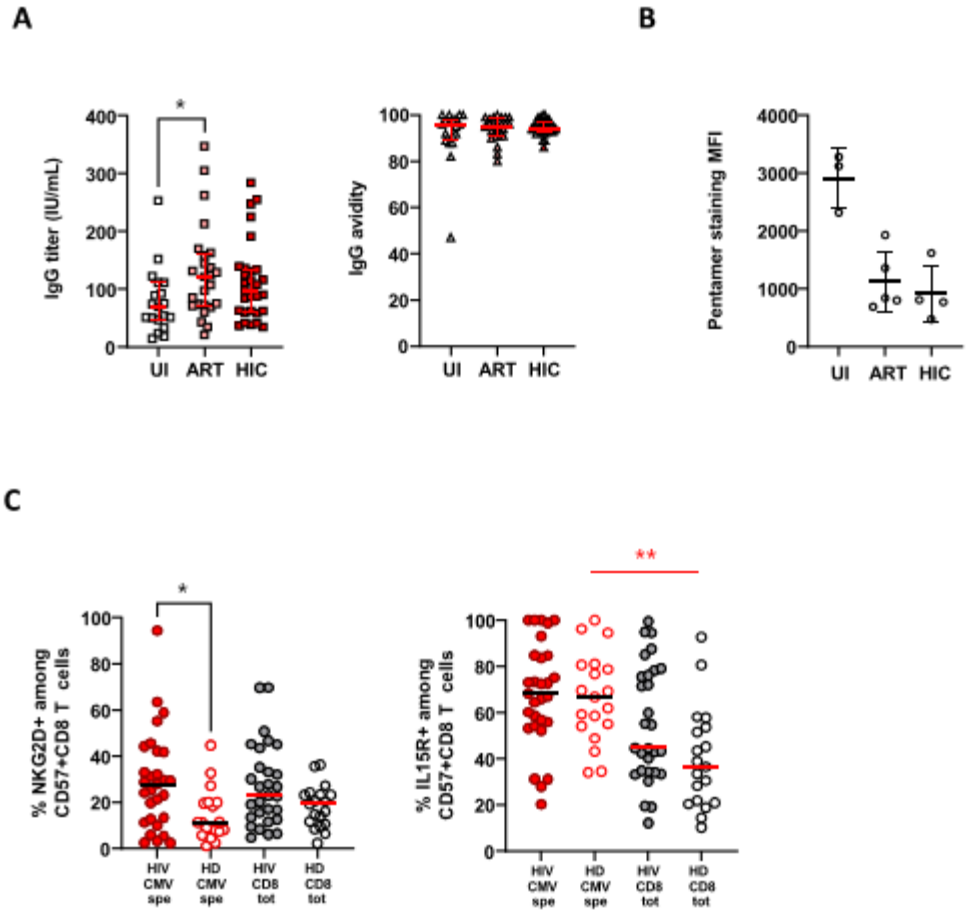
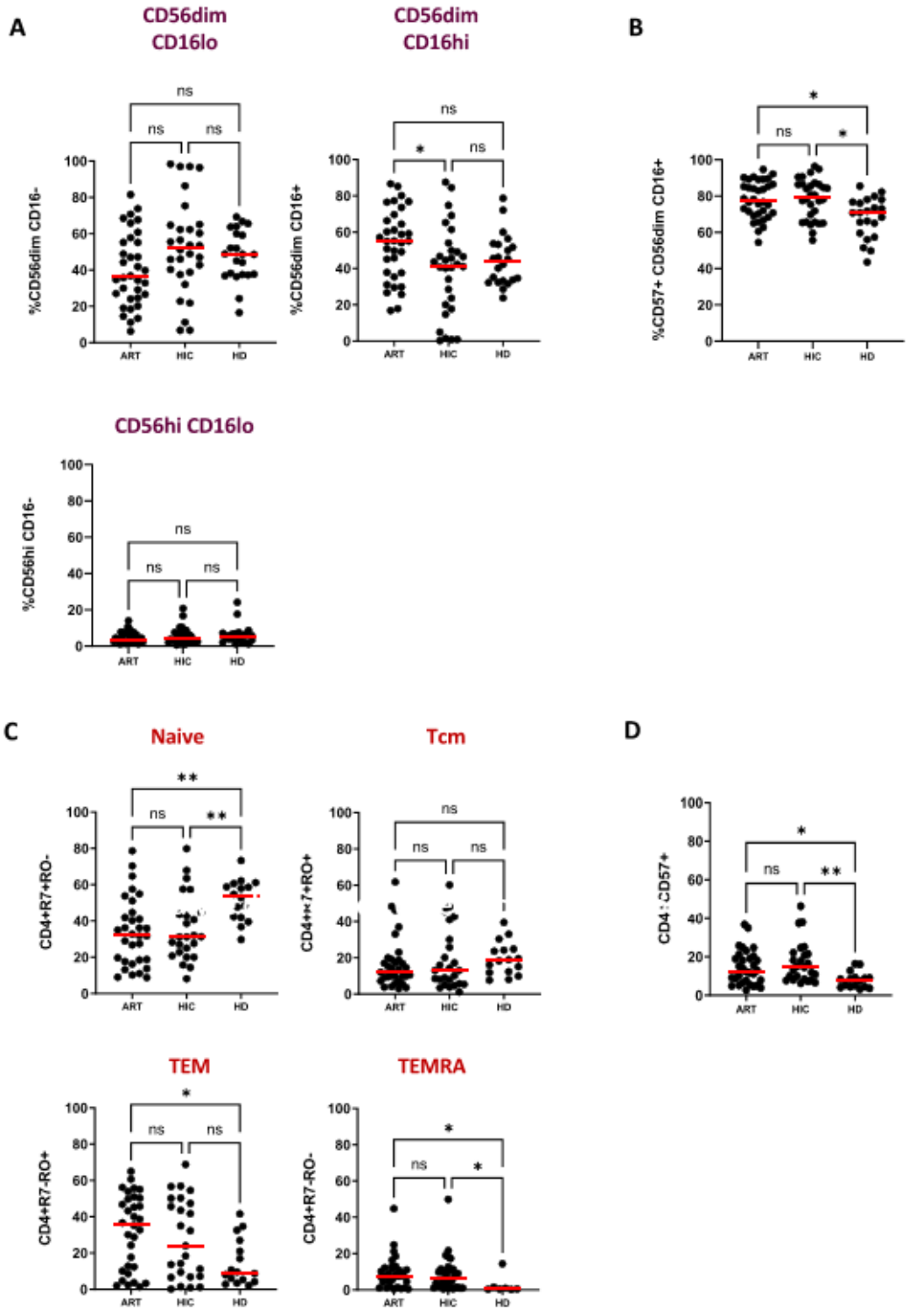


