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Interactions between Human Immunodeficiency Virus Type 1 and Polymorphonuclear Neutrophils

Sarah Casulli Carole Elbim

Université Pierre et Marie Curie-Paris 6, INSERM UMR-S 945, Immunité et Infection, Hôpital Pitié Salpêtrière, Paris, France

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Human immunodeficiency virus · Neutrophils · Virus-host cell interactions · Inflammatory diseases

Abstract
Polymorphonuclear neutrophils (PMN) are the most abundant circulating leukocytes. They represent a first line of innate immunity against a large panel of microbial pathogens, pending development of specific immune responses. The role of PMN in human immunodeficiency virus type 1 (HIV-1) disease has mainly been investigated from the point of view of the increased susceptibility of HIV-1-infected patients to bacterial and fungal infections. However, it is now clear that the relationship between PMN and HIV-1 is far more complex. This review examines both the beneficial and the detrimental effects of PMN during HIV infection.

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Introduction

Polymorphonuclear neutrophils (PMN) are the most abundant blood leukocytes and are key components of the early innate response to bacterial and fungal pathogens. Several lines of evidence also suggest a key role of PMN in controlling viruses [1–3]. In response to pathogens, PMN rapidly migrate from the blood to inflamed tissues, where their activation triggers microbicidal mechanisms such as the release of proteolytic enzymes and antimicrobial peptides, and rapid production of reactive oxygen species (ROS) in an 'oxidative burst'. PMN are usually short-lived cells, dying spontaneously by necrosis or apoptosis, and apoptotic PMN are recognized and phagocytosed by tissular macrophages. PMN activation by circulating microbial products, endogenous cytokines, and other proinflammatory mediators increases their lifespan and is critical for their antimicrobial efficacy. A newly identified form of ROS-dependent death, distinct from necrosis and apoptosis, leads to the generation of neutrophil extracellular traps (NETs), which are also crucial for PMN antimicrobial activity [4].

Human immunodeficiency virus type 1 (HIV-1) establishes persistent infection in humans. The pathogenesis of HIV infection is highly complex and involves numerous components of the immune system. In particular, the virus targets and replicates inside CD4+ T cells. Infection of CD4+ T cells and their resulting depletion is the main mechanism of HIV pathogenesis, as the immunodeficiency it causes exposes patients to an escalating risk

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Dr. Carole Elbim
INSERM UMR-S 945, Immunité et Infection
Hôpital Pitié Salpêtrière, 83 Bd de l'Hôpital
FR–75013 Paris (France)
E-Mail carole.elbim@upmc.fr

