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Summary

Systemic chronic immune activation is considered today as the driving force of CD4+ T-cell depletion and acquired immunodeficiency syndrome (AIDS). A residual chronic immune activation persists even in HIV-infected patients in which viral replication is successfully inhibited by antiretroviral therapy, with the extent of this residual immune activation being associated with CD4+ T-cell loss. Unfortunately, the causal link between chronic immune activation and CD4+ T-cell loss has not been formally established. This article provides first a brief historical overview on how the perception of the causative role of immune activation has changed over the years and lists the different kinds of immune activation that have been observed to be characteristic for human immunodeficiency virus (HIV) infection. The mechanisms proposed to explain the chronic immune activation are multiple and are enumerated here, as well as the mechanisms proposed on how chronic immune activation could lead to AIDS. In addition, we summarize the lessons learned from natural hosts that know how to ‘show AIDS the door’, and discuss how these studies informed the design of novel immune modulatory interventions that are currently being tested. Finally, we review the current approaches aimed at targeting chronic immune activation and evoke future perspectives.

Keywords

HIV; SIV; pathogenesis; immune activation; inflammation; natural hosts

Introduction

Systemic chronic immune activation is considered today as the driving force of CD4+ T-cell depletion and acquired immunodeficiency syndrome (AIDS). However, the causal link between these two phenomena has not been formally established. In this article, we discuss how the perception of the causative role of immune activation has changed over the years, list the different kinds of immune activation described during human immunodeficiency virus (HIV) infection, enumerate the mechanisms proposed to explain the chronic immune activation, and propose how they could lead to acquired immunodeficiency syndrome (AIDS). In addition, we summarize the knowledge learned by studying natural simian immunodeficiency virus (SIV) hosts that learned how to ‘show AIDS the door’ and discuss how these studies informed the design of novel immune modulatory interventions that are currently being tested. Finally, we review the current approaches aimed at targeting chronic immune activation and evoke future perspectives.
immune activation and evoke future perspectives. Chronic immune activation persists even in HIV-infected patients in which viral replication is successfully inhibited by antiretroviral therapy. However, since the pathogenic role of this residual immune activation is discussed in another review of this special issue on HIV Immunology, our manuscript mainly focuses on the natural history of HIV infection.

**Historical review on immune activation in HIV**

HIV was first isolated 30 years ago from a lymph node biopsy taken from a patient with generalized hyperplasic lymphadenopathy (1). The isolation of the virus was attempted on cells from lymph node, based on the presumption that the amount of virus would be higher in this organ. Lymphadenopathy was a recognized symptom of patients with AIDS, and it was already acknowledged by clinicians as a sign of immune activation in AIDS patients. Since it had been shown that HIV is cytopathic in vitro, uses CD4 as the major receptor and predominantly replicates in CD4^+ T lymphocytes, it was obvious to speculate that HIV causes the progressive depletion of CD4^+ T cells by direct cytopathic effects. Yet even early on, pathogenic models that hypothesized indirect mechanisms and immune system activation as important contributors to progressive immunodeficiency were proposed (2). For example, the levels of inflammatory markers in the plasma during the asymptomatic phase, such as of neopterin, were demonstrated to be predictive for the development of AIDS (3, 4). Moreover, high levels of activated CD8^+ T cells were shown to predictive of shorter survival (5).

A few years later, however, support emerged for direct virus involvement in CD4^+ T-cell loss. In particular, the analysis of viral dynamics under HAART demonstrated that the asymptomatic phase of HIV infection is not silent but rather characterized by a massive and continuous viral replication, with as many as 50 billion viruses produced every day in the body of an infected individual (6, 7). This view was further supported by reports highlighting the gastrointestinal tract as a major site of viral replication in HIV and SIV infections and the associated massive and rapid CD4^+ T-cell depletion (8, 9).

Meanwhile, tools were developed to quantify viral load in the natural hosts of SIV, African nonhuman primates (NHPs) that do not progress to AIDS, i.e. sooty mangabeys (SMs), African green monkeys (AGMs), and mandrills (see ‘Learn from the experts’ section below). Natural hosts showed sustained high levels of plasma viremia, high viral load in gut and an early, massive intestinal CD4^+ T-cell depletion (10, 11). While viral replication pattern and cytopathicity were similar in nonpathogenic and pathogenic infections, natural hosts did not show high chronic immune activation (10, 11). These observations have been central to refocus attention on the role of immune activation in HIV infection. Indeed, more recent studies are in full support of immune activation being the major driving force of CD4^+ T-cell loss and AIDS: (i) the levels of activated CD8^+ T cells were shown to be more closely associated with a shorter survival than viral load or CD4^+ T cells (12); (ii) comparative studies on HIV-2-infected individuals are consistent with a direct causal relationship between immune activation and CD4^+ T-cell depletion but show only an indirect relationship of these parameters with the virus replication rate (13); (iii) a few HIV-1-infected individuals do not progress to AIDS despite displaying high viremia (14, 15); (iv) successfully HAART-treated patients with undetectable viremia still show higher levels of T-cell activation than healthy controls, with the extent of this residual immune activation being associated with increased morbidity and mortality (16); and (v) elite controllers of HIV (HIC), i.e. individuals who spontaneously control viral replication to below detection levels, show levels of T-cell activation higher than healthy donors and even higher than HAART-suppressed individuals (17). Of note, those HIC with higher T-cell activation display slow but progressive CD4^+ T-cell loss and can develop AIDS. The fact
that antiviral drugs do not target immune activation and that a residual immune activation persists despite successfully suppressed viremia highlights the importance of identifying, and then targeting, the mechanisms that cause immune activation.

**Immune activation in distinct cells and tissues**

**Markers of abnormal immune activation during HIV infection**

The first descriptions of HIV-associated immune activation were based on clinical observations (lymphadenopathy) and analyses of blood samples collected from chronically HIV-infected individuals. These analyses revealed increased levels of markers of activation (HLA-DR, CD38) and proliferation (Ki-67) on both CD4+ and CD8+ T cells (Table 1). Increased numbers of HLA-DR+, CD38+ CD8+ T lymphocytes were associated with a fall in CD4+ T-cell counts and the development of AIDS (18). Later on, even in HIC, higher CD4+ and CD8+ T-cell activation was associated with lower CD4+ T-cell counts (17). For each individual, the level of T-cell activation stabilizes for a period of time after the acute infection. This time point of stabilization is established early in infection (generally 6 months post-acute infection) and called ‘set-point’. Interestingly, the T-cell activation at the set-point, and even at earlier time points, is predictive of the rate at which CD4+ T cells are lost over time (5, 19, 20).

The expressions of the activation markers CD38 and HLA-DR are also increased on HIV-specific CD8+ T cells and, although to a lesser extent, on CD8+ T cells specific for other viruses (21). CD38 expression on HIV-specific as well as EBV-, CMV-, and FLU-specific CD8+ T cells correlates with HIV viral load (21).

This persistent pathological level of T-cell activation is associated with inflammation, microbial translocation (MT) and increased levels of coagulation markers (Table 1). The first plasma proteins documented to be increased in chronic HIV infection were neopterin, β2-microglobulin (β2M), tumor necrosis factor-α (TNF-α), soluble TNF receptor II (TNFRII), soluble interleukin-2R and interferon-γ, with β2M generally being the most stable marker (3, 22-24). Many other proteins, including anti-inflammatory cytokines IL-10 and TGF-β1, have been shown to also be increased in the plasma of chronically HIV-1-infected individuals and to predict the rate of progression towards AIDS (25). More and more data point towards an association between monocyte/macrophage activation and disease progression. In viremic, untreated patients, the numbers of pro-inflammatory CD14+CD16+ monocytes are increased (26, 27). In SIV-infected MAC, massive turnover of peripheral monocytes predicted progression towards AIDS better than viral load (28). Less is known on HIV-2, but sCD14, a marker of monocyte activation and an indirect marker of microbial translocation, is also negatively correlated with CD4+ T-cell counts in HIV-2 infected individuals (29).

In acute infection, a massive increase of cytokines, called cytokine storm, is characteristic of HIV-1 infection (30). The levels of some of these are predictive of the rate of CD4+ T-cell loss and of T-cell activation levels, and plasma levels of the IFN-inducible protein-10 (IP-10) during acute infection are predictive of rapid disease progression (31).

**Cellular sources of factors associated with chronic immune activation**

Most of the cytokines involved in chronic immune activation and inflammation during HIV-1 and SIVmac infections can be produced by multiple cell types. Not all of them have been defined. It has been reported that (i) T cells are the major sources of IP-10 (32); (ii) TGF-β is mostly produced by T-regulatory (Treg) cells (33); and (iii) plasmacytoid dendritic cells (pDCs) are the predominant IFN-1 secreting cells, although other cells might serve as another important source in later stages of infection (34, 35). Blood mononuclear cells from
viremic patients produce more TNF-α in response to lipopolysaccharide (LPS) than those from virologically suppressed patients, with M-DC8+ monocytes being predominantly responsible for this overproduction (27). Excessive production of TNF-α could act at mucosal sites to compromise the integrity of the epithelial barrier (see ‘Proposed mechanisms inducing chronic immune activation’ section below).

Other cells of the immune system, such as B cells, neutrophils, or natural killer T (NKT) cells, contribute to the inflammatory or immunosuppressive milieu, as well as secreting chemokines able to modify cellular distributions. Finally, some non-immune cells, such as cervical epithelial cells, are capable of secreting chemokines after exposure to HIV-1 (36, 37).