Figure 5. Contribution of both chronic Ag stimulation and bystander activation of CMV-specific CD8 T cells

(A) CMV serology. Graphs showing IgG titers and IgG avidity for the three groups. (B) MFI of the CMV-specific pentamer staining on CD8 T cells among the three groups. (C) Percentage of NKG2D+ cells (left graph) and IL15R+ cells (right graph) among CD57+ CD8 T cells. Median are shown. Statistics for the comparison between PLWH and UI were performed using the Kruskall-Wallis test. P value (indicated in black): * <0.05. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was performed using Mann-Whitney test. P value (indicated in red): ** <0.01.

(Data in Fig B generated by Souhila Boucif (M2 student))



Supplemental Figure 4. data regarding the NK and CD4 T cell subsets

(A, B) NK cell profile. Graphs showing (A) the relative percentage of the NK cell subsets (CD56dimCD16-, CD56dimCD16+ and CD56hiCD16low) in the three groups.; (B) the percentage of CD57+ cells among the CD56dimCD16low NK cells. (C, D) CD4 T cell profile. Graphs showing (A) the proportion of naïve (CD45RO-CCR7+), TCM (CD45RO+ CCR7+), TEM (CD45RO+ CCR7-) and TEMRA (CD45RO- CCR7-) and (B) the percentage of CD57+ cells among total CD4 T cells. The median are shown. Statistics for the comparison between PLWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001.

Legends

Figure 1. Perturbation of the CMV-specific CD8 T compartment in the blood of PWH

(A) Percentage of CD8 T cells stained by CMV specific pentamer. On the left, dop plot showing representative CMV-specific pentamer staining (sample from the UI group). On the right, graphs showing the percentage of CMV specific pentamer in the different groups. For the participants exhibiting 2 to 3 relevant alleles, all values are shown. Analyses showing results for 39, 54 and 44 HLA/pentamer detection (respectively based on 20 UI, 46 and 40 HIC participants). (B) Phenotypic profile of CMV-specific compared total CD8 T cells in PWH and UI. Graphs showing the percentage of HLA-DR+CD38+ fraction, CX3CR1+ fraction, CD57+KLRG1+CD28- fraction and PD1+ fraction. Statistics for the comparison between PWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was performed using assay relevant for paired and nonparametric data, i.e. the Mann-Whitney test. P value (indicated in red): ** <0.01, *** <0.001, ****<0.0001.

Figure 2. Phenotypic analysis of the CMV-specific CD8 T compartment in the blood of PWH, integrating the memory differentiation profile

Graphs showing the percentage of CD57+KLRG1+CD28- fraction, CX3CR1+ fraction and PD1+ fraction among TEM (left column) and TEMRA (right column) CD8 T cells, in CMV specific and total CD8 T cells from PWH and UI. Statistics for the comparison between PWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was

performed using assay relevant for paired and nonparametric data, i.e. the Mann-Whitney test. P value (indicated in red): ** <0.01, *** <0.001, ****<0.0001.

Figure 3. Association between the percentage of CMV-specific CD8 T cells and the percentage of CD57+ cells.

Graphs showing the percentage of CMV-specific CD8 T cells as a function of the percentage of CD57+ cells. Correlation assay using nonparametric Spearman correlation, based on 78 XY pairs.

