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To cite this version:
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Purpose of review
This review summarizes studies in natural hosts, with a particular focus on the control of immune activation and new insights into viral reservoirs. We discuss why these findings are relevant for HIV research today.

Recent findings
AIDS resistance in natural hosts is characterized by a rapid control of inflammatory processes in response to SIV infection despite persistent viremia. While CD4+ T cells are dramatically depleted in the intestine in primary infection, Th17 cells are preserved and natural hosts lack microbial translocation. Thus, viral replication in the gut is not sufficient to explain mucosal damage, but additional factors are necessary. Natural hosts also display a lower infection rate of Tscm, Tcm and Tfh. The follicles are characterized by a lack of viral trapping and the viral replication in the T zone of secondary lymphoid organs is rapidly controlled. Hence, the healthy status of natural hosts is associated with preserved lymphoid environments.

Summary
Understanding the underlying mechanisms of preservation of Th17 cells and of the low contribution of Tscm, Tcm and Tfh to viral reservoirs could benefit the search for preventive and curative approaches of HIV. Altogether, the complementarity of the model helps to identify strategies aiming at restoring full capacity of the immune system and decreasing the size of the viral reservoirs.

Key words
SIV, inflammation, natural hosts, microbial translocation, reservoirs.
Introduction

Progression towards AIDS in HIV-1 infected humans is closely associated with chronic immune activation (IA). How some individuals are able to more effectively control than others remains a key question. Over the past decades, important insights for HIV/AIDS research were achieved from studies involving non-human primate (NHP). These studies include both “experimental” or “non-natural” hosts, such as Asian macaques, that reproduce all disease progression profiles seen in humans infected by HIV, and “natural” hosts of SIV, such African Green Monkeys (AGMs) and Sooty mangabey (SMs), where infection is generally non-pathogenic. One of the striking hallmarks of all studied natural SIV infections is the high viremia both in the acute and the chronic phase of infection as high as that observed during HIV-1/SIVmac infections (Figure 1)[1]. SIV in their natural hosts also replicate at high levels in the gut and the distribution of the virus in many other tissues (e.g. thymus, cerebro-spinal fluid, lungs) is also similar to pathogenic infections[1-4]. Similarly to HIV-1/SIVmac, SIV replication in its hosts, occurs preferentially in activated CD4+ lymphocytes [5-7] and the primary infection is characterized by a rapid and dramatic depletion of mucosal memory CD4+ T cells[8]. However, in contrast to pathogenic infection, mucosal CD4+ T cells are partially recovered after the acute phase of infection and the epithelial barrier remains intact[8, 9]. We will summarize recent insights into natural SIV infections and debate what make natural hosts of SIV so interesting for current major challenges in HIV research, i.e. the search for a vaccine and HIV cure.
Lack of chronic immune activation in natural hosts of SIV

The most striking feature of SIV infection in natural hosts is the absence of aberrant chronic IA[10]. Long-term infected natural hosts display normal lymphocyte turnover and no increases of pro- or anti-inflammatory proteins in the plasma and tissues during chronic phase[11, 12]. Their LN exhibit (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 [13]. Natural hosts also lack microbial translocation, and hypercoagulability and cardiovascular pathology, which are consequences of excessive IA in HIV-1 infections, are not observed in natural hosts [14].

The studies in natural hosts provided perhaps the strongest evidence that the inflammatory response to the virus is a critical determinant of pathogenesis [15]. They also demonstrated that high viral replication in the gut is not sufficient to explain the mucosal damage, but that other additional factors are responsible for this phenomenon.

