Monitoring cellular immune markers in HIV infection: from activation to exhaustion

Delphine Sauce, Carole Elbim, and Victor Appay

Purpose of review
The pathogenesis of HIV infection is highly complex and involves numerous actors of the immune system. On the one hand, our immunity has a predominant role in limiting HIV replication and the depletion of its targets, but on the other hand, the persistent infection established by the virus is associated with chronic immune activation and inflammation, potentially resulting in the progressive exhaustion of the host immune resources, and in the onset of non-AIDS-defining comorbidities. The thorough study of HIV pathogenesis is increasingly more challenging.

Recent findings
New knowledge together with technological advances offers the possibility to monitor a constellation of cellular immune markers. Here, we discuss the relevance of studying these markers in order to assess the efficacy to control HIV, the inflammatory response to HIV infection, and the alteration and exhaustion of the immune compartments.

Summary
Monitoring these cellular immune markers is important to reach a deeper understanding of HIV pathogenesis and to perform a comprehensive clinical follow-up of HIV-infected patients.

Keywords
exhaustion, function, immune activation, lymphocytes, phenotype

INTRODUCTION

Despite HIV is a rather small virus, consisting of only nine genes, it establishes a persistent infection in humans, and its pathogenesis is highly complex, involving numerous actors of the immune system. Most importantly, HIV targets and replicates in $CD4^+$ T cells (as well as, but to a lesser extent, macrophages and dendritic cells). The infection and depletion of $CD4^+$ T cells represent the most fundamental facet of HIV pathogenesis, and the $CD4^+$ T-cell count is the primary feature to monitor in infected patients, in order to inform on the progress toward disease progression and the onset of immunodeficiency. More precisely, memory $CCR5^+CD4^+$ activated T lymphocytes represent the privileged targets of the virus. Up to 80% of the gut-associated lymphoid tissue $CD4^+$ T cells, which consist predominantly of $CCR5^+$ activated cells, can be depleted in the first 3 weeks of primary HIV infection [1]. As the main target of HIV, memory $CCR5^+CD4^+$ T cells are particularly important to monitor. Moreover, studies even suggest that CCR5 expression levels on memory $CD4^+$ T cells are inversely associated with the development of AIDS in nonhuman primate models [2,3]. Low CCR5 expression levels may actually be linked to the preservation of the central memory $CD4^+$ T-cell pool [4], which is associated with long-term survival in vaccinated simian immunodeficiency virus (SIV)-challenged monkeys [5], as well as in the HIV-infected donors [6▪,7].

Apart from the target of the virus itself, a number of immune populations are also key in HIV pathogenesis, in particular HIV-specific effector cells endowed with potent antiviral functions. The importance of B cells and T cells in fighting HIV has long been established. In recent years, genetic and functional evidence have also indicated a potential role of the natural killer (NK)-cell population in controlling HIV replication. Similarly to the

INSERM UMR-S 945, Infections and Immunity, Université Pierre et Marie Curie-Paris 6, Paris, France

Correspondence to Delphine Sauce, INSERM UMR-S 945, Infections and Immunity, Hôpital Pitié-Salpêtrière, 75013 Paris, France. Tel: +33 1 40 77 98 89; fax: +33 1 42 17 74 90; e-mail: delphine.sauce@upmc.fr

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KEY POINTS

- Effective control of HIV replication involves an increasing number of cellular immune compartments, of which the attributes are important to monitor.
- Monitoring multiple cellular markers of immune activation is key to further our understanding of HIV pathogenesis.
- The balance between specific immune responses and systemic chronic activation is delicate in HIV infection and can be associated with exhaustion of multiple cellular compartments, including hematopoietic progenitors.

emergence of viral variants that escape recognition by neutralizing antibodies and T cells, HIV appears to mutate and adapt as a result of the immune pressure exerted by NK cells [8▪▪]. Last, the relevance of studying cellular actors of the systemic inflammatory response established from the early phase of primary infection and throughout chronic infection has also emerged in recent years. On the one hand, the immune system has a predominant role in controlling this persistent virus, but on the other hand, this long-term control is associated with chronic immune activation and inflammation, potentially resulting in the progressive exhaustion of the host immune competence and the onset of non-AIDS-defining comorbidities such as osteoporosis, atherosclerosis and neurocognitive decline, and premature aging [9–11]. In the present article, we review some of the recent developments in the field and discuss the relevance of monitoring cellular markers to assess the efficacy to control HIV, the inflammatory response to HIV infection, and the alteration and exhaustion of the immune compartments (Fig. 1).

