Association between increased CD177+ neutrophils and chronic activation in people living with HIV

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To the Editor: Neutrophils, as innate cells, play an important role in the immune defensive response against invading pathogens and provide signals for the proliferation and activation of T and B cells to initiate adaptive immunity.[1] Neutrophils from human immunodeficiency virus (HIV)-1 patients exhibit multiple functional defects, such as impaired antimicrobial killing activity and impaired production of reactive oxygen species (ROS), and an altered phenotype.[2] CD177+ neutrophils were identified as a functionally activated cell population.[3] It releases high levels of ROS and antimicrobial peptides compared with CD177− neutrophils under inflammatory conditions. This increased inflammatory cytokine production and ROS generation may have detrimental impacts on the site of stimulation leading to tissue damage, such as epithelial barrier damage resulting in microbial translocation, which is a critical contributor to chronic immune activation during HIV-1 infection.

From 2018 April to 2021 December, 19 acute HIV-infected, 11 antiretroviral therapy (ART)-naïve, 11 ART-treated, and 22 HIV-negative subjects (as healthy donor [HD] group) at Beijing Ditan Hospital, Capital Medical University and Tianjin Second People's Hospital, NanKai University were enrolled in this study. This study was approved by the local Ethics Committee of Tianjin Second People's Hospital (No. 2020-49). All human samples were collected with written informed consent, and the study conformed to the tenets of the *Declaration of Helsinki*. Acute HIV-infected was defined as individuals with negative or indeterminate HIV antibody but positive HIV RNA.[4] Other methods could be found in the supplementary section, http://links.lww.com/CM9/B829. The demographic and laboratory data for the patients are provided in Supplementary Table 1, http://links.lww.com/CM9/B829. The median age of the study participants was 36 years, the sex ratio (men/women) was 59:4, and the median CD4+ T cell count was estimated to be 245 (45, 475) cells/μL in HIV-infected patients and 1015 (377, 1279) cells/μL in HD. Multicolor flow cytometry (BD Biosciences; San Diego, CA, USA) was used to determine the percentage of CD177+ neutrophils in the peripheral blood [Figure 1A, B]. Compared with HD, acute HIV-infected patients and ART-naïve patients displayed significantly increased percentages of CD177+ neutrophils (66.2% *vs*. 89.4%, 86.6%, P <0.001). Meanwhile, a significant decrease in the proportion of segmented neutrophils was observed in these patients [Figure 1C]. To gain an overview of granulopoiesis of CD177 granulocytes, we also investigated the percentage of granulocyte subsets in bone marrow. CD177 is expressed on myelocytes, metamyelocytes, bands, and segmented neutrophils during granulopoiesis in all groups. The percentage of CD177-expressing cells was greatest on the most mature CD16+CD10+ segmented neutrophils. Next, we evaluated the CD177 neutrophils in paired bone marrow and blood samples and the percentage of CD177 neutrophils was increased more significantly in blood than in bone marrow in HIV-infected patients (60% *vs*. 82%, P <0.001). To determine whether there was a difference in function between CD177+ and CD177− neutrophils, ROS production and phagocytosis by the neutrophils were examined. Lipopolysaccharides (LPS)-triggered CD177+ neutrophils produced significantly higher levels of ROS compared with CD177− neutrophils [Figure 1D,E]. We found that the percentage of CD177+ neutrophils was positively correlated with the CD8+ T cell counts (r = 0.4, P = 0.014). To further determine whether CD177+ neutrophils

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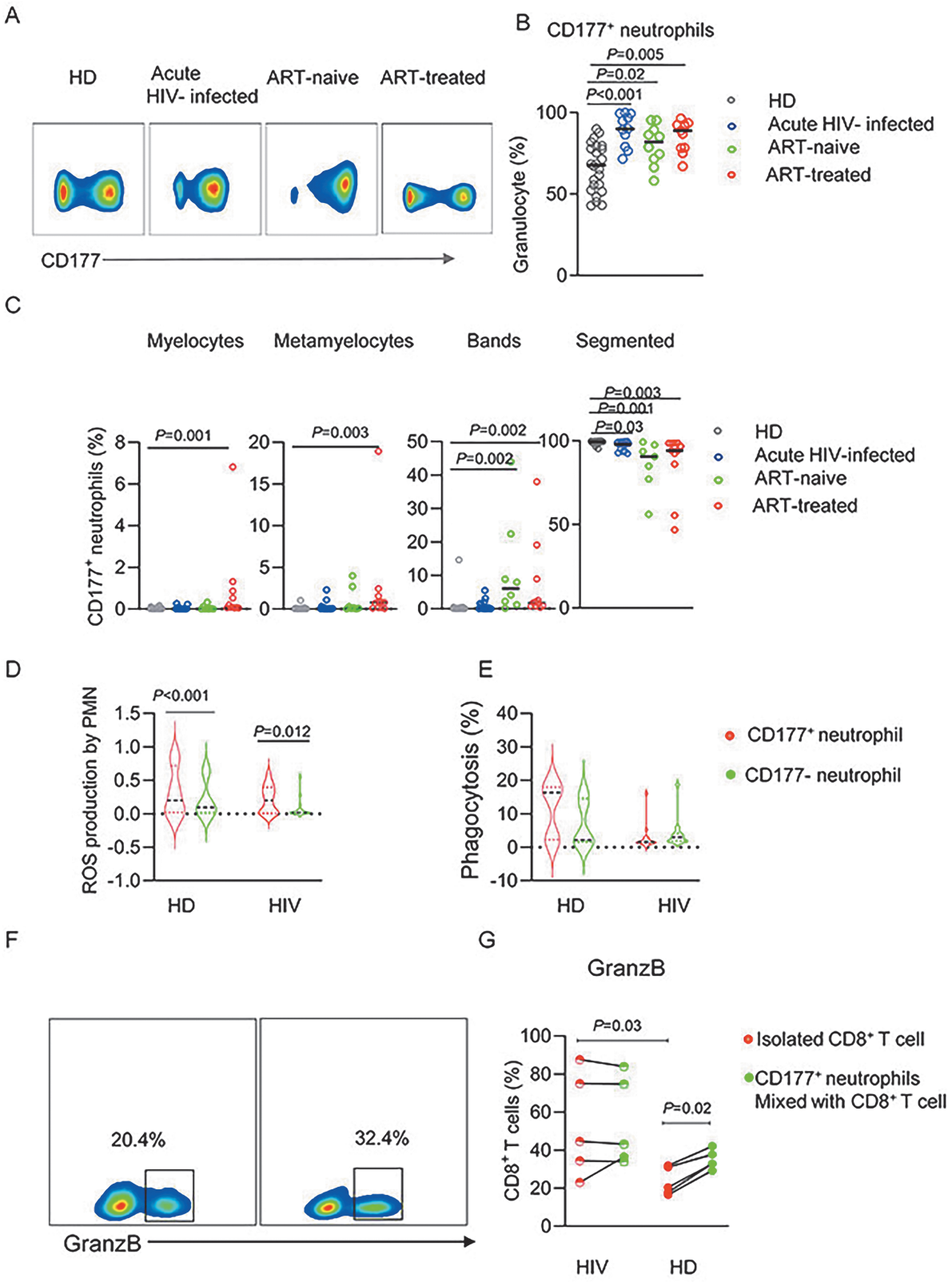

