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QUARTERLY

High molecular mass dextran sulfate increases expression of HIV-1 coreceptor CCR-5 in macrophage-monocytes in culture*°

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It has been reported that high molecular mass dextran sulfate (HMDS) enhances the infection of monocyte-macrophages by HIV-1. We observed that in monocyte-macrophages maintained in the presence of HMDS the expression of HIV-1 coreceptor CCR-5 was increased approximately 5-fold at the transcriptional level. We postulate that the increased expression of CCR-5 might be responsible for HMDS-enhanced infectivity of monocyte-macrophages by HIV-1.

The human immunodeficiency virus (HIV-1) is a principal pathogen which causes the acquired immunodeficiency syndrome (AIDS). The virus binds to protein CD4 on the surface of the target cells and, during the fusion process, infects the cells [1, 2]. HIV-1 can be divided into two major species of viruses: lymphotropic and monotropic. The lymphotropic viruses infect $CD4^+$ lymphocytes and established leukemia cell lines, but do not infect monocyte-macrophages. On the other hand, the monotropic viruses infect $CD4^+$ lymphocytes and monocyte-macrophages but do not infect established leukemia cell lines [3]. It has been observed that any cells obtained from animals which do not belong to the primate family, when transfected with cDNA encoding CD4, do not become infected by HIV-1 [4]; this suggests a requirement for an additional, yet poorly defined, cofactor(s) for infection. Two major coreceptors indispensable for HIV-1 entry into the target cells have recently been discovered. These proteins designated CXCR-4 and CCR-5 are chemokine re-

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Abbreviations: CRDS, 1,3-β-glucan sulfate; HMDS, high molecular mass dextran sulfate; LMDS, low molecular mass dextran sulfate.

ceptors. CXCR-4 is a coreceptor for lymphotropic viruses and binds chemokine SDF-1 while CCR-5, which binds MIP-α, MIP-β and RANTES, is a coreceptor for monotropic viruses [5-7]. It has been reported that high molecular mass dextran sulfate (HMDS) increases infectivity of HIV-1 in macrophages [8]. In our study we have demonstrated that the addition of HMDS to the culture media increases expression of CCR-5 in macrophage cells, at a transcriptional level. In contrast, low molecular dextran sulfate (LMDS) and 1,3-β-D-glucan sulfate (CRDS) had no effect on CCR-5 expression in these cells.

MATERIALS AND METHODS

Sulfated polysaccharides. HMDS (500 kDa) was obtained from Pharmacia Biotech (Uppsala, Sweden). LMDS (8 kDa) and heparin (low molecular mass) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Glucan sulfate – CRDS (molecular mass 79 ± 0.6 kDa, sulfur content 15.2 ± 0.3%) was obtained from Ajinomoto Co. (Tokyo, Japan).

Isolation of monocyte-macrophages. Peripheral blood mononuclear cells were obtained from HIV-1 seronegative donors after centrifugation over Ficoll-Hypaque (density 1.007 g/cm^3) and were incubated in RPMI 1640 (Gibco BRL, Gaithersburg, MD, U.S.A.) culture medium for 1 h at 37°C. Under these conditions the monocyte macrophage cells became attached to the plastic flask (Corning Costar, MA, U.S.A.) used. The unattached cells were discarded. The macrophages were grown for 5 days in RPM-1640 medium supplemented with 10% human serum and antibiotics [9] in the absence or presence of HMDS, LMDS, or CRDS at concentrations of 3, 10, 25 or 50 μg/ml.