IMMUNE CONTROL OF HIV-1 INFECTION

The NK-cell population can be divided into multiple subsets based on the expression of different inhibitory and activating receptors (including killer immunoglobulin or natural cytotoxicity receptors, NKG2A, C, or D, and the Fcγ receptor CD16), which determine the specificity and function of the cells [12]. The activity of NK cells on viral replication, through cytolysis of virally infected cells and production of antiviral cytokines and chemokines, may occur very early during HIV infection [13]. Genetic studies indicate that slow HIV disease progression is associated with a particular killer immunoglobulin receptor (KIR3DS1) and human leukocyte antigen (HLA-Bw4-80I) combination [14]. In this context, NK cells display enhanced functionality and persistent anti-HIV activity *in vitro* [15,16]. Moreover, evidence suggests that NK cells from HIV-infected controllers display a strong antibody-dependent cellular cytotoxic (ADCC) activity [17,18]. These encouraging results support the role of NK cells in the control of HIV; however, a comprehensive understanding of the mechanisms involved and the exact role of each NK-cell subset is still necessary in order to facilitate the immunomonitoring of this specific compartment.

The role of B cells is to secrete specific antibodies upon activation with their cognate antigen, and give rise to plasma cells. Anti-HIV antibodies that neutralize autologous virus develop slowly, arising several weeks after HIV primary infection, and usually do not cope with the fast rate of HIV mutation [19]. Nonetheless, antibodies that show some degree of neutralization against heterologous viruses eventually arise in about 20% of patients after years of infection [20]. Nonneutralizing antibodies (e.g., ADCC antibodies) are also detected within the first 3 weeks of infection both in plasma and in mucosal sites [21]. Beyond the dissection of the total B-cell compartment into naive, memory subset, plasmablast populations (based on the surface expression of receptors such as IgD, CD10, CD20, CD21, and CD27), there is presently no simple approach to study the HIV-specific B-cell response. Monitoring the anti-HIV humoral immunity currently involves measuring total or neutralizing antibody titers, or embarking into high-throughput screening and characterization of immortalized memory B cells [22]. However, the recent development of antigen tetramers to identify and characterize memory B cells *ex vivo* [23▪▪] may represent a major advance in the HIV field.

This should be coupled to the analysis of T follicular helper (TFH) cells, which represent a subset of $CD4^+$ T cells known to interact with antigen-specific B cells, promoting memory B-cell and plasma cell development, as well as inducing antibody affinity maturation [24]. TFH cells are characterized by high expression of CXCR5, Bcl-6, and programmed death-1 (PD-1), and for secreting IL-21. HIV-specific TFH cells have recently been studied in HIV-infected donors [25▪] as well as SIV-infected nonhuman primates [26▪]. $CD4^+$ T lymphocytes are nonetheless not confined to the sole role of helping other cells in their fight against HIV. In addition to producing a number of effector cytokines [e.g., interferon gamma (IFN-γ) and tumor necrosis factor α (TNF-α)] and chemokines (e.g., Regulated And Normal T cell Expressed and Secreted (RANTES), Macrophage Inflammatory Protein (MIP)-1α and β), a subset of highly differentiated $CD4^+$ T cells ($CD27^-$, $CD57^+$) is also known to