**Immune activation in tissues**

The large majority of studies on immune activation were performed in blood, as this compartment is the most accessible. Studies that could assess immune activation in tissues, particularly at earlier phases of infection, are rare (38). They consistently showed that HIV-1 and SIVmac infections are characterized by marked hyperplasia in lymph nodes with significant expansion of plasma B cells (39). Moreover, all stages of infection are characterized by infiltrations of CD8+ T cells into germinal centers (40). The T-follicular helper (Tfh) subset is also expanded according to several recent studies (see ‘Proposed mechanisms inducing chronic immune activation’ section below). The T zone of lymph nodes is marked by infiltrations of CD163+ macrophages, polymorphonuclear leukocytes, as well as TGF-β and IL-10-producing Treg cells (33, 41).

Utilizing the longitudinal collection of mucosal tissues and the comparison between pathogenic and nonpathogenic SIV infections, studies on nonhuman primates showed that the level of Ki-67 expressing T cells was increased in both MAC and natural hosts during the acute phase of infection, but only remained sustained throughout the chronic phase in pathogenic SIVmac infection (42). In chronically HIV-infected patients with high proviral loads, pDCs accumulate in the spleen and traffic to mucosal sites (34, 43, 44). In the brain, patients with encephalitis can, for example, display intraparenchymal infiltrations by CD8+ lymphocytes (45). Thus, HIV infection is associated with a modification of the distribution of immune cells. This is probably due to increased and sustained expression of chemokines in the respective tissues where the cells accumulate, but also to the modification of the expression of homing receptors. Indeed, in SIVmac infection, pDCs capable of producing pro-inflammatory cytokines significantly upregulated the gut-homing marker integrin α4β7 (34, 43, 44), and in vivo blockade of α4β7 dampened pDC recruitment to the colorectum and resulted in reduced immune activation. Remarkably, upregulation of β7-integrin expression on circulating pDCs was observed in HIV-infected humans but not in chronically SIV-infected SMs that show low levels of immune activation.

Collectively, these findings clearly illustrate that HIV infection is characterized by an immune activation status that encompasses many cells and tissues, with T-cell- and monocyte/macrophage-associated markers as well as inflammatory soluble plasma molecules being predictive of disease progression. Although the general consensus is for a link between inflammation and T-cell activation, the exact mechanisms binding these two phenomena still need to be clearly defined.

**Proposed mechanisms inducing chronic immune activation**

In the previous section, we discussed how extended and generalized chronic immune activation is in the setting of HIV infection. That being established, the next burning question is what mechanisms contribute to chronic immune activation during HIV infection.
Unfortunately, and despite intense research efforts, there is no clear response to this question. Given the complexity of the interaction between HIV and the host immune system, there are multiple molecular and cellular mechanisms by which HIV infection, at least in theory, can induce immune activation. To make things even more complicated, it is possible that several of the proposed mechanisms synergistically contribute to cause aberrant chronic immune activation. Moreover, it is conceivable, and in our opinion very likely, that the relative contribution of the different mechanisms changes significantly in different subsets of HIV-infected individuals, in different phases of HIV-infection (early vs. chronic vs. late), and in naive versus HAART-treated patients. In this section, we discuss the mechanisms that are considered key players in chronic immune activation in the literature (Fig. 1). For each of these mechanisms, we summarize the available experimental data supporting or questioning their contribution.

**HIV replication and immune response to the virus**

The most obvious cause of immune activation in the context of HIV infection is the direct innate and adaptive immune responses against the virus and its antigens. Not only are HIV antigens recognized by, and thus activate, T cells expressing virus-specific T-cell receptors and B cells bearing virus-specific surface immunoglobulins, but HIV components also bind to pattern recognition receptors, such as the Toll-like receptors 7 and 9 (46-49). In addition, during its process of entry and fusion, HIV might activate target cells by signaling through CD4 and its coreceptors, such as CCR5.

Fully supporting the important contribution of HIV replication, immune activation and inflammation correlate with the level of viremia and are significantly lower in HIV-infected patients who control viral replication spontaneously (HIC) or by HAART. Although the direct contribution of HIV replication to chronic immune activation is well recognized, several lines of evidence indicate that high levels of HIV replication are neither sufficient nor necessary to induce pathological levels of immune activation. First, the frequency of activated T cells largely exceeds the frequency of HIV-infected CD4+ T cells and HIV-specific CD4+ and CD8+ T cells (21, 50, 51). Furthermore, other cell types, including B cells, NK cells, pDCs, and monocytes, show increased levels of activation, turnover, and/or cell death (28, 52, 53). Second, levels of immune activation predict CD4+ T-cell decline better than, and independently of, viral load (12, 19). Third, in ART-treated HIV-infected patients as well as in HIC, the main markers of immune activation and inflammation are still significantly higher than in HIV-uninfected individuals despite undetectable viral loads (16, 54). Thus, inhibition of viral replication is still associated with non-physiologic levels of immune activation. Fourth, high viral loads are not sufficient to induce pathogenic levels of immune activation, as indicated in studies of natural hosts for SIV, such as SMs and AGMs. Indeed, SIV infection in natural hosts is non-pathogenic and does not result in chronic immune activation despite viral loads similar to those present in chronically HIV-infected individuals (10, 11) (see ‘Proposed mechanisms for the pathogenic role of HIV-associated chronic immune activation’ section below). Further evidence comes from studies of immune activation in a rare subset of HIV-infected individuals named virologic non progressors (VNP). VNP maintain a high and remarkably stable CD4+ T-cell count for many years despite levels of viral replication similar to those found in non-controllers. Despite high viral loads, VNP show a profile of non-activation, remarkably similar to those found in SIV-infected natural hosts (15, Klatt N, personal communication). Consistent with all these findings, it has been confirmed that viral load is only an indirect contributor to the rate of progression to AIDS, that immune activation predicts changes in CD4+ T cells stronger and independent of viral load, and that the effect of anti-retroviral therapy in increasing CD4+ T-cell counts better correlates with the decrease in immune activation than the suppression of viral load (17, 55-57). Collectively, the body of available data clearly supports a direct role.
of HIV replication in inducing chronic immune activation but also indicates that high levels of HIV replication are neither sufficient nor necessary to induce pathological levels of immune activation.

**Viral proteins and immune activation**

HIV gene products, such as Env, Tat, and Nef, have been proposed to be involved in HIV-induced immune activation. The Nef protein of HIV-1 has lost the ability to downmodulate the CD3-TCR complex from the surface of infected T cells (58). As a consequence, HIV-1 Nef may directly contribute to immune activation by rendering infected CD4⁺ T cells highly sensitive to re-stimulation through the T-cell receptor. The potential contribution of this mechanism is supported by the finding that the Nef proteins of SIV-infected SMs and AGMs can downmodulate the CD3-TCR complex from the cell surface, thus making infected CD4⁺ T cells more refractory to further antigenic stimulation (59). However, Nef from HIV-2 and SIVmac can also downmodulate CD3-TCR from the cell surface. To better define the role of this viral feature on immune activation *in vivo*, further studies in which AGMs will be infected with SIV<sub>agm</sub> containing an ‘HIV-1-like’ Nef are under investigation (F. Kirchhoff, personal communication).

**Immune response to reactivated infections**

Since HIV infection induces immunodeficiency, a second obvious mechanism contributing to chronic immune activation is represented by opportunistic infections and environmental pathogens (Fig. 1). This mechanism may be of particular importance in the late phase of HIV-infection but may also occur during acute infection. The vast majority of HIV-infected individuals contain other chronic viral infections, and highly prevalent latent viruses, such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV), reactivate more frequently during HIV infection due to the depletion of CD4⁺ T cells. Through stimulation of a large numbers of cells, opportunistic pathogens may play a central role in sustaining chronic activation of the immune system in HIV-infection (21). Consistently, chronic CMV infection or CMV reactivation have been found to promote immune activation, inflammation, the release of angiogenic growth factors, and hypercoagulability; in addition, a role for CMV-specific CD4⁺ T cells in inducing HIV-associated atherosclerosis has been recently described (60). Moreover, it has been shown that in HIV-infected women, CMV antibody titers are increased and associated with subclinical cardiovascular disease (61). The contribution of CMV to immune activation and inflammation is also evident in HAART-treated patients with sustained virological suppression, in which CMV-induced immune response associates with chronic immune activation and CMV viremia predicts increased mortality (62). As perhaps the strongest piece of evidence for CMV (and/or other herpesvirus) replication being an important mechanism of immune activation in HIV-infected individuals, a recent clinical study showed that Valganciclovir significantly reduced the levels of activated (CD38<sup>+</sup>HLA-DR<sup>+</sup>) CD8⁺ T cells in HAART-treated HIV-infected individuals with incomplete CD4⁺ T-cell reconstitution (63). Thus, an intervention able to suppress a hypothesized cause of immune activation did indeed decrease chronic immune activation in ART-treated HIV-infected individuals.

