6. Chronic inflammation and immune activation in HIV disease

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Abstract. Chronic activation of the immune system and related tissues is a pervasive characteristic of HIV infection at all stages of disease progression. It is well established that measures of immune activation are strong independent predictors of CD4+ T cell depletion and immune suppression. Mechanistic insights of its role in immune pathogenesis are emerging, which point to a direct participation of chronic activation in the development of immune suppression, as well as in other inflammation-related pathologies that accompany HIV infection. Extensive further research has found that activation spans a broad range of cells and functions of the immune system, and is present at all stages of HIV disease, even under control of viral replication. Important advances in the mechanisms underlying immune activation have been made in the last years, revealing diverse triggers ranging from HIV itself to toll-like receptor ligands. Of particular importance, the passage of bacterial products to the blood of HIV-infected patients is a cause of immune activation that has greatly influenced the study and management of HIV pathogenesis. This chapter reviews the evidence for the central pathogenic role of activation, the mechanisms that cause it, its role in immune...
suppression, and the consequences of chronic inflammation. Attention is made on non-AIDS-defining illnesses, which have emerged with the control of immunodeficiency by highly active antiretroviral therapy. Recent findings support current efforts to control activation/inflammation as part of effective treatment of HIV infection.

Introduction

A state of immune suppression may seem at odds with the presence of underlying inflammation or chronic activation of the immune system. However, there is ample evidence that these conditions coexist in persons infected with human immune deficiency virus (HIV). It is well established that HIV is the etiological agent of acquired immune deficiency syndrome (AIDS). However, the way in which this lentivirus alters immune functions and eventually depletes the CD4\(^+\) T cell pool is complex and includes mechanisms other than the cytopathic effect of direct infection of CD4\(^+\) T cells, its principal target cell (1, 2). An early finding during the HIV epidemic was that persons presenting AIDS, in addition to a depletion of CD4\(^+\) T cells in blood, had chronically elevated levels of different soluble proteins and T cell surface molecules associated with inflammation and immune activation. As it will be reviewed here, chronic immune activation and subclinical or overt inflammation are essential to understand HIV pathogenesis.

T Cell activation as a predictor of HIV disease progression

CD38 and HLADR expression on CD4\(^+\) and CD8\(^+\) T cells

Soon after the recognition of acquired immune deficiency syndrome (AIDS), reports appeared describing the abundance of T cells that expressed CD38 in the blood of patients with AIDS (1, 2). Specifically, it was early found that CD8 T cells were mainly the ones with an increased expression of CD38 (at the time known as OKT-10), particularly in advanced AIDS cases (3). CD38 is an ectoenzyme expressed on the surface of immature naive T cells and in activated memory T cells (4, 5). The incomplete understanding of its function in T cells contrasts with the extensively demonstrated capacity of the expression of CD38 to predict disease progression in HIV-infected people. For instance, statistical evaluation of a six-year follow up cohort of 98 HIV\(^+\) patients showed that the counts of CD38\(^+\) CD8\(^+\) T cells added to the capacity of CD4\(^+\) T cell count (the main marker of HIV disease progression) to predict the time elapsed between HIV infection diagnose and the occurrence of AIDS defining illnesses (6). Activation of CD4\(^+\) T cells, as measured by the percentage of cells co-expressing CD38 and HLADR (one of the class II MHC molecules, also indicative of activation) also predicts AIDS progression (7, 8).
The amount of CD38 molecules expressed per cell was clearly a strong predictor, even more than HLADR (9, 10). When compared with HLADR surface expression and with several soluble indicators of immune activation and inflammation, CD38 on CD8 T cells was the strongest predictor of time to AIDS onset and time to death (11), sometimes independently of blood CD4+ T cell count and viral load (viral RNA copies/mL blood) (11–15). Thus, T cell activation, as measured by the expression of surface HLADR, and very especially the expression of CD38, is not only a hallmark of HIV infection but a strong predictor of HIV disease progression, suggesting a role of chronic activation in HIV pathogenesis.

It has also been established that the correlation between T cell activation and HIV disease progression is not due to an effect of immune suppression on CD38 expression, because increased CD38 expression predicted later CD4+ T cell loss and ensuing immune suppression (16). The frequency of CD38 expression among the memory subpopulation of CD4+ T cells also predicts subsequent CD4+ T cell count decay (10). While the capacity of CD4+ T cell counts to predict the median time to AIDS was stronger in individuals with more years of HIV infection, the predictive capacity of CD38 expression was independent of the time of infection (17). Interestingly, in individuals with more than eight years of HIV infection, viral load (HIV RNA copies/mL blood) lost its capacity to predict disease progression, while CD38 expression remained predictive. Research in recently infected individuals has verified that both CD4+ T cell and CD8+ T cell activation levels predict time to AIDS (18). CD8 T cell activation (measured as median density of CD38 molecules on the surface of CD4+ and CD8+ T cells) established early during infection (set-point activation) is largely constant and predicts future CD4+ T cell loss (19).

Additional variables have verified the activated state of T cells in HIV infection. Of particular importance, the presence of the intra-nuclear protein Ki67, which is expressed at all phases of cell cycle except C0 (20), and may thus indirectly indicate proliferation (21), has been found to predict HIV disease progression better than viral load in patients with HIV-2 infection (22). Further characterization of phenotypically defined activated T cells from HIV infected patients has verified their increased expression of Ki67 (23). In fact, this protein has replaced CD38 and HLADR as indicator of activation in recent studies that link activation with mucosal depletion of CD4+ T cells (24). When proliferation was measured directly by the in vitro incorporation of the thymidine analogue 5-bromo-2'-deoxyuridine (BrdU), it was found that more than 90% of proliferating CD4+ and CD8+ T cells from HIV+ patients expressed CD38 (25). Additionally, it has been reported that blood CD38+ HLADR+ T cells have the highest proliferation rates, as measured by BrdU incorporation, among different blood cell subsets from HIV-infected subjects (26).
The potentially causal relation between immune activation and HIV pathogenesis is suggested in settings where the numbers of HIV virions in blood (viral load, measured as number of viral RNA copies/mL of blood) are almost negligible. Such is the case of elite controllers, patients that maintain undetectable viremia without antiretroviral treatment. These patients show, despite undetectable blood HIV, increased T cell activation (measured as frequency of CD38 and HLADR co-expression), which inversely correlates with CD4+ T cell blood counts, and predicts progress to AIDS despite undetectable viremia (27, 28).

Highly active antiretroviral therapy (HAART) controls HIV replication and normally lowers HIV RNA copies to less than 50 per mL of blood. This is normally accompanied by recovery of circulating CD4+ T cell counts and a decrease in immune activation (29). However, activation is not completely normalized, and evidence suggests that remnant activation is related with unsatisfactory CD4+ T cell recovery. Along with other factors, remnant activation of CD8 T cells (% CD38+ HLADR+ of CD8 T cells) showed to be a significant predictor of CD4+ T cell count change in a group of 99 patients with HAART-suppressed viremia (defined as sustaining a viral load equal or lower than 1000 HIV RNA copies/mL). These patients showed a mean decrease of 35 CD4+ T cells/µL blood for each 5% increase in activated CD8 T cells (30). In patients followed up for 12 months under HAART, the activation of total CD8+ T cells (measured as the density of CD38 molecules per cell), as well as activation of either naive or memory subsets were significant predictors of lower CD4+ T cell counts in patients under HAART achieving less than 50 HIV RNA copies/mL. Interestingly, among the CD4+ T cells, only the activated memory subpopulation was significantly predictive (31). However, CD4+ T cell recovery was not dependent of T cell activation after the first follow up year (31). It was later shown that after this first year on viral suppressive HAART, CD38 expression on CD8+ T cells was indeed predictive of lower recovery of central memory CD4+ T cells, and that the lack of correlation in the former study could be due to the differential representation of CD4+ T cell subpopulations at different stages of immune recovery (32). The capacity of activated CD4+ T cells (% of HLADR+ CD4+ T cells) to predict a low CD4+ T cell recovery, particularly among naive CD4+ T cells, was demonstrable in patients stratified according to the lowest and the highest CD4+ T cell counts achieved with treatment (33).

Even in the presence of viremia under unsuccessful HAART in HIV-infected children, the frequency of activated (HLADR+ CD38+) CD8+ and CD4+ T cells was correlated with lower CD4+ T cell counts independently of viral load (34). Further understanding of the relationship of activation with unsatisfactory reconstitution was gained by studying antiretroviral treated
patients that had sustained undetectable blood HIV levels (<50 copies/mL) for more than seven years. Among them, patients with less than 500 CD4+ T cells per µL blood showed significant increases in Ki67 expression, along with other variables correlated with activation, like the frequency of regulatory T cells and the degree of apoptosis (35). Thus, the setting of sustained viral suppression reveals the independent relationship of CD8 T cell activation with an insufficient recovery of CD4+ T cells, possibly due to a role of activation on CD4+ T cell depletion.

