Similarity of Chemokines Charge and the V3 Domain of HIV-1 env Protein

To the Editor: Most clinical HIV-1 isolates can infect $CD4^+$ peripheral blood T lymphocytes, monocytes, and cultured macrophages (macrophage or M-tropic) but not transformed T-cell lines. In contrast, HIV-1 strains adapted for growth in transformed T-cell lines (T-cell line or T-tropic) do not infect primary monocytes or macrophages. This difference in tropism appears to be a consequence of specific amino acid changes in the env protein. Changes in env responsible for an M- to T-tropism shift often involve the acquisition of multiple positively charged residues in the hypervariable V3 loop domain (1). However, some non-V3 determinants are also important for viral tropism. Although both types of viruses use CD4 as receptor, the CXCR4 chemokine receptor (previously designated LESTR/fusin) is the unique cofactor for entry of T-tropic HIV-1 strains (2). The CCR5 chemokine receptor was subsequently demonstrated to be the cofactor for M-tropic HIV-1 isolates (3). Although some direct evidence for cell surface association of the CD4-env complex and the CXCR4 coreceptor was obtained (4), little detail is available on the molecular forces responsible for these protein-protein interactions. In particular, there is no direct evidence to indicate that the V3 loop binds to the chemokine receptor.

Jiang (5) reported that the extracellular domain of the CXCR4 coreceptor for T-tropic HIV-1 isolates is more negatively charged than the CCR5 coreceptor. Because T-tropic isolates have evolved a positively charged V3 domain, it was suggested that coreceptor-env binding involves the interaction between oppositely charged residues. We now expand this analysis by showing that the chemokines corresponding to the different receptors have a similarly unbalanced composition of charged amino acids. So far, the CXCR4 receptor has been demonstrated to bind only the SDF-1 chemokine (6). The CCR5 receptor binds more than one chemokine, including RANTES, MIP-1α, and MIP-1ß (7). The amino acid sequence of these four chemokines is presented in the Figure. We listed the number of positive residues (arginine [R] and lysine [K]) and negative residues (aspartic acid [D] and glutamic acid [E]), and calculated the net charge. SDF-1 appears to have the highest number of positive residues and the lowest number of negative residues, resulting in a net charge of +11. All other chemokines have much less positively charged amino acids, resulting in a net charge for MIP-1α and MIP-1ß of -2 and -1, respectively. The RANTES chemokine has an intermediate charge of +7, which may correlate with the unique receptor use of this chemokine (e.g., RANTES, but not MIP-1α and MIP-1ß, binds the CCR3 receptor [7]). These results are consistent with the idea that positive charges in SDF-1 interact with negative charges in the CXCR4 receptor, and this binding may thus resemble the HIV-1 env-CXCR4 interaction.

Early evidence that both the chemokines and HIV-1 bind to the same domain of the chemokine receptor comes from virus inhibition studies. Several ß-chemokines suppress infection with M-tropic but not T-tropic HIV isolates (8), and SDF-1 specifically blocks entry of T-tropic isolates (6). Although direct blocking of the receptor may explain part of this chemokine-mediated inhibition, it has also been proposed that internalization of the receptor contributes to the antiviral effect (9). Irrespective of the precise antiviral mechanism, the combined results of this analysis

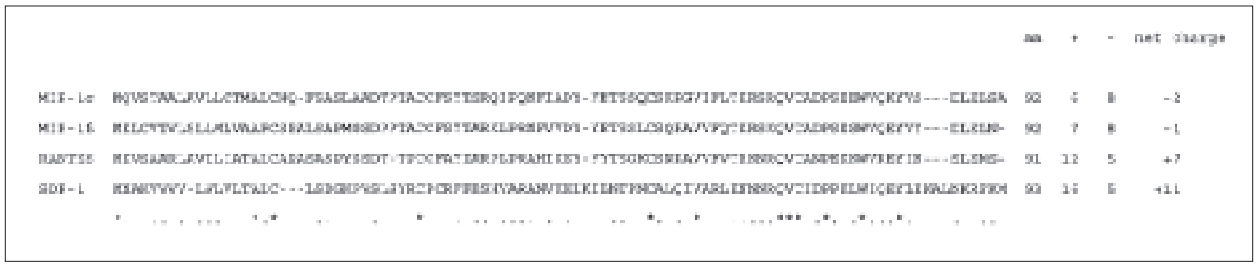

Figure. Amino acid alignment of the four chemokines was performed with the PC/Gene program. An overall identity and similarity of 12.4% and 40.2% was calculated, respectively. The initiator methionine contained within each sequence is removed in the processing of the chemokine. The SDF-1 form shown is the ß-form; the α-form lacks the C-terminal amino acids RFKM, thus reducing the net charge to +9.
* = a perfectly conserved residue.
. = a conservative change.

and the one presented by Jiang (5) suggest that both the SDF-1 chemokine and the V3 loop of T-tropic HIV-1 viruses use positively charged amino acids for an electrostatic interaction with the negatively charged CXCR4 receptor. To examine whether the similarity between the chemokine and env V3 domain is also apparent at the primary sequence level, we performed an amino acid alignment; however, we found no conserved motifs (data not shown). A detailed mutational analysis is required to further our understanding of the env-coreceptor interaction.

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Detection of Glycoprotein of *Burkholderia pseudomallei*

To the Editor: Melioidosis, a potentially fatal disease that is difficult to diagnose and treat, is common in areas with subtropical climate (e.g., Singapore, the southern provinces of China) and is hyperendemic in Thailand. The etiologic agent, *Burkholderia pseudomallei* (*Pseudomonas pseudomallei*), is widely distributed in Southeast Asia and northern Australia. The agent has the potential to become established in regions with similar climate conditions, particularly if animals infected with *B. pseudomallei* are imported from endemic-disease zones (1-3).

Rapid and reliable detection of *B. pseudomallei* and its antigens has many potential applications. Recently, we developed a monoclonal antibody immunoenzyme test system for the detection of minimal concentrations of a *B. pseudomallei* glycoprotein, which is considered one of the pathogenicity factors for this microorganism. This glycoprotein, called Ag8 by N.N. Piven and V.I. Ilyukhin (4), is present in different strains of *B. pseudomallei* and *B. mallei* but not in other *Burkholderia* spp. (*B. aeruginosa, B. putida, B. cepacia, B. malthophilia, B. fluorescens, B. pseudoalcaligenes*). Ag8 is composed of 10% protein and 90% carbohydrate, has molecular mass 800 kDa, and is localized in an extracellular capsulelike substance surrounding *B. pseudomallei* cells (5).

We developed an immunoenzyme test system with three monoclonal antibodies (Mab) to different epitopes of Ag8 (Mab 2A6-IgG3, Mab 2H7-IgG1, Mab 1G2-IgG2b) and one antibody to epitopes common for Ag8 and LPS of *B. pseudomallei* (mab 1ES-IgG2b). A sandwich enzyme-linked immunosorbent assay (ELISA) was used for the detection of Ag8 in different test samples (6). The sensitivity of the immunoenzyme test system was determined with a standard antigen sample. Minimal sensitivity (37 ng/ml of carbohydrate) was observed when polyclonal immunoglobulins were used as "catching" antibodies. Maximal sensitivity (0.37 ng/ml of carbohydrate) was noted when either Mabs 2A6 or mixtures of Mabs were used as catching antibodies.

The test system was further evaluated with samples of extracellular antigens (extracts of cultural media, fractions after gel chromatography of extracellular antigens) and bacterial suspensions of *B. pseudomallei* and *B. mallei* strains isolated in different regions of the world.