Induction of a strong but only transient immune activation in natural hosts

In order to understand the lack of chronic immune activation in natural hosts, the early steps necessary to induce immune activation following HIV/SIV infections where analyzed, i.e. the sensing by and activation of the innate immune system. AGMs display no or only weak increases of inflammatory cytokines such as TNF-a and IL-6 in blood, LN and mucosa associated lymphoid tissue[12]. Also, only macaques exhibit an up-regulation of Th1-associated markers, whereas AGM do not [16-18]. However, some inflammatory cytokines
are strongly increased upon infection, in particular IFN-I, IL-15, MCP-1 and CXCL10/IP-10[19-24]. The latter are cytokines, which are induced very early on during primary infection (Fiebig stages I - III), before the other inflammatory cytokines [24]. Plasmacytoid DC (PDC) are indeed recruited in AGM LN from day 1 p.i. during the acute phase of infection [20, 21]. Natural hosts’ pDC have a normal capacity to produce IFN-a upon exposure to SIV [21-23, 25, 26]. There even seems to be a selective pressure in natural hosts to maintain capacity by PDC to sense species-specific SIV [26]. NK cells are highly activated during primary infection in natural hosts [24]. Therefore, the lack of chronic inflammation is not due to ignorance or tolerance. Indeed, T and B cell responses raised against SIVs can be readily detected [27, 28]. Altogether, natural hosts develop a strong early inflammatory response, but are able to efficiently control immune activation by the end of the acute phase of infection despite persistent virus replication.

These data on the rapid control of inflammation in non-pathogenic SIV infection have raised the hypothesis that individuals infected by HIV-1 displaying lower levels of inflammation at the end of the acute phase of infection could have a higher probability to become long-term non-progressors [29]. Indeed, the inflammatory profile in primary HIV-1 infection is associated with T cell activation levels at set-point, CD4+ T cell loss and disease progression [29-31]. In general, many studies in non-human primates have indicated that the very early virus-host interactions, during primary infection, are determinant for the outcome of infection [32]. In macaques it was shown that ART initiation before the peak of viremia results in lower tissue viral reservoirs [33]. Altogether, studies in non-human
primates provided strong arguments that treating early upon infection may be beneficial for HIV-infected individuals.

*Natural SIV infections are not associated with microbial translocation*

Despite chronic highly active viral replication and the destruction of mucosal CD4+ T cells very early during the acute phase of infection, natural hosts of SIV maintain mucosal immune function[8, 9, 34]. Lipopolysaccharide (LPS) administration into SIV-infected AGM led to increased IA and viral replication [35]. To investigate the interconnection between microbial translocation, inflammation and pathologic consequences, experimental damage to the intestinal epithelial barrier was induced by administering dextran sulfate sodium to chronically SIV-infected AGMs[36]. The dextran sulfate sodium treatment of SIV-infected AGM SIV resulted in colitis with elevated levels of plasma SIV RNA, sCD14, LPS, CRP and mucosal CD4+ T-cell loss. Studies in natural SIV infections thus provided the first direct link between microbial translocation and IA.

*Natural SIV infections spare specific target cells*

It has been shown that Th17 cells in the gut mucosa are spared in non-progressive SIV infections in sharp contrast to pathogenic HIV/SIV infections[18, 34, 37]. The dramatic loss of CD4+ T cells in gut in natural hosts upon SIV infection accompanied by a remarkable preservation of Th17 cells gut reinforced the hypothesis that protecting Th17 might be particularly beneficial [38]. Early initiation of ARV after HIV infection helps preserve Th17 [39]. In macaques treated during chronic infection, IL-21 treatment together with probiotics improved Th17 frequencies, reduced microbial translocation and morbidities [40].
Many CD4+ T cells from AGM downregulate CD4 \textit{in vivo} as they enter the memory pool. The loss of CD4 expression protects these memory CD4+ T cells from infection by SIV\textsubscript{agm} \textit{in vivo}\cite{41, 42}. Studies in SM revealed a relative protection of two CD4+ T cell subsets, the central memory (Tcm) and stem-cell memory (Tscm)\cite{43, 44}. In particular, SM CD4+ Tcm cells present reduced susceptibility to SIV infection – with up to 10-fold lower levels of cell-associated SIV-DNA - when compared with CD4+ Tcm cells of rhesus macaques \cite{44}. SM Tcm are also significantly more stable than their macaque counterparts \cite{45}. It is interesting that particularly central memory cells are protected. Memory cells are endowed with resistance to death and self-renewing properties, which make them an important source of antigen-experienced cells to maintain long-term immunological memory. These observations contributed to shift attention to the role of Tcm as viral reservoir. It is remarkable that patients with distinct types of HIV control (i.e. individuals with early cART initiation during PHI, post-treatment Controllers, HLA-B27/57+ HIV Controllers and LNTP) all have in common that Tcm contribute less to the viral reservoir\cite{46, 47}.