A link between immune activation and immune suppression is strongly suggested by an AIDS animal model, the infection of Rhesus monkeys (*Macaca mulatta*) by simian immune deficiency virus (SIV). While SIV infection of its natural host, the sooty mangabey (*Cercocebus atys*) yields high viremia and even a certain loss of CD4+ T cells without important immune suppression, the experimental infection of rhesus macaques yields immune suppression. The only feature that distinguishes non suppressive infection in sooty mangabeys from immunosuppressive infection in rhesus macaques is a lower degree of immune activation in sooty mangabeys (36, 37).

There are also strong correlations between cellular dysfunction and immune activation. It has been described that the percent of CD8+ T cells with the CD38+ HLADR+ phenotype correlated positively with the percent of CD8 cells expressing PD-1 (a co-inhibitory molecule associated with functional exhaustion), and negatively correlated with the proportions of regulatory cells (38). Neopterin is a small non-peptidic molecule produced by activated T cells, monocytes, macrophages and dendritic cells (39–42) and predicts the course of multiple trauma, cardiovascular diseases and some cancer forms (43) (42). The concentration of neopterin in plasma from HIV+ subjects was a significant predictor of decreased proliferative responses of bulk peripheral blood mononuclear cells challenged with concanavalin A (ConA), phytohemagglutinin (PHA), and pokeweed mitogen (PWM) (44). Interestingly, while CD4+ T cell count and viral load (HIV RNA copies /mL blood) were also significant predictors of ConA and PHA responses, only neopterin levels significantly predicted lowered PWM responses, further underscoring the independent capacity of immune activation to predict particular immune dysfunctions.

**Soluble mediators: Evidence of underlying inflammation**

Soluble molecules in blood produced by different arms of the immune system during inflammatory responses or activation have been found since early studies with patients infected with HIV. Among the most commonly found are the soluble interleukin-2 receptor (sIL-2R), neopterin (see above),
and β2-microglobulin (B2-M) (45). Plasma levels of soluble tumor necrosis factor-α receptor (sTNFR), β2-microglobulin (b2M), and neopterin correlated with the number of copies of HIV/mL blood (viral load) in individuals not showing opportunistic infections (46). Soluble IL-2R is released by activated T and B lymphocytes and by monocytes (39, 47). β-2 microglobulin is a component of class I HLA molecules, and is released by lymphocytes and hepatocytes during inflammatory processes (48). Their elevated levels in HIV-infected individuals (49, 50) provide additional evidence that a broad range of cells and tissues are activated, and strongly suggest the existence of an underlying inflammatory state in HIV infection, despite the inefficiency of the immune system to control opportunistic diseases.

The prognostic capacity of soluble mediators of inflammation has been statistically assessed. In a retrospective analysis of patients from the Multicenter AIDS Study Cohort (MACS) (50) a survival analysis regression model, with time to AIDS onset as predicted variable, allowed to quantify and compare the precision of the blood risk markers sTNFR-II, neopterin, sIL-2R, CD4+ T cell counts, and viral load (46). While all markers had predictive capacity, adding a soluble marker to CD4+ T cell counts, sTNFR-II yielded a significantly greater prediction precision. Interestingly, the most precise predictor during chronic infection (defined as CD4+ T cells/mL > 350) was viral load, which was replaced in more advanced stages by the combination of CD4+ cell counts and sTNF-RII concentrations (46). As it occurs with T cell activation, even under HIV replication control by antiretroviral therapy, activation, measured by the concentrations of sTNFR-II, still predicted the onset of AIDS (51). These studies show that sTNFR-II originated in cells other than T cells also predicts HIV disease progression.

Other studies have consolidated the view of soluble inflammation markers as predictors of HIV infection outcome. In a smaller cohort, proportional hazard analysis of the development of AIDS or death in a one-year follow up, neopterin, β-2 microglobulin, and soluble TNF receptor were better predictors than HIV RNA copies in blood (47).

Since early studies, neopterin emerged as a powerful prognosis marker. Using proportional hazard analysis of the AIDS-free time in a cohort, it was found that blood concentrations of neopterin, β-2 microglobulin, and to a lesser degree, sIL-2R were as useful as the CD4+ T cell count in blood in predicting the risk of progress to AIDS (49). Additionally, in a cohort followed up for 3-years, neopterin was a particularly good predictor of risk of AIDS and risk of death in HIV-infected patients (52). The capacity of neopterin to predict AIDS onset in a 54 week follow-up was independent of specific anti-HIV p24 protein antibodies, and was comparable to initial CD4+ T cell counts (53). A great capacity of neopterin to predict HIV disease stage has been reported, in
comparison with β2-microglobulin, endogenous interferon, interleukin-6 (IL-6), sTNF-RII, and HIV-1 RNA (54). In contrast, while sIL-2R levels correlate well with neopterin, they show a smaller predictive capacity (52). Nevertheless, increased levels of sIL-2R in HIV infection, while not directly predictive seem related with progression. Soluble IL-2R was found to increase with time and with AIDS onset in HIV-infected patients (55).

In line with the increased numbers of activated CD8+ T cells in HIV infection, soluble CD8 (sCD8) levels (indicating CD8+ T cell activation) in early infection added to the prognostic value of CD4+ T cell counts, and correlated with neopterin and β2-microglobulin levels (56). The strong correlation of sCD8 with the surface expression of CD38 in CD8 T cell lymphocytes supports its role as indicator of CD8+ T cell activation in HIV-infected people (57).

When cellular and soluble immune activation markers that predicted AIDS were adjusted for CD4+ T cell counts, sIL-2R, neopterin, β2 microglobulin, and CD25 on CD19 cells (activated B cells) remained independently predictive in a proportional hazards model (15). Considering the diverse cellular origin of these molecules, it is to be expected that different mechanisms trigger immune activation in HIV infection.

In subsequent years, other soluble indicators of inflammation have been added to the list of correlates of HIV disease progression, like soluble CD27 (58), intercellular adhesion molecule-1 (sICAM-1), soluble E-selectin (sEs) (59), and L-selectin (60). Of clinical relevance, molecules related with non-AIDS defining illnesses are also increased by HIV infection and correlate with its progression. Soluble intercellular adhesion molecule I (sICAM-I), soluble vascular cell adhesion molecule I (sVCAM-I), and von Willebrand factor are released by activated endothelium (61–63). The plasma concentrations of these three molecules are increased in HIV-infected patients, and correlate with levels of TNF-α, and soluble TNF-α receptor (64). Likewise, the cardiovascular risk marker high-sensitivity C-reactive protein (hsCRP) prospectively predicts the risk of opportunistic diseases in patients with HIV, along with the pro-inflammatory cytokine IL-6 (65).

**Activation of B cells and innate immunity**

Chronic immune activation is not restricted to T cells. Almost every component of the immune response, both adaptive and innate has been shown to be chronically activated in HIV infection. Polyclonal activation of B cells in subjects with HIV infection was an early finding of the HIV epidemic (66). It is reflected in an increased spontaneous in vitro production of IgD by blood mononuclear cells from HIV-infected subjects, throughout different stages of
infection, as well as by an increased spontaneous production of IgG and IgM by cells from HIV+ subjects with apparently more advanced disease (as indicated at the time by generalized lymphadenopathy and Kaposi’s sarcoma) (67). This polyclonal activation is also sustained in gut mucosal tissue, as reflected by high IgG, IgM and IgA production by cells derived from this tissue, and by the increased production of non-HIV-related responses (anti-keyhole limpet hemocyanine and anti-dog serum albumin responses) (68). Mucosal B cell polyclonal activation coexists with a strong anti-HIV immunoglobulin response, which is greater than blood response by orders of magnitude (68). This strong mucosal B cell activation very likely reflects the preferential infection of mucosal CD4+ T cells by HIV (69). Unspecific immunoglobulin production comprises also IgE, which was found significantly associated with progression to AIDS when adjusted for age and CD4+ T cell count (70). Thus, progression of HIV infection can be predicted by activation of both T and B cells.

As described with T cells, B cell activation is greatly decreased by HAART, but not completely, since both IgG and IgA blood levels remain higher than in HIV- controls despite control of viral replication (71). Mechanisms triggering B cell activation in HIV infection are discussed below. As it occurs with T cells, chronic activation of B cells in the context of HIV infection is accompanied by diminished presence of B cell subpopulations and impaired anamnestic antibody responses (72).