Figure 4. Chronic HIV infection impacts the survival capacity of CD57- CMV specific CD8 T cells

Graphs showing the survival capacity of total CD8 and CMV specific CD8 T cell fractions after 24h incubation of PMBC without stimulation. Percentage of survival was based on the numeration of the cell at day 0 and 24 hours later. Culture assay were generated in triplicates. The median and interquartile are shown. Statistics for the comparison between PWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001.

Figure 5. Contribution of both chronic Ag stimulation and bystander activation of CMV-specific CD8 T cells

(A) CMV serology. Graphs showing IgG titers and IgG avidity for the three groups. (B) MFI of the CMV-specific pentamer staining on CD8 T cells among the three groups. (C) Percentage of NKG2D+ cells (left graph) and IL15R+ cells (right graph) among CD57+ CD8 T cells. Median are shown. Statistics for the comparison between PWH and UI were performed using

the Kruskall-Wallis test. P value (indicated in black): * <0.05. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was performed using Mann-Whitney test. P value (indicated in red): ** <0.01.

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Supplemental materials

Supplemental Figure 1. Additional data on the impact of the HLA allele on CMV specific percentage

(A) Analysis of the percentage of CMV-specific CD8 T cells detected as a function of the HLA allele among the UI participants. Fifteen out of the 20 participants expressed more than one relevant HLA alleles. We analysed whether the percentage of CMV-specific differed depending on the HLA allele. Each participant is shown with a different color. The median value and interquartile are shown. (B) Analyses of the percentage of CMV-specific CD8 T cells when cumulating values obtained for 2 alleles. Percentages calculated as the sum of the percentage obtained for 2 alleles, or as 2 times the mean value for samples from participants with 3 alleles. (C) Analysis of the percentage of CMV-specific CD8 T cells in the context of HLA-A*02, HLA-A*03, HLA-B*07 or HLA-B*35. The median values and interquartile are shown. Statistics for the comparison between PWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001.

Supplemental Figure 2. Analyses of the senescent profile, based on CD57 expression

(A) Graphs showing the percentage of CD57+ cells among CMV-specific and total CD8 T cells on the same samples as shown in Figure 1B. On the left, analyses distinguishing the three groups: ART, HIC and UI. On the right, analyses comparing PWH and UI. Statistics for the comparison between PWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value: ** <0.01, ***<0.001. (B) Analyses of the percentage of CD57+ cells among CMV-specific CD8 T cells as a function of the HLA

among PWH (left graph) or UI (right graph). The median values are shown. Statistics for the comparison between the percentage CMV-specific CD8 T cells among participants exhibiting different HLA was performed using the same assay.

Supplemental Figure 3. Differentiation profile of CMV-specific CD8 T cells

Graphs showing the relative percentage of naïve, central memory, effector memory and terminally differentiated memory CD8 T cells among CMV-specific and total CD8 T cells, PWHIV and UI. Statistics for the comparison between PWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001. Statistics for the comparison between CMV-specific and total CD8 T cells among each participant was performed using assay relevant for paired and nonparametric data, i.e. the Mann-Whitney test. P value (indicated in red): ** <0.01, *** <0.001, ****<0.0001.

Supplemental Figure 4. data regarding the NK and CD4 T cell subsets

(A, B) NK cell profile. Graphs showing (A) the relative percentage of the NK cell subsets (CD56dimCD16-, CD56dimCD16+ and CD56hiCD16low) in the three groups.; (B) the percentage of CD57+ cells among the CD56dimCD16low NK cells. (C, D) CD4 T cell profile. Graphs showing (A) the proportion of naïve (CD45RO-CCR7+), TCM (CD45RO+ CCR7+), TEM (CD45RO+ CCR7-) and TEMRA (CD45RO- CCR7-) and (B) the percentage of CD57+ cells among total CD4 T cells. The median are shown. Statistics for the comparison between PWH and UI were performed using assay relevant for unpaired and nonparametric data, i.e. the Kruskall-Wallis test. P value (indicated in black): * <0.05, ***<0.001.

