Chemokines play diverse roles in inflammatory and non-inflammatory situations via activation of heptahelial G-protein-coupled receptors. Also, many chemokine receptors can act as cofactors for cellular entry of human immunodeficiency virus (HIV) in vitro. CCR5, a receptor for chemokines MIP-1α (LD78α), MIP-1β, RANTES, and MCP2, is of particular importance in vivo as polymorphisms in this gene affect HIV infection and rate of progression to AIDS. Moreover, the CCR5 ligands can prevent HIV entry through this receptor and likely contribute to the control of HIV infection. Here we show that a non-allelic isoform of human MIP-1α (LD78α), termed LD78β or MIP-1αP, has enhanced receptor binding affinities to CCR5 (~6-fold) and the promiscuous β-chemokine receptor, D6 (~15–20-fold). We demonstrate that a proline residue at position 2 of MIP-1αP is responsible for this enhanced activity. Moreover, MIP-1αP is by far the most potent natural CCR5 agonist described to date, and importantly, displays markedly higher HIV suppressive activity than all other human MIP-1α isoforms examined. In addition, while RANTES has been described as the most potent inhibitor of CCR5-mediated HIV entry, MIP-1αP was as potent as, if not more potent than, RANTES in HIV-1 suppressive assays. This property suggests that MIP-1αP may be of importance in controlling viral spread in HIV-infected individuals.

LD78β, A Non-allelic Variant of Human MIP-1α (LD78α), Has Enhanced Receptor Interactions and Potent HIV Suppressive Activity*

(Received for publication, December 16, 1998, and in revised form, March 22, 1999)

Robert J. B. Nibbs‡, Jinying Yang§, Nathaniel R. Landau§, Jinhua Mao, and Gerard J. Graham**

From the ‡Beatson Institute for Cancer Research, Department of Medical Oncology, CRC Beatson Laboratories, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD Scotland, United Kingdom and the §Aaron Diamond AIDS Research Center, Rockefeller University, New York, New York 10016

Chemokines are a family of structurally and functionally related proteins that play a central role in the regulation of hematopoietic cell migration during development, immune surveillance, and the establishment of inflammatory and immune responses (1, 2). These biological effects are mediated through a family of cell surface G-protein-coupled heptahelial receptors (3). Recently, these receptors have been implicated in HIV1 pathogenesis with the discovery that a large number of the members of this family are able to act as cofactors for the entry of HIV into cells in vitro (for reviews, see Refs. 4 and 5). The ligands for these receptors are able to compete for binding with the HIV envelope protein to abrogate viral entry (6–8). While an in vivo role for many of these receptors has yet to be demonstrated in the context of HIV infection, CCR5, a receptor for RANTES (regulated on activation, normal T cell expressed and secreted), MCP2 (monocyte chemotactic protein-2), and MIP-1α and -1β (macrophage inflammatory protein-1α and -1β) appears to be critical in HIV pathogenesis as individuals who are homozygous or heterozygous for a CCR5 null mutation are relatively resistant to HIV infection and progression to AIDS, respectively (9–11). Moreover, the CCR5 ligands are produced by activated HIV-specific cytotoxic and helper T cells, whereby they are likely to play an important role in controlling viral spread (12–16). Current data show RANTES to be the most effective antagonist of CCR5-mediated HIV entry, with MCP2 and MIP-1α and -1β being less effective (17–19).

We have been interested in characterizing receptors mediating the biological effects of MIP-1α, a member of the β subfamily of chemokines able to induce chemotaxis of many mature leukocyte types (1, 2) and a potent inhibitor of hematopoietic stem cell proliferation (20, 21). Mice lacking this gene display dramatic alterations in responses to several infectious agents (22) likely due to leukocyte chemotaxis abrogation. We have recently cloned several receptors for this β chemokine from mouse and human sources (23, 24) and found a number of discrepancies with respect to their interaction with murine MIP-1α and its presumed human homologue, LD78α. Thus, while the murine and human forms of the promiscuous β-chemokine receptor D6 (23, 24) bind murine MIP-1α with high affinity, LD78α interacts poorly with these receptors. Similarly, murine CCR5 binds murine MIP-1α with high affinity but does not recognize the putative human homologue.