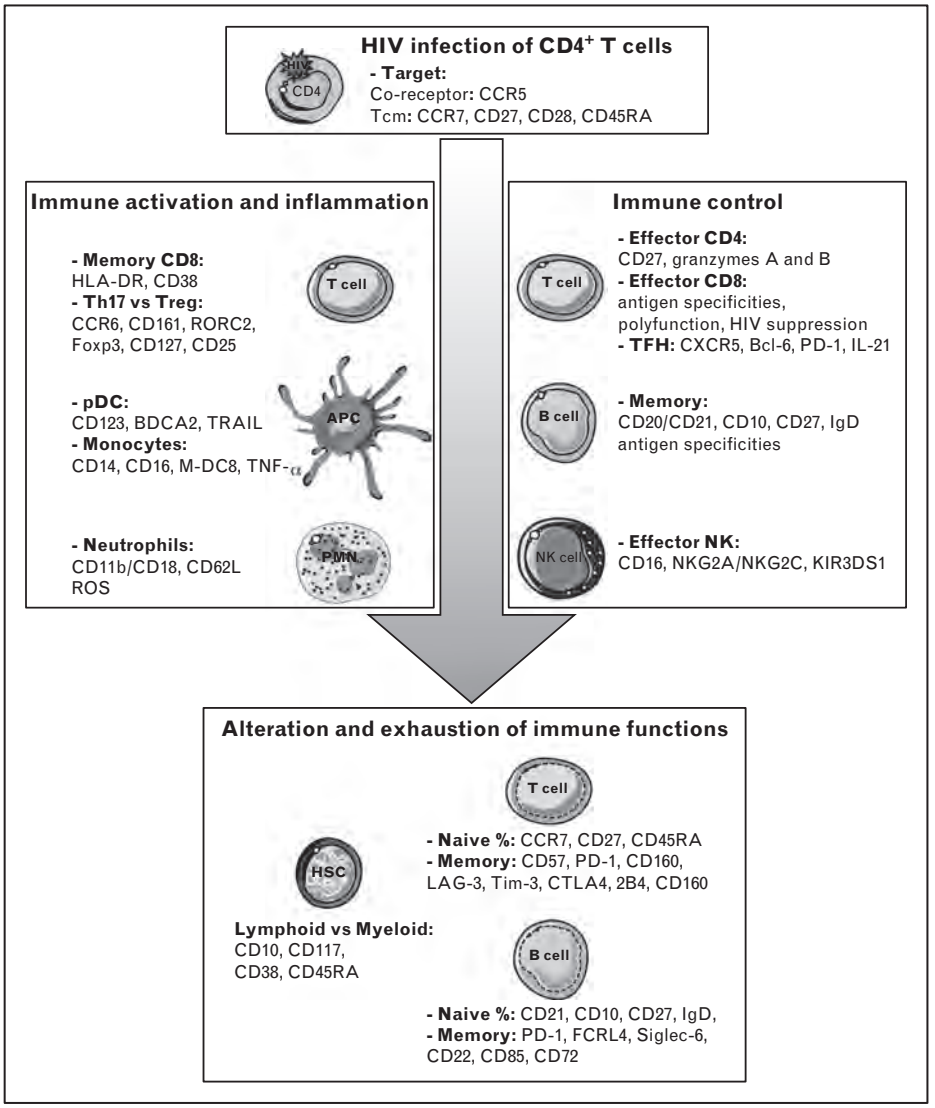

FIGURE 1. Monitoring cellular markers in HIV infection. Nonexhaustive list of cellular markers to assess HIV pathogenesis, in particular the actors involved in the immune control of HIV-1 infection, the immune activation and inflammatory responses, and the alteration of the immune functions. TCM, central memory T-cells.

express molecules such as perforin and granzymes A and B, and to present direct cytolytic activity [27]. Recent reports support an active role of cytotoxic $CD4^+$ T cells in controlling HIV. HIV-specific cytotoxic $CD4^+$ T cells were shown to suppress viral replication in HIV-infected $CD4^+$ T cells and macrophages *in vitro* [28]. Moreover, spontaneous control of HIV replication in untreated HIV-infected patients was shown to be associated with a significant expansion of HIV-specific CD4 T-cell responses endowed with direct cytolytic activity [29].