DISCUSSION

Despite long-term viral control, either spontaneous (in HIC) or under treatment (in ART), immune defects persist in PWH in the form of chronic residual inflammation, immune exhaustion and senescence. These dysfunctions are commonly linked to HIV persistence, microbial translocation due to intestinal mucosa alterations, ART toxicity or viral co-infections. All these factors contribute to accelerated ageing and comorbidities. Studying the anti-CMV response is particularly interesting because the population of PWH is ageing naturally, and CMV infection is associated with an 'immune risk profile' (increased infectious complications and mortality) in the elderly. The CMV and HIV coinfection may be two parts of a vicious circle favoring inflammation. The scar of the HIV infection notably in the gut, leading to CD4 depletion may favor CMV reactivation and/or non-silent latency, which in turn may favor senescence and senescent associated inflammation, that will entertain the chronic low-grade inflammation. We hypothesised that HIV infection may aggravate the deleterious impact of CMV infection on ageing, thus contributing to the immune defects and comorbidities that the PWH are suffering. Our results reveal quantitative and qualitative changes in CMV-CD8 cells in PWH compared to HIV-uninfected participants. We described that PWH (ART or HIC) have a lower percentage of CMV-CD8 cells than uninfected donors, despite similar anti-CMV IgG titers and avidity. The percentage of senescent cells (assessed by CD57+ or CD57+KLRG1+CD28-) among CMV-CD8 cells (either overall or in memory effector or terminally differentiated fractions) was lower in PWH. We next wanted to evaluate the mechanisms that could explain this reduction of the size of the CMV-specific compartment in the blood. We reasoned that it could be either due to CMV reactivation that may lead to activation-induced cell death, and/or bystander activation of CMV-specific CD8 LT, as it has been described that these cells were sensitive to IL-15. IL-15 has been associated to the CMV-specific memory inflation. However, the reduced percentage in the blood may also reflect migration of these towards inflamed sites (and again this migration may be either driven by CMV reactivation or not). A closer study of the senescent profile of CMV-CD8 cells revealed a higher percentage of CX3CR1+ and NKG2D+ cells among CD57+ CMV-CD8 cells in PWH. Some publications associate the expression of these markers to bystander mechanisms, indicating a central role of IL-15 in maintaining the CMV-CD8 cell compartment (Kim et al., 2018). Lastly, we also hypothesize that the lower percentage of CMV-specific CD8 LT in the blood may reflect altered survival of

this fraction on PWH. This was the case with the surprising result of a specific impact of the chronic HIV infection on the CD57- CMV-specific CD8 T cell fractions.

Some aspects of these results will be discussed below.

1 The heterogeneity of senescence markers may reveal some degree of heterogeneity in senescent profile

Replicative senescence is characterised by an irreversible loss of T cell proliferative capacity and is associated with telomere shortening (Bellon & Nicot, 2017; Brenchley et al., 2003a; Henson & Akbar, 2009; Rodriguez et al., 2020). However, senescent T cell phenotypic characterisation remains uneasy for different reasons:

- The senescent phenotype is difficult to define as it partially overlaps with terminal differentiation, which is characterised by the progressive loss of CD28 and CD27. (Nga et al., 2024). Terminal differentiation $CD28^{-}CD27^{-}$ TEMRA cells and senescence ($CD57^{+}$ cells with short telomeres) have some features in common, such as residual cytotoxicity and the expression of inhibitory molecules (PD-1 and TIGIT), but differ in terms of their proliferative capacity and inflammatory impact (Ramello et al., 2021). Unlike terminally differentiated cells, senescent cells display a SASP, producing cytokines such as IL-6 and TNF-α. This contributes to inflammaging and age-related comorbidities (A. Kumar & Thirumurugan, 2023).

- The project used typical surface markers and established characteristics of exhausted and senescent CD8+ LT according to Donovan et al. (Donovan et al., 2021) . The expression of CD57 or the CD57+ KLRG1+ and CD28- combination have been alternately used to describe senescent LT in humans. While CD57 is a robust indicator of senescence, linked to proliferative arrest and apoptosis, KLRG1 plays a more ambiguous role.

CD57 is a key marker of replicative senescence. It is expressed by CD4 and CD8 T LT, NK cells and neural crest cells. It identifies cells that are unable to divide despite the presence of cytokines such as IL-2 or IL-15 and that are prone to cell death after activation. However, these cells retain the ability to secrete cytokines (γ-IFN) during antigen recognition. Regarding its function, CD57 does not appear to have any specific activity; rather, it reflects terminal differentiation (Brenchley et al., 2003b). Importantly, the expression of CD57 is also modulated and can be dissected in CD57hi and CD57int subsets, with different functional

properties (Simonetta et al., 2014). In PWH, the frequency of senescent CD57+CD8+ LT decreased after initiation of ART in TCM but not in TEM cell populations TemRO and TemRA. This indicates a partial recovery of these immune cells during treatment (Eberhard et al., 2016). One of the complexities of the interpretation of the CD57 profile is the absence of such molecule in murine model, thus precluding exhaustive analyses of the modulating factor of CD57 expression, and the specific activity of this molecule.

KLRG1 signals both immunological ageing and functional inhibition via the PI3K/Akt pathway without being exclusive to senescence. KLRG1, which is linked to soluble cadherins (E-, N- and R-cadherin), is expressed by non-naive LT and NK cells. The specific functional role of KLRG-1 is to lose proliferative capacity via its intracellular ITIM domain. This results in the recruitment of SHP-1 and SHP-2 phosphatases, which prevent the activation of PI3K and the phosphorylation of Akt (Henson & Akbar, 2009). It blocks proliferation by interfering with cyclins via the Akt pathway and reduces the cytolytic activity of CD8 LT by causing a loss of perforin/granzymes. It also suppresses cytokine production (like γ-IFN), as observed in mouse models. While CD57 reflects irreversible proliferative arrest, KLRG1 signals impaired differentiation function (Brenchley et al., 2003c; Henson & Akbar, 2009).