Antigen-presenting cells (APC) are also abnormally activated during HIV infection. In consonance with elevated levels of plasma neopterin in HIV infection (produced by activated macrophages), macrophages obtained by bronchioloalveolar lavage show increased surface expression of HLA-DP, HLA-DQ, and HLADR, which indicates activation, a state verified by their increased spontaneous in vitro production of superoxide radical (73), in comparison with alveolar macrophages from HIV- controls. The multiple consequences of macrophage activation in HIV infection are discussed in a separate section.

Activation of macrophages and dendritic cells from HIV+ subjects has been studied as a facilitator of cell infection by HIV (74, 75). Activation has other effects. Activation of dendritic cells is detected by the expression of molecules that allow their antigen presenting function, which include co-stimulatory molecules and major histocompatibility molecules. Among these, the expression of the co-stimulatory molecule CD40, which interacts with T cells during antigen presentation, is increased in myeloid dendritic cells from blood of untreated patients with HIV, concurring with altered capacity of migration to lymph nodes (76), and levels of Bcl-2 and caspase-3+ expression indicating a pro-apoptotic state (77).
A state of generalized immune activation is demonstrated by this long list of indicators of activation or inflammation in diverse arms of the immune function. The fact that activation is not restricted to CD4+ T cells in HIV infection is in consonance with the existence of indirect mechanisms of immune pathogenesis in HIV infection.

**The long reach of chronic activation/inflammation in HIV infection**

As seen with T cell activation, control of viral replication and viremia with HAART is not able to fully normalize the levels of soluble indicators of inflammation. Very notoriously, six years of viremia control by HAART (<40 HIV-1 RNA copies/mL) significantly reduced the serum concentration of IgG, IgM, interferon gamma-induced protein 10 (IP-10), sTNF-RII, neopterin and soluble TNF-related apoptosis-inducing ligand (sTRAIL) (78). However, despite this long period of viral control, concentrations of IFN-α and sTNF-RII were still significantly higher than in HIV controls (78). The causes of this residual activation are not clear. Among patients under HAART and showing viremia control, those developing immune reconstitution inflammatory syndrome, a paradoxical worsening of AIDS-related inflammatory symptoms in the setting of effective HAART had increased levels of sTNF-RI and sIL-1Ra (79), which suggests that variables other than residual viremia (58, 80) can be related with remnant activation. Remnant activation underscores the need of controlling immune activation in addition of HIV replication control.

**Triggers of chronic immune activation in HIV infection**

After the initial studies linking immune activation with HIV pathogenesis, it became clear that the mechanisms triggering this activation should be diverse, and not restricted by specific response against HIV epitopes.

**Activation by HIV components**

Immune activation is reduced, albeit not eliminated, by HAART (81), and HAART interruption allows a rebound in immune activation (82). In the same way, insufficient control of viremia during treatment yields increased immune activation in blood (83) and in cerebrospinal fluid (84). Further, residual viremia, even during a good response to HAART has been found to correlate with activation (80). This demonstrates that the presence of HIV is necessary at least in part for chronic activation.
From these evidences, it would seem reasonable that immune activation in HIV infection is mainly driven by specific adaptive immune response to HIV. Indeed, CD38$^+$ HLADR$^+$ CD8 T cells, which strongly predict infection progress show the strongest specific cytotoxic activity against HIV antigens, compared with not activated or partially activated cells (83). In this same regard, there is a significant correlation between the frequency of HIV-specific CD8 T cells and the frequency of CD38$^+$ CD8 T cells, particularly in patients with high levels of CD8 T cell activation (84). HIV-specific CD8$^+$ T cells from HIV$^+$ patients that progress to AIDS at a normal rate (progressors), more frequently co-express CD38 and PD-1 (85). However, there is evidence that not all activated T cells are specific to HIV. Even though HIV-specific CD8$^+$ T cells were significantly enriched among CD38$^+$ HLADR$^+$ CD8$^+$ T cells, they constituted only about 4-18% of all the CD38$^+$ CD8$^+$ T cells, and were present among other CD8$^+$ T cell subsets, thus not accounting for most of the CD8$^+$ T cell activation (86). Likewise, the frequency of HLADR$^+$ CD38$^+$ CD8$^+$ T cells in patients with HIV has been found to correlate with the frequency of CD8$^+$ T cells specifically responding to HIV protein Gag and with an intermediate differentiation phenotype (87), which is only a fraction of all CD8$^+$ T cells. Moreover, there is no consensus about the activation state of HIV-specific CD8$^+$ T cells. CD8 lytic activity against antigenic determinants of the HIV protein nef, pol and gag (as area under the curve) had a clear correlation with the frequency of non-activated (CD38$^-$ HLADR$^-$) CD8$^+$ T cells (88). It is clear from these contrasting studies that a specific response against HIV can contribute to CD8$^+$ T cell activation, but it does not account for the whole amount of activated cells.

**HIV Proteins: Nef**

Several HIV components and gene products can induce activation of different immune cells, without mediation of specific response. Nef, a product of HIV genome (89) was initially studied by *in vitro* induction of its expression in different cell lines. It was found in Nef-transfected CD4$^+$ T cell lines, that when expressed with CD8$^+$ on the cell surface of Jurkat cells, nef activated early events of TCR (T cell receptor)-mediated early signaling events (90). This was verified by the gene expression profile of transfected cells, with the effect requiring TCR-ξ and ZAP-70 proteins (91). Activation that depends on upstream TCR signaling events has also been evidenced by IL-2 production in Nef-transfected CD4$^+$ T cell lines (92), an event mediated by nef association with membrane lipid rafts (92, 93). Nef transfection also sensitizes primary resting CD4$^+$ T cells to new stimuli (94), and elicits MIP-1α and MIP-1β production in macrophages (95). While evidencing an activating role, these
findings have the limitation of only emulating HIV-infected cells. Since only up to 1% of all CD4+ T cells can be infected by HIV in vivo at a certain time (reviewed in (16)), it is hard to infer a possible biological meaning of these in vitro expression effects on in vivo chronic activation.

Studies using recombinant Nef protein are closer to resemble in vivo interactions. Free Nef protein activates immature dendritic cells (DC), inducing mixed lymphocyte reaction, and maturation to an antigen expressing cell (APC) phenotype. This APC state is evidenced by expression of HLADR, CD40, CD83, secretion of TNF-α, IL-1β, IL-12 and IL-15, and by showing reduced phagocytosis mediated by mannose receptor (95). Free recombinant Nef also activates monocytes, increasing their production of IL-1α, IL-6, TNF-α and, importantly MIP-1α and MIP-1β (96). These Nef-induced APCs could indirectly activate T cells during antigen presentation. Finally, a role of Nef in activation induction is suggested by the finding that Nef, while inducing a down regulation of CD3 in natural hosts of simian immunodeficiency virus (SIV), has lost this activity in the HIV lineage, thus facilitating TCR-mediated activation (97).

**HIV Proteins: Gp120**

Gp120 is the component of HIV envelope that forms virion spikes, and is therefore highly exposed on the viral particle (98). In addition to be an important immunogen, it has activating effects on numerous cell types. In self-reactive T cells and in T cells specific to the M. tuberculosis antigen PPD, a peptidic fraction of this protein enhanced proliferation. This effect could have been mediated by the great homology of this peptide with the HLA β chain (99). Gp120 also activates T cells unspecifically, inducing the expression of Vβ subunit families 2 and 3, in a manner resembling superantigens (100). The endothelium can also be activated by gp120, as shown by a study in which the adhesion molecules ICAM-1 and VCAM-1 and substance P were induced by this protein in the blood-brain barrier vascularity, which could lead to greater permeability of this compartment (100). Cardiomyocytes can also be influenced by gp120, which enhances IL-1β-induced nitric oxide (NO) production by engaging p38 MAP and NFκB intracellular signaling (101). Induction of NO production by gp120 has also been observed in dorsal spinal cord, NO in turn induces IL-1β and IL-6, and enhances pain (102).

Glycoprotein 120 (gp120) of HIV can also activate antigen presenting cells (APC), like microglia (103). The mechanism of macrophage activation by gp120 is known to some extent. This protein mimics CCR5 and CXCR4, chemokine ligands of macrophages, which induces extracellular Ca2+ influx, inducing c-Jun amino-terminal kinase/stress-activated protein kinase and p38
MAPK intracellular signaling. This in turn leads to the secretion of the chemokines monocyte chemotactic protein-1 (MCP-1), and MIP-1β phosphorylation. The effect requires phosphorylation of tyrosine kinase Pyk2 (104). CCL2 secretion is also elicited in macrophages by gp120, an effect mediated by CCR5 binding, engagement of phosphatidylcholine-specific phospholipase C and the inflammation mediating activation of NFκB (105). Induction of B cell differentiation by the gp120 precursor gp160 has been reported, and is dependent on their interaction with T cells (106).