It is well established that cytotoxic $CD8^+$ T cells kill infected cells as well as secrete an arsenal of cytokines with antiviral properties (e.g., IFN-γ, TNF-α, RANTES, MIP-1α and β, perforin, and granzymes). Assessing HIV-specific $CD8^+$ T-cell functional properties, rather than their phenotype, has proven to be most informative and relevant with regard to its association with HIV control. The capacity to suppress HIV replication *in vitro* [30,31] or to produce multiple effector molecules simultaneously (referred to as polyfunctionality) upon stimulation with cognate antigens [32], is commonly monitored, and has been associated with slow HIV disease progression. Of note, the possibility to assess the expression of transcription factors using flow cytometry directly *ex vivo* led to the observation that increased T-bet expression may influence HIV-specific $CD8^+$ T-cell cytotoxic potential associated with potent HIV control [33▪]. The sensitivity of $CD8^+$ T cells for HIV antigens, which determines, at least in part, the functional properties of the cells, has also emerged as a potential correlate of HIV control [34]. Recently, monitoring cellular

functionality has been improved owing to the definition of a novel polyfunctionality index that numerically evaluates the degree and variation of polyfunctionality, thus allowing comparative and correlative statistical analyses of multiparametric datasets in association with the control of HIV replication [35▪].

Other cell subsets, including regulatory T cells, NKT cells as well as γδT cells have been studied in HIV-1-infected patients in recent years [36–42]. However, there is currently no clear understanding or general consensus with regard to their exact role in HIV pathogenesis.

IMMUNE ACTIVATION AND INFLAMMATORY RESPONSE

Immune activation levels have become a focal point of HIV-infected patient immunomonitoring, as they are strongly associated and predictive of HIV disease progression, even in HIV-infected patients with sustained antiretroviral therapy (ART)-mediated viral suppression [43]. The expression of the receptors CD38 and HLA-DR on memory T cells, which reflects the activity of the immune system against viral antigens, is commonly monitored and assimilated to the degree of systemic immune activation. In addition to viral antigens, an important trigger of immune activation are type I IFNs, mainly produced by plasmacytoid dendritic cells (pDCs) that can be identified by their expression of CD123 (IL-3Rs chain) as well as BDCA-2 (CD303). *Ex vivo*, a severe decrease of pDCs in blood from HIV-infected viremic patients is observed [44▪▪]. This apparent blood depletion is probably due to migration of HIV-activated pDCs from blood to lymphoid organs [45], in which massive $CD4^+$ T-cell depletion occurs. Furthermore, activated pDCs also express the apoptotic ligand TRAIL (TNF-related apoptosis inducing ligand) providing them potential killer activity (IFN-producing killer pDCs) [45], and activated pDCs and IFN-α have been recently reported to contribute to chronic immune activation and $CD4^+$ T-cell depletion [46]. Although a signature of increased IFN-α production is observed in HIV-1 infection, the response of circulating pDC to Toll-like receptor (TLR) stimulation is substantially impaired in relation to the enhanced interaction of CD40 ligand and its receptor CD40, which are both upregulated upon immune activation [47▪]. Interestingly, it has been recently reported that HIV controllers patients maintained high blood number of pDCs, which were producing high levels of IFN-α in response to HIV exposure; in addition, pDCs from controllers did not express activation (CD40, HLA-DR), maturation (CD80, CD83, CD86), migration markers (CCR7), or TRAIL [48▪].