Figure 1: Flow cytometry analysis of CD177⁺ neutrophils in the peripheral blood from HD and HIV-infected patients. (A) Scatter diagram of the flow cytometry data of neutrophils classified by the expression of CD177. (B) The percentage of CD177⁺ neutrophils in the blood of HD, patients with acute infection, and ART-naïve and ART-treated participants. (C) The percentage of myelocytes, metamyelocytes, bands, and segmented neutrophils among the blood CD177⁺ neutrophils from HD, patients with acute infection, and ART-naïve and ART experienced participants. (D) Summary scatter plots of the percentage of CD177⁺ cells positive for DCFH. (E) Summary scatter plots of the percentage of CD177⁺ cells positive for phagocytes. (F) Representative histograms of granzyme assays of leukocyte activation cocktail-stimulated CD8⁺ T cells. (G)Summary scatter plots of the percentage of GranzB CD8⁺ T cells. ART: Antiretroviral therapy; DCFH: Dichloro-dihydro-fluorescein; GranzB: Granzyme B CD8⁺ T cells; HD: Healthy donor; HIV: Human immunodeficiency virus.

induced CD8+ T cell function, we used isolated CD177+ neutrophils and CD8+ T cells from people living with HIV (PLWH) and HD and stimulated the cells with leukocyte activation cocktail at an effector-to-target cell ratio of 4:1 for 4 h. We found that the level of granzyme B was higher in CD177+ neutrophils co-cultured with CD8+ T cells than in CD8+ T cells isolated from HD, but this phenomenon was not detected in PLWH [Figure 1F,G]. The percentage of granzyme B-positive isolated CD8+ T cell was increased in PLWH than in HD, which indicated increased hyperactive CD8+ T cells in PLWH.

Our findings demonstrated that the increased percentage of CD177+ neutrophils in the blood of PLWH during the acute infection stage was associated with the CD8+ T cell count. Similar to the HD, CD177 was expressed by myelocytes in PLWH. When CD177+ neutrophils from HD were co-cultured with CD8+ T cells, the killing function of CD8+ T cells was elevated. By comparison, co-culturing with CD177+ neutrophils from HIV-infected patients resulted in impaired killing by CD8+ T cells.

CD177 is a neutrophil-specific glycoprotein that has been shown to be involved in the pathogenesis of many diseases. Activated CD177+ neutrophils negatively or positively regulate disease progression using different pathways.[5] However, the function and mechanism of action of CD177+ neutrophils remained unclear. The ability of CD8 T cells to release granzyme and perforin is considered to represent their cytotoxic activity.[6] In HIV-infected patients, granzyme+ CD8+ T cell clones expanded, and this negatively correlated with the HIV latent reservoir.[7,8] However, granzyme B release by CD8+ T cells mediated by CD177+ neutrophils was impaired in PLWH compared with HD. Taken together, the results indicated that other mechanisms may be involved in CD8+ T cell cytotoxic activation in PLWH. This study had several limitations. First, it was a cross-sectional study, but HIV infection is a disease that progresses over a lifetime. Second, the study was carried out in two health centers, and the conclusions drawn from this study may not be fully representative of China as a whole. Therefore, our data should be further validated. Third, the cause of the increase in CD177 expression requires further investigation. In summary, our study reveals that CD177+ neutrophils, as a functionally activated subset of neutrophils, were increased in PLWH, but ROS production and activated T cell function were impaired. Thus, targeting CD177+ neutrophils might provide a novel approach in the treatment of HIV infection.

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Conflicts of interest

None.

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