The basis for this marked selectivity has not previously been addressed, however, it is notable that, while the murine genome contains only one copy of MIP-1α and -1β, in humans these two genes have been duplicated and mutated to produce two different non-allelic isoforms which still retain >90% homology (25, 26).‡ For MIP-1α, these have been called LD78α and -β, with LD78α being the predominant experimentally used isoform and the one shown to interact poorly with the cloned chemokine receptors. Both forms are transcribed (26) and can be secreted from mammalian cells (6, 21, 27, 28). Interestingly, consideration of differences in the putative signal sequences of the two LD78 isoforms by predictive algorithms,‡ suggests that there is variation in the site of signal peptidase cleavage between the two isoforms during secretion, and that LD78α is in

‡ This work was supported by the Cancer Research Campaign (to G. J. G), National Institutes of Health Grants CA72149, AI36057, and AI1384 (to N. R. L.), and AmFar Grant 02580-23-RGV (to J. Y. and N. R. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby indicated this fact.

‡ Elizabeth Glaser Scholar of the Pediatric AIDS Foundation.

** To whom correspondence should be addressed. Tel.: 44-141-330-3982; Fax: 44-141-942-6521; E-mail: graham@beatson.gla.ac.uk.

† The abbreviations used are: HIV, human immunodeficiency virus; MCP2, monocyte chemotactic protein-2; MIP-1α and -β, macrophage inflammatory protein-1α and -1β, CHO, Chinese hamster ovary.

‡ R. J. B. Nibbs, unpublished data.
fact produced without the four anticipated amino-terminal amino acids, ASLA, and was used in the amino-terminal sequence ADTPT (6, 27). We have worked extensively with the LD78β isoform (21, 28), and numerous amino-terminal sequencing exercises have consistently revealed a "full-length" amino terminus ADTPT.

Using full-length and truncated variants of LD78α and -β, we show here that the murine/human MIP-1α binding discrepancies are resolved by studying the properties of LD78β which consistently behaves more like murine MIP-1α than does LD78α. It appears therefore that LD78β more accurately represents the functional human homologue of murine MIP-1α and that LD78α should be considered to be a related but functional distinct chemokine. Importantly, we also show that LD78β is the most potent natural CCR5 agonist described to date. Furthermore, LD78β exhibits a much greater ability to antagonize HIV entry through CCR5 than other forms of human MIP-1α. In fact, LD78β is consistently better at HIV antagonism than RANTES, previously described as the most potent CCR5-dependant HIV entry inhibitor. We demonstrate that the enhanced activity of LD78β is solely due to the presence of a proline residue at position 2 of the mature protein. We propose renaming LD78α and -β to MIP-1αS and MIP-1αP, respectively, to reflect the importance of this residue in the functional differences between these two proteins.

**EXPERIMENTAL PROCEDURES**

**Chemokines**—MIP-1αS is "human MIP-1α" purchased from Peprotech, London, United Kingdom. It is derived from the LD78α cDNA and has the amino-terminal sequence of ADTPT. MIP-1αS is a human MIP-1α purchased from R&D Systems, Abingdon, UK. It is derived from the LD78α cDNA and starts at ADTPT. MIP-1αP is derived from the LD78β cDNA and was prepared as described previously (28). Sequencing revealed ADTPT at the amino-terminal. MIP-1αP was produced from a modified LD78β cDNA in bacteria with the amino terminus of ADTPT.

