HIV INFECTION

Hard-to-kill macrophages lead to chronic inflammation in HIV

Evasion of the immune system and global activation of the immune system are hallmarks of human immunodeficiency virus (HIV) infection. Studies reveal that macrophages might be responsible for HIV-associated pathogenesis via resistance to killing and induction of chronic inflammation.

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Activation of the immune system is a prominent feature of infection with human immunodeficiency virus type 1 (HIV-1), which is associated with a poor clinical outcome in untreated and treated disease. Various causes of such activation have been identified in HIV-1 infection, including innate and adaptive immune responses to HIV-1, co-infection with other viruses (such as cytomegalovirus and hepatitis C virus), increases in microbial translocation and changes in the composition of the intestinal microbiome[1]. In this issue of *Nature Immunology*, Clayton et al. identify another potential cause of increased inflammatory immune responses in HIV-1 infection[2]. They show that the delayed killing of HIV-1-infected macrophages relative to that of infected CD4$^+$ T cells by CD8$^+$ cytotoxic T lymphocytes (CTLs) is associated with increased secretion of the cytokine IFN-γ by CTLs due to constant antigenic stimulation and subsequent induction of the production of pro-inflammatory chemokines by macrophages (Fig. 1). The authors suggest that this might result in chronic activation of the immune system typical of HIV-1 infection and show that similar results are obtained with other viral antigens, which suggests that resistance to being killed by CTLs is an intrinsic property of macrophages.

A major strength of this paper[2] is the comprehensive set of experiments performed to elucidate the mechanistic basis of differential CTL-induced killing of target cells. The in vitro kinetics of the killing of macrophages by CTLs are much slower and are associated with greater HIV-1 replication relative to the killing of CD4$^+$ T cells. Macrophage death is associated with activation of the caspase-3 pathway, whereas the death of CD4$^+$ T cells is characterized by increases in reactive oxygen species. Further support for the proposal of a role for caspase-independent killing of CD4$^+$ T cells comes from experiments with CTL populations that have been expanded with HIV-1 peptide and then co-cultured with target cells; these show that pan-caspase inhibitors and inhibitors of caspase-3 inhibit only the killing of macrophages but do not affect the depletion of CD4$^+$ T cells. The killing of both macrophages and CD4$^+$ T cells by CTLs is mediated by recognition via major histocompatibility complex class I–restricted T cell antigen receptors that triggers granule exocytosis; however, specific blockade of granzyme B activity inhibits only the death of macrophages, not that of CD4$^+$ T cells. This suggests that the slow killing of macrophages is mediated mainly by granzyme B and subsequent activation of caspase-3, in contrast to the death of CD4$^+$ T cells, which occurs in a caspase-independent manner. More importantly, the authors show that prolonged formation of synapses between CTLs and macrophages and constant antigenic stimulation, a proxy for inefficient cell killing, is correlated with increased secretion of IFN-γ by CTLs and subsequent induction of the production of pro-inflammatory chemokines by macrophages. The findings of this study have two main implications for HIV-1 research: first, they will promote renewed interest in the potential contribution of macrophages in HIV-1 persistence; and second, they will stimulate understanding of the role of macrophages in chronic inflammation in patients on long-term antiretroviral therapy (ART). A key issue is how far the results of this in vitro study can be applied to patients infected with HIV-1.

Macrophages have long been recognized as having a key role in anti-microbial defense through phagocytosis, the clearance of apoptotic cells and the induction of secondary immune responses; however, more recent findings have clearly shown that they make a major contribution to the detection of tissue damage, resolution of inflammation and initiation of tissue repair and possess distinctive specialized tissue function, such as neuronal support in the brain and clearance of pulmonary surfactant in the lungs[3]. The traditional view that tissue macrophages originate from hematopoietic stem cell precursors has been superseded by evidence largely derived from murine studies showing that there are two distinct sources of macrophages: a self-renewing tissue-resident population derived from embryonic precursors, and a population that originates from infiltrating blood monocyte populations. The development of tissue macrophages is complex; however, it is believed that it consists of an initial irreversible differentiation step that governs broad characteristics of macrophage biology and tissue identity, followed by reversible, polarization signals into different macrophage types, M1 (induced by IFN-γ)

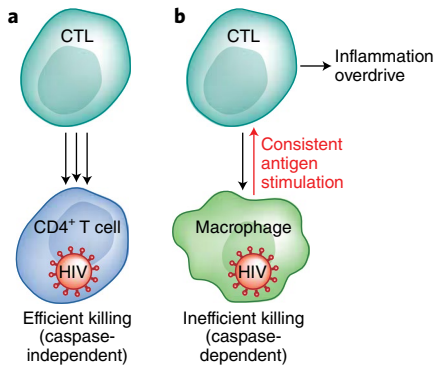

Fig. 1 | The killing of HIV-infected cells by CTLs. **a**, The killing of HIV-infected CD4$^+$ T cells by CTLs is more efficient and rapid, and occurs in a caspase-independent manner. **b**, The killing of macrophages by CTLs is less efficient and depends on activation of caspase-3 pathway; this results in consistent antigen stimulation of CTLs and the production of more inflammation cytokines such as IFN-γ, which can 'overdrive' the inflammation of other cells, including macrophages.

and M2 (induced by the cytokines IL-4 and IL-13), which facilitate local tissue functional demands[4].