The immune activating properties of gp120 are not restricted to immune cells. It can bind to the complement proteins C4, C3d, C5b and properdin, which in turn activates complement (107). Activation mediated by CCR5 binding by gp120 is also related with apoptosis induction. Peptides from the hypervariable domain (V3) of gp120 enhance apoptosis induced by *Staphylococcus aureus* toxin A in effector memory CD4+ T cells (108).

Despite the above described *in vitro* activating effects of gp120, opposite findings make it hard to infer a final *in vivo* contribution of the gp120 envelope molecule to chronic immune activation in HIV infection. In fact, gp120 has been postulated as a cause of immune suppression in HIV infection (109). The presence of gp120 in conjunction with anti-gp120 antibodies inhibits *in vitro* CD4+ T cell activation with an agonistic anti-CD3 monoclonal antibody, as seen by a reduction on Ca2+ mobilization. Concomitantly, surface expression of CD4+ is reduced, which is seen as an inhibiting effect of gp120/anti-gp120 ligation (110). This apparent co-internalization of CD4+ and gp120 gained experimental support by the determination of binding kinetics, and by the demonstration of intracellular localization of gp120 with parallel kinetics of CD4+ internalization (111). This study additionally found a simultaneous loss of p56lck, a tyrosine kinase associated with CD4+ signaling, and a parallel loss of TCR-mediated responses (111). TCR-mediated responses require the concourse of co-stimulatory molecules interacting with their ligands in the immunological synapse (112). Such is the case of CD40 and CD28 (109). It was demonstrated that the inhibitory activity of gp120 upon TCR-mediated stimulation is mediated by alteration in the expression of the co-stimulatory molecules CD40L (on T cells) and B71 (on B cells functioning as APCs) (109). Moreover, there is evidence that the impairment in CD40L induction is actually due to the above mentioned engagement of CD4+ by gp120 (113). Among the intracellular signaling events that are altered by gp120, downstream of CD4 engagement previous to co-stimulatory molecule induction, is the phosphorylation and activation of lck and lyn molecules (114), and the induction and activation of the Janus kinase associated with the IL-2 receptor (JAK 3) (115). This inhibitory activity has also been observed using the whole HIV Env protein precursor of the smaller gp120 fraction (116).
The inhibitory activity of gp120 includes other immune cells. Upon contact with plasmacytoid dendritic cells, it inhibits functions mediated by the toll-like receptor 9 (TLR-9), like the expression of the surface molecule CD83, and the secretion of IFN-α, TNF-α, IL-6 and IP-10 (117).

The dual activating and inhibitory activities of HIV gp120 should not be seen as paradoxical, since the co-existence of activation and dysfunction has already been described in T cells from HIV-infected patients (86, 118). Another way in which activation by gp120 may lead to immune suppression is by activation of T regulatory cells, which can in turn diminish a number of immune functions (119). Therefore, there exists sufficient evidence supporting a role of gp120 as a promoter of abnormal activation in which functional alterations can be present.

**HIV Proteins: Tat**

Tat is a nonstructural protein of HIV, with a gene expression inducing function (120). Tat has shown in vitro activating properties on monocytes, inducing IL-1β, IL-6, IL-8 and TNF-α, of which IL-1, TNF-α are involved in endothelial damage (121). In contrast, monocytes can also be induced to produce the immunomodulatory cytokine IL-10 (122). The overall contribution of HIV Tat to activation of circulating monocytes remains unclear. In T cells, Tat’s activating capacity seems to be more indirect, as a facilitator, rather than as a trigger of activator. It has been found to potentiate nuclear factor κB (NFκB, central in many inflammatory pathways) induction by TNF-α, by down-regulating manganese superoxide dismutase (123). Tat induces phosphorylation of cAMP response element-binding (CREB) in T cells, possibly potentiating upregulation of activation-related genes (124). Tat also enhances IL-2 and proliferative responses of CD4+ T cells to T cell receptor stimulation with anti CD3 antibodies and co-stimulation with anti CD28 antibodies (125).

The endothelium and the central nervous system are apparently better targets of Tat-mediated pathogenesis by activation. By binding αVβ3 integrin, Tat initiates NFκB-mediated endothelial proliferation and neovascularization (126). It also promotes migration at the endothelium, as well as migration of Kaposi’s sarcoma by activating the Ras-ERK intracellular cascade (127), and by controlling MAP kinase pathways (128). Related with its endothelial effects, Tat is able to disrupt the blood-brain barrier, thus participating in HIV dementia. Tat reduces the expression of zona occludens 1 protein of tight junctions, which increases the infiltration of inflammatory cells to the brain (131). HIV neuropathogenic effects by activation also involve microglia, astrocytes and neurons. It affects microglia cells electrophysiologically through an NFκb-mediated mechanism (129). In conjunction with HIV protein
gp41, it increases superoxide levels, and is also able to increase intracellular Ca\(^{2+}\) levels, as well as the production of IL-1\(\beta\), IL-6, MIP-1\(\alpha\), TNF-\(\alpha\), and the chemokine RANTES (130). In astrocytes, Tat increases the frequency of CCL2 expression (131), a process mediated by CCL5 and RANTES (135). When the protein is expressed in astrocytes, it induces activation, as detected by the expression of fibrillar acidic protein (GFAP), parallel to a decrease in glutamate uptake, which leads to neuron death (132). A direct effect on neurons is the induction of TNF-\(\alpha\) production (133).

**HIV Genome**

Toll-like receptors (134) are a central link between HIV infection and chronic immune activation. HIV genome, composed of two single-stranded ARN molecules (135), can bind TLR 7 and TLR 8, and directly activate plasmacytoid dendritic cells, one of the cell types that can internalize HIV and be infected by it (136). One result of TLR 7 activation is the production of IFN-\(\beta\). Even though this induction was initially proposed as a potentially adjuvant in protective anti-HIV immunity, it has been proposed, given the important drop in CD8\(^+\) T cell activation that accompanies viremia control, that it can be seen as an additional trigger of HIV pathogenesis (137). Additionally, HIV-derived single stranded RNA moieties able to bind TLR 7 and TLR 8 induce monocytes and plasmacytoid dendritic cells (pDC) to produce TNF-\(\alpha\) and IL-6. The participation of TLR 7/8 was verified by the requirement of MyD88, an indispensable step of TLR7 and TLR8 intracellular signaling pathway (137). TLR7/8-binding ssRNA from HIV was also able to activate CD8 T cells in a TCR-independent way, both directly and via pDC activation (137) which is of importance, given the strong correlation of CD8\(^+\) T cell activation and HIV disease progression. Activation by HIV ssRNA is further explained by the expression of TLR 4, 5, 7, and 8 on CD8\(^+\) T cells, among which TLR 7 is additionally over-expressed in CD8\(^+\) T cells from patients infected with HIV, making them over-responsive to HIV ssRNA (138). Differential expression of TLR 7 also explains the higher levels of in vitro CD8\(^+\) T cell activation secondary to pDC activation with HIV-derived TLR 8 ligands in women, compared with men (139). Importantly, this greater proneness to TLR 7/8 activation was accompanied by relatively greater levels of in vivo CD8\(^+\) T cell activation in women, who tend to advance more rapidly to AIDS (137). Thus, TLR 7/8-mediated activation in HIV-infected people has a great influence in overall chronic immune activation. Furthermore, TLR 7 engagement potentiates the immune activation induction via other TLRs, like TLR 4 (140), which have a great contribution to chronic immune activation in HIV infection, as is explained in the following section.
The microbial translocation paradigm

Albeit important, the above mentioned mechanisms of immune activation by HIV proteins are not sufficient to explain chronic immune activation in HIV infected persons, since drastic control of viral replication and viremia does not revert activation completely (141). A drastic turn in the research of immune activation in HIV infection occurred when it was reported that an important cause of immune activation was the passage of bacterial products from the gut to the blood (142). Lipopolysaccharide (LPS or bacterial endotoxin), a component of bacterial membranes, was found to be present in increased concentrations in blood from HIV-infected individuals (142). LPS in blood was found to chronically activate monocytes in vivo, as evidenced by the increased presence of soluble CD14 (a product of monocytes in response to LPS) and LPS-binding protein, a product of LPS-challenged gastrointestinal and hepatic epithelia. Simultaneously, LPS induced tolerance in monocytes, a result of chronic TLR 4 (LPS ligand) stimulation, as evidenced by a lower in vitro TNF-α and IL-1β responses to LPS challenge. An altered capacity to control the deleterious effects of endotoxemia was evidenced by lowered concentrations of the IgM antibody specific to LPS-core (EndoCab). Of note, the participation of bacterial products translocation in HIV pathogenesis by immune activation was established by the strong correlation of LPS concentrations and the frequencies of CD38+ HLADR+ CD8 T cells, and by the positive correlation of CD4+ T cell recovery with lowered concentrations of LPS as a result of antiretroviral therapy. In turn, the partial control of endotoxemia and activation by antiretroviral therapy shows a role of HIV infection in perpetuating this mechanism of bacterial product translocation and activation (142). The importance of LPS in HIV pathogenesis is underscored by the finding that endotoxemia and monocyte activation persist even when antiretroviral therapy controls viremia successfully (143).