**Receptor Binding Studies**—CHO cells expressing chemokine receptors were prepared, and binding assays performed, as outlined previously (23). In short, binding assays were performed using 35S-labeled murine MIP-1α at a constant concentration of 600 pM (for D6), 3 nM (for CCR1), or 9 nM (for CCR5), while varying the concentration of unlabeled human MIP-1α competitor protein. Remaining radioactivity bound after 90 min, and three ice-cold phosphate-buffered saline washes, was determined. Each point was done in triplicate, the average taken, and converted into a percentage of radioactivity bound in the absence of any unlabeled competitor chemokine. Data were analyzed using the LI-GAND software (29).

**Ca2+ Flux Assay**—HEK 293 cells stably expressing human receptors CR1 and CCR5 were derived by subcloning the cDNAs into pcDNA3 and transfection using Transfectam (Promega, Southampton, UK) according to the manufacturer’s methods. Stably transfected cells were selected in 0.8 mg/ml G418. To detect ligand-induced calcium ion fluxes, cells were loaded with Fura-2-AM, then, ~6 × 10⁶ cells were incubated at 37 °C in a continuously stirred cuvette in a Perkin-Elmer LS50 Spectrometer (340 nm (λex); 500 nm (λem)) and fluorescence emission recorded every 100 ms. After 2 min, ligand was added to a defined concentration and fluorescence recorded every 100 ms for a further 2 min. To control for day-to-day experimental variation, a full dose-response curve for LD78β was performed each time a different ligand was tested.

**HIV Entry Assays**—5 × 10⁶ CEMx174-CCR5 cells, pretreated with chemokine for 30 min, were incubated for 4 h with luciferase virus (5 ng of p24), pseudotyped by either JR.FL or ADA according to a previously described protocol (17). Medium was then changed and the luciferase activity measured 3 to 5 days post-infection. The extent of inhibition of HIV entry was determined by comparing luciferase activity of chemokine-treated cells, with untreated controls. For studies with the replication-competent SF162 virus, protocols were similar to those described elsewhere (30). Briefly, CEMx174-CCR5 cells, or PHA/IL-2-activated PBMC from HIV1 seronegative donors, were pretreated with chemokine then infected overnight at 37 °C with SF162 virus (m.o.i. ~0.04). The medium was then changed, and the p24 concentration subsequently determined after 5 days at 37 °C using enzyme-linked immunosorbent assay. Percentage inhibition of HIV entry was calculated relative to chemokine-untreated controls.

**Statistical Methods**—Analysis of the dose-response relationship between the MIP-1α isoforms and other chemokines in binding, calcium flux, and HIV suppressive assays were tested by "log likelihood" methodology essentially as described previously (31).

**RESULTS**

In our recent studies of MIP-1α receptors (23, 24) that revealed discrepancies between the binding of murine and human MIP-1α, we used the LD78α isoform of human MIP-1α with the amino acids ASLAADTPT at the amino terminus (Fig. 1). The close sequence similarity between LD78α and murine MIP-1α led us to examine the possible amino acid residues that may be responsible for the discriminatory binding of these ligands. The only consistent feature present in the mCCR5 and hD6 receptors but absent in human LD78α is a proline residue at position 2 of the mature protein (Fig. 1). The serine residue at position 2 of the LD78α we have used may therefore be preventing optimal receptor interaction. The alternative human MIP-1α isoform, LD78β, however, does have a proline residue in position 2, with two reciprocal serine/glycine swaps in the region between cysteines 3 and 4 being the only other differences between the LD78α and -β proteins (Fig. 1) (25, 26). To simplify the nomenclature, and emphasize the serine/proline residue difference at position 2 in the two isoforms, we henceforth refer to LD78α and -β as MIP-1αS and MIP-1αP, respectively.

