Impact of gp120 on Dendritic Cell-Derived Chemokines: Relevance for the Efficacy of gp120-Based Vaccines for HIV-1

Dendritic cells (DC) are the professional antigen-presenting cells and act as sentinels of the immune system. Therefore, the activation status of DC upon an encounter with vaccine antigens determines the intensity and duration of protective immune responses. DC are also critical for the transmission of HIV-1 to $CD4^+$ T cells (2).

gp120 mediates binding of HIV-1 to DC and is one of the major antigens implicated in the pathogenesis of AIDS (12). In about 62% of individuals, gp120 can induce interleukin 10 (IL-10) in monocyte-derived DC (Mo-DC) (13). Although gp120-mediated abnormal maturation and functional alteration in Mo-DC was reported (7), we found that gp120 did not impart any functional abnormalities to Mo-DC (14). In plasmacytoid DC, gp120 can induce indoleamine 2,3-dioxygenase (4) and can inhibit TLR-9-mediated alpha interferon (IFN-α) secretion (11).

During HIV infection, immature DC capture HIV at mucosal sites of initial infection and are facilitated by gp120 (6). Therefore, gp120 has been explored as one of the vaccine candidates for HIV-1 (8, 9). However, the repercussion of interaction with gp120 on the secretion of DC-derived chemokines is not known. Chemokines mediate trafficking of diverse immune cells and, hence, are critical for the efficacy of the vaccines and for inducing protective immune responses. Upon maturation and activation, DC secrete a wide range of chemokines that dictate the migration of T cells, monocytes/macrophages, and granulocytes and, hence, can influence the course of HIV-1 infection and immune response to vaccines. Therefore, we hypothesized that gp120 vaccination can affect the immune response by modulating the chemokine expression pattern of human DC.

Immature Mo-DC were obtained from healthy donors as previously described upon ethical committee approval (3). We first performed a dose-response analysis of recombinant gp120 protein from HIV-1 on DC maturation by using various concentra-

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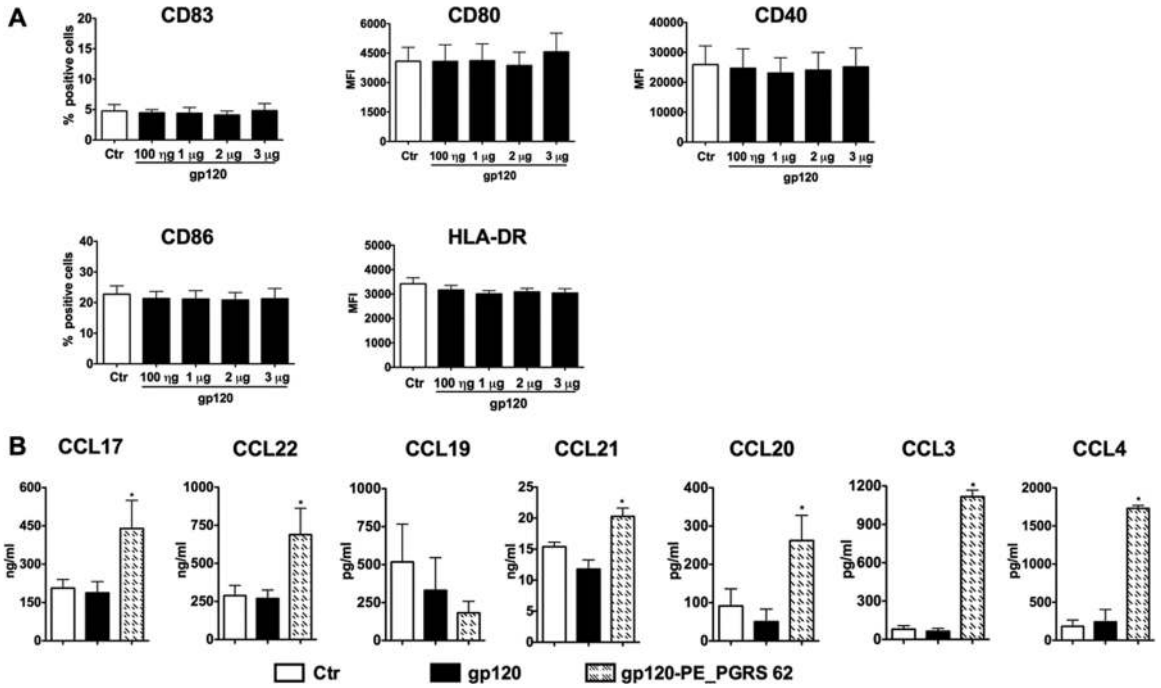