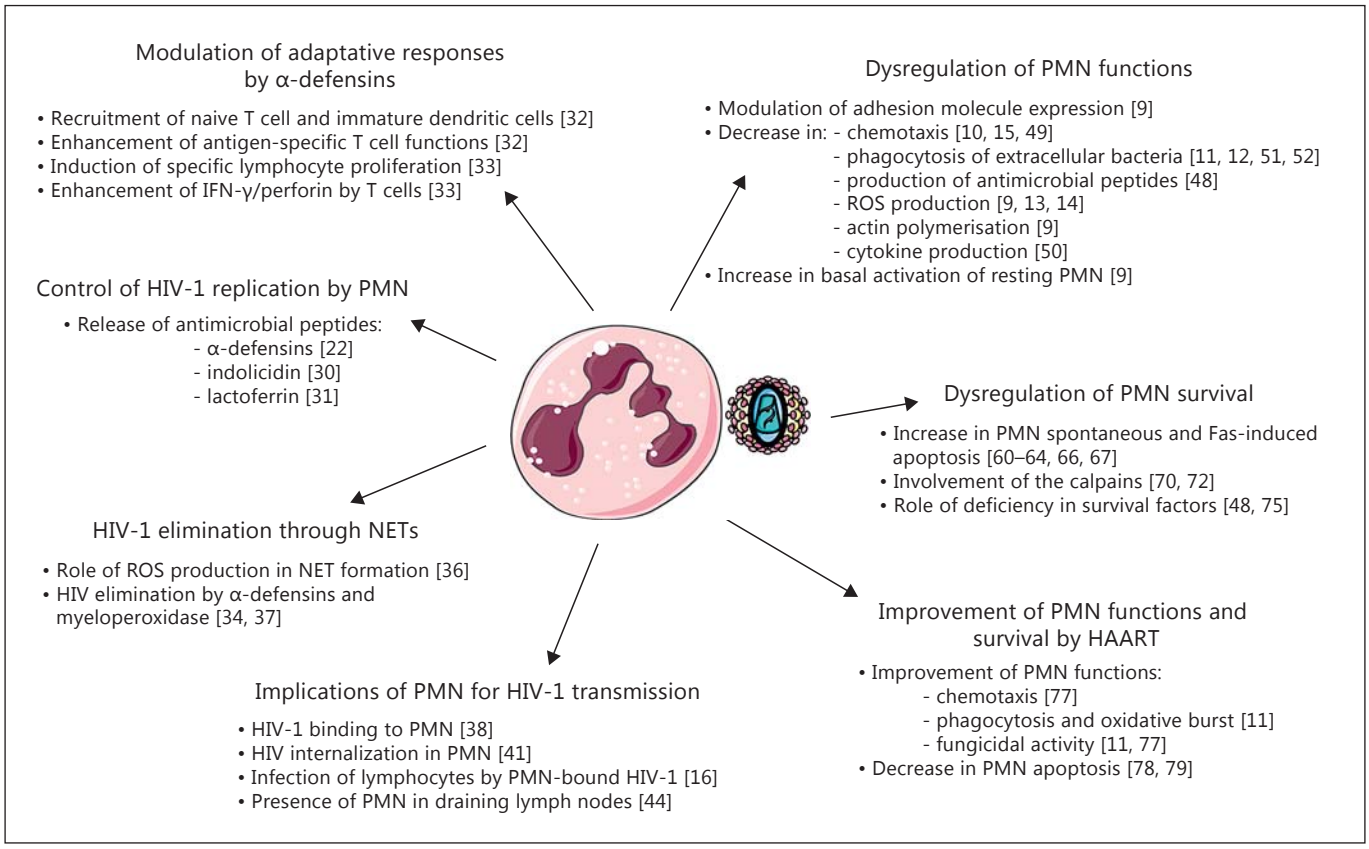

Fig. 1. Summary of the different facets of the interactions between PMN and HIV-1, i.e. the role of PMN in the control of HIV-1 infection, the role of PMN in HIV-1 transmission, the dysregulation of PMN functions and survival in HIV-infected patients, and the effects of HAART on PMN functions and survival.

of opportunistic infections. Antiretroviral therapy (ART) prevents AIDS-related complications and prolongs patients' life expectancy. However, despite sustained viral suppression by ART, HIV-infected patients still have a higher basal level of immune activation than do healthy individuals [5], an abnormality implicated in non-AIDS-defining comorbidities such as osteoporosis, atherosclerosis, neurocognitive decline, and premature aging [6–8]. B cells, T cells, and natural killer cells also play a key role in controlling HIV replication.

The role of PMN in HIV-1 disease has mainly been examined from the point of view of patients' increased susceptibility to bacterial and fungal infections. HIV-1 does not infect PMN but leads to impaired PMN responses (phagocytosis, oxidative burst, and bacterial killing) and a higher rate of apoptosis [9–15]. The immune defects associated with HIV infection, and especially CD4 cell depletion, are largely corrected by ART [11].

It is now becoming clear that PMN can have both beneficial and detrimental effects in HIV infection. For example, PMN play an active role in controlling HIV-1, whereas PMN to which HIV particles have bound may provoke further mucosal inflammation and thereby enhance HIV-1 transmission [16].

This article reviews recent findings on the beneficial and detrimental consequences of PMN interactions with HIV (fig. 1).