Among other target cells, macrophages from SMs have recently been shown to be relatively more resistant to SIV infection \textit{in vitro} compared to RM macrophages\cite{48}. It has been suggested that this is due to a combination of entry and post-entry restriction mechanisms. In contrast to macrophages, pDC seem to be highly infected in natural hosts, as much as in macaques \cite{26}. Further studies in the non-human primates are needed to better understand the contribution of antigen-presenting cells as viral reservoirs.

Altogether these observations highlight the entente cordiale set up between SIV and their natural hosts. Preserving Tcm from massive rounds of infection may partly sustain the immuno-competent state of natural hosts of SIV. In parallel,
productive infection of SIV in effector cells of the gut lamina propria concomitantly to preserved Th17 cells ensure an environment for the virus to persist without the deleterious bacterial leakage which contributes to the generalized immune activation seen in pathogenic HIV/SIV infections.

**The type I IFN file: the truth is out there**

High viremia and disease progression during HIV-1/SIVmac infections are associated with sustained expression levels of IFN-stimulated genes (ISG, interferon-stimulated genes)[19, 23, 49-53]. Type I IFN has therefore been suggested as one of the main culprits of chronic IA and HIV pathogenesis. Manipulations of IFN-I during pathogenic and natural SIV infections shed light onto the complex role of IFN-I in the setting of lentiviral infections. IFN-I treatment during chronic infection in natural hosts leads to reduction of viremia to similar extents as previously reported for HIV-infected humans[24, 54]. IFN-I injections in natural hosts however result neither in sustained ISG expression nor in T cell activation, whether injected during primary or chronic SIV infection, ruling out a major role of IFN-I in chronic immune activation[24, 54]. Blockade of the IFN-I receptor in acute SIVmac infection caused reduced antiviral gene expression, increased SIV reservoir size and accelerated disease progression further highlighting the antiviral role of IFN-I[55]. In line with this, IFN-I injection at the time of SIV transmission in macaques upregulated expression of antiviral genes and reduced the risk of infection. In sharp contrast, however, the animals that got infected displayed an accelerated disease progression, probably due to IFN-I desensitization subsequent to the IFN-I treatment, decreased antiviral gene expression and increased cell-associated SIV DNA load in lymphoid tissues.
These results are important with respect to future trials based on IFN-I in cART-treated patients.

**Role of immune activation in the persistence of viral reservoirs**

Strikingly, while the levels of IFN-I decrease after primary infection in both pathogenic and non-pathogenic SIV infections, ISG expression remains elevated in pathogenic infections[19, 23, 56]. It is unclear why in natural hosts ISG returns to normal levels. The triggering of ISG may be multifactorial. During the acute phase of infection, the ISG expression profiles are strongly correlated with type I IFN (IFN-I) levels [57]. In chronic phase of infection, microbial translocation as well as the expansion of the enteric virome, as observed in SIVmac infection, might be responsible for triggering ISG expression in pathogenic infections[58].

Among these ISG, CXCR3-ligands (IP-10, ITAC and MIG) are responsible for the recruitment of circulating activated immune cells. In HCV, IP-10 attracts cytotoxic T lymphocytes to the site of infection (liver) resulting in better control of HCV infection[59]. In natural hosts, these chemokines are rapidly downregulated to basal levels in LNs and gut, but remain elevated during SIVmac infection where they are responsible for the intense recruitment of CXCR3+ T cells to these tissues[50, 60, 61]. Among the recruited T cells, many could be potential target cells for the virus as CXCR3 is increased on memory CD4+ T cells, which also express higher levels of CCR5[62]. Of note, chemokines such as IP-10 can also promote viral latency in resting CD4+ T cells and thus enhance the establishment of viral reservoirs[63]. The studies in the non-human primates therefore suggest, that these ISG might modify the tissue distribution of cellular targets (Figure 2).
Secondary lymphoid organs are protected in natural hosts