FIG 1 Effect of gp120 from HIV-1 on human DC chemokine responses. Monocytes from buffy bags of healthy blood donors were isolated by using CD14 microbeads (Miltenyi Biotec, France). Monocytes were then differentiated into immature DC by culturing for 6 days in the presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) (1,000 IU/10^6 cells) and IL-4 (500 IU/10^6 cells). (A) Dose-response analysis of the effect of gp120 on DC maturation. Immature DC (0.5×10^6 cells/ml) were cultured in the presence of GM-CSF and IL-4 alone (Ctr) or the cytokines and 100 ng, 1 μg, 2 μg, or 3 μg of recombinant, CHO cell-expressed gp120 from HIV-1 strain JR-FL (gp120) ($n = 3$) for 48 h. The phenotype of the DC was analyzed by flow cytometry (LSR II; BD Biosciences) by using fluorochrome-conjugated monoclonal antibodies to the indicated markers. MFI, mean fluorescence intensity. (B) Immature DC (0.5×10^6 cells/ml) were cultured in the presence of GM-CSF and IL-4 alone (Ctr), the cytokines and 3 μg/ml of recombinant gp120 (gp120) ($n = 8$ to 10 donors), or a combination of the cytokines, gp120, and 5 μg/ml of the recombinant PE_PGRS 62 protein for 48 h (gp120-PE_PGRS 62) ($n = 4$ to 5 donors). Chemokines were quantified in the cell-free culture supernatants by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, France). We confirm that the stimulatory capacity of PE_PGRS 62 is not due to endotoxin contamination. Statistical significance as determined by a two-tailed Student *t* test is indicated (*, $P < 0.05$).

tions: 100 ng, 1 μg, 2 μg, and 3 μg. We found that irrespective of the concentration used in the assay, gp120 did not significantly modify the maturation of DC (Fig. 1A). Therefore, for analyzing the stimulatory effect on DC-derived chemokines in subsequent experiments, we used gp120 at a concentration of 3 μg. This concentration of gp120 is also compatible with the amount of gp120 used for immunization purposes (8–10).

Immature DC (0.5×10^6) were cultured with 3 μg of gp120 for 48 hours, and the secretion of various chemokines was analyzed. The chemokines that were analyzed include the following: CCL22 and CCL17, the chemokines that induce migration of $CCR4^+$ cells, including Th2 cells and subsets of macrophages, DC, Th17 cells, and regulatory T cells; CCL19 and CCL21, which bind to CCR7, and CCL20, which binds to CCR6, all of which can mediate effective T cell responses to HIV-1 and vaccines; and CCL3 and CCL4, chemokines that target the recruitment of CCR5-positive immune cells, including T cells and natural killer cells, and, hence, are critical for effective immune responses toward vaccines and infectious agents (1).

We found that gp120 did not modulate any of the analyzed DC-derived chemokines. Thus, the levels of secretion of CCR5, CCR4, CCR7, and CCR6 ligands by gp120-stimulated DC were similar to that of unstimulated cells (Fig. 1B). However, gp120-exposed DC are neither defective nor tolerogenic, as stimulation of cells with a combination of gp120 and one of the immunogenic proteins, PE_PGRS 62 (Rv3812), of *Mycobacterium tuberculosis* (5) induced significant amounts of several chemokines, including CCL3, CCL4, CCL17, CCL20, and CCL22 (Fig. 1B). Also, we observed neither synergy nor antagonism between gp120 and PE_PGRS 62 in their capacity to stimulate DC chemokines.

Thus, a lack of enhancement of CCR5, CCR7, and CCR6 ligands in DC by gp120 indicates that gp120 does not induce migration and trafficking of inflammatory cells and T cells and, hence, does not promote effective cellular responses to HIV-1 and vaccines. As immunogenicity of vaccine antigens is one of the major criteria for inducing effective immune response to vaccines, these results, along with our previous data on the inability of gp120 to stimulate DC maturation, cytokine response, and DC-mediated T cell responses (14), might explain, in part, the possible reasons for the failure of gp120-based HIV-1 vaccines to confer protection in clinical trials (8, 9).

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