Active Role of PMN in the Control of HIV-1 Infection

Involvement of PMN Products in the Control of HIV Infection

Control of HIV Replication. Although PMN-derived mediators such as ROS, TNF-α, and IL-8 have been reported to enhance HIV-1 replication [17, 18], an active role of PMN in controlling HIV-1 replication through the

release of defensins has also emerged. Human α-defensins are cysteine-rich cationic peptides with antimicrobial activity. Humans express 6 α-defensins: proteins 1–4 are produced by PMN, while defensins 5 and 6 are produced by Paneth cells. The anti-HIV-1 activity of α-defensins has been actively studied since Zhang et al. [19] first reported that HNP-1, HNP-2, and HNP-3 were the main components of the CD8+ cell-derived soluble antiviral factor. However, subsequent studies showed that HNP-1–3 are not produced by CD8 cells and that their presence is due to contamination with PMN [20, 21]. These α-defensins have been implicated in several steps of the HIV-1 replication cycle, notably blocking both virus entry into target cells and HIV-mediated fusion by inhibiting viral envelope glycoprotein gp120 binding to CD4 receptors and coreceptors [22–24], and by downregulating CD4 and CXCR4 expression [22, 23, 25]; α-defensins also inhibit HIV-1 replication at the reverse transcription and integration steps [23–25]. α-Defensins have been reported to inhibit HIV-1 infection by upregulating chemokine expression and secretion [26] and to directly inactivate HIV-1 virions in serum-free medium [24].

Increased α-defensin levels have been reported in HIV-infected patients [27], notably in breast milk, and play a role in preventing HIV transmission [28]. This α-defensin upregulation in PMN, which is not modified by ART, has been linked to the chronic immune activation seen in HIV infection [27].

No human θ-defensins have been isolated to date, but humans have 3 θ-defensin pseudogenes that contain premature stop codons. In nonhuman primates, θ-defensins have been isolated from PMN and from bone marrow. Naturally occurring and synthetic θ-defensins have anti-HIV-1 activity. θ-Defensins interact with viral gp41, thus preventing HIV env-mediated fusion with the cytoplasmic membrane [25, 29].

Finally, other antimicrobial peptides stored in PMN granules, such as indolicidin and lactoferrin, have been reported to exhibit potent inhibitory activities against HIV-1 [30, 31].

Modulation of Adaptive Responses. In addition to their direct role in host defense against microbial infection, α-defensins are thought to contribute to adaptive immunity. Indeed, α-defensins exhibit immunostimulatory activities, including recruitment of naive T cells and immature dendritic cells to sites of infection, as well as enhancement of antigen-specific T cell functions [32]. A recent study showed that defensins induce specific lymphocyte proliferation to HIV antigens and enhance IFN-γ/perforin secretion by CD4+/CD8+ T cells [33].

Involvement of PMN in HIV-1 Elimination through NETs

Activated PMN produce NETs – extracellular structures composed of genomic DNA and histones/chromatin released during PMN death – that capture and kill invading bacteria and fungi [4]. Saitoh et al. [34] recently demonstrated that NETs can also capture HIV-1 and promote HIV-1 elimination. PMN detect HIV-1 via Toll-like receptor (TLR)-7 and TLR-8, which recognize viral nucleic acids [35]. Engagement of TLR-7 and TLR-8 in PMN generates ROS production that triggers NET formation and promotes HIV-1 elimination through the actions of α-defensins and myeloperoxidase [4, 36, 37]. However, HIV has developed mechanisms to counteract this PMN antiviral response. For example, HIV-1 stimulates IL-10 production by dendritic cells via DC-SIGN and thereby suppresses TLR-mediated NET formation [34].

HIV-1 Binds to PMN: Implications for HIV-1 Transmission

Binding of the HIV envelope to CD4 is followed by its binding to other coreceptors, namely the chemokine receptors CCR5 and CXCR4, followed by membrane fusion and cell entry. One study has shown unconventional CD4 expression on the surface of peripheral blood PMN in some HIV-1-infected patients and uninfected individuals. The molecular conformation of PMN-expressed CD4 is very similar to that of CD4 expressed on the surface of lymphocytes, and CD4+ PMN have been shown to bind HIV-1 gp120 [38]. HIV-1 binding has also been reported on the surface of CD4-negative PMN, independently of gp120 [16]. In addition, PMN constitutively express CXCR4 and may selectively bind X4 strains of HIV [39]. FPLRI, a formyl peptide receptor belonging to the 7-transmembrane G protein-coupled receptor family, has also been reported to be expressed on the PMN surface and to be an efficient coreceptor for HIV-1 primary isolates [40]. HIV internalization by PMN can also occur through phagocytosis after opsonization with specific anti-HIV antibodies present after the seroconversion phase, and through binding to Fcγ receptors on the PMN surface [41].