Natural carriers of SIV exhibit high viremia in blood and high viral load in gut. Surprisingly, the number of productively infected cells in LNs during chronic SIVagm and SIVsm infection is remarkably low as compared to HIV-1 and SIVmac infections [4, 13, 64, 65, 66-71]. The replication levels in LN are similar to pathogenic HIV/SIVmac infections in acute infection but under control during the chronic phase. Strikingly, virus-producing cells in the germinal centers are rare at any stage of infection in natural hosts [4, 13, 65, 70]. Thus, follicular helper CD4+ T cells (Tfh) residing in the germinal centers are largely spared of active infection in natural hosts [64]. The latter have recently been shown to constitute one of the major reservoirs of HIV [64]. Moreover, viral trapping in the follicular DC network is rarely observed in natural hosts [4, 13, 65, 70]. In line with the viral RNA and DNA loads, the average titer of infectious virus in AGM LNs is also weak (21 TCID50/10^6 LN mononuclear cells) [72]. Inflammation seems to be controlled more rapidly and more strongly in LN than in other compartments in natural hosts [12, 23, 73]. It is unclear so far if the lower viral load in LN could be a cause or a consequence of lower IA in LN.

LNs are a major organ for the education of adaptive immune responses. During pathogenic HIV/SIV infections, regulatory CD4+ T cells (Treg) accumulate in LNs as one arm of the immunosuppressive regulatory response shortly after HIV/SIV infections [74]. This results into elevated TGFβ1 expression levels and extensive collagen deposition leading to collateral fibrotic lymphoid tissue damage [74]. This phenomenon was suggested to lead to progressive survival impairment of CD4+ T cells [75]. In natural hosts, the absence of elevated TGFβ expression and collagen deposition help preserve immune functions [74]. The extent of collagen deposition at
the time of initiation of suppressive ART was found to predict the magnitude of recovery of the peripheral CD4+ T-cell pool [76]. The efficacy of treatment is stronger in the gut than in LNs; probably due to a less efficient tissue diffusion of the antiretroviral drugs [33, 77]. TGFβ-associated immunosuppressive mechanisms initially in place to counteract chronic activation in HIV/SIVmac infections, by promoting disruption of the LN integrity, most likely contribute to limit antiviral drug penetration and thus to viral persistence in these compartments.

Altogether, the studies have shown that natural hosts protect secondary lymphoid organs, and in particular follicles, from viral replication. In the future, it will be important to decipher the mechanisms of how natural hosts succeed in efficiently controlling infection in LNs, in particular in Tfh cells.

**Conclusion**

Natural hosts seem to have co-evolved with their respective SIVs in order to shape an equilibrium whereby mounting transient inflammatory processes, such as the early IFN-I response, allow the one hand the host to mount some anti-viral responses leading to partial control of viremia after the peak. On the other hand, this equilibrium allows the virus to spread where it then persists notably in the gut. Natural carriers also showed that LNs are rapidly devoid of active viral replication in the long run. Preserving Tcm and Tfh in LNs from infection and long-term active viral replication is a rather smart trade-off between the virus and its natural host. Further, natural hosts have proven that mechanisms preventing AIDS progression are in place very early after infection. This calls for further studies evaluating the beneficial impact of early anti-viral treatment upon HIV infection as observed in the VISCONTI and START studies [47, 78]. Therefore understanding early mechanisms leading to
control of viral replication in secondary lymphoid organs and/or chronic immune activation may be one of the next promising goals to achieve amongst others in order to secure an HIV cure [79]. HIV induced chronic inflammation may also be an obstacle to vaccine strategies as it impairs immune responses and furthermore potentially leads to the recruitment of target cells to sites of infection enhancing viral dissemination and establishment of viral reservoirs. Further comparative studies would definitely help understand the early events or check points that need to be overcome in order to induce an efficient control.
Key bullet points