As to how HIV infection enables chronic bacterial translocation, it has been determined that in the first weeks of HIV infection, there is a massive depletion of CD4+ T cells residing in the gut mucosa (144), which are not restored in the chronic phase (69), nor with antiretroviral therapy (69). Parallel to mucosal CD4+ T cell depletion, there is a disruption of the gut epithelium and a decrease in its capacity to limit the passage of bacteria and their components to the blood (145).

It is important to mention that these findings do not rule out a contribution of direct activating effects of HIV to overall immune activation in HIV infection, as suggested by the fact that activation in the early acute phase of HIV infection does not correlate with blood LPS levels (142). Likewise, LPS does not seem to be the only bacterial inducer of activation. The passage of
bacterial products that bind other TLRs (e.g. peptidoglycan, flagellin, bacterial DNA) was strongly suggested by increased concentrations of IFN-α, mainly produced by plasmacytoid dendritic cells, which do not express TLR4, the LPS receptor (142).

Increased levels of bacteria-specific DNA was demonstrated by the presence of DNA encoding for 16s RNA in the blood from untreated viremic HIV+ patients (146). Its concentration correlated with LPS concentrations, viral load (HIV RNA copies /mL blood), and, importantly, with CD8+ T cell activation, as measured by their % CD38+ HLADR+ (146). A pathogenic role of bacterial DNA-driven immune activation was evidenced by the strong negative correlation of %CD38+ HLADR+ CD8+ T cells and 16rDNA with CD4+ T cell recovery under antiretroviral therapy (146). Immune activation by bacterial DNA may be explained by TLR 9 engagement. Bacterial DNA is greatly enriched in six-base long DNA motives formed by unmethylated CpG (deoxycytidylate-phosphate-deoxyguanylate) dinucleotide with two purines at the 5’ end and two pyrimidines at the 3’ end. CpG DNA is able to induce B and T cells to secrete IL-12, IFN-γ and IL-6 (147), thanks to CpG binding to TLR 9 expressed by these cells (148). In B cells, TLR 9 engagement by CpG DNA oligonucleotides induces proliferation and differentiation, with induction of IgM secretion, as well as CD40, HLADR, and CD80 expression, rendering them more able to function as antigen-presenting cells (149).

The possible participation of other TLR ligands derived from bacteria in chronic immune activation is supported by the induction of CD38 (but not HLADR) expression on CD4+ and CD8+ T cells upon stimulation of peripheral blood mononuclear cells with ligands of TLR3 (polyinosinic:polycytidylic acid or Ploy I:C), TLR4 (LPS), TLR5 (Flagellin A), and TLR-9 (CpG DNA) (150). Additionally, CD4+ T cells were induced to enter cell cycle by these ligands (except LPS), as well as by imiquimod (TLR7 ligand) and ssPoly U (TLR 8 ligand), while CD8+ T cells were more frequently induced to express CD69 (also associated with T cell activation) by TLR ligands, including the above mentioned, plus imiquimod and ssPoly U (150). The extent to which each of these TLRs are involved in chronic activation in HIV infection remains to be determined, and will indicate new possible strategies to control chronic inflammation in HIV infection.

**Opportunistic infections**

Having described the important contribution of bacterial translocation to chronic activation in HIV disease, it is reasonable to think that the increased presence of microbial products during opportunistic infections may increase immune activation, by providing more specific antigens as well as TLR
ligands. As found in a study including HIV\(^+\) patients with acute infections of diverse etiology (\textit{Pneumocystis jirovecii} pneumonia, bacterial pneumonia, lung aspergillosis, toxoplasmosis, and bacteremia), infection was related with a significant increase in HIV viremia, while recovery was accompanied by a decrease in blood viral load. Parallel to this decrease, decreases in TNF-\(\alpha\) and soluble sTNF-RII were significantly greater in patients that recovered from the opportunistic infection than in those not fully recovering. Changes in the levels of sTNFR-I, sTNFR-II, and sIL-2R correlated significantly with viremia throughout infection onset and resolution (151). The heterogeneity of the opportunistic infections in this study precludes assigning particular contributions to infectious agents. However, it is interesting to note that PCP pneumonia was accompanied by greater plasma concentrations of sTNFR-I, sTNFR-II, IL-2, sIL-2R, IL-6, and IL-19 than bacterial pneumonia (151), which may suggest that the inflammatory processes of PCP pneumonia contribute to the activated state and/or viremia.

Some parasitic infections might influence the activation state of HIV\(^+\) patients, as suggested by the increased plasma HIV load in HIV\(^+\) subjects co-infected with intestinal helminthes, in proportion with the amount and diversity of worms (152). However, there is important inconsistency of results reported by different groups (153). Studies standardizing parasitic infection evaluation and measuring immune activation are required to clarify this possible association. In contrast, patients co-infected with HIV and the microscopic parasite \textit{Leishmania} had significantly higher frequencies of CD8\(^+\) T cells expressing CD38 (154). However, caution should be used at inferring from this observation, since it was reported only at the remission phase of leishmaniasis.

Findings in patients co-infected with HIV and HCV are consistent with a net contribution of HCV infection to chronic activation in HIV disease. Activation of T and B cells correlated with activation of hepatic stellate cells (as assessed by the expression of the \(\alpha\)-isotype of actin), which are involved in inflammation and fibrosis of the liver, in patients with HIV/HCV co-infection (155). Accordingly, frequencies of circulating CD8\(^+\) T cells co-expressing CD38 and HLADR are significantly higher in female patients infected with HIV and HCV, as assessed by the presence of both anti-HCV IgG and viral RNA, compared with women infected with HIV only (156). An independent study found that the percent CD38\(^+\) HLADR\(^+\) of CD8 T cells from persons with both HCV and HIV was higher than that of subjects singly-infected either by HIV or HCV (157). This study provides further evidence of the association of co-infection and activation with the finding that effective control of HCV with IFN-\(\alpha\) and ribavirin in dually infected people significantly reduces CD8\(^+\) and CD4\(^+\) T cell activation (157). This finding is sustained when activation is
determined by plasma concentrations of the soluble indicators of inflammation sTNF-R1, sE-selectin and sICAM-1 (158).

Herpes simplex virus type 2 (HSV-2) co-infection is simultaneously associated with HSV-2-specific immune dysfunction and with a significantly increase in the frequency of CD8+ and CD4+ T cells expressing CD38 (159). As explained before, lack of HIV replication control with antiretroviral therapy is a cause of incomplete control of immune activation. One of the causes may be insufficient control of opportunistic infections, like cytomegalovirus (CMV). Control of active infection with CMV with the antiviral agent valganciclovir resulted in a significant drop in the frequencies of CD38+ HLADR+ of CD8+ T cells, compared with nested controls receiving placebo (160). In this study, the control of other herpes virus (Epstein Barr virus, HHV-6, and Kaposi’s sarcoma-associated HHV-8) by valganciclovir was not evident, and thus their contribution to immune activation remains unclear. Additionally, the correlation between control of herpesvirus replication and immune activation was not clear in this study. In a nested case-control study in which patients had achieved controlled HIV replication, but had residual herpesvirus replication, this relation with CD8+ T cell activation was not observed (161). This suggests a complex relationship between these opportunistic viruses and immune activation, and an increased capacity of opportunistic infections to drive immune activation when HIV replication is not controlled.

**Role of immune activation in pathogenesis of HIV disease**

**Activation-driven immune dysfunction**

The above described body of evidence has been taken to reveal an independent role of immune activation, particularly T cell activation, in CD4+ T cell depletion and dysfunction. However, only recent studies have provided direct evidence linking both phenomena at the single-cell level; that is, cases in which a given dysfunction of immune cells is conferred by their activated state. By microarray transcription analysis it was shown that activated (CD25+ or HLADR+) CD4+ T cells from patients with untreated HIV infection have increased transcription of genes related with proliferation, and with genes that responded to type I interferon stimulation. Further validation showed that in vivo activated cells from HIV+ individuals more often expressed cyclin A and B, and are were more frequently in S or G2/M phases of the cell cycle (162). These studies show alterations that are unique of activated cells and point to a role of type I interferon in the functional alterations of activated T cells.