As PMN are the most abundant peripheral leukocytes, HIV binding to the PMN surface might favor the spread of HIV. PMN-bound HIV-1 virions have been shown to infect activated PBMC and to transfer the infection to lymphocytes more efficiently than free HIV-1 [16, 42]. Furthermore, HIV binding to PMN is enhanced by in-

flammatory stimuli produced by T lymphocytes, such as TNF-α, in turn increasing the rate of PBMC infection [16]. Interestingly, sustained contact between viable HIV-1-bearing PMN and CD4 lymphocytes may be facilitated through GM-CSF production by HIV-1-infected PBMC, thus prolonging PMN survival and increasing the percentage of HLA-DR+ PMN [43]. PMN are abundant in the inflamed oral and genital mucosae and have also been observed in draining lymph nodes of HIV-1-infected patients [44]. Thus, HIV-1 binding to human PMN and the ability of PMN-bound HIV-1 to infect activated PBMC represent an additional mechanism by which mucosal inflammation may enhance HIV-1 transmission [45].

Dysregulation of PMN Functions and Apoptosis in HIV-Infected Patients

PMN Functional Abnormalities in HIV-Infected Patients

Although some authors have reported an increase in PMN phagocytosis, ROS production, and intracellular bactericidal activity in HIV-infected patients as compared to healthy individuals [46, 47], there is general agreement that PMN from HIV-infected patients, and particularly those with AIDS, exhibit a variety of functional defects resulting in impaired bacterial and fungal killing [9–15]. These defects are not always accompanied by neutropenia or other leukopenias. They result in increased susceptibility to gram-negative and gram-positive bacterial infections and mycoses, and are likely to be a major contributor to the increased morbidity/mortality associated with HIV infection.

PMN activities negatively affected by HIV infection include the regulation of adhesion molecule expression [9], production of antimicrobial peptides such as leukotrienes [48], chemotaxis [10, 15, 49], phagocytosis of extracellular bacteria [11, 12], ROS production [9, 13, 14], actin polymerization [9], and cytokine production [50].

The mechanisms underlying PMN functional defects in HIV-infected patients are not fully understood but could be related to a direct effect of HIV or HIV proteins on PMN. HIV alters FcγR-mediated phagocytosis by downmodulating the γ signaling chain of FcγR [51] and HIV inhibits the formation of the phagosome through Nef-dependent alteration of recycling endosomal compartment membranes [52]. Continued exposure to HIV or viral products such as Tat protein reduces the expression of chemotactic receptors on the PMN surface [53]. An inappropriate cellular distribution of the PMN NADPH oxidase complex, which is the main source of superoxide anion, has also been described in HIV-infected patients [14], although the mechanism remains unknown.

Defective PMN responses could also be related to developmental defects during hematopoiesis. This has been implicated in the profound alteration of PMN chemotaxis observed in HIV-infected patients, a defect independent of neutropenia and associated with decreased expression of chemotactic receptors [15]. Potential mechanisms include an altered bone marrow cytokine environment, direct suppression of hematopoiesis by viral proteins, myelosuppressive effects of antiretroviral drugs, bone marrow infection by viruses, bacteria, or fungi, and, possibly, HIV infection of hematopoietic stem cells.

An altered plasma cytokine environment might play a role in some PMN abnormalities. In particular, a decrease in IL-15 production has been reported in untreated HIV-infected patients and in patients with ART failure as compared to healthy subjects [54]. Interestingly, IL-15 has been shown to enhance PMN functions in vitro [55] and to induce monocytes to produce IL-8, a chemokine specifically involved in PMN recruitment [54].

