Myeloid Dendritic Cell Dysfunction During Primary HIV-1 Infection Is Independent of Interaction With gp120

Dendritic cells (DCs) have a critical role in sensing the invasion of pathogens, including human immunodeficiency virus type 1 (HIV-1) and in presenting antigen-derived peptides to T cells to initiate adaptive cellular and humoral immune responses. In addition, DCs secrete a wide range of cytokines that regulate T-cell polarization and inflammation. Previous studies have indicated that chronic HIV-1 infection is associated with loss of peripheral DC populations. These DCs also exhibit defective maturation and functions [1, 2]. However, it is not known whether these defects are a consequence of chronic exposure to HIV-1, opportunistic infections, and associated therapies or a result of modulation of DC population during primary HIV-1 infection.

Huang et al [3] recently addressed these issues by analyzing DCs during primary HIV-1 infection and found that primary HIV-1 infection results in substantial defects in DC functions, such as antigen-presenting ability and secretion of cytokines. Upon infection with HIV-1, the viral envelope is the first to be recognized by DCs. The HIV-1 envelope is a huge reservoir of various pathogen-associated molecular patterns that can regulate DC functions either positively or negatively. Among these patterns, envelope glycoprotein gp120 is a major antigen of HIV-1 [4] and plays a crucial role in the pathogenesis of HIV-1 infection by facilitating binding of HIV-1 to DCs and by helping HIV-1 to infect CD4$^+$ T cells. Therefore, we wished to determine whether the defective functions of DCs following primary HIV-1 infection observed by Huang et al are due to interaction of gp120 with DCs.

To address our hypothesis, we investigated the effect of recombinant gp120 from HIV-1 strain JR-FL [5], a well-characterized clinical strain belonging to HIV-1 clade B, on maturation and functions of human monocyte-derived DCs. Six-day-old immature DCs $(0.5 \times 10^6/$ mL) were cultured with 3 μg of gp120 for 48 hours and analyzed for the expression of various surface markers (Figure 1A and 1B). We found that gp120 did not alter the expression of either costimulatory molecules CD80, CD86, and CD40 or terminal maturation marker CD83 or of antigen-presenting class II molecule HLA-DR. These results were further substantiated by analysis of secretion of MHC class I chain-related protein A (MICA), a glycoprotein related to HLA class I molecules, and secretion of which is associated with the maturation of DCs. We could not detect any MHC class I chain-related protein A (MICA) secretion in the supernatants of JR-FL gp120-stimulated DCs
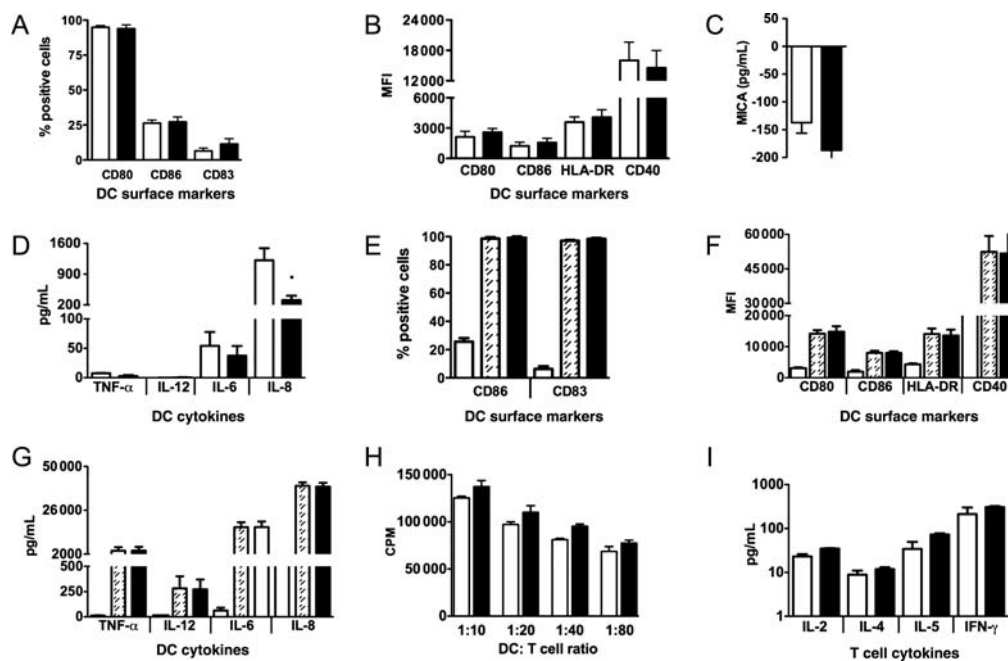
Figure 1. Effect of gp120 from JR-FL strain on human dendritic cell (DC) functions and T-cell responses. *A–D*, Peripheral blood monocytes were isolated from buffy coat bags of healthy blood donors using CD14 microbeads (Miltenyi Biotec). Monocytes were cultured for 6 d in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF; 1000 IU/10^6 cells) and interleukin 4 (IL-4; 500 IU/10^6 cells) to obtain immature myeloid DCs. These immature DCs (0.5 × 10^6 cells/mL) were cultured in the presence of GM-CSF and IL-4 alone (control, *open bars*) or cytokines plus 3 μg/mL of recombinant, Chinese hamster ovary cell–expressed gp120 from HIV-1 strain JR-FL (*dark bars*) for 48 h. The phenotype of DCs (% positive cells [*A*] and mean fluorescence intensities [MFIs; *B*]), MICA secretion (*C*), and cytokines in the cell-free culture supernatants (*D*) were determined. MICA was quantified by enzyme-linked immunosorbent assay (R&D Systems); cytokines were quantified by inflammatory cytokine bead array (CBA) kit (BD Biosciences). All results (mean ± standard error of the mean [SEM]) are from 5–8 experiments using cells from different donors. Statistical significance as determined by Student *t* test is indicated (*$P < .05$). *E–G*, Immature DCs were cultured in the presence of GM-CSF and IL-4 alone (*open bars*) or cytokines plus 10 ng/mL of lipopolysaccharide (LPS; *hatched bars*) or cytokines plus combination of 10 ng/mL of LPS and 3 μg/mL gp120 JR-FL (*dark bars*) for 48 h. The phenotype of DCs (% positive cells [*E*] and MFI [*F*]) and amount of cytokines in the cell-free culture supernatants (*G*) were determined. Results (mean ± SEM) are from 4 donors. *H* and *I*, Control DCs (*open bars*) and gp120-educated DCs (*dark bars*) were washed and cocultured with 0.1 × 10^6 allogeneic CD4$^+$ T cells at various DC:T-cell ratios for 4 d. CD4$^+$ T cells were isolated by using CD4 microbeads (Miltenyi Biotec). After 4 d, the cells were pulsed for 16 h with 1 μCi (1 μCi = 37 GBq) of (^{3}H) thymidine to quantify T-cell proliferation (*H*). The amount of T-cell cytokines, as determined by human Th1/Th2 CBA kit (BD Biosciences), in the mixed lymphocyte reaction (DC:T-cell ratio of 1:10) after 4 d of culture (*I*). Results (mean ± SEM) are from 3 donors.