**MIP-1αP Interacts with High Affinity with Both Murine and Human CCR5 and D6**—To test the importance of these isoform differences, we have now compared the ability of MIP-1αP and MIP-1αS to interact with known murine MIP-1α receptors stably expressed on CHO cells (Table I). In addition, we have tested the −4 presumed naturally secreted form of MIP-1αS

[3] A. N. Parker, G. J. Graham, A. R. Sim, S. C. Clark, and I. B. Pragnell, manuscript in preparation.
Characterization of a Potent MIP-1α Isoform

TABLE I
MIP-1αP shows high affinity binding to murine CCR5 and D6, but not CCR1

| Dissociation constants (in nM) for displacement of 125I-mMIP-1α from CHO cells expressing murine MIP-1α receptors, by different forms of human and murine MIP-1α. Dissociation constants were calculated using LIGAND software (29) from data derived from displacement experiments performed as described under “Experimental Procedures.” |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| MIP-1αS         | MIP-1αS-4        | MIP-1αP         | MIP-1αP-4        | mMIP-1α         |
| mCCR-1          | 13              | 4               | 2               | 2               |                 |
| mCCR-5          | 136             | 133             | 3.1             | 0.54            |                 |
| mD6             | >200            | >200            | 5.5             | 0.11            |                 |

(see Introduction) as well as the corresponding –4 variant of MIP-1αP to examine the impact of this truncation on receptor interactions. Our results demonstrate (Table I) that in contrast to MIP-1αS and MIP-1αS-4 (Kₐ values of 136 and 133 nM, respectively), MIP-1αP binds with high affinity to murine CCR5 (Kₐ of 1.1 nM). The importance, for this high affinity binding, of the proline residue at position 2 of MIP-1αP is indicated by the fact that MIP-1αP binds with high affinity to murine CCR5 (Kₐ of 1.1 nM). Murine MIP-1α, which carries a proline residue at position 2 of the mature protein, binds to murine CCR5 in a manner similar to that seen with MIP-1αP, suggesting that this human variant behaves more like the murine MIP-1α on CCR5 than does the more common MIP-1αS isoform.

Similarly, the presence of the proline residue at position 2 of MIP-1αP allowed it to bind with high affinity to murine D6 (Kₐ of 5.5 nM) in contrast to the other two isoforms tested (Kₐ > 200 nM). Again the comparison with murine MIP-1α (Kₐ of 5.5 nM) demonstrates a substantially higher binding affinity to that seen with murine MIP-1α indicating that the apparent equivalence of MIP-1αP and mMIP-1αP on murine CCR5 and D6 does not extend to muCCR1.

CHO cells expressing human receptors were also tested and showed similar results (Fig. 2 and Table II). Thus, with human D6, while the MIP-1αP isoform binds with high affinity (Kₐ of 5.5 nM), the –4 variants of the two isoforms bind only weakly (Kₐ of 77 nM for MIP-1αS-4 and Kₐ of 124 nM for MIP-1αP-4) and MIP-1αS does not exhibit an enhanced binding affinity over its –4 variant (Fig. 2A). Again, as seen with the murine receptors, the Kₐ of MIP-1αP for D6 is more similar to that of murine MIP-1α than any of the other isoforms tested (Table II). The differences between MIP-1αS and MIP-1αP are, however, somewhat less stark than those seen with the murine receptor. With human CCR5, there is an approximately 4–6-fold higher binding affinity of MIP-1αP (Kₐ of 5.2 nM) than seen for the –4 variant of the two isoforms (Kₐ of 25 and 36 nM), and the addition of the ASLA amino acids to MIP-1αS results in a further reduction in binding affinity (Fig. 2B). Curiously, mMIP-1α binds in a manner more similar to that seen with either of the –4 variants than that seen with MIP-1αP. The clear requirement for the proline residue in position 2 for enhanced binding of human MIP-1α to human CCR5 suggests that the simple presence of the analogous residue in murine MIP-1α is insufficient in the context of the other evolutionary changes in this protein, to mediate high affinity binding to human CCR5. Again, in a manner similar to that observed for murine CCR1, the proline at position 2 of MIP-1αP appears to be unnecessary for the interaction of this ligand with human CCR1, with the full-length versions of MIP-1αS and MIP-1αP, showing a consistently lower affinity interaction with human CCR1 than either of the –4 variants (Fig. 2C). Again, in common with the data obtained using murine CCR1, murine MIP-1α binds more like the human –4 variants than either of the two full-length human MIP-1α isoforms (Table II).