Loss of mucosal integrity of the gastrointestinal tract and microbial translocation Another potential key contributor to HIV-associated chronic immune activation is the loss of immune function in the gastrointestinal (GI) tract. Many studies have shown that in HIV-infected humans and SIV-infected MACs, CD4⁺ T cells, particularly those expressing CCR5, are severely depleted from GI tract in the first weeks of infection (9, 64, 65). Due to the large surface area of the GI tract, this massive depletion of CD4⁺ T cells reflects loss of most of the CD4⁺ T cells within the body and associates with loss of intestinal epithelial cells, disruption of tight junctions and compromised integrity of the mucosal intestinal barrier.
It is commonly accepted that loss of mucosal integrity favors translocation of bacterial and fungal products – including peptidoglycan, lipoteichoic acid, LPS, flagellin, ribosomal DNA and unmethylated CpG-containing DNA from the intestinal lumen to the systemic circulation (66). Through the stimulation of several TLRs, these microbial products may activate and induce production of pro-inflammatory cytokines, such as TNF-α, IL-6, IL-1β, and type I interferons, in various immune cell types (23, 24, 38). While essential to the host in the setting of acute infection, these responses may significantly contribute to aberrant immune activation in chronic HIV infection. Overproduction of TNF, for example, can be particularly deleterious for the integrity of the epithelial barrier of the GI tract by inducing enterocyte apoptosis (67), and therapeutic interventions that reduced TNF levels in vivo resulted in improved integrity of the structural barrier of the GI tract in individuals with Crohn’s disease (68).

An association between HIV infection and high LPS levels has been described in the vast majority of studies in chronic HIV infection (69). Although it is widely accepted that during HIV infection the numerous insults to the mucosal immune system result in microbial translocation, the relative contribution of microbial translocation to immune activation is not completely understood and still a matter of intense discussion. In our opinion, data generated in studies of natural hosts for SIV are among the strongest evidence supporting the role of microbial translocation in maintaining immune activation. Indeed, SIV-infected SMs and AGMs, that avoid chronic immune activation, do not show increased level of LPS in plasma or in the colon (42, 70, 71). Thus, microbial translocation is specific for progressive HIV/SIV infection. In addition, systemic injections of LPS increased immune activation in SIV-infected AGMs, thus supporting the conclusion that maintenance of mucosal integrity and lack of microbial translocation is an important mechanism for the absence of chronic immune activation in natural hosts for SIV (72). In a recent study aimed at reducing microbial translocation, MAC were treated with rifaximine (a luminal antibiotic) and Sulfasalazine (an anti-inflammatory drug used in patients with inflammatory bowel disease). This treatment was effective in reducing plasma levels of LPS, and treated SIV-infected macaques showed significantly lower levels of immune activation and cardiovascular complications compared to control animals (73). Finally, in a recent study exogenous administration of Interleukin (IL)-21 significantly reduced plasma level of LPS and sCD14 in SIV-infected MAC. Although there were no differences in the activation or proliferation levels of circulating T cells, reduced LPS levels correlate with lower levels of several markers of systemic activation, including sTNFR II, Neopterine, D-Dimer (Paiardini M, unpublished data). However, it should be noted that not all currently generated evidence supports the working model in which microbial translocation is a key cause of chronic immune activation. For example, some studies in chronic HIV infection described immune activation in the absence of increased level of LPS (69). Despite these few contrasting findings, we believe that the bulk of evidence is consistent with the working model in which loss of CD4+ T cells, compromised mucosal integrity and microbial translocation are critical contributors to chronic immune activation in HIV infection, both in the natural history of infections as well as in the context of ART-treatment (as discussed in a different article in this issue of Immunological Reviews).

Loss of specific CD4+ T-cell subsets

CD4+ T cells, the main target of HIV and SIV, are a heterogeneous population of immune cells based on phenotype, function, and anatomic distribution. CD4+ T cells can be phenotypically classified in broad subsets of naive, central memory, transitional memory and effector memory cells based on their differentiation status, and into Th1, Th2, Th17, Thf, and regulatory T-cell subsets based on their function, cytokine, and transcriptional profile. This list is not exhaustive, with more subsets being better characterized (stem T
cells, Th22, Th9) while we are writing. HIV/SIV infections are associated with major perturbations of the different CD4+ T-cell subsets. Interestingly, some CD4+ T-cell subsets are perturbed differently in natural and non-natural hosts for HIV/SIV, thus suggesting they may play an important role in the progressive or nonprogressive outcome of the infection, as well as in the establishment and/or maintenance of immune activation. A description of the main CD4+ T-cell subsets that are differentially regulated in natural and non-natural hosts is provided below.

Th17 cells

In both humans and MAC, the HIV/SIV-induced depletion of intestinal CD4+ T cells preferentially involves Th17 cells, a lineage of CD4+ T cells characterized by the production of IL-17 and IL-22 (66, 74-76). IL-17 and IL-22 function in vivo to promote recruitment of neutrophils to areas of bacterial infection, epithelial regeneration, production of tight junction proteins and antibacterial defensins. Therefore, Th17 cells are thought to be crucial for mucosal immunity, and their specific loss may render chronically HIV/SIV-infected subjects less capable of maintaining the physical and immunological integrity of the mucosal barrier. While the mechanisms underlying the preferential loss of Th17 cells remain unclear, the importance of this loss is highlighted by several pieces of evidence. First, Th17 cells are relatively preserved in the GI tract of chronically SIV-infected SMs and AGMs (66, 77), which, as noted above, avoid microbial translocation and chronic immune activation, as well as in HIC and long-term nonprogressors (78-80). Second, the severity of Th17 cell depletion correlates with microbial translocation, chronic immune activation, and disease progression in HIV/SIV-infected subjects (74, 76, 81, 82), as well as with effective CD4+ T-cell restoration in gut-associated lymphoid tissue of HIV-infected patients on HAART (83). Third, a recent study showed that the size of the Th17 cell compartment prior to infection limited viral replication in SIV-infected RMs (84). Finally, we recently found that in SIV-infected RMs, preservation of intestinal Th17 cells associates with reduced levels of intestinal T cell activation and plasma LPS (Paiardini, unpublished data), thus confirming directly in vivo a molecular link between loss of Th17 cells and microbial translocation in pathogenic SIV-infection. Although Th17 cells are the predominant source of IL-17 and IL-22, recent studies have demonstrated that these cytokines can be produced also by subsets of CD8+ T cells and innate lymphocytes (ILCs) (85). As described for Th17 cells, the frequencies of CD8+ T cells and ILCs that produce IL-17 and/or IL-22 are significantly lower within the GI tracts of chronically SIV-infected macaques (75, 86, 87). The fact that neither CD8+ T cells nor ILCs express CD4 on their surface suggests that the general loss of IL-17 and/or IL-22 producing cells within the GI tracts of SIV-infected macaques is likely due to complex mechanisms and not simply related to preferential infection by HIV/SIV.

Central memory CD4+ T cells

Central memory CD4+ T cells (TCM) express CD62L and CCR7, reside in the lymph nodes and other inductive lymphoid tissues, and show limited effector functions but strong proliferation in response to antigenic re-stimulation. CD4+ TCM are of particular importance for immune function since they are longer-lived, self-renewing cells that maintain CD4+ T-cell homeostasis by replenishing the pool of shorter-lived, non-self-renewing CD4+ TEM cells (88). CD4+ TCM represent the largest reservoir of infected CD4+ T cells in HIV-1 infection (89-91). In SIV-infected MAC, progressive depletion of CD4+ TCM is the key factor dictating the tempo of progression to AIDS, both in the natural history of infection and in the context of vaccination (92-94). In vivo comparative studies in nonhuman primates recently showed that the infection frequencies of CD4+ TCM cells of SMs are remarkably lower as compared to both CD4+ TEM of SMs and CD4+ TCM of MACs in both blood and lymph nodes (95, 96). Consistent with this relative preservation from infection, in SIV-infected SMs CD4+ TCM cells are significantly more stable than in

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SIV-infected MAC (Paiardini M, unpublished data). Remarkably, recent data suggest that a phenotype of CD4+ TCM protection similar to those that we described in SMs is also present in nonprogressive HIV-infection. Specifically, low levels of CD4+ TCM infection have been described in (i) long-term non-progressors with protective HLA alleles, (ii) earlytreated patients that show a prolonged control of viremia and preserve CD4+ T cells after ARTinterruption, and (iii) VNPs, a rare subset of HIV-infected patients that preserve CD4+ T-cell counts despite high levels of viral load, thus resembling the SM phenotype (97, 98, Klatt N, personal communication).

Infection and depletion of CD4+ TCM cells is hypothesized to contribute to the establishment of chronic immune activation more significantly than does infection of CD4+ TEM cells, and by at least two different processes (11). First, since CD4+ TCM cells are critical to maintaining CD4+ T-cell homeostasis, their depletion may trigger T-cell homeostatic proliferation. Second, with CD4+ TCM cells concentrated in LN, their higher levels of infection may translate to higher viral replication, and thus activation, in the main anatomic sites in which immune responses are initiated (Fig. 2). In our opinion, it is likely that higher infection of CD4+ TCM cells and higher levels of immune activation feed into each other in a vicious cycle whose establishment is central to the immune deficiency that follows HIV/SIV infections of humans and RMs.

Regulatory T cells

An additional CD4+ T-cell subset whose function and/or homeostatic balance is perturbed by HIV/SIV infection is that of CD4+ regulatory T cells (Tregs). Classical Tregs, immunophenotypically defined as being CD25hiCD127low and by their intracellular expression of Forkhead Box P3 protein (FoxP3), are specialized in downmodulating the effector activity of other immune cell types via secretion of cytokines like IL-10 and TGF-β, as well as through a poorly-defined mechanism dependent on cell-to-cell contact. The importance of Tregs for maintaining immune homeostasis has been highlighted by signs of autoimmune pathology that occur when Treg activity is deficient. Tregs also facilitate early protective responses to local viral infection by allowing a timely entry of immune cells into infected tissue and diminishing pro-inflammatory chemokine levels in the LNs (99).