Persistent HIV-1 replication during ART has been well documented, but the role of tissue macrophages in HIV persistence has remained unclear. Studies of HIV-1 infection in a macrophage-only human mouse model have shown that in some mice, HIV-1 replication can occur when successful ART is withdrawn[5], which suggests that macrophages might contribute to the latent reservoir of HIV-1. In addition, intact infectious viral particles of HIV-1 assembled in macrophages can successfully persist in an intracellular compartment called the 'virus-containing compartment', which inhibits degradation of the virus by lysosome-associated reactive oxygen species and proteases and facilitates the dissemination of HIV infection via cell-to-cell contact[6]. Future experiments should aim to address mechanism(s) showing how macrophages could 'protect' pathogens while initiating immune responses. The idea that resistance to being killed by CTLs is an intrinsic property of macrophages might reflect their role in maintaining tissue homeostasis and the recruitment of inflammatory cells in response to pathogens and tissue damage. Further experiments to show whether resistance to being killed by CTLs is seen in response to bacterial and fungal pathogens and tissue injury and is a property of other antigen-presenting cells, such as dendritic cells, will clarify whether the findings reported by Clayton et al.[2] highlight a unique aspect of tissue macrophage biology or represent a more general feature of the function of antigen-presenting cells.

Extrapolation of these findings to the root causes of the activation of the immune system in vivo in patients infected with HIV-1 is complex, given the heterogeneity of macrophage subsets, their differential susceptibility to HIV-1 infection and the absence of tissue environmental cues that influence macrophage phenotype and function. In vitro studies of macrophage function in HIV-1 are based mainly on the model that macrophages are derived from hematopoietic stem cell precursors. That assumption is now known to exclude a self-renewing tissue-resident macrophage derived from embryonic precursor cells, and it will be important as well to clarify if this macrophage population also resists being killed by CTLs. It is clear that monocyte function is altered in patients on suppressive ART, and further experiments with this model system could evaluate whether CTL-induced killing of macrophages elicits the production of monocyte activation markers such as sCD14, sCD163 and neopterin, which have all been linked to inflammatory organ disease and mortality in HIV-1 infection[7].

Another area of future study would be to investigate whether, compared with CTLs, HIV-infected macrophages are resistant to being killed by HIV-specific $CD4^+$ CTLs and drive activation of the immune system. Virus-specific cytotoxic $CD4^+$ T cells have been reported to have a key role in control of viral infections, including infection with HIV[8,9]. HIV-specific $CD4^+$ T cell responses appear much earlier than do CTLs during acute HIV infection[10]. A study of the interaction between cytotoxic $CD4^+$ T cells and macrophages is even more relevant to activation of the immune system in patients during early ART, as most $CD4^+$ T cells are preserved and protected from HIV infection.

The ability of HIV-1 to persist in a transcriptionally latent form in multiple cell types is a major barrier to a cure for HIV-1 infection[11]. Most investigators have concentrated on finding ways to eliminate the latent reservoir of HIV-1 in $CD4^+$ memory T cells through the use of a 'shock-and-kill' strategy in which latent reservoirs are reactivated ('shock') by HIV latency–reversing agents so that infected cells can become vulnerable to immune-system clearance mechanisms ('kill'). Given the unique feature of macrophages in HIV infection in terms of their resistance to being killed by CTLs and harboring HIV in the virus-containing compartment, it could be predicted that the 'shock-and-kill' approach would be less effective for HIV-infected macrophages, which might be one of the reasons why clinical trials based on this approach have failed to have a substantial effect on the HIV reservoir during ART. An alternative approach to overcoming the resistance of macrophages to being killed should be considered, such as chimeric antigen receptor–based T cell immunotherapy, a powerful technology that enhances the killing of tumor cells by CTLs and bypasses conventional T cell recognition and signaling pathways[12]. Indeed, a published study has shown that $CD8^+$ T cells expressing CD4-based chimeric antigen receptor–based T cell constructs exhibit over 50-fold more potency in controlling HIV replication and spread than do conventional T cells[13]. In conclusion, the findings from Clayton et al.[2] may inspire future studies focused on understanding of the peculiar features of HIV-infected macrophages and may promote new effective approaches for the rapid elimination of HIV-infected macrophage in addition to infected $CD4^+$ T cells, which could help inhibit the persistent inflammation observed during long term ART. ❐

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Competing interests

The authors declare no competing interests.