It has been experimentally investigated if CD38 expression on effector memory (EM) and central memory (CM) CD4+ T cells from HIV+ patients
conferred decreased cytokine responses to polyclonal activation through the T cell receptor (TCR). It was found that the IL-2 and IFN-γ responses of CD38+ memory CD4+ T cell subsets were uncoordinated with the normally simultaneous induction of the co-stimulatory molecule CD154 (CD40 ligand) (118), and this disconnection was enhanced in samples from HIV+ donors. This alteration could render cells unable to respond to CD40 from antigen-presenting cells. Interestingly, IFN-γ response of patients’ CD38+ effector memory cells was not increased by agonistic ligation of the co-stimulatory molecule CD28, and the same lack of response to co-stimulation was seen in IL-2 production by central memory cells (118). Likewise, after in vitro induction of apoptosis with Fas ligand, it was seen that CD38+ CD8+ T cells from HIV patients more frequently entered apoptosis and expressed CD95 (Fas receptor), especially cells from patients with a high frequency of CD38+ CD8+ T cells (86). However, in this experimental setting (in vitro stimulation in the absence of Golgi blocker), it is not possible to know if CD38 levels are representative of ex vivo values, since de novo CD38 expression could have been induced by the experimental procedure. Whether intrinsic dysfunctions of CD38+ T cells can also explain CD4+ T cell depletion requires further investigation.

Role of immune activation in HIV replication and transmission

The main cellular target of HIV is constituted by the effector memory subpopulation of CD4+ T cells, which home mainly to the mucosal tissues. These cells express the chemokine receptor CCR5, which is used as co-receptor (the CD4 molecule being the main receptor) by the strains of HIV that are transmitted (reviewed elsewhere (163)). As a lentivirus, HIV has a replication cycle that involves reverse transcription of its single stranded RNA genome to DNA, and integration of this DNA to the target cell’s DNA. Some of the infected cells will then harbor a transcriptionally dormant pro-viral DNA. Latent pro-virus reactivation in CD4+ T cells is mediated by both viral and cell promoters, the latter normally involved in T cell activation or inflammation signaling pathways (reviewed elsewhere (164, 165)). More specifically, the initiation step of HIV pro-viral transcription requires an initiation complex that includes the intracellular signaling protein NFκB, a concurrence point of signaling cascades of pro-inflammatory molecules like IL-1β, TNF-α, TLR4 ligands and TLR9 ligands (166), as well as NFAT, JAN, and SP1, which are involved in T cell activation by cytokines like IL-15 and IL-7, by T cell receptor binding, CD4+ binding, and the binding of the co-stimulatory receptor CD28 (165). As described in previous sections, the concentrations of many of these activators are increased in HIV infection, and can therefore be expected to participate in maintaining viral replication.
Additionally, as previously described in this chapter, Nef and Tat proteins of HIV are immune activators, and activate both NFAT and NFκB (92, 167).

There is evidence that activation may enhance viral replication in vivo. Experimental activation of lymphoid tissue preparations with HIV induces productive infection in CD4+ T cells, mainly in activated ones. Importantly, the activation state after infection (measured as % cells expressing either CD25 or HLADR) predicts the viral production of these preparations (168). Other activation mechanisms could enhance viral replication in CD4+ T cells in vivo, as demonstrated by the ability of Neisseria gonorrhoeae to increase infection by HIV in resting CD4+ T cells, while also inducing their activation, an effect mediated by toll-like receptor 2 (TLR2) (169).

In monocytes and macrophages, a secondary target of HIV, enhancement of productive HIV infection by the pro-inflammatory mediators macrophage colony stimulating factor (M-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF), TNF-α, and prostaglandin E 2 (PGE2) was not demonstrable; however, the addition of anti-inflammatory cytokines IL-10, IL-4, and TGF-β decreased the normal infectivity (74). The complex interaction of activating mediators with productive infection of macrophages has been reviewed elsewhere (176). Viral entry can be enhanced by IL-10, MC-SF, and by the up-regulation of CCR5 expression. However, HIV transcription is increased by TNF-α, IL-1β, IL-6, GM-CSF, and IL-18. Post transcriptional events (virus assembly and budding) can be decreased by IFN-α, IFN-β and IL-10 and IL-27 (170). The M1 activation pattern of macrophages, which requires priming by IFN-γ followed by TLR-4-mediated activation, facilitates pro-virus transcription (170). However, experimental infection of macrophages with HIV does not trigger activation by itself, and the addition of IFN-β or the TLR3 ligand poly I:C actually reduces the number of infected cells (171). Thus, while being an important HIV reservoir, the role of activated macrophages in virus replication is less clear.

HIV expression reactivation can be induced in a model of latent infection in macrophages by Fusobacterium nucleatum and Porphyromonas gingivalis, (Gramm-negative bacteria involved in periodontal disease) via ligation of TLRs 2 and 9. In agreement with previous reports, TLR4 ligation by bacterial lipopolysaccharide did not reactivate HIV expression in macrophages (172). This shows again the importance of activation, in this case elicited by bacterial translocation or infection, in HIV replication. In this line of evidence, gram-positive bacteria may also increase HIV cellular infectivity and replication. Culture-derived and skin-originated Langerhans cells’ susceptibility to HIV infection is increased by TLR2 ligation with heat-killed gram-positive bacteria, whole gram-positive bacteria, and synthetic TLR2 agonists, while their activation was increased (measured as surface CD86
expression) and decreasing the expression of the cellular protein APOBEC 3G that restricts HIV replication (173). Taken together, these studies suggest that activation mechanisms increasing viral infectivity and replication in cells that constitute their first encounter in mucosal tissue could in turn increased the probability of acquiring HIV infection.

An opportunity for studying the mechanisms of HIV infection is given by persons that despite being exposed to HIV remain uninfected (29). Highly-exposed individuals that remain seronegative, when compared with a group at risk that acquired HIV infection, showed significantly lower frequencies of CD4+ T cells in cycle, as assessed by expression of the intranuclear factor Ki67, an indicator of activation (174). In the same way, mother to child transmission of HIV was independently predicted by the percentage of CD8+ T cells that co-expressed CD38 and HLADR (175). A strong association between the incidence of sexually transmitted diseases with the risk of HIV acquisition has been repeatedly found (reviewed elsewhere (183)); however, this association could be due to shared behavioral risk factors. There is evidence; however, indicating that the infectious process generates an inflammatory milieu that facilitates viral infection. Vaginal trichomoniasis and herpes simplex virus infection are significantly correlated with cervicovaginal lavage concentrations of IL-1β and IL-8, which in turn are predictive of cervicovaginal HIV shedding (176, 177). This finding points to a link between genital tract infections, inflammation, and HIV transmission.

Inflammation and non-AIDS-defining illnesses

The advent of highly active antiretroviral therapy allowed to control HIV replication and revert to a good extent immune suppression (reviewed elsewhere (29)). The decline in AIDS incidence revealed the existence of many other complications of HIV infection, collectively called non-AIDS-defining illnesses. Central to HIV pathogenesis, these other consequences of HIV infection are related to inflammation and chronic immune activation. These inflammation-related consequences of HIV infection comprise atherosclerosis, deep vein thrombosis, stroke, diabetes, neuropathology, and others.

Cardiovascular disease and thrombosis

Atherosclerosis and myocardial infarction

A higher incidence of myocardial infarction was found in a group of HIV+ patients compared with a group of HIV- patients, both already with previous cardiovascular events (178). HIV infection is a strong independent predictor of atherosclerosis, along with traditional markers, as smoking (179). The
thickness of the intima media layer of the carotid artery, a quantitative measure of atherosclerosis, was significantly greater in HIV+ patients than in HIV- controls after adjusting for traditional cardiovascular risk factors, and HIV infection predicted atherosclerosis independently of other factors (180). Several other studies have found similar associations (reviewed by Fisher et al. (181)). However, several antiretroviral drugs that constitute highly active antiretroviral therapy (HAART), particularly protease inhibitors (PI), have been found to increase cardiovascular risk by altering lipid metabolism and distribution (lipoatrophy) (182)). Therefore, it has been difficult to determine the contribution of HIV infection itself to cardiovascular disease. Further complicating this, traditional cardiovascular risk markers (e.g. body-mass index, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, etc.) may be strong predictors in some cohorts, to the extent of hampering the assessment of the contribution of IP antiretrovirals (183)).

