Neutrophils in antiretroviral therapy–controlled HIV demonstrate hyperactivation associated with a specific IL-17/IL-22 environment

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Background: Despite control of HIV infection under antiretroviral therapy (ART), immune T-cell activation persists in patients with controlled HIV infection, who are at higher risk of inflammatory diseases than the general population. PMNs play a key role in host defenses against invading microorganisms but also potentiate inflammatory reactions in cases of excessive or misdirected responses.

Objective: The aim of our study was to analyze PMN functions in 60 ART-treated and controlled HIV-infected patients (viral load, <20 RNA copies/mL; CD4 count, ≥350 cells/mm^3) with (HIV[I] group) and without (HIV[NI] group) diseases related to an inflammatory process and to compare them with 22 healthy control subjects.

Methods: Flow cytometry was used to evaluate PMN functions in whole-blood conditions. We studied in parallel the activation markers of T lymphocytes and monocytes and the proinflammatory cytokine environment.

Results: Blood samples from HIV-infected patients revealed basal PMN hyperactivation associated with deregulation of the apoptosis/necrosis equilibrium. Interestingly, this hyperactivation was greater in HIV(I) than HIV(NI) patients and contrasted with a lack of monocyte activation in both groups. The percentage of circulating cells producing IL-17 was also significantly higher in HIV-infected patients than in control subjects and was positively correlated with markers of basal PMN activation. In addition, the detection of IL-22 overproduction in HIV(NI) patients suggests that it might contribute to counteracting chronic inflammatory processes during HIV infection.

Conclusions: This study thus demonstrates the presence of highly activated PMNs in HIV-infected patients receiving effective ART and the association of these cells with a specific IL-17/IL-22 environment. (J Allergy Clin Immunol 2014;134:1142-52.)

Key words: *Neutrophils, HIV, inflammation, IL-18, IL-17, IL-22*

HIV-1 establishes persistent infection in human subjects. Although antiretroviral therapy (ART) prevents AIDS-related complications and prolongs life expectancy, HIV-1–infected patients have several physiologic impairments and comorbidities (eg, atherosclerosis, osteoporosis, malignancies, cognitive impairment, and frailty) that are usually observed during the human aging process.[1] These clinical manifestations are thought to result from chronic increased immune activation that occurs despite effective HIV control by ART.[2] Activated adaptive (eg, T lymphocytes) and innate (mainly monocytes) cells secrete proinflammatory mediators, leading to the chronic low-grade inflammation that is a hallmark of these non-AIDS comorbidities.[2]

PMNs are a key component of the early innate response to bacterial and fungal pathogens.[3] In response to pathogens, PMNs rapidly migrate from the blood to inflamed tissues, where their activation triggers such microbicidal mechanisms as rapid production of reactive oxygen species (ROS) in oxidative bursts.[3] ROS can be harmful to their environment, including cells and tissues, especially in cases of excessive release, extracellular release, or both.[3] After they kill microbes, PMNs die spontaneously, mainly through apoptosis. Although they have a very short lifespan, their activation by circulating microbial products, as well as by proinflammatory mediators, promotes their survival and is a critical mechanism in their effectiveness against pathogens.[4] Nevertheless, inappropriate PMN survival might lead to a chronic persistent inflammatory state with ongoing release of inflammatory mediators and damage-associated molecular patterns through PMN necrosis.[4] Thus although PMNs are primarily protective, their inappropriate, excessive, or prolonged activation presents the risk of tissue injury and organ dysfunction and has been involved in various inflammatory diseases, including cardiovascular and osteoarticular disorders.[5-7] The severity of these inflammatory processes is controlled, at least in part, by factors that regulate PMN functions, cell death, and survival, mainly proinflammatory cytokines, such as IL-8, TNF-α, IL-1β, IL-6, GM-CSF, and IL-18.[4,8]

The role of PMNs 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 PMNs but leads to impaired PMN responses (phagocytosis, oxidative burst,

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Abbreviations used
7-AAD: 7-Amino-actinomycin D
APC: Allophycocyanin
ART: Antiretroviral therapy
FITC: Fluorescein isothiocyanate
HC: Healthy control subject
HIV(I): HIV-infected patients with inflammatory diseases
HIV(NI): HIV-infected patients without an inflammatory disease
MDC-8: 6-Sulfo LacNAc (Slan) DC
NRTI: Nucleoside reverse transcriptase inhibitor
PE: Phycoerythrin
ROS: Reactive oxygen species
sCD14: Soluble CD14
TLR: Toll-like receptor

and bacterial killing) and a higher rate of PMN death.[9-11] ART basically corrects these abnormalities associated with HIV infection, especially CD4 cell depletion.[10] However, despite the persistence of increased levels of proinflammatory cytokines[2] that can, as stated above, stimulate PMN activities, the possible link between excessive PMN responses and chronic inflammation in ART-treated HIV-infected patients has never been explored. One reason might be the difficulty of studying PMN functional responses, especially given the need to handle these samples immediately after taking them.

The aim of our study was to analyze PMN functions and survival in ART-treated patients with controlled HIV-1 infection, as well as activation markers of T lymphocytes and monocytes and the proinflammatory cytokine environment. We also investigated associations between these indicators and the presence of clinical disorders related to inflammatory processes.