It is important to note that while all the above binding data was obtained from displacement studies utilizing radiolabeled murine MIP-1α and the relevant cold competitor, very similar dissociation constants have been obtained from direct binding studies using radiolabeled human MIP-1α isoforms.

**MIP-1α Isoform Binding Variations Are Reflected in Signaling Potency**—To test whether the observed isoform binding variations are also reflected in the dose response for signaling through human CCR5 and CCR1, ligand-induced mobilization of Ca²⁺ was studied. The transfected CHO cell lines used in the above binding studies are inefficient at fluxing Ca²⁺ and for this reason we have used stably transfected HEK cells in the

![Table I](image)

![Table II](image)

**Fig. 2.** The proline residue at position 2 of MIP-1αP enhances affinity for human CCR5 and D6, but not CCR1. Displacement of 125I-mMIP-1α by unlabeled human MIP-1α proteins, from CHO cells expressing: A, human D6; B, human CCR5; and C, human CCR1. Experiments were performed on 10⁶ cells incubated at room temperature in the presence of 0.4% azide for 90 min before washing with ice-cold phosphate-buffered saline. Cells were lysed in 1% SDS and the remaining 125I-mMIP-1α in the lysate counted. □, MIP-1αP; ◇, MIP-1αS-4; ○, MIP-1αS; △, MIP-1αS-4.
Ca\textsuperscript{2+} fluxing studies reported in this paper. Binding analyses using these cell lines indicates that the tested ligands for CCR1 and CCR5 bind with equivalent affinities to the receptors expressed in either CHO cells or HEK cells suggesting the likely equivalence of the receptors expressed on these different heterologous cell types. With full-length proteins on CCR5, half-maximal signaling potency is seen at approximately 10 nM for MIP-1\textalpha{}S and at approximately 500 pM for MIP-1\textalpha{}P (Fig. 3A). The dose-response curves obtained for these two full-length chemokines are highly significantly different (\(p < 0.0002\)) indicating, as was seen with the binding studies, that MIP-1\textalpha{}P is a markedly better ligand for CCR5 than MIP-1\textalpha{}S. The MIP-1\textalpha{}S-4 variant shows a slight, but significant (\(p < 0.001\)), increase in signaling potency through CCR5 compared with MIP-1\textalpha{}S, while removal of the terminal 4 amino acids from the MIP-1\textalpha{}P isoform significantly reduces its activity approximately 10-fold (\(p < 0.0002\)). Dose-response curves obtained using the two –4 variants were not significantly different and half-maximal activity was observed for both at concentrations of approximately 5 nM. The potency of signaling with MIP-1\textalpha{}P is significantly higher (approximately 10-fold; \(p < 0.001\)) than that seen with RANTES (half-maximal activity seen at 2.5 nM) and –100-fold higher than that seen with MIP-1\textbeta{} (\(p < 0.0002\)). MIP-1\textalpha{}P is therefore the most active natural CCR5 agonist described to date. We have detected Ca\textsuperscript{2+} fluxes with MCP2, which has recently been identified as a CCR5 ligand (32), but this ligand is not as potent as MIP-1\textalpha{}P (Fig. 3B).

In summary, these results show that the proline residue at position 2 of MIP-1\textalpha{}P is responsible for the enhanced binding of this isoform to murine and human D6 and CCR5, and for its strong activation of human CCR5. Conversely, interaction with CCR1 is lessened in the presence of APLA or ASLA at the NH\textsubscript{2} termini of SLAADTPT, LAADTPT, and AADTPT, respectively. In short, on human CCR1 and -5, and D6, the –2 and –3 forms behave like the –4 variants, while -1MIP-1\textalpha{}S acts like full-length MIP-1\textalpha{}S protein (data not shown). Truncation beyond the –4 position reduces CCR1 and -5 activation properties further (not shown).