Isolation of RNA, reverse transcription and amplification of cDNA by PCR. Total RNA was isolated according to Chomczynski & Sacchi [10], reversely transcribed into cDNA and a 202 bp CCR-5 coreceptor fragment was amplified by PCR in the presence of internal standards (mimics). The following primers were used: 5'-CAA GTG TCA AGT CCA ATC TA-3' (forward) and 5'-TGA GCA GGT AGA TGT CAG -3' (reverse). We used a 548 bp β-actin fragment as an external stan-

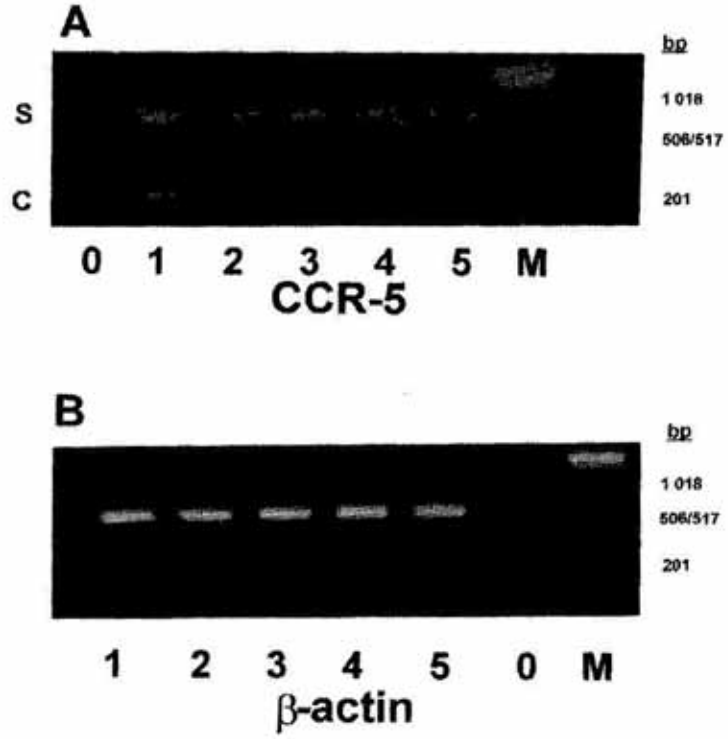

Figure 1. Semiquantitative PCR of monocyte-macrophage cDNA.

Total RNA was isolated, reversly transcribed into cDNA and amplified by PCR in the presence of internal standards (mimics) and the PCR products were subjected to electrophoresis in 2% agarose gel and visualized by ethidium bromide staining as described in Materials and Methods. Panel A represents amplification of CCR-5 cDNA (c) and amplification of internal standard (s), whereas panel B shows the PCR product of amplification of β-actin cDNA used as an external control. Lanes 1 through 5 correspond to samples incubated with HMDS at the concentration of 50, 25, 10, 3 μg/ml and without HMDS, respectively. 0, negative control; M, molecular size marker.

dard. To amplify the β-actin fragment we used the following primers: 5'-GTG GGG CGC CCC AGG CAC CA-3' (forward) and 5'-CTC CTT AAT GTCACG CAC GCA CGA TTT C-3' (reverse). The PCR products were subjected to electrophoresis in 2% agarose gel and visualized by ethidium bromide staining.

RESULTS AND DISCUSSION

We observed that HMDS at the concentration of 50 μg/ml increased CCR-5 mRNA expression as compared to the control sample (Fig. 1, panel A). In contrast, under the same conditions LMDS and CRDS had no effect on the expression of CCR-5 (not shown). The application of internal standards (mimicks) and monitoring of the expression of β-actin as an external standard (Fig. 1, panel B) allowed to semi-quantify the results by scanning the gel. Our results revealed that HMDS increases the expression of the HIV-1 coreceptor CCR-5 about 5-fold. This indicates that HMDS may bind to an unknown factor present on the surface of macrophage-monocytes and trigger by an unknown way the expression of the HIV-1 coreceptor CCR-5 needed for HIV-1 entry.

It has been reported that various dextran sulfates and other sulfated polysaccharides can act as mitogens for human T-cells and activators of murine polyclonal B-lymphocytes [11] which suggests that HMDS might also interact with other types of cells, among them monocyte-macrophages.

We have shown that HMDS can induce coreceptor CCR-5 expression in macrophage-monocytes in culture. The increased expression of CCR-5 can explain, at least in part, the enhanced infectivity of monocyte-macrophage cells in the presence of HMDS. However, future investigations are needed to study the molecular mechanism which underlies the observed effect of HMDS.

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