The data on the levels of Tregs during HIV-infection are conflicting, mainly due to the lack of Treg-specific markers (with different markers used in different studies), the multiplicity of distinct Treg subsets and to whether frequencies or absolute numbers were assessed. The majority of findings indicate that the relative frequency of CD25+ Tregs is increased in blood, while their absolute counts are reduced as a consequence of lower total CD4+ T-cell counts during progressive HIV-1 infection. In parallel, Foxp3+ Tregs are maintained or accumulate in the gut and lymph nodes according to some but not all studies (33, 100, 101). The ratio of Th17 to Treg cells is decreased in both blood and GI tract of HIV-infected individuals and correlates with increased plasma markers of microbial translocation and higher level of activated CD8+ T cells (102).

How Tregs impact HIV-associated chronic immune activation is still intensively debated. On one hand, Tregs seem to be ‘harmful’, since they are able to suppress HIV-specific immune responses (103-105). Studies of acute SIVmac infection have identified a distinct Treg subset (CD8+CD25+FoxP3+ T cells) that expands during the acute phase of infection and that also has a negative impact on the infection outcome (106). In this scenario, interventions aimed at decreasing Tregs numbers and functions may limit chronic immune activation. On the other hand, Tregs could be ‘beneficial’, since they might reduce the pro-inflammatory environment typical of HIV infection. In this case, interventions aimed at increasing their numbers and functions would limit chronic immune activation and thus ameliorate the course of HIV-disease. This latest hypothesis is supported by studies showing
that (i) depletion of Treg numbers strongly correlate with both CD4+ and CD8+ T-cell activation in HIV-infected individuals; (ii) high levels of double negative Treg cells in acute HIV-1 infection are predictive of low T-cell activation at set-point, and (iii) the in vitro suppressive activity of blood and lymph node-derived Tregs correlates with high CD4+ T-cell counts and low levels of T-cell activation in SIV-infected cynomolgus macaques (107-110). Tregs, however, might be efficient in limiting residual immune activation in the context of controlled viral load but unable to suppress strong immune activation in viremic patients (111).

**T-follicular helper cells**

Tfh are a specialized subset of CD4+ T cells that reside within the germinal centers of secondary lymphoid tissues and play a critical role in the activation, differentiation, and survival of B cells. Expression of CXCR5, together with loss of the T-cell zone homing chemokine receptor CCR7, allows Tfh cells to relocate from the T-cell zone to the B-cell follicles, where they are positioned to directly support B cell expansion and differentiation. Moreover, Tfh cells express high levels of the surface receptors ICOS, CD40 ligand, PD-1, BTLA, as well as high levels of the cytokine IL-21 and the transcription factors Bcl-6 and c-Maf. Recent papers investigated the levels, function and susceptibility to infection of Tfh cells in HIV-infected humans and SIV-infected macaques (96, 112-115). These studies consistently showed that the frequency of Tfh cells within secondary lymphoid tissues is preserved or even increased in viremic HIV/SIV infected subjects. Expansion of Tfh cells associates with an increase in activated germinal center B cells and plasma cells (112, 113). Interestingly, expansion of Tfh cells does not result from reduced susceptibility to HIV/SIV infection, since Tfh cells were infected at frequencies similar to or higher than non-Tfh cells (96, 113, 115). Less is known on how HIV/SIV infection impacts the function of Tfh cells. One study showed that SIV infection altered the gene profile and decreased the survival, cycling, and trafficking of Tfh cells, thus suggesting a loss of function, while another study showed that Tfh cells remain capable of stimulating B cells’ Ig production (113, 115).

These findings suggest that Tfh cells are expanded and infected at high frequencies in chronic HIV/SIV infection and thus critically contribute to HIV replication and production. If and how the expansion of Tfh cells contributes to the maintenance of chronic immune activation still remains to be determined.

In summary, a large body of evidence generated in the last few years is consistent with a mechanism of HIV pathogenesis in which the changes in the balance of the different CD4+ T-cell subsets (Fig. 1) are more important than the total number of infected cells or the level of plasma viremia in causing the immune deficiency.

**Type I interferons and pro-inflammatory mediators**

Recognition of HIV products by pathogen-recognition receptors (PRRs) leads to the activation/maturation of innate immune cells and to the release of multiple inflammatory molecules, such as IFN-I. Recent findings generated in HIV-infected humans and SIV-infected non-human primates highlighted the potential role of type I interferon response as a contributor to HIV-associated chronic immune activation. Interferons, which include at least 14 different isoforms, induce the expression of hundreds of genes [interferon-stimulated genes (ISGs)], resulting in a number of clearly beneficial outcomes, including restriction of intracellular pathogens. However, in the context of chronic HIV/SIV infection, persistent upregulation of ISGs can have profound and possibly harmful effects, among others the chronic production of chemokines and the upregulation of IDO. Indeed, comparative studies of natural and non-natural hosts for SIV showed that the production of IFN-α and the expression of ISGs are remarkably lower during chronic SIV infection of SMs and AGMs.
than in MAC (35, 116, 117). Thus, chronic high expression of ISGs is a key distinguishing factor between pathogenic and nonpathogenic SIV infection. Interestingly, it also distinguishes long term non-progressors from progressors among HIV-1 infected individuals (15).

Based on these findings, several studies were recently designed to directly address in vivo the impact of type I interferons on viral load and immune activation. AIDS Clinical Trials Group (ACTG) study 5192 investigated the activity of 12 weekly administrations of IFNα to HIV-infected individuals (without HCV infection) not receiving ART (118). IFN-α was well tolerated, increased the expression of the activation markers CD38 and HLA-DR on CD8+ (but not CD4+) T cells and significantly reduced plasma viral load (118, 119). Of note, no signs of accelerated progression to AIDS have been observed in the thousands of HIV-infected individuals that were treated with IFN-α due to concomitant HCV infection. In two recent studies IFN-α was administered to SIV-infected natural hosts. When administered to chronically SIV-infected SMs, IFN-α induced a significant anti-viral effect (consistent with the data in human) but did not increase chronic immune activation (120). When administered to AGMs in the acute phase of infection, IFN-α did not lead to chronic ISG expression, nor impact the outcome of infection (Jacquelin B, personal communication). These latest findings are more consistent with a mechanism in which high IFN-α levels may be a consequence of high viral load and not sufficient alone to cause HIV-associated immune activation. However, further preclinical and clinical studies are warranted to better understand the cause-effect relationship between IFN-α and chronic immune activation in the context of HIV-infection.

Several processes have been suggested to contribute to the establishment and maintenance of HIV-associated immune activation. Their relative contribution may change in different subsets of HIV-infected patients and/or in different phases of infection. It will be necessary to evaluate the relevance of each alone and in combination as some of these processes might synergize in their promotion of immune activation.

**Learn from the experts: how to maintain low chronic immune activation in the setting of HIV/SIV infections**

One important approach to better identify the key mechanisms regulating chronic immune activation is to define the immunologic and genetic features distinguishing HIV/SIV-infected subjects who spontaneously limit chronic immune activation from those who fail to do so. HIV-infected humans who spontaneously limit chronic immune activation in the presence of viral replication (VNP) are extremely rare. In addition, most of the complex virus-host interactions take place in secondary lymphoid organs and mucosal tissues, and thus are difficult to study in humans. Finally, in humans, it is often impossible to study the early virus-host interactions. This is an important limitation, since studying these early interactions may allow for time sensitive identification of the factors that initiate and regulate T cell activation and also could provide key insights on what distinguishes harmful from desirable immune activation (that needed to induce antiviral responses) (121). Many of these limitations, including the possibility to study virus-host interaction at early stages of infection and in multiple tissues, are overcome by using animal models of HIV infection.

The best animal model so far that fully recapitulates the physiopathology of HIV infection is that of monkeys infected with SIV. The first SIV was isolated from an Asian monkey (MAC). Their infection resulted from an accidental transmission of SIV from West-African monkeys (SMs). African NHPs represent the only reservoir of SIV in the wild. These lentiviruses are the ancestors of HIV-1 and HIV-2. The first species identified as natural carriers of SIVs were SMs, AGMs, mandrills and chimpanzees. In most cases, the SIV is
specific for the primate species from which they have been isolated and they are designated as such (e.g., SIV of AGMs is named SIVagm; SIV of SMs is called SIVsm). The average SIV seroprevalence of AGMs, SMs and mandrills in the wild has been reported to be 40-50% (10). In this section, we mainly focus on SIV infection in AGMs and SMs, which have been studied the most with respect to correlates of protection against AIDS (11).