**FIG. 3.** The proline residue at position 2 of MIP-1\textalpha{}P enhances agonist activity through CCR5, but not CCR1. A, CCR5–293 cells; B, CCR1–293 cells. Dose-response curves for ligand-induced calcium ion fluxes in HER293 cells expressing human MIP-1\textalpha{} receptors and loaded with Fura-2. Readings were taken every 100 ms for 2 min (340 nm (\(\lambda_{em}\)); 500 nm (\(\lambda_{ex}\))) and ligand added after ~40 s. The peak of ligand-induced fluorescence increase is presented as a percentage of the maximum achievable peak with each particular transfected cell line. □, MIP-1\textalpha{}P; ○, MIP-1\textalpha{}P-4; ●, MIP-1\textalpha{}S; ∆, MIP-1\textalpha{}S-4; ×, hRANTES; ◆, hMIP-1\textbeta{}.

We have so far been unable to demonstrate a signaling role for human D6 (24) and, despite initial encouraging data (23), have been unable to reproducibly confirm a signaling role for murine D6. Thus the functional consequences of the high affinity binding of MIP-1\textalpha{}P to either the murine or human orthologues of this receptor remain to be determined.
RANTES has been reported to be a much more potent antagonist of HIV entry through CCR5 than MIP-1α, MIP-1β, and MCP2 (17–19). Therefore, further experiments were performed using replication-competent virus entry into CEMx174-CCR5 cells, and peripheral blood mononuclear cells (shown in Fig. 4, C and D, respectively), to compare MIP-1αP to RANTES. These studies suggest that in addition to being by far the most effective human MIP-1α variant in suppressing HIV entry into target cells, MIP-1αP is also at least as potent as RANTES. In fact in repeated experiments, MIP-1αP was consistently better than RANTES as an HIV suppressive chemokine, however, these differences did not reach levels of statistical significance. Again, while MIP-1αP was consistently less active as an HIV suppressive chemokine than the NH2-terminal modified form of RANTES, AOP-RANTES (33), this difference did not reach levels of statistical significance (Fig. 4, C and D, and see “Discussion”). Note that RANTES and MIP-1αS-4 can enhance HIV entry into peripheral blood mononuclear cells at the lower concentrations tested, an effect not seen with MIP-1αP or AOP-RANTES (Fig. 4D). In contrast to the CCR5-dependent viruses, no inhibition of CXCR4-dependent viral entry was observed using any of the MIP-1α isoforms tested thus confirming the CCR5 dependent selectivity of the MIP-1αP inhibition (data not shown). These results present MIP-1αP as one of the most potent naturally occurring inhibitors of HIV1 entry through CCR5.

**DISCUSSION**

Studies from our own and other laboratories have demonstrated the curious inability of human MIP-1α to bind with high affinity to CCR5 and D6 to which the closely related (74% identity at the amino acid level) murine MIP-1α protein binds strongly (23, 24, 34–36). We now reveal that the basis for this is the absence of a proline residue at position 2 of the most commonly used isoform of human MIP-1α (MIP-1αS/LD78α). Our studies on the presence or non-allelic variant of human MIP-1α (MIP-1αP/LD78β) which has a proline at position 2, reveal this to be active as a potent CCR5 and D6 ligand. It therefore more closely resembles murine MIP-1α than do any of the other human MIP-1α isoforms, suggesting that MIP-1αP may be a closer functional homologue of murine MIP-1α than MIP-1αS. It appears, therefore, that despite the similar levels of identity between MIP-1αS and P and murine MIP-1α, MIP-1αS is a functionally evolving variant of MIP-1α. Our results suggest that MIP-1αS should be regarded as a structurally related but functionally distinct chemokine which has substantially lost its ability to interact with the CCR5 and D6 receptors but which has apparently enhanced binding to CCR1 due to the proposed truncation of MIP-1αS by 4 amino acids during release from the cell. Neither murine MIP-1α nor MIP-1αP are released from mammalian cells as –4 variants (20, 28, 37) and thus this putative alternative signal peptidase cleavage may be regarded as a component of the functional evolution of this human chemokine. The importance of the amino terminus to chemokine function is well documented (32, 38–41) and it is therefore an ideal position for evolutionary modulation of function.