Chronic stimulation of pDC driven by continual HIV production can also lead to alteration of Th17/regulatory T-cell balance [49]. In fact, during HIV infection, a significant loss of Th17 cells and an increase of Tregs have been reported in progressive patients, although HAART treatment partially normalized Th17/Treg ratio [50▪]. Massive depletion of Th17 cells, particularly in gut mucosa, results in loss of barrier integrity, causing leakiness and translocation of microbes and microbial products into circulation [51,52]. In addition, it has been recently described that many of $CD4^+$ T cells expressing the gut-homing receptors CCR9 and integrin α4β7, a population that included most gut-homing Th17 cells, remained in the circulation rather than repopulating the mucosa of the small intestine. Interestingly, the defective gut homing of $CCR9^+\beta7^+$ $CD4^+$ T cells correlated with high plasma concentrations of markers of mucosal damage, microbial translocation, and systemic T-cell activation [53▪]. Furthermore, circulating $CD4^+CD90^+$ cells, which share similarities with Th17 cells because they express the Th17-specific transcription factor RORC2, produce IL-17A, and express the gut mucosal markers CCR6 and CD161, are decreased with an imbalance of the $CD4^+CD90^+$/regulatory T-cell ratio in nontreated patients compared with treated patients and healthy donors [54]. Therefore, it would be of interest to study the frequency of $CD90^+$ T cells in the gut of HIV-infected patients and whether there is a correlation with microbial translocal emergence.

Several serum components that reflect microbial translocation are available for use as biomarkers such as soluble CD14, a marker of monocyte activation, which is an important predictor of outcome and is the only one correlated with all-cause mortality [55▪]. In addition, whereas circulating classical $CD14^{++}CD16^-$ monocyte numbers are normal during HIV infection, $CD14^+CD16^{++}$ monocyte numbers were found to be higher in AIDS or AIDS-related dementia [56,57] as well as in asymptomatic but viremic patients [44▪▪]. In addition, Dutertre *et al.* [44▪▪] pointed to the M-$DC8^+$ subset, which plays a role in several inflammatory diseases [58], as the main responsible for the elevation in $CD14^+CD16^{++}$ monocyte number. The proinflammatory M-$DC8^+$ monocytes were responsible for a large part of the overproduction of TNF-α *in vitro* in response to lipopolysaccharide (LPS) of peripheral blood mononuclear cells from viremic patients. Moreover, patients with high M-$DC8^+$ cell number exhibited a significant increase in sCD14 levels compared to the other viremic patients. This TNF-α-producing M-$DC8^+$ monocyte population might, thus, be considered as a major act in the

immune hyperactivation fueling HIV infection progression.

Microbial translocation can also lead to activation of polymorphonuclear neutrophils (PMNs) inducing the release of many inflammatory mediators such as reactive oxygen species (ROS). We recently demonstrated that resting PMNs from HIV-1-infected aviremic patients are activated, as reflected by increased β2-integrin (CD11b/CD18) expression, decreased L-selectin (CD62L) expression, and increased ROS production (CE, unpublished data). Such an excessive PMN activation, in the absence of monocyte activation, may play a key role in the chronic systemic pro-inflammatory state observed in HIV-infected patients despite unstained ART-mediated viral suppression and may participate in the development of immunosenescence [59].

ALTERATION AND EXHAUSTION OF IMMUNE FUNCTIONS

Antigen-mediated activation of lymphocytes is associated with the upregulation of a number of coinhibitory receptors, which are negative regulators of the cell activity. Strong correlations between the expression of activation markers CD38 or HLA-DR and receptors like PD-1 are evidence of this feedback regulation [60,61]. In the context of chronic HIV infection, the upregulation of coinhibitory receptors has been assimilated to an exhaustion of the HIV-specific response, as the functionality (e.g., cytotoxicity, proliferation, and cytokine secretion) of the activated lymphocytes expressing such receptors is significantly decreased. Upregulation of receptors such as PD-1, lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin and mucin domain-containing molecule-3 (Tim-3), cytotoxic T-lymphocyte antigen 4, 2B4, or CD160 is now commonly monitored on both $CD4^+$ and $CD8^+$ T cells during the course of HIV infection [62]. The combined expression of some of these markers by HIV-specific $CD8^+$ T cells has been proposed to define a subset with advanced dysfunction and linked to disease progression [63,64]. The function of these 'exhausted T cells' can usually be restored by blocking the relevant coinhibitory receptor/ligand pairs, as shown in in-vitro experiments and, more recently, in nonhuman primate models [65,66]. Similarly, recent studies show that memory B cells upregulate also a number of inhibitory receptors such as Fc-receptor-like-4, sialic acid-binding Ig-like lectin 6 , CD22, CD72, CD85j, CD85k, as well as PD-1 in HIV-infected viremic patients [67,68]. Their expression may affect B-cell receptor signaling and the capacity of memory B cells to proliferate and secrete antibodies during HIV infection, which could be reversed by blocking signaling through these receptors [69▪].