METHODS

Study design and patients

This cross-sectional study took place in a cohort of 60 HIV-1–infected subjects from the Infectious Diseases Department of the Pitié Salpêtrière Hospital (Paris, France). General inclusion criteria included documented HIV infection, age of 18 years or greater, stable ART treatment, and viral (viral load, ≤20 RNA copies/mL) and immunologic (CD4 count, ≥350 cells/mm^3) control. Exclusion criteria included current opportunistic infections, HIV/hepatitis B virus/hepatitis C virus coinfection, acute inflammatory or infectious conditions, and corticoid or immunosuppressive therapy.

Next, we classified the included patients according to the presence of diseases related to an inflammatory process. Because chronic inflammatory diseases are a heterogeneous group of conditions that can affect any organ or system, we decided to select patients with (1) at least 1 of the active conditions (major criteria) of cardiovascular disease (atherosclerosis, coronaropathy, stroke), chronic obstructive pulmonary disease, vasculitis, or chronic arthritis or (2) at least 2 of the (minor criteria) conditions of hypertension, metabolic syndrome, lipodystrophy, diabetes type 2 with vascular complications, or osteoporosis/osteopenia. Patients meeting either criteria were assigned to the group of HIV-infected patients with inflammatory diseases (HIV[I] group, n = 18). The other group of HIV-infected patients (HIV[NI] group, n = 42) included those with no inflammatory conditions, according to our inclusion criteria.

All HIV-infected patients were enrolled after a physical examination at which blood pressure, height, weight, and temperature were measured and recorded. An extensive review was conducted of each patient's chart (Table I). Demographic characteristics were collected, including smoking status and ethnicity. For comparison, peripheral blood from 22 aged-matched healthy control subjects (HCs) was collected at the Etablissement Français du Sang (Pitié Salpêtrière Hospital, Paris, France) after ethical approval for the use of such material by the institutional review committees of INSERM and Etablissement Français du Sang (convention 12/EFS/079). Exclusion criteria in the control group included acute or chronic inflammatory conditions and corticoid or nonsteroidal anti-inflammatory drugs; smoking status and ethnicity were unavailable for these patients. The study was approved by the local institutional ethics committee (Comité de Protection des Personnes of the Pitié Salpêtrière Hospital). After written informed consent had been obtained from the patients and control subjects, whole blood was sampled, kept on ice, and transported immediately to the laboratory.

Immunophenotyping of T cells and monocytes

The expression of cell-surface molecules in the T-cell and monocyte subpopulations was assessed by using 10-color flow cytometry (Gallios Flow Cytometer; Beckman Coulter, Fullerton, Calif). The detailed staining procedure is described in the Methods section in this article's Online Repository at www.jacionline.org.

Determination of surface molecule expression on PMNs

Heparin whole-blood samples were either kept on ice or incubated at 37°C with PBS, Pam_3CSK_4 (Toll-like receptor [TLR] 1/2 ligand, 1 μg/mL; InvivoGen, San Diego, Calif), LPS from *Escherichia coli* serotype R515 (TLR4 ligand, 10 ng/mL; Invivogen), or TNF-α (5 ng/mL; R&D Systems, Abingdon, United Kingdom) for 45 minutes. Samples were stained with phycoerythrin (PE)–anti-human CD11b (DakoPatts, Glostrup, Denmark), allophycocyanin (APC)–anti-human CD62L (BD Biosciences, San Jose, Calif), and fluorescein isothiocyanate (FITC)–anti-human CD16b antibodies (Beckman Coulter).[12] Samples were then analyzed by means of flow cytometry, as described in the Methods section in this article's Online Repository.

Measurement of oxidative burst of PMNs and monocytes

Superoxide anion ($O_2^{-\circ}$) production by PMNs and monocytes was measured with a flow cytometric assay derived from the hydroethidine oxidation technique.[12] Heparin whole-blood samples (500 μL) were loaded for 15 minutes with 1500 ng/mL hydroethidine (Sigma Aldrich, St Louis, Mo) at 37°C and then incubated for 45 minutes with PBS or various stimuli, as described above. Samples were then treated with PBS or 10^{-6} mol/L formyl-methionyl-leucyl-phenylalanine (Sigma Chemical, St Louis, Mo) for 5 minutes. Samples were then analyzed by means of flow cytometry, as described in the Methods section in this article's Online Repository.

Measurement of PMN apoptosis/necrosis

PMN cell death in whole blood was quantified with Annexin V and the impermeant nuclear dye 7-amino-actinomycin D (7-AAD). Whole-blood samples were incubated for 20 hours at 37°C with PBS or various stimuli, as described above. Samples were incubated with APC–anti-CD15, FITC–Annexin V, and 7-AAD (BD Biosciences).[12] Samples were then analyzed by means of flow cytometry, as described in the Methods section in this article's Online Repository.

Cytokine and soluble mediator assays

Intracellular analyses of IL-17 and IL-22 production were performed by means of flow cytometry. A detailed procedure is described in the Methods section in this article's Online Repository. Cytokines and chemokines were detected from serum with the Cytometric Bead Array kit (FACSCanto II, BD Biosciences) or ELISA assays (Platinum ELISA; eBioscience, San Diego, Calif). Soluble CD14 (sCD14) was detected from serum with an ELISA assay (Quantikine ELISA, R&D Systems).