Finally, untreated HIV-infected patients exhibit increased basal activation of resting PMN, as reflected by increased CD11b and decreased L-selectin expression, increased basal actin polymerization, and increased ROS production, which could explain the reduced capacity of PMN to respond to stimulation [9]. Basal PMN activation may be related at least in part to increased plasma levels of extracellular mitochondrial DNA released from damaged or dead cells [56]. In addition, a significant loss of Th17 cells and an increase in regulatory T cells (Tregs) has been reported in patients with progressive HIV disease, while ART treatment partially normalizes the Th17/Treg ratio [57]. Perturbations of Th17 cells during HIV infection could compromise mucosal defenses against resident and pathogenic microbes. The loss of Th17 cells observed during HIV infection might also contribute to microbial translocation of bacterial products from mucosal tissues, resulting in systemic immune activation [5, 58, 59].

Increased PMN Apoptosis in HIV-Infected Patients

PMN are unique in their susceptibility to undergo rapid spontaneous apoptosis once released from the bone marrow, resulting in their clearance from the circulation within a few hours. Two major pathways that regulate apoptosis have been documented in various cell types, in-

cluding PMN. The first depends on so-called death receptors such as TNFR and Fas (CD95) that can directly trigger a caspase cascade via activation of caspase-8, an initiator caspase. The second, called the intrinsic apoptosis pathway, involves mitochondria and Bcl-2 family members and results in caspase-9 activation.

PMN apoptosis is accelerated as soon as the early stages of untreated HIV infection [60–64]. Downregulation of proinflammatory capacity has also been reported during PMN apoptosis [65]. This increased PMN apoptosis might be involved in the PMN functional impairment observed in HIV-infected patients. Shortened PMN survival due to apoptosis could also contribute to the neutropenia observed in the later stages of HIV infection [66, 67].

Some authors observed increased spontaneous PMN apoptosis in HIV-infected patients but found no correlation with viral load [60, 61, 64]. This spontaneous PMN death is dependent on caspase-3 but independent of caspase-8, suggesting that the intrinsic pathway is involved in PMN death. Spontaneous death of PMN from HIV-infected patients was also reduced upon incubation with catalase/SOD, which is known to reduce ROS levels, suggesting that the underlying mechanism may be related to increased basal ROS production [63]. Several studies suggest that ROS affect the intrinsic apoptotic pathway, probably by targeting mitochondria. ROS might also promote apoptosis by interfering with the activation of survival pathways mediated by NF-κB and MAPKs [68]. This process might affect the ERK [61] and P38 MAPK [63] pathways, resulting in a PMN apoptosis/survival imbalance.

Salmen et al. [61] reported increased PMN susceptibility to apoptosis following Fas cross-linking and found that this correlated with both viral load and coexpression of Fas/FasL surface molecules. HIV is not known to infect PMN but, like other bystander cells, PMN may be targeted by HIV proteins secreted by infected cells, and this could enhance their susceptibility to Fas-induced apoptosis. It has been reported that incubation of the cell line HL60 (PMN promyelocytic leukemia) with purified Nef induces apoptosis [69].

PMN apoptosis in untreated AIDS patients has also been correlated with increased activity of calpains [70], a family of noncaspase cysteine proteases involved in PMN apoptosis [71]. Increased PMN death during SIV infection is prevented by inhibiting calpain activation but not caspase activation [72].

Apoptosis is an intrinsic cellular process that can be regulated by external factors such as endogenous cytokines. Increased PMN apoptosis during HIV infection might thus be related to changes in circulating levels of various cytokines, some of which have a crucial role in PMN survival. In particular, immune activation in HIV infection is associated with increased levels of various proinflammatory mediators such as TNF-α. Although TNF-α, at low concentrations, has been shown to have an antiapoptotic effect on PMN [73], it can also trigger the death-receptor-dependent pathway via TRADD recruitment and subsequent caspase-8 activation. In addition, TNFRI signaling can result in sequential activation of p38 MAPK and class IA PI3Ks, leading to ROS production and subsequent activation of effector caspases via a novel caspase-8- and mitochondria-independent apoptotic pathway [74].