Natural history of SIV infection in natural hosts: early transient T-cell activation In SIV-infected natural hosts, the levels of plasma viremia are similar to those associated with disease progression in HIV/SIV-infected humans and macaques. In addition, similar to pathogenic HIV/SIV-infection in humans and macaques, SIV also replicates at high levels in the gut of natural hosts, both in the acute and the chronic phase of infection (42, 122, 142). A key feature of SIV infection in natural hosts is the absence of aberrant chronic immune activation, as manifested by many parameters. Chronically infected natural hosts display normal lymphocyte turnover and no increases of pro- or anti-inflammatory proteins in the plasma and tissues (123). Their lymph nodes show (i) a normal architecture; (ii) absence of marked lymphadenopathy or extensive follicular hyperplasia; (iii) no infiltration of CD8+ T cells into germinal centers; and (iv) a normal fibroblastic reticular cell (FRC) network (124) (Müller-Trutwin, unpublished data). Another distinguishing feature of non-progressive SIV infection is the maintenance of mucosal barrier integrity and the absence of microbial translocation (42, 70, 71). Therefore, fundamental clues regarding the role of immune activation in AIDS and the mechanisms that protect (or do not) against it lie in the natural hosts of SIV.

It is clear that the lack of chronic immune activation is not simply due to an ignorance of the virus by the host. For instance, natural hosts mount SIV-specific T and B-cell responses (125-127). Binding anti-SIV antibodies start to be detectable by two weeks post-infection, and seroconversion is completed within the same time frame as in MAC (124, 128). In line with the lack of efficient viral control, the levels of neutralizing antibody and SIV-specific T cells in blood do not exceed those in humans and macaques (129). Longitudinal studies in natural hosts clearly show an increase in T-cell activation around the time of viral peak, which then returns to baseline levels by the end of the acute infection and remains stable thereafter (123, 130). Thus, in sharp contrast to progressive HIV and SIV infections, SIV-infection of natural hosts is characterized by an early resolution of acute T cell activation.

Differences at the level of target cells

Recent studies have highlighted several differences in the nature of cells targeted by the virus in natural versus non-natural hosts, which could contribute to their better capacity to control immune activation. In both natural hosts and pathogenic HIV/SIV infections, the main cell type supporting virus replication is a population of short-lived, activated CD4+ T cells (131, 132). Interestingly, CD4+ T cells of natural hosts show lower frequencies of CCR5 expression (133, 134). However, SIVsmm and SIVagm, which largely use CCR5 as a co-receptor, still induce high viremia in the context of these low CCR5 levels (135, 136). Thus, it may be possible that SIV infects distinct target cells in natural hosts, or that the levels of CCR5 expression on cells may still be sufficient to allow infection, or that the SIVs use additional co-receptors in vivo. Indeed, SIVagm and SIVsm can, at least in vitro, also use the co-receptors Bonzo and Bob and more rarely CXCR4 (134-137). A recent report showed that SMs lacking a functional CCR5 on their cell surface still had robust plasma viral loads and was capable of using CXCR4 (134). The low level of CCR5 expression is more pronounced in TCM than other CD4+ T cells. We recently showed that SM CD4+ T cells fail to upregulate CCR5 after in vitro stimulation (95). CD4+ T cell activation was similarly uncoupled from CCR5 expression during acute SIVsm infection and as a result of the homeostatic proliferation that follows antibody-mediated CD4+ T-cell depletion. Remarkably, SM CD4+ TCM cells showed reduced susceptibility to SIV infection both in
vivo and in vitro when compared to CD4+ TCM cells of rhesus MAC. These data suggest that SM are protecting the ‘precious’ CD4+ TCM cell subset from direct virus infection. AGMs have also developed a mechanism to protect memory CD4+ T cells. Indeed, their CD4 molecules get downregulated from the surface of the CD4+ T cells as these cells get activated (138, 139). The functional properties usually associated with memory CD4+ T cells seem to be maintained and/or assumed by these CD4+ T cells (140). This has also been reported in a subset of SMs in which a significant loss of CD4+ T cells is partially compensated by a population of double-negative T cells, which perform CD4+ T-cell function (141).

In addition to those for TCM, major differences in natural and non-natural hosts for HIV/SIV have been reported for three other CD4+ T-cell subsets. Specifically, natural hosts are characterized by a low infection rate of Tfh cells, a maintenance of intestinal Th17 cells and a conservation of intestinal IL-21 producing CD4+ T cells (66, 81, 96) (see ‘Proposed mechanisms inducing chronic immune activation’ section above).

While SIVs also have the potential to replicate in macrophages, such as those in the lung and brain, few studies have been performed on other target cells in natural hosts (142). The susceptibility of dendritic cells to SIV infection in natural hosts is not known, but it has been shown that these cells have the ability to efficiently attach and transmit the virus through DCSIGN (143).

These findings suggest that during their fight to cope with SIV infection, natural hosts have acquired mechanisms of protection for specific, and probably the most critical, CD4+ T-cell subsets such as CD4+ TCM, Tfh, and Th17 cells. Better preservation of these cells may be critical for the maintenance of CD4+ T-cell homeostasis, the lack of chronic immune activation, and the preservation of normal immune competence.

Low viral load in lymph nodes during the chronic phase of SIV infection in natural hosts

Similar to progressive HIV/SIV infections in humans and macaques, SIV can be found in many tissues in the early and late infection of natural hosts (e.g. lymph nodes, intestine, thymus, cerebrospinal fluid, lung) (122, 142, 144). Additionally, the major site of viral replication in both natural and unnatural hosts is the gut (42, 122, 142).

While the viral load is similar in the blood and gut between natural and unnatural hosts, viral replication is significantly lower in the lymph nodes of the former (Fig. 2B). Thus, only a few productively infected cells can be detected in the lymph nodes during chronic SIVagm infection (145-147). The proviral load in AGM lymph nodes is also very low during the chronic phase (124, 144, 146). This contrasts with humans infected with HIV-1 and macaques infected with SIVmac, which both show a five to ten times more extensive proviral burden in the LNs than in blood cells (148). Recent studies reveal that this is also the case in SMs, thus pointing towards a common feature of natural hosts (96, 149). The mechanism responsible for the lower viral load in the LN of natural hosts is not known but likely to be multifactorial. The lack of viral trapping by follicular dendritic cells (FDC) contributes substantially to the lower total viral burden. Moreover, while Tfh cells are a major cellular target for the virus in pathogenic HIV/SIV infections, this is not the case in natural hosts (96). In addition, central memory CD4+ T cells, whose frequency is superior in LNs than in blood or mucosa, are less susceptible to SIV infection compared to RM (95). Finally, the lower viral load in lymph nodes may also be related to a more rapid immune clearance of virus from lymphoid tissue in natural hosts (149).

The lower viral load could be responsible for a lower immune activation in this tissue. However, one cannot exclude that the lower viral load in LNs is not the cause but instead the consequence of better control of immune activation in this tissue. Indeed, less activated T
cells would deliver a lower number of targets cells to viral replication. In line with this, the expressions of CXCL9, CXCL10 (IP-10), and CXCL11, the ligands of CXCR3, are not upregulated in chronic infection in natural hosts, in contrast to macaques, in which their levels associate with disease progression (32, 116). CXCR3-ligands are responsible for the recruitment of circulating activated immune cells (such as Th1 cells). Although essential for effective host defense against infection, the sustained recruitment of activated cells can fuel inflammation and replication. Altogether, natural hosts have found a way to allow for preferential immune preservation of secondary lymphoid organs, i.e. those organs that have the most crucial role in the induction of immune responses.

Natural hosts are able to resolve inflammation

The study of early events following experimental SIV infection of SM and AGM have conclusively shown that the absence of chronic immune activation is not a failure of the innate immune system to respond to SIV infection but rather the result of a rapid downmodulation of inflammation. Indeed, natural hosts mount an innate immune response post-SIV-infection manifested by rapid and robust increases in pro-inflammatory cytokines, such as IFN-α, IP-10, and MCP-1, during the first two weeks that follow SIV infection (35, 116, 150, 151). In all cases, the elevation was only transient and there was no persistent increase of inflammatory cytokines during the chronic phase in natural hosts. The early but transient inflammation could also be measured at the level of gene expression. Many of the genes which were up-regulated during acute infection in natural hosts were associated with inflammation, such as the genes known to be inducible by IFN (ISGs). The gene expressions returned to normal levels at the end of the acute infection (116, 117). Similarly, LTNP and even viremic LTNP (VNP) display significantly lower levels of ISG expressions than HIV-infected individuals progressing to AIDS (15). Studies on IFN and ISGs in natural hosts contributed to the change in view on IFN. While a defect in IFN-I production had been considered to be associated with disease progression, studies in natural hosts emphasized the potential harm of chronic IFN-associated inflammation. The controversial results both in SIV and HIV infections encouraged the deeper studies of IFN-I regulation and function in HIV infection. The processes inducing the down-modulation of inflammation in natural hosts, which is lacking in pathogenic SIV infection, are currently a major field of investigation. Of note, some other pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-8, are only weakly elevated in natural hosts (123, 152, 153). The lack of sustained inflammation in natural hosts might play a key role in the preservation of epithelial barrier integrity (Fig. 1).