A reason to postulate HIV association with cardiovascular disease risk, independent of drug contribution, is that several mediators of inflammation and immune activation that are increased in HIV infection are also known markers of cardiovascular risk. Thus, in HIV+ patients with increased atherosclerosis, plasma hs-CRP, CD4+ and CD8+ T-cell activation, and CMV-specific interferon-γ CD8+ T-cell responses are increased, relative to controls (180). Stroma-derived factor 1 (SDF-1), associated with cardiovascular disease, is expressed significantly more in peripheral blood mononuclear cells (measured as mRNA) of HIV+ patients than in controls (184). SDF-1 and its receptor CXCR4 have a role in pathogenesis of myocardial infarction. The participation of SDF-1 in heart risk specifically in HIV+ patients was demonstrated by the protective effect of the mutant allele of SDF-1 gene SDF-3’A, determinant of lower plasma concentrations of SDF-1, on incidence of atherosclerosis, as measured by intima media layer thickness of the carotid artery (185). The products of activated endothelium sVCAM-1, sICAM-1, which mark atherosclerosis and predict coronary heart disease, are elevated in HIV-infected patients, and are lowered by HAART, independently of whether the drug combination contained protease inhibitors or not (186), pointing to an independent role of HIV infection. In the same line, when hemostasis markers related to cardiovascular disease were compared between HIV+ patients with three different antiretroviral regimes, 2 nucleoside reverse transcriptase inhibitors (2NRTI), 2NRTI plus a non-nucleoside reverse transcriptase inhibitor (NNRTI), and 2NRTI plus protease inhibitors (PI), no differences between groups were found. However, each group showed characteristic increases of different hemostasis markers when compared to HIV- controls (187). Finally, asymmetric dimethylarginine, a marker of endothelial dysfunction also predicting cardiovascular disease, was significantly increased
in the blood of HIV+ and correlated significantly with the activation marker neopterin (188).

HIV infection is an independent predictor of intima media thickness (IMT), and progression of atherosclerosis (one year increase in IMT) is significantly predicted by blood CD4+ T cell count (189). A better picture of the contributions of both HIV infection per se and HAART to atherosclerosis was gained with the inclusion of HIV patients not receiving HAART but controlling HIV (showing less than 75 HIV RNA copies/mL blood), as well as viremic patients, and patients under HAART. A contribution of HIV infection to atherosclerosis was demonstrated by the greater intima media thickness (IMT) of untreated HIV controllers compared with HIV− subjects, as well as the higher IMT thickness of viremic patients compared with viral controllers (190). Additionally, a contribution of HAART to atherosclerosis was shown by the greater IMT of HAART treated patients than untreated ones, even those with lower viral load (190).

The independent role of HIV infection in increased cardiovascular risk is attributable to HIV infection-triggered immune activation, as shown by further studies. Untreated HIV+ patients without HAART had significantly higher levels of the endothelial activation markers sVCAM and von Willebrand factor than HAART-treated subjects, even after controlling for HIV disease progression markers. These endothelial markers were in turn very well correlated with plasma concentrations of TNF-α, sTNFR-I and II, IL-6, C-reactive protein, and myeloperoxidase c. In contrast, patients on HAART showing good viral control had levels of endothelial activation markers comparable to those of HIV− controls, even if they showed higher lipid levels (a side effect of antiretroviral drugs). This has been seen as an indication that endothelial dysfunction was driven by viremia, while high blood lipids were an independent risk factor driven by HAART (64). Increased carotid intima media thickness in HIV+ patients on HAART was predicted by age, and sVCAM, myeloperoxidase, TNF-α, and CRP plasma concentrations. CRP was the only predictor shared with HIV− (191). In summary, the evidence strongly suggests that the increased levels of some endothelial and inflammatory markers of cardiovascular risk in HIV+ subjects are independent of the effects of HAART on cardiovascular disease.

**Thrombosis**

As described in previous sections, many of the cardiovascular disease determinants that are increased in HIV infection are produced by the activated endothelium, and also have a role in coagulation. Among them, soluble intercellular adhesion molecule-1 (sICAM-1) and the indicators of thrombotic
events fibrinogen and D-dimer are significantly increased in untreated HIV patients, compared with HIV\(^-\) controls (192). Endothelium soluble vascular cell adhesion molecule-1 (sVCAM-1) is also increased in HIV\(^+\) patients compared with controls (191).

As expected from these predictors, thrombotic events are more frequent in HIV\(^+\) patients. There is an increased risk of deep venous thrombosis and venous thromboembolism in hospitalized HIV\(^+\) patients compared with all hospitalized HIV\(^-\) patients (193). Likewise, it has been reported that HIV\(^+\) patients that are not under HAART have six times more risk of venous thrombosis (deep vein thrombosis, pulmonary embolism, or vein thrombosis at other parts) than the general population, and this risk increases further with the use of antiretrovirals (194). Arterial thrombosis is indicated by cardiovascular events like myocardial infarction, ischemic stroke, transient ischemic attack and symptomatic peripheral artery occlusive disease (194). As described above, several of these events have an increased incidence in HIV\(^+\) people, and are not completely explained by the use of HAART.

Among the different thrombophilic abnormalities found in HIV\(^+\) patients, increased concentrations of Factor VIII (coagulation promoter) and lowered S protein (coagulation cascade blocker) have been found in patients in advanced HIV disease (CD4\(^+\) T cells counts <200/µL) (195). Also indicating a relationship between HIV disease progression and thrombosis risk, a case-control study found that HIV disease progression (measured as blood CD4\(^+\) T cell counts lower than 500 cells/µL) independently predicted the occurrence of thrombotic events, along with age, hospitalization, and central venous catheterism (196). Likewise, effective HAART significantly decreased the plasma levels of von Willebrand factor, thrombin-antithrombin complex, protein C and D-dimer (markers of endothelial activation and coagulation), while increasing the levels of the total and free S protein. Similar to observations on immune activation markers, these coagulation markers remain elevated, relative to HIV\(^-\) controls despite HAART, with the exceptions of protein C and D-dimer (197). Further linking HIV with the increase in coagulation markers, it was observed in patients undergoing structured interruption of HAART that increases in viral load (HIV RNA copies/mL blood) were related with increases in D-dimer level, in turn significantly associated with increased mortality. The particular causes of death were not reported (198).

The study of surface expression of tissue factor on monocytes from HIV\(^+\) subjects revealed a definitive link between HIV infection progress, immune activation, and increased pro-thrombotic state. Tissue factor expression on the surface of monocytes and tissue factor bioactivity were significantly increased in HIV\(^+\) patients, in which CD38\(^+\) HLADR\(^+\) CD8\(^+\) T cells were over represented.
The expression of this coagulation mediator correlated with CD8+ T cell activation and increased with disease progression, as measured by increased viremia and decreased blood CD4+ T cell counts. Interestingly, tissue factor expression may be linked with bacterial translocation (see *The Bacterial Translocation Paradigm*), as strongly suggested by its correlation with the plasma concentrations of CD14 (indicating *in vivo* exposure of monocytes to bacterial LPS), which were increased in the studied patients, as well as by its experimental induction in monocytes after exposure to LPS and flagellin. These findings are biologically relevant, since the patients had evidence of *in vivo* increased coagulation, as shown by increased levels of D-dimers, which also were well correlated with CD14 expression on monocytes (199). Studies like this, linking immune activation with inflammation or activation-mediated disorders in HIV infection are needed to fully determine the consequences of immune activation, and their reversibility.

**Central nervous system alterations**

Central nervous system alterations due to HIV infection are a broad spectrum of disorders among which the most severe is HIV-associated dementia or HAD. Their characteristics, risk markers, and mechanisms have been broadly reviewed elsewhere (200, 201). HIV-associated dementia (HAD) is associated with neuropathological abnormalities collectively known as HIV-encephalopathy. HIV-1 encephalopathy is mainly mediated by brain mononuclear phagocytes, microglia, and perivascular macrophages. The secretions of these cells have neurotoxic activity, (Reviewed by McArthur (201)). Direct neurotoxicity of HIV products on CNS cells is also involved (200). In both mechanisms, activation is involved. For instance, Tat protein induces TNF-α production in monocytes and microglia, an effect synergized by the increased presence of the soluble form of CD40 ligand (a product of activated T cells) in plasma and cerebrospinal fluid of patients infected with HIV (202). Prospective memory loss, one of the milder CNS alterations also found in HIV patients, is predicted by increased plasma levels of MCP-1 and sTNFR-II, suggesting an association between immune activation and HIV-1-related CNS alterations (203).