(Figure 1*C*). Also, except for interleukin 8 (IL-8), gp120 did not alter the basal level of any of the DC inflammatory cytokines (Figure 1*D*). Together, these results demonstrate that gp120 does not modify the functions of immature DCs.

We then analyzed whether gp120 mediates abnormalities in DCs when cells are stimulated in combination with Toll-like receptor (TLR) ligands. We found that gp120 neither imparts refractoriness nor augments lipopolysaccharide (LPS; from *Escherichia coli*) (TLR4 ligand)–driven maturation of DCs (Figure 1*E* and 1*F*). In addition, DCs did not display any abnormalities in the

secretion of inflammatory cytokines such as tumor necrosis factor α, interleukin 12p70, interleukin 6, and IL-8 when the cells were stimulated in combination with gp120 and LPS (Figure 1*G*). These results suggest that the abnormalities in the myeloid DC inflammatory cytokines observed by Huang et al [3] during primary HIV-1 infection do not implicate gp120.

Because primary HIV-1 infection leads to reduced ability of DCs to stimulate T cells, we investigated the capacity of "gp120-educated" DCs to induce proliferation and secretion cytokines of allogeneic CD4$^+$ T cells in a mixed lymphocyte

reaction. We did not detect any defects in the ability of gp120-educated DCs to stimulate T cells; the level of CD4$^+$ T-cell proliferation was similar to that of the control (Figure 1*H*), indicating that the antigen-presenting functions of DCs were not altered by gp120. In addition, there were no differences in the production of CD4$^+$ T-cell cytokines interferon γ, interleukin 2, IL-4, and interleukin 5 (Figure 1*I*). Thus, our results demonstrate that myeloid DC dysfunction in primary HIV-1 infection is not due to the interaction of DCs with gp120.

Although gp120 is one of the most explored antigens for vaccination against

HIV infection [6–9], gp120-based vaccines have failed to confer protection in clinical trials. The gp120 vaccines neither prevented the acquisition of HIV nor altered the viral loads in virus-exposed vaccinated individuals. The protective immune responses induced by vaccines depend largely on the immunogenicity of vaccine antigens. Because activation of DCs upon encounter with vaccine antigens drives effective immune responses, the inability of gp120 to induce either maturation or activation of DCs might explain, in part, the failure of gp120-based vaccination approaches.

Notes

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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