- Studies in natural hosts have contributed to a major extent in highlighting the essential role of chronic IA in AIDS pathogenesis and in orientating the attention to the role of IA in non-AIDS morbidities and mortality.
- The generalized IA is most likely multifactorial and studies in natural hosts help to distinguish between factors, which are simply associated with uncontrolled viral replication from those, which might be driving pathogenesis.
- Despite strong replication in the gut during SIV infection in natural hosts, the integrity of the gut mucosa is preserved.
- In natural hosts, SIV spare some specific target cells, notably the central memory CD4+ T cells, follicular helper CD4+ T cells and IL-17-producing helper CD4+ T cells.
- In sharp contrast to HIV/SIVmac infections, virus-producing cells are only rarely detected in germinal centers during chronic SIV infection in natural hosts and secondary lymphoid organs lack signs of fibrosis.

Acknowledgements

We acknowledge the Center for Infectious Disease Models and Innovative Therapies (IDMIT) regarding the studies in non human primate models.

Financial support and sponsorship

The authors are supported by the Fondation AREVA, the French National Agency for
Research on AIDS and Viral Hepatitis (ANRS), SIDACTION, the National Institute of Health and Institut Pasteur.

**Conflict of interest**

None
Figure 1. Main differences between pathogenic HIV/SIV infections and SIV infections in their natural hosts. Natural hosts of SIV exhibit similar viremia as observed during pathogenic HIV/SIVmac infections. SIVs also quickly target the gut mucosa upon infection. However, in contrast to humans and macaques, natural hosts display preserved epithelial barrier integrity, Th17 immunity and lack of microbial translocation despite continuous viral replication. Another striking difference between progressive HIV/SIV and non-pathogenic SIV infections resides in the secondary lymphoid organs. Chronic SIV infection in macaques is associated with persistent viral replication in germinal centers and viral trapping by FDC. The inflammation in the lymph nodes induces anti-inflammatory regulatory mechanisms, which progressively leads to disruption of the lymphoid architecture. In sharp contrast, life-long SIV infection in their natural hosts is characterized by a rapid control of viral replication in secondary lymphoid organs and very rare detection of viral trapping and viral replication in germinal centers. It remains unclear whether during the acute phase of natural SIV infection, a more rapid control of viral replication in secondary lymphoid organs leads to a more easy control of inflammatory processes. As the virus needs activation of its target cells for productive infection, it is not excluded that a more efficient control of immune activation in secondary lymphoid organs leads to a reduction in viral replication in these tissues. The intimate early interplay between viral and host determinants that dictates the efficiency at which viral persistence and immune activation will be either controlled or sustained will need to be unraveled. Studies of the early events/signals shaping these two distinct outcomes of SIV infections in non-human primates are therefore warranted. BZ = B-cell zone, GC = Germinal center, FDC = follicular dendritic cells, IFN-I = type I interferons, ISG = interferon-stimulated genes, pDC = plasmacytoid dendritic cells, Tcm = central
memory T cells, TGF-β 1 = Transforming growth factor beta 1.
Figure 2. Intertwined key features of HIV/SIV infections: immune activation and viral persistence. Upon infection, the virus rapidly spreads from the port of entry and is produced preferentially in activated antigen-experienced CD4⁺ T cells. In parallel, a cytokine storm is triggered to shape and orchestrate the immune warfare, including cells of the innate and adaptive immune systems, in response to the viral insult. Chemokines induced by activation of the immune system will attract immune cells to the site of infection, among them CXCR3+ CD4+ memory T cells. These cells present a target for viral infection in tissues. During the early stage of infection, the race between viral spread/replication and host defenses is critical, as this will impact the subsequent outcome of infection.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

★★ of outstanding interest


This work confirms that the control of immune activation in natural hosts is not a consequence of lower levels of IFN-α production.


This study shows that plasmacytoid dendritic cells of natural hosts display reduced CD4 and/or CCR5 expression, unlike macaque pDC. This do not preclude their ability to be infected or to produce IFN-I.