HIV dementia involves trafficking of activated monocytes from the periphery to the brain. It is thus very relevant that blood levels of LPS, an activator of monocytes via TLR4, predict HAD on HIV+ patients independently of viral load and degree of CD4+ T cell depletion (204). Further indicating monocyte activation as a mechanism leading to HAD, sCD14, a product of activated monocytes, correlated with LPS levels and had increased plasma concentration in patients with HAD that showed minor cognitive and
motor disorder (MCMD), compared with HIV+ controls without CNS impairments. Monocytes from HAD patients were activated, as indicated by their increased surface expression of HLADR and the increased frequency of CD69 expression. Additionally, the relation of HAD with HIV disease progression was evidenced by higher viral load and lower CD4+ T cell counts in HAD patients, compared with HIV+ patients not presenting HAD (204).

Depression is another neuropsychiatric disorder that can be associated with generalized immune activation/inflammation in HIV+ patients, as suggested by its correlation with plasma levels of neopterin and IL-6 (reviewed elsewhere (205)).

Other consequences of HIV-induced chronic inflammation

Other disorders with an underlying inflammatory origin can be enhanced by HIV infection. Diabetes incidence has been associated with chronic inflammation in HIV+ patients (206). The soluble indicators of immune activation sTNFRI, and sTNFRII are increased in HAART-naive HIV+ patients along with other markers, and is reduced with HAART. Despite this decrease, plasma concentrations of sTNFRI1 significantly predict type II diabetes, even after adjusting for several other factors (207).

Osteoporosis is associated with aging and inflammation (for a review see (208)). HIV+ patients have been reported to have reduced plasma concentrations of the bone formation indicator osteocalcin and increased levels of the indicator of bone resorption c-telopeptide, in association with elevated plasma levels of indicators of the TNF-α-mediated activation markers p55 and p75-TNF receptors, which were negatively correlated with osteocalcin, while p75-TNF was positively correlated with c-telopeptide (209).

Immune reconstitution inflammatory syndrome

A portion of HIV+ patients who initiate highly active antiretroviral therapy (HAART) show a transient clinical deterioration recognized as immune reconstitution inflammatory syndrome (IRIS) (210). Different IRIS reports concur in defining it as the appearance or worsening of an inflammatory condition or an AIDS defining illness, not attributable to newly acquired infections or drug side effects that, in the context of a satisfactory response to HAART, the condition is attributable to recovery of immune cells and functions (211–216). Recent evidence supports the idea that IRIS is not merely due to the recovery of normal effector functions against remnant opportunistic infectious agents or their products, but rather a consequence of an abnormally activated immune system already present before HAART initiation. CD4+ T
cell activation, as defined in this chapter, has been found as a general feature of patients developing IRIS (217). The increased presence of inflammation mediators produced by cells of the innate immune response has also been described as a distinct characteristic of IRIS (218). The existence of risk factors that specifically predict certain IRIS manifestations (i.e. tuberculous IRIS) warrant the investigation of a possible additional involvement of activated CD8 T cells in IRIS pathogenesis (219).

**Therapeutic control of chronic immune activation and inflammation in HIV disease**

Highly active antiretroviral therapy is very successful in suppressing HIV replication and reducing AIDS mortality, but is limited in its capacity to control chronic activation. This has prompted clinical research aimed at controlling activation, some of which have shown success. A therapeutic vaccine composed of HIV protein Tat lowered CD4+ and CD8+ T cell activation (CD38 and HLADR expression) while increasing the amounts of T regulatory cells and reducing plasma levels of neopterin and β2-microglobulin in patients under HAART (220). Control of opportunistic infections (reviewed here as a trigger of immune activation) may also diminish activation. Suppression of hepatitis C virus in HIV/HCV co-infected patients under HAART using ribavirin plus IFN-β reduced plasma concentrations of sTNF-RI, soluble E-selectin and sVCAM (158). Control of herpesvirus (mainly cytomegalovirus) with valganciclovir reduced CD8 T cell activation in HAART-treated patients who had an insufficient recovery of CD4+ T cells (160).

Immunomodulatory drugs with diverse modes of action are being tested clinically. Leflunomide, which reduces the proliferation of T cells by reducing pyrimidine availability (221), was shown in a randomized, double-blind placebo-controlled trial to reduce CD8+ T cell activation (measured as frequency of CD38 and HLADR co-expression and as frequency of cycling cells), and reduced the expression of HIV co-receptors CCR5 and CXCR4, with a transient reduction in viral load, in patients still not undergoing HAART (222). The endosome blockers chloroquine and hydroxychloroquine have received attention, since they reduce the in vitro induction of IFN-β response by blocking TLR-mediated activation and MyD88 signaling (223). In a randomized double-blind placebo-controlled trial, chloroquine reduced the frequency of CD4+ and CD8+ T cells expressing CD38+, the frequency of cycling T cells, and even plasma LPS levels (indicator of bacterial translocation, a cause of chronic activation) in patients that were not receiving HAART (224). The less toxic hydroxychloroquine has been used in HIV+ patients under HIV-suppressive HAART who had not achieved satisfactory recovery of CD4+ T cell counts
(immunological non-responders). Patients showed a reduction in TLR-mediated response of monocytes to single-stranded RNA along with lower TLR expression on these cells, and a reduced their expression of CD69, denoting lower degree of activation. This was parallel to a decrease in plasma LPS and a reduction in plasmacytoid dendritic cells expressing IFN-α. At the same time, they showed a reversion of immune activation, as seen by a reduction of plasma levels of IL-6, and a reduction of cycling CD4+ T cells (Ki67+). Of clinical relevance, % CD4+ T cells among T cells, an indicator of CD4+ T cell preservation, was significantly increased, along with an increase in the frequency of T regulatory cells (225). Caution should be taken regarding the exact setting in which these positive effects of chloroquine and hydroxychloroquine have been found. In patients not under antiretroviral therapy, hydroxychloroquine failed to decrease CD8+ T cell activation, and actually caused a decline in CD4+ T cell counts (226). It is possible that control of viral replication by antiretroviral therapy, which partially controls some causes of immune activation, is necessary for the positive effects of chloroquine.

Maraviroc is a clinically proven antiretroviral drug (227) that blocks the chemokine receptor CCR5, a co-receptor of HIV on CD4+ T cells (228). Its CCR5-blocking activity makes it an immunomodulatory drug (228). In a case control sub-study comparing Efavirenz (a non-nucleoside reverse transcriptase inhibitor) and Maraviroc as components of HAART, patients receiving Maraviroc had a significantly greater recovery of circulating CD4+ T cells than patients receiving Efavirenz, despite equally good viral control. Patients on Maraviroc showed no increase in C-reactive protein levels, in contrast to patients on Efavirenz. Relevant to the role of activation, increases in CD4+ T cells were predicted by the decrease in CD38 expression on CD8+ T cells only among patients on Maraviroc (229), which suggests that the better immunological response had a link with control of immune activation by Maraviroc.

Statins, like atorvastatin, are a group of drugs with 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase-inhibiting activity that deplete cholesterol, and show anti-inflammatory effects (reviewed elsewhere (230)). In a case-control study, virologically suppressed HIV-positive patients receiving atorvastatin for 48 weeks showed a significant decrease in the CD38 expression of CD8+ T cells (231). This evidence poses the possibility of controlling remnant activation (related with poorer health outcomes) in successfully HAART-treated patients. Atorvastatin administration significantly reduced the frequency of activated CD4+ and CD8+ T cells (according with their co-expression of CD38 and HLADR) in patients not receiving HAART, as reported in a randomized, double-blind, placebo-controlled crossover trial (232). This activity was independent of its cholesterol-reducing activity.

Finally, the broadly used cyclooxygenase-2 inhibitor celecoxib was able to reduce the surface density of CD38 on CD8+ T cells, as well as the expression
of the co-inhibitory molecule PD-1. An increased response to recall antigen was a promising clinical correlate of these effects (233). While demonstrating a role of activation in T cell functionality, this agent adds to the spectrum of drugs that can be used in the future to complement the control of HIV replication with strategies aimed at controlling chronic immune activation.

**Concluding remarks**

Cumulative evidence gives chronic immune activation and inflammation a direct role in the mechanisms leading to CD4+ T cell depletion and immune suppression, as well as a number of non-AIDS defining alterations in persons infected with HIV. The whole spectrum of manifestations of HIV disease seems related with chronic activation of the immune system, sometimes with underlying inflammation despite immune dysfunction. The independence of chronic activation relative to HIV replication once the infection is stable, even under suppression of replication, calls for an integration of immune modulation as a therapeutic goal, in addition to suppression of viremia.

**References**


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