- The expression of CD57, KLRG-1 and CD28 was used to identify senescent cells. Older people had significantly higher proportions of both CD28-CD57+ and CD28+CD57+ cells, most of which co-expressed CD57 and KLRG-1 (Onyema et al., 2012). Importantly, the CD28+CD57+ fraction exhibited the highest expression of p16, p21, Bcl-2, CD95, CD45RO, CCR5 and PD-1, suggesting this fraction was the closest to a senescent phenotype (Onyema et al., 2015). Thus, there is still debate on the relevance of using CD28 to identify senescent cells.

2 The CMV and HIV coinfection, as a factor contributing to senescence in PWH ?

HIV infection accelerates immunological ageing via mechanisms combining chronic immune activation, systemic inflammation and persistent cellular alterations, even under ART. HIV induces the depletion of CD4 LT and the accumulation of terminally differentiated CD8 LT (TEMRA, $CD28^{-}CD57^{+}$), which are characteristic of immunosenescence. These disturbances are accompanied by over-expression of pro-inflammatory markers (IL-6 and TNF-α) and

oxidative stress, which are similar to the effects of natural ageing, but more severe. Despite ART, residual immune activation persists. This increases the risk of age-related comorbidities, such as cardiovascular disease and cancer. The mechanisms of immune ageing in HIV have been investigated by M. Chauvin and D. Sauce Delphine (Chauvin & Sauce, 2022). PWH show over-expression of genes associated with cellular senescence, oxidative stress and inflammation (CDKN2A/p16, NF-κB), which is comparable to that observed in older uninfected individuals. They also show a reduction in genes associated with DNA repair and mitochondrial function. Alterations in the high-dimensional T-cell profile and gene signature of immune ageing in HIV-infected older adults without viremia have also been observed (Shin et al., 2022).

It is known that CMV is a factor in accelerated senescence and a predominant contributor to the CD57+ compartment. However, Sulggi A. Lee et al. demonstrate that HIV disrupts this process by accumulating less differentiated CD28- transitional memory LT (TTM) and reducing the proportion of $CD57^{+}CD28^{-}$ cells in untreated PWH (Lee, Sinclair, Hatano, et al., 2014). These abnormally low proportions of $CD28^{-}$ $CD57^{+}$ CD8 LT predict increased mortality during treated HIV infection. However, early initiation of ART can partially restore these proportions, thereby reversing the dysfunctional phenotype and improving patient prognosis (Lee, Sinclair, Jain, et al., 2014). Another study also describes that in chronically ART-treated PWH, CMV/HIV coinfection is associated with lower proportion of CD57+ CD28-CD8+ LT compared to CMV seropositive patients (Mueller et al., 2021). This suggests that the anti-CMV immune response is impacted by the HIV infection. Interestingly, these data were not performed on the CMV-specific CD8 T cell response. Our data are in accordance with a change in the differentiation profile of CMV-CD8 LT.

3 The pentamer staining and the HLA selection

As described in the methods section, we selected samples from participants exhibiting similar HLA profile. Due to the need for HLA matching, we specifically targeted individuals carrying HLA-A*02, HLA-A*03, HLA-B*07 and HLA-B*35 alleles, all selected for their technical compatibility with available pentamers used to detect CMV-specific CD8 LT. The distribution of HLA-A*02 and HLA-A*03 alleles was found to be homogeneous between the PRIMO and CODEX cohorts, reflecting the predominantly European ethnic profile of the patients studied. These alleles predominate in the general French population. According to epidemiological

data, HLA-A*02 is present in approximately 25-27% of Europeans and dominates in clinical studies. HLA-A*03 varies between 11% and 17%, reflecting geographical heterogeneity (Arrieta-Bolaños et al., 2023; Olivier et al., 2023). Regarding the HLA-B, we firstly observed a high diversity but also high heterogeneity between the PRIMO and the CODEX cohort. In the CODEX control patients, there was a high representation of certain protective HLA class I alleles (alleles associated with resistance to infection), such as HLA-B57, HLA-B27, known for their strong correlation with a better clinical prognosis. In PRIMO patients, the HLA-B*35 and HLA-B*07 alleles which are associated with more rapid progression of infection, were dominant. This divergence reflects the impact of genetic factors on the course of HIV infection between PRIMO and CODEX. However, statistical adjustment and targeted selection of common alleles (B*07 and B*35) made it possible to establish a basis for comparison between the two cohorts by HLA.

Similarly, a control group of HIV-negative healthy donors was designed to provide equivalent profile to chronically infected patients (PRIMO and CODEX cohorts) according to three key criteria: sex, age and HLA status. This strategy ensures a balanced comparison between patients and controls, methodological consistency and technical feasibility. The rarity of certain alleles (e.g. HLA-B*35 in the general population) complicated the recruitment of matched controls, reducing the proportion of this allele in the study. The lack of a CMV-specific pentamer for non-targeted HLA alleles B*57/B*27 (protective alleles in HIV controller patients) limits the analysis to mechanisms related to B*07/B*35.