Pigtailed macaques (Maccaca nemestrina), which lack an HIV-1–restricting TRIM5 protein, were inoculated with HIV-1 clones encoding SIVMAC Vif, which antagonizes several macaque APOBEC3 proteins that would otherwise restrict HIV-1 replication in the macaque. During adaptation over four passages in macaques, the modified HIV-1 also acquired the ability to antagonize the macaque restriction factor tetherin. The virus induces a persistent infection, but the macaques have a controller phenotype. Transient treatment with an antibody to CD8 during acute infection caused rapid progression to AIDS in contrast to non CD8-depleted animals. Thus, an adapted HIV-1 can cause AIDS in macaques, and stark differences in outcome can be determined by immunological perturbations during early infection.


   This work reports a dramatic loss of mucosal Th17 and their functional capabilities occurring even earlier during acute HIV infection than previously described and that initiation of ART in Fiebig I/II, before this loss occurs, prevented alterations in Th17 numbers and functionality. If antiretrovirals were initiated at Fiebig III, near the peak of viremia, Th17 polyfunctionality was not restored. These findings argue for early and aggressive intervention for therapeutic benefit and a potential functional cure.


   Probiotic and IL-21 supplementation of ARVs are associated with enhanced polyfunctional Th17 expansion and reduced markers of microbial translocation and dysbiosis as compared with infected controls receiving ARVs alone. Importantly, treatment resulted in fewer morbidities compared with controls. We propose that combining ARVs with therapeutics aimed at restoring intestinal stasis may significantly improve disease prognosis of ARV-treated, HIV-infected individuals. These findings support the concept that early therapeutic interventions aiming at limiting microbial translocation may substantially affect levels of immune activation and inflammation.


48. This manuscript represents the first comparative analysis of the susceptibility of macrophages to SIV infection between rhesus macaques and natural hosts. Monocyte-derived macrophages (MDMs) from sooty mangabeys were more resistant to SIV infection in vitro compared to macaque-derived MDMs.


This elegant work demonstrates that treatment with an antagonist of the interferon receptor leads to accelerated disease progression. Administering interferon IFNα2a to rhesus macaques before exposure to SIV reduces the risk of infection. However, in those animals that got infected, the latter treatment had a detrimental effect leading to higher viral load and accelerated progression towards AIDS. This seminal work highlights the importance of the timing and duration of interferon administration, and underscores the difficulty to predict the exact potential of intervening in the interferon signalling pathways during HIV infection.


This study demonstrates that vaccine regimens that elicit CXCR3-CCR5-biased TFH cell responses favor Ab persistence and avidity but may predispose to higher acute viremia in the event of breakthrough infections.


This study clearly shows that HIV continues to replicate in the lymphatic tissues of some individuals taking antiretroviral regimens considered fully suppressive, based on undetectable viral loads in peripheral blood, and that one mechanism for persistent replication in lymphatic tissues is the lower concentrations of the antiretroviral drugs in those tissues compared with peripheral blood. This work emphasizes the need to initiate antiretrovirals as early as possible before HIV-mediated inflammation infection led to irreversible modifications of the lymphoid tissues.

Initiating antiretroviral therapy immediately after HIV diagnosis rather than waiting until a patient’s CD4+ count has declined is of considerable benefit. The study found a 72% relative reduction in serious AIDS related events in the immediate initiation group largely from reductions in the rates of tuberculosis, Kaposi’s sarcoma, and malignant lymphomas. A 39% relative reduction was seen in serious non-AIDS related events largely because of a reduction in non-AIDS defining cancers. All these events are associated with chronic inflammation. This study support the concept that initiating early antiretrovirals would limit the long-term detrimental effects of chronic inflammation. The beneficial effect of immediate antiretroviral therapy was independent of age, sex, race, region of the world, CD4+ count, viral load, or risk factors for serious non-AIDS diseases. These findings have global relevance, since the study was conducted in 215 clinics in 35 countries and represents a broad population of HIV-positive patients.