The function of individual lymphocytes is not the sole parameter influenced during HIV infection; phenotypic analyses show that the representation and distribution of whole subpopulations within the total lymphocytes are also strongly altered. With HIV disease progression, the $CD4^+$ and $CD8^+$ T-cell compartments present an accumulation of highly differentiated memory T cells ($CD57^+$) [70], and importantly, a profound loss of $CCR7^+$ $CD27^+$ $CD45RA^+$ naive T cells [71]. The latter reflects best the premature development of an immunosenescent phenotype during HIV infection [72]. Similarly, the B-cell compartment is characterized by an overrepresentation of activated and terminally differentiated cells ($CD27^-CD20^{hi}CD21^{lo}CD10^-$), and a reduced number of naive and resting memory B cells [68,73–75]. Finally, progressive HIV disease is associated with NK-cell subset redistribution, evidenced with dramatic alterations of the NK-cell pool phenotype, potentially reflecting the overrepresentation of highly differentiated NK cells [76]. Overall, T-cell, B-cell, and NK-cell compartments are all characterized by an unbalanced representation of end-stage memory or highly differentiated cells over naive or early differentiated cells with HIV disease progression.

This is the likely consequence of peripheral activation and differentiation of existing cells, together with reduced *de novo* production of new cells. Indeed, decreasing numbers of total T cells, B cells, and NK cells in HIV-infected progressors indicate that the general capacity to produce new lymphocytes is defective with progressive disease. Impaired thymopoiesis on its own cannot account for the general failure to maintain adequate production of lymphocyte populations, indicating that upstream elements of lymphocyte development may be affected. Circulating hematopoietic progenitors (HPCs) have been recently analyzed in a large set of HIV-1-infected patients [77▪▪]. This study shows that patients progressing toward AIDS have decreased numbers of circulating HPCs compared to healthy donors or HIV-1-infected nonprogressors, and that the remaining $CD34^+$ cells present both functional alterations and a preferential reduction in cells with lymphoid precursor capacity (based on $lineage^{neg}$ $CD34^+$ $CD117^-$ $CD10^+$ $CD45RA^+$ $CD38^+$ phenotype). Although the exact mechanisms are still unknown (e.g., HIV depletion of HPCs [78] or bone marrow stromal auxiliary cells [79], or indirect effect of chronic systemic inflammation [77▪▪]), studying the exhaustion of lymphopoiesis through the monitoring of circulating HPC attributes may be highly relevant for the long-term follow-up of

HIV-infected patients, in particular related to immune reconstitution with antiretroviral treatment.

CONCLUSION

A broader understanding in the HIV immunology field over the last 30 years implies the need to study an increasing numbers of immune compartments and attributes importance to HIV pathogenesis. This knowledge, together with significant technological advances, in particular related to multiparametric flow cytometry analyses, offers the possibility of monitoring cellular immune markers that are relevant for control of replication, immune activation and inflammation, and the alteration or exhaustion of the immune functions. Our review focuses primarily on the monitoring of such markers in the blood of patients. However, the primary sites of HIV transmission and infection are mucosal, and the primary target of the virus, the $CD4^+$ T cells, resides mainly in lymphoid tissues, such as the lymph nodes, and in particular the mucosal lymphoid tissues, such as the gastrointestinal tract. It is, thus, important to focus increasing efforts on monitoring cellular immune markers directly in the sites of interest and relevance for HIV pathogenesis.

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Conflicts of interest

There are no conflicts of interest.

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