4 Quantitative change in the proportion of senescent CD8 LT: Reduction of the CMV-CD8 compartment in PWH.

The CMV infection is commonly characterised by a massive clonal expansion of CD8 LT. In CMV-seropositive individuals, CMV-specific CD8 LT represent on average 10% of the repertoire of memory LT and can reach >40% (Sylwester et al., 2005).

The reduction in the percentage of CMV-specific CD8 LT (CMV-CD8) in PWH compared to uninfected donors underscores the profound impact of chronic HIV infection on antiviral immune responses. Although anti-CMV IgG titers and their avidity are similar between groups, this dissociation between humoral and cellular responses suggests that HIV specifically

disrupts the compartment of CD8 LT targeting CMV. Several mechanisms may explain this observation. Firstly, CMV-CD8 cells in PWH may be more susceptible to apoptosis due to the chronic pro-inflammatory environment or metabolic exhaustion induced by HIV. A long-term survival defect could also result from disruptions in lymphocyte maintenance signals, such as IL-7 or IL-15, which are essential for the persistence of memory cells. Secondly, competition for immune resources between anti-HIV and anti-CMV responses could limit the expansion or maintenance of CMV-CD8 LT. HIV-specific CD8 LT might monopolize lymphocyte niches or co-stimulatory molecules, reducing the available space for CMV-CD8 cells. Thirdly, preferential migration of CMV-CD8 cells to inflammatory tissue sites, such as the intestinal mucosa or bone marrow, could explain their underrepresentation in peripheral blood. The bone marrow, a known reservoir for HIV and CMV, could act as a niche for these cells, retaining them outside the bloodstream. Finally, HIV could alter the differentiation of CMV-CD8 cells, promoting less mature or functionally exhausted phenotypes, which would limit their detection by classical markers of senescence or activation.

To confirm these hypotheses, targeted experimental approaches are necessary. Studying apoptosis via markers (such as Annexin V or the expression of anti-apoptotic proteins Bcl-2, Mcl-1) would help assess the survival of CMV-CD8 cells. Tissue migration analysis could be performed by measuring the expression of migration markers such as CX3CR1 or CCR5 or by quantifying these cells in intestinal, lymph node, or bone marrow tissues. Additionally, isolating monocytes to determine CMV viral load (via quantitative PCR) in blood could reveal whether CMV replication increased. The same approach in tissues may reveal a redistribution of CMV-CD8 cells out of the blood. These studies are obviously not easily doable in humans, but could be performed in nonhuman primates' model, that show the same proportion of CMV-seropositive individuals. Functional tests, such as proliferation after stimulation with CMV peptides or measuring cytotoxicity (degranulation via CD107a, γ-IFN production), would help evaluate whether residual cells retain effective antiviral activity.

Clinically, this reduction in circulating CMV-CD8 cells may reflect a suboptimal anti-CMV response, increasing the risk of CMV reactivation or complications related to immunosenescence, such as cardiovascular or neurodegenerative diseases. Increased migration to tissues could also contribute to local inflammation, exacerbating pathologies such as HIV-associated enteropathy. These findings argue for combined therapeutic strategies targeting both HIV persistence, CMV control, and modulation of chronic inflammation. Finally,

an integrated analysis of blood and tissue compartments is essential to understand the global dynamics of these cells and their role in the pathogenesis of HIV-associated comorbidities. This alteration of the CMV-CD8 compartment illustrates the complex disruptions in antiviral immunity induced by HIV, highlighting the need for personalised approaches to mitigate accelerated aging and improve the quality of life for PWH.

5 Qualitative modulation of the CMV-specific compartment :

Our results reveal an unexpected paradox in the senescence profile of CMV-specific CD8 LT in PWH. Contrary to expectations, fewer CMV-CD8 cells from PWH exhibited a senescent phenotype, particularly in the memory effector or terminally differentiated subpopulations. A reduction in the percentage of cells expressing the senescence marker CD57 (CD57+KLRG1+CD28-) was observed in CMV-specific CD8 LT, whereas this percentage increases in non-CMV-specific CD8 LT. This finding challenges the traditional link between CMV infection and the accelerated ageing of T lymphocytes. Several hypotheses could explain this result: Chronic HIV infection triggers systemic inflammation (microbial translocation, co-infections, viral reservoir) that could accelerate the turnover of CMV-specific CD8 LT. Chronic antigenic stimulation may induce rapid clonal expansion followed by early apoptosis, preventing the accumulation of senescent cells (CD57+). Furthermore, senescent CMV-CD8 cells may be more vulnerable in the context of HIV. Additionally, senescent cells may progressively lose their function (γ-IFN production, cytotoxicity) and be eliminated, leaving a residual compartment dominated by less differentiated cells (CD57−). These aspects need to be further investigated.