A deficiency in survival factors such as proinflammatory cytokines or colony-stimulating factors could also contribute to the accelerated PMN apoptosis observed in HIV infection. Indeed, G-CSF and GM-CSF administration can promote PMN survival in this setting [75] and also reverses neutropenia [48]. We found that PMN death was increased during the chronic phase of SIV infection in Asian rhesus macaques (RMs) and was significantly more frequent in RMs that progressed rapidly to AIDS [72]. Interestingly, levels of the inflammatory cytokines IL-8 and IL-1β, which prevent PMN death in vitro, were lower in RMs progressing towards AIDS than in nonprogressors.

SIV-infected RMs have been used to investigate the relationship between PMN susceptibility to death during the acute phase of infection and subsequent disease severity. We found that PMN death increased early during the acute phase of SIV infection in RMs, and that it was significantly more severe in RMs that progressed rapidly to AIDS and also coincided with neutropenia. In contrast, PMN death and PMN counts were not modified in African green monkeys, a nonpathogenic model of SIV infection, despite similar high-level viral replication [76]. These findings suggest that increased PMN apoptosis may play a key role in early viral replication and dissemination within the host.

Effects of Highly Active Antiretroviral Therapy on PMN Functions and Survival

The advent of highly active ART (HAART) has led to a significant decline in HIV-related morbidity and mortality. This standard treatment of HIV infection consists of various combinations of nucleoside reverse transcriptase inhibitors, nonnucleoside reverse transcriptase in-

hibitors, protease inhibitors, integrase inhibitors, and fusion inhibitors.

HAART significantly improves PMN functions, including phagocytosis, the oxidative burst [11], chemotaxis, and fungicidal activity against *Candida albicans* [77]. In addition, the larger the fall in viral load, the better the restoration of PMN functions. HAART also markedly reduces PMN apoptosis, possibly contributing to the observed recovery of PMN counts during treatment [78, 79]. In particular, treatment with protease inhibitors induces a rapid and significant decrease in PMN apoptosis, which correlates with improved chemotactic function [62]. These results were obtained in patients with both an immunological and a virological response, and also in patients with an isolated immunological response [62]. Several lines of evidence suggest that this can be attributed to a direct effect of protease inhibitors via calpaine inhibition [70, 80]. Nevertheless, PMN defects are still observed in some patients who respond positively to HAART [81].

Basal immune activation persists despite effective HAART and is a critical factor in HIV pathogenesis [5]. Indeed, this immune activation is postulated to be the leading cause of non-AIDS-defining comorbidities such as atherosclerosis, osteoporosis, and cognitive impairment [6], accelerating the replicative senescence of T cells that would otherwise accumulate [82]. These abnormalities are associated with increased levels of inflammatory mediators such as high-sensitivity C-reactive protein, IL-6, and D-dimer [83], which can be referred to as inflammaging [6].

PMN can also contribute to the tissue injury associated with autoimmune and inflammatory diseases. Indeed, excessive or inappropriate PMN stimulation can trigger excessive ROS production and thereby amplify the inflammatory response [84], damaging lipids, proteins, and DNA. In addition, inappropriate PMN survival and persistence at sites of inflammation are thought to contribute to the pathology of chronic inflammatory diseases through the release of cytotoxic contents into the extracellular environment. α-Defensins exhibit potent antimicrobial activities but also exert immunomodulatory effects by inducing cytokine and chemokine production, inflammatory and immune cell activation, and prolongation of PMN survival [85].

We recently demonstrated that basal activation of circulating PMN persists in HIV-1-infected patients despite effective HAART, along with a decrease in spontaneous PMN apoptosis [pers. data]. Such excessive PMN activation might play a key role in the chronic systemic proinflammatory state observed in HIV-infected patients despite sustained ART-mediated viral suppression [6] and could participate in T lymphocyte senescence [86].

Conclusion

ART significantly improves PMN functions and survival, but basal immune activation and inflammation nonetheless persist. We recently observed persistent PMN activation in HIV-infected patients on effective HAART, a phenomenon that might play a key role in the chronic systemic proinflammatory state and also participate in T lymphocyte senescence. We are currently investigating PMN activation status in HIV-infected patients during effective ART, together with the activation and senescence status of CD4+ and CD8+ T lymphocytes and monocytes. If these parameters correlate with the onset of inflammatory disorders, it may be possible to identify new predictive markers.

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