The rapid control of inflammation in natural hosts had led us to raise the hypothesis that HIV-infected individuals with lower levels of inflammation by the end of acute HIV-1 infection would have a higher probability to become long term non progressors. We have quantified 28 plasma proteins in HIV-1 infected individuals with a known disease progression profile (31). Already during primary infection, rapid progressors showed a higher number of inflammatory cytokines than progressors or slow progressors. The plasma levels of TGF-β1 and IL-18 in acute HIV-1 infection were able to predict 74% of the T-cell activation variation at set-point. Plasma IP-10 was positively and negatively associated with, respectively, T cell activation and CD4+ T cell counts at set-point and capable of predicting 30% of the CD4+ T-cell count variation. Moreover, plasma IP-10 levels during acute infection were predictive of rapid progression. This study showed for the first time that the inflammatory profile in acute HIV-1 infection is predictive of subsequent T-cell activation levels (31). It is urgent to identify the mechanism responsible for the resolution of inflammation in natural hosts and to understand how this impacts the maintenance of normal tissue structure and function.
Proposed mechanisms for the pathogenic role of HIV-associated chronic immune activation

Although the mechanisms inducing chronic immune activation are still not completely defined, the paradoxical hypothesis that aberrant immune activation is an essential contributor to the immunosuppressive state associated with pathogenic HIV/SIV infection is widely recognized and supported by a growing number of observations. When foreign antigens are recognized, the cells of the immune system proliferate and produce molecules that will eliminate the pathogen as well as the cells that harbor these pathogens. While crucial for host defense, this process can become very destructive to the host itself if protracted for long periods of time, as in the case of chronic infection with HIV and SIV. As for the mechanisms that may trigger immune activation, the hypotheses which suggest how chronic immune activation may cause damage to the immune system are multiple and may vary in different classes of patients.

One mechanism by which generalized immune activation may damage the immune system is by providing available targets for HIV replication. Indeed, activation, proliferation and differentiation of naïve and memory CD4+ T cells lead to increased CCR5 expression that renders these cells more susceptible to infection. The concomitant presence of high levels of CD4+ T-cell activation and a virus infecting and killing activated CD4+ T cells may help to sustain a vicious cycle, in which infection stimulates activation and activation stimulates infection, which may lead to a severe loss of CD4+ T cells. Due to the critical role of CD4+ T cells for the host immune response, the increased virus-mediated killing of activated CD4+ T cells may eventually translate to the inability to successfully control a wide range of potential pathogens. This phenomenon is even more pronounced for HIV-specific CD4+ T cells that are indeed particularly susceptible to HIV infection (154).

Furthermore, the depletion of CD4+ T cells may trigger a homeostatic response of the immune system that induces activation and proliferation of the remaining cells, particularly those with a naïve and central memory phenotype. While triggered in an attempt to replenish the depleted compartment, this homeostasis-driven CD4+ T-cell proliferation may paradoxically have harmful effects during pathogenic HIV/SIV infection by increasing the number of cells targeted by the virus. In addition, it is widely admitted that the chronic stimulation of these regenerative compartments that occurs during HIV infection translates into a premature aging of the immune system (155). Of note, the use of homeostatic cytokines, particularly interleukin-7 (IL-7), is very promising when HIV replication is inhibited, a context in which this cytokine may exert its beneficial effect (proliferation with reconstitution of the compartment) without providing more target to the virus (see ‘Targeting immune activation’ section).

An additional key mechanism by which HIV-associated chronic immune activation negatively impacts the immune system is via the destruction and/or dysregulation of the architecture of tissues that are crucial for T-cell homeostasis and function, such as the bone marrow, thymus, and lymph node (156, 157). Immune activation in HIV/SIV infections is associated with chronic TGF-β1 production in secondary lymphoid tissue leading to collagen deposition which damages the fibroblastic reticular cell (FRC) network (156) (Fig. 2A). Loss of the FRC network results in reduced availability of IL-7, impairing the survival of naïve T cells. This leads to a decreased availability of CD4+ T-cell-produced lymphotoxin-β by the FRC and to a vicious cycle of further destruction of the FRC network. The FRC damage limits immune reconstitution after antiretroviral therapy in HIV infection (156).
Another way in which chronic immune activation can exert suppressive effects on the immune system is by direct inhibition of the normal functions of B cells, NK cells, dendritic cells, and monocytes, leading to less efficient viral control, more virus replication, and thus further immune activation (158, 159). Furthermore, the proliferation and activation of immune cells (i.e. monocyte/macrophages) together with increased levels and a prolonged presence of many proteins (IL-6, sCD14, C-reactive protein, D-dimer, TGF-β1 etc.) can induce damage in the vasculature, as well as lipid abnormalities, and result in cardiovascular diseases (160, 161). They also facilitate the proliferation of pre-malignant cells and malignant cells, thus predicting development of non-AIDS associated cancers. A mechanistic link between HIV-associated microbial translocation and cardiovascular disease has been recently proposed (161). LPS induces the expression of tissue factor on monocytes, which in turn activates the coagulation cascade. Consistent with this finding, an association between the levels of LPS and those of D-dimer has been described in HIV infection and may potentially contribute to the increased risk of cardiovascular disease (162, 163). Strong support for the link between microbial translocation, chronic immune activation and vascular disease was generated from a recent study in non-human primates. As observed in HIV-infected individuals, chronically SIV-infected pigtail macaques develop atherosclerosis and thrombotic disease, whose severity associates with increased levels of monocyte/macrophage activation and coagulation markers (164). Remarkably, vascular disease was not observed in SIV-infected natural hosts that lack microbial translocation, and administration of LPS to SIV-infected AGMs increased both monocyte/macrophage activation and D-dimer levels, providing strong direct evidence that chronic immune activation, and particularly microbial translocation, can cause cardiovascular disease in the setting of HIV/SIV infection (164).

Chronic immune activation established during pathogenic HIV and SIV infections in humans and RMs appears to undermine the integrity and functionality of the host immune system by utilizing at least three interrelated mechanisms: (i) generation of activated CCR5+ cells that are more susceptible to infection by HIV/SIV; (ii) disruption of secondary lymphoid tissue niches required for the long-term homeostasis of the naive and memory CD4+ T-cell compartments, which then severely undermines the capacity of the host to mount effective adaptive immune responses; and (iii) contributing to premature aging and non-AIDS related events. The surest way to prove that activation/inflammation is driving HIV morbidity and mortality is by blocking them and testing whether that is beneficial.

**Targeting chronic immune activation: where are we, and where are we going?**

We hope that we have convinced the reader that targeting chronic immune activation and thus reducing its negative impact on overall immune function is of the highest importance to optimize the treatment of HIV-infection in humans. Importantly, it is now clear that HIV-associated chronic immune activation is often not fully resolved even in the setting of long-term, successful HAART and that this residual immune activation may be largely responsible for the increased ‘non-AIDS’ morbidity and mortality observed in treated HIV-infected individuals (165). Based on this fact, a role for immune suppressive interventions in the clinical management of HIV-infected individuals is increasingly being considered. Believing on the paradox of treating an immune deficiency with immune suppressive drugs, from the early 1990s several investigators indeed started to test immunomodulatory compounds having the potential, at least theoretically, to reduce chronic immune activation during HIV infection (166). Overall, the vast majority of these original immunomodulatory interventions had limited and/or temporary effects on disease progression. The main reason for these limited effects, in our opinion, was that "generic" immune suppressive drugs were originally used, due to our incomplete understanding of the molecular mechanisms.
responsible for the HIV-associated chronic immune activation. In recent years, however, more specific immunomodulatory approaches have been designed and, although still at the early steps of investigation, more encouraging results have been generated. These immunomodulatory interventions are discussed in this section.

**Chloroquine and Hydroxychloroquine**

Chloroquine (CQ) and hydroxychloroquine (HCQ), two antimalarial drugs that have also been used to reduce inflammation in the treatment of systemic lupus erythematosus and rheumatoid arthritis, have recently captured the attention of HIV researchers. Interest for CQ in the setting of HIV-infection arose when two of its properties were better elucidated. First, CQ may interfere with the intracellular processing of antigens and cytokines and inhibits Toll-like receptor signaling, thus reducing T cell proliferation (167). Second, through modification of the gp120 glycosylation patterns, CQ stimulates the production of neutralizing antibodies and inhibits in vitro viral replication (168). Several recent studies investigated the ability of CQ/HCQ to reduce immune activation in HIV infection. In one study, CQ was administered for two months to nine HIV-infected patients not receiving antiretroviral therapy (169). CQ-treated patients showed reduced levels of activated (CD38^+ HLA-DR^+) CD8^+ T cells during the complete course of treatment, as well as decreased levels of proliferating (Ki-67^+) T cells and of plasma LPS in the first month of treatment (169). In a second study, HCQ was administered to 20 HIV-infected immunologic non-responders for 6 months (170). This treatment resulted in the significant reduction of several markers of immune activation and inflammation, including plasma LPS, percentages of CD4^+Ki-67^+ T cells, IL-6, and TNF-α. These HCQ-induced effects on immune activation were stable (maintained up to two months after treatment interruption) and increased the percentages but not the absolute numbers of CD4^+ T cells (170). These encouraging results were recently contrasted by a larger double-blind, placebo-controlled trial in which HIV-infected individuals not receiving ART were treatment for 48 weeks with HCQ. Not only did HCQ not reduce the levels of immune activation, but treatment also resulted in a greater decline of CD4^+ T-cell counts and increased HIV replication (171). Thus, the effects of CQ/HCQ may be very different based on the time (early vs. chronic infection) and category of patients (not in ART vs. ART-treated) in which it is used, as well as based on dose and length of treatment. In our opinion, this is a crucial message that may apply to many of the proposed immune modulatory intervention.