Although the study includes both patients on ART and spontaneous HIV controllers, some effects may be common. ART reduces HIV replication and systemic inflammation, potentially favoring the maintenance of less senescent CD8 LT. Spontaneous controllers, on the other hand, may exhibit optimised immune regulation, limiting terminal differentiation of CMV-CD8 cells. Conversely, the increased senescence of non-CMV-specific CD8 LT in PWH suggests distinct mechanisms depending on antigen specificity. Non-CMV-specific cells may be more exposed to viral (HIV) or bacterial antigens (translocation), leading to accelerated aging.

Clinically, reduced senescence in CMV-CD8 cells might reflect an ineffective anti-CMV response, increasing the risk of CMV reactivation or complications linked to

immunosenescence (e.g., cardiovascular diseases). At the same time, these less senescent but activated cells (via CX3CR1, NKG2D) could contribute to chronic inflammation and comorbidities (atherosclerosis, neurodegeneration). These mechanisms illustrate the complex interplay between HIV infection, residual inflammation, and antiviral responses.

Longitudinal and functional studies, including tissue analysis, will be essential to clarify the precise role of these disturbances in the accelerated ageing of PWH. The survival assay already performed by the team is in favor of a specific alteration of the CD57- fraction of the CMV-specific compartment. We may thus speculate that the reduction in the percentage of CMV-specific CD8 LT may reflect the lower viability of the CD57- fraction, thus preventing proper replenishment of the CD57+ fraction. The mechanisms for the specific alteration of the CMV-specific CD57- CD8 LT fraction remain to be determined. It may reflect either defective survival after antigen-specific activation or bystander activation.

Role of the bystander expansion in maintaining the CMV-CD8 compartment

The study also showed an increase in CX3CR1 and NKG2D markers among CMV-CD8 CD57+ cells in PWH, suggesting an increased use of Ag-independent mechanisms to maintain this compartment.

CX3CR1, the receptor for fractalkine (CX3CL1), is expressed on CD8 LT (Combadiere et al., 1998). In the context of CMV infection, CX3CR1 is also associated with an inflammescent profile, i.e. cells combining senescent phenotype and pro-inflammatory secretory profile. This fraction is especially sensitive to IL-15 in vitro (Morris et al., 2020a), as the incubation in presence of IL-15 alone allows expansion of this CX3CR1+ fraction, whereas peptide-specific proliferation does not. This study refers to the expansion capacity of the CX3CR1+ CD57+ fraction, but does not discuss the mechanism ensuring the differentiation of the CD57- fraction towards CD57+.

NKG2D, an activating receptor associated with recognition of stressed cells, may reflect an adaptive response to a chronically inflammatory environment. As previously mentioned for CX3CR1, it is difficult to define whether NKG2D should be considered only as a marker of bystander activation. However, if this is the case, then our data suggest a contribution of bystander activation to the dynamic of the CMV-specific compartment. The expression of NKG2D on bystander memory T cells is nevertheless associated with the control of viral load

(Chu et al., 2013) and pathophysiology related to bystander activation (Balint et al., 2024; Crosby et al., 2014).

These changes are part of a vicious circle in which HIV infection, even when controlled, alters the immune balance. The reduction in functional CMV-specific cells could promote asymptomatic CMV reactivation, thereby perpetuating inflammation. At the same time, bystander activation and increased expression of pro-inflammatory markers exacerbate tissue damage. Non-CMV-specific LT accumulate senescence markers, probably in response to continuous exposure to viral or bacterial antigens (microbial translocation), reflecting an increased overall immune load.

Activation profile of CMV-CD8 LT: mild activation

The slight increase in the expression of activation markers (CD38/HLA-DR) within total and CMV-CD8 LT in CMV-seropositive PWH (ART or HIC), compared to healthy CMV-seropositive donors, suggests persistent residual immune activation despite virologic control. Additionally, the lower level of CMV-specific pentamer staining on CMV-specific CD8 LT in PWH is also in favor of antigen-specific activation. This activation could be explained by several mechanisms. Chronic inflammation induced by HIV, even attenuated by treatment, persists through phenomena such as intestinal microbial translocation, the presence of viral reservoirs, or co-infections like CMV. The latter, even in a latent state, generates intermittent antigenic stimulation that, combined with the inflammatory environment of HIV, may exacerbate activation of CMV-LT. In PWH, immune regulation is impaired, unlike in uninfected CMV+ donors, where a balanced response limits chronic inflammation.

The synergistic interaction between HIV and CMV creates a hyperactive microenvironment: HIV disrupts lymphocyte homeostasis, while CMV drives persistent clonal expansion. CMV-CD8 LT, already prone to terminal differentiation, may become further activated in this context. In PWH on ART, although viral replication is controlled, tissue immune activation (in the gut, lymph nodes) persists, potentially contributing to residual CD38 and HLA-DR expression on CMV-specific CD8 LT (and other CD8 LT with other specificities). Spontaneous controllers, meanwhile, maintain efficient immune response to control HIV, that still persist in reservoir which may explain their similar profile. The tissue specific activation is less known in HIC.

