Chemokines are cytokines that activate and induce the migration of leukocytes. Stroma-derived factor (SDF-1) is a novel chemokine that blocks the entry of T-tropic HIV-1 mediated by fusin/CXCR4/LESTR (leukocyte-derived seven-transmembrane domain receptor). In this work we demonstrate that SDF-1 triggers increases in intracellular calcium and inhibits the proliferation of myeloid progenitor cell line 32D. By contrast, SDF-1 neither triggers a calcium response nor affects the proliferation of the myeloid progenitor cell line 32D-GR that is deficient in CXCR4. Responsiveness to SDF-1 was rescued by transfection of 32D-GR cells with a cDNA encoding the human CXCR4. The data indicate that SDF-1 induces myelosuppression by activation of CXCR4. The constitutive production of SDF-1 by bone marrow stromal cells argues for a major role of SDF-1 on the regulation of myelopoiesis.

Chemokines are peptides of 70–100 amino acids secreted by many cell types in response to injury and infection. Two major subfamilies of chemokines are distinguished according to the position of the first two cysteines, the CXC or CC. Chemokines activate and induce migration of leukocytes in a cell-specific fashion (1). SDF-1 is a CXC chemokine secreted constitutively from several cell types. Two isoforms of SDF-1 have been identified, α and β, which are generated by differential splicing (2). SDF-1 was initially characterized as a pre-B-cell stimulatory factor (3). Recently, SDF-1 has been identified as a highly efficient chemotactic factor for T-cells, monocytes (4), and CD34+ human progenitor cells (5). The constitutive expression of SDF-1 by many tissues has suggested that SDF-1 plays a key role in homing T-cells and monocytes under basal conditions (4). Targeted disruption of the SDF-1 gene in mice was shown to be lethal with severe abnormalities in B-cell lymphopoiesis, bone marrow myelopoiesis, and development of the cardiac ventricular septum (6). The signaling of SDF-1 is mediated by CXCR4/LESTR (leukocyte-derived seven-transmembrane domain receptor), a G protein-coupled receptor expressed preferentially in leukocytes (4, 7–9). Recent studies have demonstrated that CXCR4 is the coreceptor for the entry of T-tropic HIV-1 (10) and HIV-2 (11) into CD4+ and CD23 cells, respectively. Of importance is the observation that SDF-1 blocked the entry of T-tropic HIV-1 strains (7, 8). Since SDF-1 is constitutively expressed by bone marrow cells we investigated whether SDF-1 also regulates myelopoiesis. Our data indicate that SDF-1 induces myelosuppression of bone marrow-derived cell line 32D by activation of CXCR4.
level of intracellular \( \text{Ca}^{2+} \). We found that SDF-1a or -b increased the intracellular \( \text{Ca}^{2+} \) in 32D cells (Fig. 1), a non-tumorigenic cell line that exhibits features of normal myeloid progenitor cells (16). In contrast to leukemic cell lines, the proliferation of 32D cells requires the presence of IL-3 (18). The \( \text{Ca}^{2+} \) response mediated by SDF-1b was concentration-dependent, and a maximal response was achieved with 100 nM SDF-1b (Fig. 1). Cells treated with SDF-1a or -b were weakly responsive to subsequent additions of SDF-1a or -b showing homologous desensitization. Other chemokines including IL-8, MIP-2, neutrophil activating peptide-2, platelet factor-4, melanocyte growth stimulatory activity, monocyte chemotactic protein-1, or ATP did not desensitize the \( \text{Ca}^{2+} \) response mediated by SDF-1b. The SDF-1-induced calcium responses in 32D cells were similar to those previously observed with T-cells and monocytes (7). In addition, SDF-1b failed to induce calcium responses in 32D cells pretreated with pertussis toxin (data not shown), demonstrating that the SDF-1b receptor is coupled to \( \text{Gi proteins} \). To evaluate the functional role of SDF-1 on myeloid progenitor cells we tested the effect of SDF-1b on the proliferation of 32D cells using agar colony assays (16). Like normal...
hematopoietic progenitor cells, 32D cells form colonies in semisolid culture that are strictly dependent on the presence of IL-3. As shown in Fig. 2, SDF-1β inhibited colony formation of 32D cells, suggesting that SDF-1β is a myelosuppressor.

To determine whether the calcium responses and suppression of proliferation of 32D cells are mediated by activation of the HIV-1 coreceptor CXCR4 we first explored the expression of CXCR4 in 32D cells by Northern blot analysis. Blots of RNA extracted from 32D cells, Jurkat T cells, and white blood cells failed to detect the mRNA of CXCR4 (Fig. 3) nor exhibit the typical ATP-dependent Ca2+ response (Fig. 4). However, similarly to 32D cells, 32D-GR cells exhibit the typical ATP-dependent Ca2+ responses (Fig. 4, A and C), form colonies in semisolid culture, and require IL-3 for proliferation. Both 32D and 32D-GR form a similar number of colonies; however, SDF-1β failed to inhibit the proliferation of 32D-GR cells (Fig. 4). Finally, responsiveness to SDF-1 was rescued by transfection of 32D-GR with human CXCR4 cDNA. These data indicate that SDF-1β induces myelosuppression by activation of CXCR4. Previous studies have shown that certain chemokines including IL-8, platelet factor-4, MIP-1α, and MIP-1β have shown myelosuppressive activity in bone marrow myeloid progenitor cells; however, the heterogeneity and low frequency of precursor cells from bone marrow has precluded the identification of receptor systems that mediate the effect of these chemokines (20–22). It is unlikely that these chemokines mediate their myelosuppressive activity via CXCR4 since they do not activate CXCR4 or desensitize the calcium responses mediated by SDF-1. Probably, these chemokines bind specific receptors.

Indeed, we have recently shown the endogenous expression of the murine homolog of the human IL-8 receptor B in 32D cells. The constitutive expression of SDF-1 by bone marrow stromal cells strongly argues for a major role of the HIV-1 coreceptor CXCR4 on myelopoiesis.

2 X. Sanchez, K. Suetomi, B. Hodges, J. Horton, and J. Navarro, unpublished results.

Acknowledgment—We thank Nancy Wilkinson for helping with the agar colony assays.

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