**Cyclosporine A**

Cyclosporine A (CsA) is a cyclic undecapeptide that has been used as an immunosuppressive agent in transplant recipients as well as in the treatment of autoimmune disorders. CsA inhibits T-cell activation, proliferation, and effector functions by inhibiting nuclear factor of activated T cells (NFAT) and the transactivation of NFAT-dependent genes. As a result, CsA reduces the gene expression of proinflammatory cytokines such as IL-2, IL-4, TNF-α, and upregulates the expression of the immune-suppressive cytokine TGF-β (172). Encouraging results were generated in early studies of CsA treatment in HIV-infected individuals. In one of such study, the mean CD4^+ T-cell counts remained remarkably stable while patients were on CsA monotherapy (median duration of CsA treatment was 11 months), while it significantly decreased after CsA interruption (173). In a subsequent study, CsA was administered in the first 8 weeks of ART in nine patients with primary HIV infection (174). Although no significant differences were found in viral replication between the CsA+ART and the ART alone cohorts, CsA treatment was associated with a persistent increase in the percentages and absolute numbers of CD4^+ T cells within the first week of treatment (174). Further support for a potentially beneficial role of CsA in the treatment of HIV infection was provided in a review of 53 transplant patients who acquired HIV (either from an infected transplant or a blood transfusion) shortly after...
transplant (175). This study showed that the 5-year cumulative risk of developing AIDS was remarkably lower (31%) in patients receiving CsA as part of their immunosuppression regimen than in those not on CsA therapy (90%). On the basis of these promising results, ACTG conducted a randomized study (ACTG 5138) on treatment-naïve, chronic HIV-infected patients starting ART with or without CsA (176). Disappointingly, CsA did not provide any sustained immunologic benefit. Similarly, CsA did not change levels of proviral DNA or CD4+ T-cell count when used with ART in acute and early HIV infection (177). Thus, despite early promising results, the enthusiasm for CsA treatment of HIV infection has declined.

**Mycophenolic acid**

Mycopenolic acid (MPA) and its ester derivative mycophenolic mofetil (MMF) have been successfully used in the last two decades for prevention of acute allograft rejection. MPA inhibits T-cell proliferation by blocking the synthesis of guanosine nucleotides through a reversible inhibition of the enzyme inosine monophosphate dehydrogenase (IMPDH). As such, MPA antiproliferative effects are specific to lymphocytes, since these cells are dependent on the de novo synthesis of purine nucleotides and preferentially targets activated T cells, since the isoform (type II) of IMPDH expressed in these cells is more sensitive than the type I found in resting T cells. Significant enthusiasm was generated following the first pilot study in which MMF was administered daily for a period of 24 weeks in eight ART-treated HIV-infected individuals with undetectable viral loads. The addition of MMF resulted in a significant decrease in the fraction of proliferating (i.e. Ki-67+) CD4+ and CD8+ T cells and in reduced virus titer in CD4+ T cells isolated from these patients (178). Based on this result, additional clinical studies were designed. Unfortunately, the results of these studies, which used different MPA/MMF and ART regimens as well as HIV-infected patients at different stages of disease, have been variable and inconsistent. Thus, it remains unclear if MPA/MMF is of any benefit for HIV-infected individuals, particularly for those in which viral load is controlled by ART (179, 180).

**Rapamycin**

Rapamycin (Rapa) is a macrocyclic lactone antibiotic, currently approved to prevent transplant rejection and as an anti-tumor agent, that exerts its immunosuppressive function by blocking mTOR, an intracellular Ser/Thr kinase that play an important role in regulating T-cell activation and proliferation. By this mechanism, Rapa reduces T-cell proliferation, inhibits the expression of CCR5 and up-regulates that of CCR5 ligands, thus it is of potential therapeutic interest for the treatment of HIV infection (181, 182). Indeed, in vitro and animal studies confirmed the inhibitory effects of Rapa on HIV replication and the synergistic effects of Rapa on the antiviral activities of CCR5 antagonists (182-185). A first prospective, nonrandomized trial of HIV-infected patients receiving Rapa monotherapy after liver transplantation (LT) has been reported (186). While primary immunosuppression was based on calcineurin inhibitors (CI), 6 out of 14 HIV-infected patients switched to Rapa. Although the treatment did not impact CD4+ T-cell counts, significantly better control of HIV and HCV replication was found among liver transplant recipients treated with Rapa vs. those on CI (186). This first clinical report of significant benefits in long-term HIV-1 control generated some hope for the utility of Rapa as an additional drug in the arsenal for HIV infection. Of note, in a previous study in 7 ART-treated HIV-infected individuals with Kaposi’s sarcoma, Rapa treatment was safe and well tolerated, but did not significantly change viral loads or CD4+ T cell counts (187).

**TNF inhibitors**

As previously discussed, in HIV-infected individuals, TNF levels are abnormally high and may significantly contribute to induction and/or maintenance of chronic immune activation
As previously described, progressive HIV infection is characterized by loss of CD4+ T cells from many anatomical locations, with a resulting increase in T-cell homeostatic proliferation of the remaining cells. It has been proposed that this proliferation may significantly contribute to the establishment and/or maintenance of chronic immune activation. Thus, it is not surprising that homeostatic cytokines are under extensive consideration in combination with ART for the treatment of HIV-infected individuals. Among these, significant interest has been generated by IL-7, a γ-chain cytokine that is critical in regulating the proliferation and survival of CD4+ and CD8+ T cells. Two main findings provided rationale for the use of IL-7 in the setting of HIV-infection. First, naïve and memory CD4+ T cells, the subsets more responsive to IL-7 due to their high expression of IL-7 receptor, are gradually depleted during progressive HIV infection; second, the IL-7/IL-7R signaling is dysfunctional in both CD4+ and CD8+ T cells from HIV-infected individuals (192). Exogenous administration of IL-7 has been recently tested in three clinical trials in ART-treated, HIV-infected individuals as a way to improve CD4+ T-cell levels and overall T-cell homeostasis. In all three studies, administration of IL-7 was well tolerated and resulted in a dose-dependent increase of CD4+ and CD8+ T cells, particularly those with a naive and central memory phenotype (193). IL-7 therapy has been tested in SIV-infected MAC to improve T-cell renewal as well as a means to overcome IFN-α-induced lymphopenia (194, 195). Although low-dose IFN-α induced strong lymphopenia, high-dose IFN-α treatment stimulated IL-7 production, leading to increased circulating T-cell counts. In addition, IL-7 therapy more than abrogated the
lymphopenic effect of low-dose IFN-α, with the administration of both cytokines resulting in an expansion of naïve T cells (195).

In summary, the ability of IL-7 to improve T-cell homeostasis may counteract the establishment of chronic immune activation by preserving a more intact immune system as well as by limiting the homeostasis-dependent T-cell proliferation. Finally, IL-7 treatment may also help in targeting viral reservoirs by promoting activation and reactivation of virus replication in latently infected CD4+ T cells with a transitional and central memory phenotype, which at present represent the best characterized cellular reservoir of HIV (90).

Another γ-chain cytokine that is catching the attention of HIV-investigators is IL-21, a pleiotropic cytokine mainly produced by activated CD4+ T cells, including Tfh, and NKT cells. IL-21 is a key factor in regulating central processes that are defective and/or compromised in HIV/SIV infected humans and RMs, including differentiation of Th17 cells, long-term maintenance of functional CD8+ T cells, differentiation of memory B cells and antibody-secreting plasma cells (196). Confirming the molecular link between availability of IL-21 and Th17 cell homeostasis, we recently described a significant association between the levels of IL-21-producing CD4+ T cells and those of Th17 cells in chronically SIV-infected RMs (81). The ability of IL-21 to stimulate differentiation and maintenance of functional Th17 cells is of particular importance in the context of HIV infection due to the contribution of Th17 cell loss to chronic immune activation. Based on these data, we hypothesized that IL-21 administration is beneficial during HIV/SIV infection, and recently tested this hypothesis in acutely SIV-infected rhMAC. IL-21-treatment was safe and did not increase plasma viral load or systemic immune activation. Consistent with the main functions of IL-21, IL-21 treated animals had higher expressions of perforin and granzyme B in total and SIV-specific CD8+ T cells and higher frequencies of intestinal Th17 cells as compared to untreated controls. Remarkably, increased levels of Th17 cells during early infection was associated with reduced levels of intestinal T cell proliferation and limited microbial translocation in the chronic infection (Paiardini M, unpublished observations). Thus, our results suggest that, by preservation of intestinal Th17 cells, IL-21 treatment may counteract HIV-induced chronic immune activation at mucosal sites. To better appreciate the potential of IL-21 in the clinical management of HIV infection, further studies are investigating the effects of IL-21 in ART-treated chronically SIV-infected RMs alone or in combination with other immune modulatory intervention such as a probiotics administration.

**Probiotic supplementation**

As discussed, compromised mucosal integrity with increased translocation of bacterial and fungal products is considered a key contributor to the persistent status of activation/inflammation both in the natural history of infection and during ART-treatment. Thus, supplementation of ART with therapeutic interventions aimed at decreasing GI tract inflammation and microbial translocation has been actively investigated in the past few years. In particular, several studies tested the hypothesis that, by decreasing activation/inflammation at mucosal level and competing with pathogenic bacteria, dietary supplementation of probiotic bacteria would be beneficial to HIV-infected individuals on ART. The first results were not very encouraging, with three studies in which supplementation of probiotics to ART-treated HIV-infected individuals induced only a modest improvement in circulating CD4+ T-cell reconstitution (197, 198). However, in more recent studies combination of two or four different probiotics resulted in moderately decreased microbial translocation and immune activation in ART-treated HIV-infected individuals (199, 200). Of note, supplementation of different strains of *Lactobacilli*, *Bifidobacteria*, and *Streptococcus* reduced fibrosis and increased reconstitution of CD4+ T cells in the GI tract of ART-treated SIV-infected rhMAC (201). This very interesting study suggests that the beneficial effects of probiotics may be particularly prominent at the...
mucosal level and, as with TNF inhibitors, is another example on how informative and critical are pre-clinical studies in nonhuman primates, in particular when the main effects of an intervention are at anatomical locations difficult to be longitudinally accessed in humans.