Clinically, this residual activation is concerning. It is associated with accelerated immune aging (immunosenescence) and an increased risk of non-AIDS comorbidities, such as cardiovascular or neurodegenerative diseases. Activated CMV-specific LT, by producing pro-inflammatory cytokines such TNF-α, γ-IFN, may contribute to tissue inflammation, such as atherosclerosis. Furthermore, excessive activation could lead to functional exhaustion of these cells, impairing their ability to control CMV or other pathogens.

However, these results must be interpreted cautiously. The cross-sectional nature of the study does not establish a direct causal link between the observed activation and the proposed mechanisms. Additionally, patient heterogeneity (infection duration, ART adherence, CMV status) may influence the data. Finally, the analysis is limited to circulating cells, which may not reflect activation in tissues where HIV and CMV persist.

To further explore these observations, longitudinal studies incorporating CMV viral load (DNA/RNA) measurements and transcriptomic or metabolic analyses of activated LT would be necessary.

6 Implications for accelerated ageing in PWH

It is difficult to assess the importance of the CMV-HIV coinfection on the pathophysiology of chronic HIV infection as most PWH are coinfected. Accordingly, there is no data demonstrating a deleterious impact of CMV specifically in PWH. Additionally, the impact may be more apparent in elderly. We may thus further investigate this process in older PWH.

The activation of CMV-CD8 LT in PWH by bystander pathways or reactivation has major functional consequences: it may exacerbate the immunopathogenesis of HIV and accelerate ageing. The non-specific activation (mediated at least partly by Ag-independent mechanisms such as CX3CR1 and/or IL-15) leads to chronic systemic inflammation and immunopathology (mediated by NKG2D). The activated cells produce pro-inflammatory cytokines (such as TNF-α and γ-IFN) and cytotoxic molecules, fueling a vicious circle of tissue damage and microbial translocation, particularly in the intestine. This persistent inflammation worsens epithelial lesions, allowing bacterial products to enter the bloodstream and further amplifying immune activation. This may contribute to comorbidities such as atherosclerosis and neurodegenerative pathologies.

The activation of CMV-CD8 LT by bystander pathways or reactivation may divert immune resources and impairs specific antiviral function, thereby increasing the risk of asymptomatic CMV reactivation, which is pro-inflammatory in itself. With fewer functional CMV-specific clones (reduced γ-IFN production and impaired cytotoxicity), it becomes more difficult to contain the virus, while chronic inflammation and oxidative stress accelerate immunosenescence. LT may adopt a senescent or exhaustion phenotype, expressing PD-1 and TIM-3, which limits their response to new infections or vaccines. While increased IL-15R expression and IL-15-mediated signaling can promote cell survival, they also lead to non-specific clonal expansion, which disrupts the diversity of the lymphocyte repertoire.

7 Limitations of the study

However, certain limitations should be borne in mind. Firstly, the analysis is limited to PBMCs, whereas tissues such as the intestine and lymph nodes could harbor cell populations with distinct phenotypes. Additionally, the absence of functional data (such as cytotoxicity, cytokine production) precludes the ability to determine the effectiveness of CMV-CD8 cells. Finally, as the study is cross-sectional, no causality can be established between the observed abnormalities and the clinical course.

In conclusion, this study shows that chronic HIV infection profoundly alters the biology of CMV-specific CD8 LT, favoring a phenotype that is less senescent and more dependent on bystander activation mechanisms. These disturbances could lead to inflammation and premature ageing. However, longitudinal and functional studies are essential for elucidating the precise role of these mechanisms in the pathogenesis of HIV-associated comorbidities.

We also crucially need for data on the CMV viral load to distinguish between Ag-specific activation or expansion by bystander activation, although both processes may act synergistically to reduce the CMV-CD8 LT pool by constant stimulation and death.

Conclusion

During my thesis, I demonstrated that chronic HIV infection in PWH coinfected with CMV is associated with significant alterations in CMV-specific CD8 LT. These observations reveal an unexpected impact of HIV on anti-CMV immunity, resulting in qualitative and quantitative changes in the anti-CMV CD8 response. We observed a reduced frequency of CMV-specific CD8 T cells, associated with phenotypic changes, notably regarding their senescence profile. This reduction may reflect accelerated ageing and increased susceptibility to apoptosis, although this remains to be formally established. These cells also show increased expression of markers linked to the bystander effect (Ag-independent activation), reflecting disruption of the mechanisms regulating the anti-CMV CD8 response.

Several mechanisms could explain these changes: transient or prolonged CMV viral reactivations, excessive production of viral proteins resulting from impaired control of latency, or excessive or dysregulated stimulation by pro-inflammatory cytokines, corresponding to a bystander effect. However, analyses carried out on peripheral blood (PBMCs) do not allow variations in CMV viral load to be quantified, or the dynamics of reactivation or latency to be studied. It may be particularly relevant to evaluate CMV viral load in tissues such as the intestine and bone marrow. Future work combining the study of local CD8 T-cell responses and CMV viral load in different tissues would provide a better understanding of the complex interactions between HIV and CMV-specific CD8 T cells.

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