Other therapeutic approaches involving administration or inhibition of cytokines, inhibition of fibrosis, and inhibition of cell trafficking that may be critical in modulating the extent of chronic immune activation are currently under active investigation in HIV-infected or SIV-infected subjects. These approaches, which include blockade of IFN-α, IFNα-receptor, IL-6 and statins, are not discussed in this review, since limited data are available at present.

Final remarks

Four critical messages arise from the enormous amount of evidence generated on the link between HIV-infection and chronic immune activation. First, effective inhibition of viral replication alone is not sufficient to restore a fully functional immune system and to prevent its premature aging. Effective immunomodulatory interventions are critical for the clinical management of HIV-infected individuals. Second, the complex nature of the mechanisms causing the establishment and maintenance of HIV-associated chronic immune activation has limited the impact of the interventions tested up to date. These interventions were too general, thus not specifically directed at the causes of immune activation, or too narrow, i.e. able to block only one of the main mechanisms driving immune activation or targeting a factor that is not an initial component of the activation chain. Third, a deeper understanding of the specific molecular, cellular, and pathophysiological mechanisms causing chronic T-cell immune activation and of the interaction between these mechanisms is crucial to targeting immune activation in HIV-infected individuals in a specific and effective way. Fourth, it is very likely that ART therapies will need to be complemented by multiple, combined immunomodulatory approaches, each specific for a particular cause of chronic immune activation. As such, studies of these combined immunomodulatory strategies should be prioritized in ART-treated SIV-infected nonhuman primates, thus allowing their quick translation to HIV-infected humans. Even if we will be unsuccessful in eradicating or substantially reducing HIV reservoirs, the design of novel therapeutic interventions that might improve the recovery of a competent immune system and reduce/eliminate activation/inflammation-induced cardiovascular disease, neurocognitive dysfunction, cancer, osteoporosis, among others, would be of major benefit to people living with HIV. A status of persistent activation/inflammation is also more and more observed in other diseases. Quoting Dr. Couzin-Frankel in the Science 2010 issue ‘Top inside of the decade’, ‘Over the past decade, it has become widely accepted that inflammation is a driving force behind chronic diseases that will kill nearly all of us’ (202). Thus, if achieved, the ability to specifically modulate chronic immune activation and inflammation will be a major advance for human health.

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Fig. 1. Proposed contributors to HIV-associated chronic immune activation
There are multiple molecular and cellular mechanisms by which HIV infection could induce generalized immune activation. Among these, as summarized in this cartoon, HIV replication; immunomodulatory functions of viral proteins and immune response to the virus; immune responses to reactivated infections; loss of mucosal integrity with consequent microbial translocation; altered balance of critical CD4+ T-cell subsets; increased homeostatic proliferation in response to CD4+ T-cell depletion; increased production of pro-inflammatory molecules. Importantly, each mechanism may feed to the others, thus creating an uncontrolled positive feedback. Furthermore, it is likely that the relative contribution of each varies among HIV-infected individuals or in distinct stages of HIV-infection, as well as
Fig. 2. Model of events in lymph nodes that could be associated with loss of CD4+ T cells
Immune activation is associated with a production of chemokines that allow immune cells to be held in secondary lymphoid organs. The number of T cells and B cells increases transiently and further contribute to secretion of chemokines. Moreover, HIV infection is characterized by infiltrations of CD8^+ T cells into germinal centers and accumulation of Treg in the T zone of lymph nodes. Treg associated TGFβ production induces collagen deposition and fibrosis. The latter causes damage of the FRC resulting in reduced access of naive CD4^+ T cells to IL-7 and increased cell death by apoptosis. A consequence of CD4^+ T-cell loss is a decreased production of LTβ, which is necessary for the FRC network. The attraction of cells also leads to the arrival of new target cells for the virus that fuels the vicious cycle. Treg, regulatory T cell; GC, germinal center; FDC, follicular dendritic cell; Tfh, T-follicular helper cell, FRC, fibroblastic reticular cell
Table 1
Biomarkers predicting progression towards AIDS independently of CD4+ T cells and viral load

The table lists the parameters of T cell activation, inflammation and coagulation quantified in the blood that have been shown to predict the levels of disease progression markers at set-point (CD4 counts, viremia, T cell activation) and/or disease progression independently of CD4+ T cells and viral load. For parameters predictive of disease progression, it is not further specified whether they refer to CD4+ T cell counts or AIDS-related events. The parameters that are solely increased in progressors or are simply associated with viremia and/or CD4+ T cell levels but were not identified as predictors are not listed here.

<table>
<thead>
<tr>
<th>STAGE OF INFECTION</th>
<th>MARKER</th>
<th>PREDICTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>acute</td>
<td>IP-10</td>
<td>CD4+ T cell levels (set-point)</td>
</tr>
<tr>
<td>acute</td>
<td>IL-7, IL-12p40, IL-1α, eotaxin, GM-CSF</td>
<td>Rapid disease progression</td>
</tr>
<tr>
<td>acute</td>
<td>IL-7, IL-12p40, IL-12p70, IFN-γ, IL-15</td>
<td>CD4+ T cell loss (&lt; 350)</td>
</tr>
<tr>
<td>acute</td>
<td>TGF-β1, IL-18</td>
<td>Viremia (set-point)</td>
</tr>
<tr>
<td>post-acute</td>
<td>CD8+ T cell activation\textsuperscript{a}</td>
<td>T cell activation (setpoint)</td>
</tr>
<tr>
<td>chronic</td>
<td>T cell activation and proliferation\textsuperscript{b}</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>neopterin</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>beta2-microglobulin</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>D-dimer</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>CRP\textsuperscript{c}</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>TNF-α</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>TNF-RII\textsuperscript{d}</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>IL-6</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>LPS</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>sCD14\textsuperscript{e}</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>IL-10</td>
<td>disease progression</td>
</tr>
<tr>
<td>chronic</td>
<td>TGF-β1</td>
<td>disease progression</td>
</tr>
</tbody>
</table>

\textsuperscript{a}CD8+ T cells expressing CD38 and HLA-DR.

\textsuperscript{b}Proliferation has in most cases been defined by the proportion of cells expressing Ki-67.

\textsuperscript{c}CRP : high sensitivity C-reactive protein.

\textsuperscript{d}TNF-RII := tumor necrosis factor-α receptor II p75.

\textsuperscript{e}sCD14 : soluble CD14, a marker of monocyte activation and indirect marker of microbial translocation.
### Table 2

Therapeutic interventions targeting HIV-associated chronic immune activation

This table summarizes immune-modulatory approaches whose ability to modulate the extent of chronic immune activation has been investigated so far the most in HIV infected individuals. Detailed description of the studies and references are included in section 6 “Targeting chronic immune activation: Where are we and where are we going”.

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Target</th>
<th>Rationale</th>
<th>Main result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>Interfere with antigen processing; inhibit TLR signaling; inhibit viral replication</td>
<td>Reduces innate responses</td>
<td>Tested in the presence and absence of ART - Discordant</td>
</tr>
<tr>
<td>Hydroxychloroquine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyclosporin</td>
<td>Inhibits NFAT activity</td>
<td>Reduces the gene expression of proinflammatory cytokines</td>
<td>Did not provide any sustained immunologic benefit when used with ART</td>
</tr>
<tr>
<td>Mycopenolic acid</td>
<td>Blocks the synthesis of guanosine nucleotides through a reversible inhibition of IMPDH.</td>
<td>Reduces T cell proliferation; preferentially targets activated T cells</td>
<td>Discordant</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>Blocks mTor</td>
<td>Reduces T cell proliferation; inhibits CCR5 expression; up-regulates CCR5 ligands</td>
<td>Few available data</td>
</tr>
<tr>
<td>TNF Inhibitors</td>
<td>TNF</td>
<td>TNF levels are abnormally high and contribute to chronic immune activation</td>
<td>Few available data</td>
</tr>
<tr>
<td>IL-7</td>
<td>IL-7R expressing cells</td>
<td>Contributes to regulate proliferation and survival of T cells; limits lymphopenia-induced T cell proliferation</td>
<td>Dose-dependent increase of naive and central memory CD4 and CD8 T cells</td>
</tr>
<tr>
<td>Probiotic supplementation</td>
<td></td>
<td>Decreases activation/inflammation at mucosal level, competes with pathogenic bacteria</td>
<td>Few available data; moderately decreased microbial translocation and immune activation during ART-treatment in recent studies</td>
</tr>
</tbody>
</table>

TLR= Toll-like receptor; NFAT= Nuclear factor of activated T cells; IMPDH= inosine monophosphate dehydrogenase; mTor= mammalian target of rapamycin; TNF= Tumor Necrosis Factor; IL-7= Interleukin-7