A major consequence of the enhanced interactions between MIP-1αP and CCR5 is that this chemokine variant is now identified as being as potent as RANTES and AOP-RANTES in antagonizing CCR5-mediated cellular entry by HIV1. It is important, however, to point out that MIP-1αP while not reaching levels of significance, was a consistently more effective HIV suppressive agent than RANTES. This coupled with the markedly and significantly lower ability of RANTES compared with MIP-1αP to flux Ca2+ following CCR5 binding, may suggest that the inability to demonstrate statistical significance in HIV suppression is a limitation of the assay system used and this is currently being investigated in more detail in our laboratories. Intriguingly, and as shown in Fig. 4, A and B, while it is consistently difficult to achieve higher than 50% inhibition of entry by JRFL or ADA pseudotyped viruses using other MIP-1α variants, MIP-1αP can mediate near 100% inhibition with reasonable ease. Similar effects have been demonstrated for the synthetic NH2-terminal variant of RANTES, AOP-RANTES, with recent data suggesting this to be a consequence of alternative subcellular deposition of internalized receptors, and associated impairment of recycling of receptors (42). This may suggest that MIP-1αP, in contrast to MIP-1αS, alters receptor trafficking post-ligand binding and this is currently being investigated in our laboratory.

These results attesting to the importance of a proline residue at position 2 of MIP-1α are likely to have implications for our understanding of the interactions between other chemokines and their receptors. For example, it is likely that proline 2 in other β-chemokines is necessary for high affinity binding to D6. This, however, is not sufficient for D6 interaction, as SDF1 also contains a proline residue at position 2 yet shows no potential to bind to D6 (data not shown). Thus, proline 2 must be presented in the context of a β-chemokine to permit high affinity D6 interaction, while with CCR5 other domains are likely important in restricting the ligands for this receptor to MIP-1α, -1β, RANTES, and MCP2. Interestingly, the NH2 terminus Xaa-Pro in chemokines has been demonstrated to be a target of dipeptidyl peptidase IV (CD26) (41, 43, 44). We would predict that MIP-1αP, but not full-length nor –4 forms of MIP-1αS, would be cleavable by this protease with dramatic changes in its properties, specifically a near total loss of interaction with D6, a reduced signaling capacity (and inhibition of HIV entry) through CCR5, and enhanced CCR1 activity. In fact, studies we have performed on a –2 form of MIP-1αS (not shown) suggest that these properties will be seen with CD26-cleaved MIP-1αP.

The role of the proline residue in CCR5 activation is of interest in light of recent data indicating the crucial role of proline 2 in SDF1 activation of its receptor CXCR4, which acts as an entry cofactor for T-tropic HIV1 strains. Indeed, mutation of this residue to glycine generates a high affinity CXCR4 antagonist (45). It is intriguing that both major HIV entry co-receptors have a strong preference for a proline residue at position 2 during receptor activation. Moreover, as proposed above for MIP-1αP, SDF1 has been demonstrated to lose its anti-HIV and chemotactic activities upon removal of the first two amino acids by CD26 cleavage (43). Targeted reduction of CD26 activity in vivo may therefore enhance the activity of these HIV suppressive chemokines.

In addition to being of general interest to workers in the chemokine and HIV fields, our data also open up a number of potential therapeutic avenues of research. For example, earlier studies have demonstrated that the MIP-1αP copy number varies between individuals, and this gene can in fact be absent from some individuals (25, 26). β-Chemokine production has been reported to be associated with a number of inflammatory and autoimmune diseases (1, 2), and deletion of the MIP-1α gene in mice dramatically alters responses to several infectious agents (22). In addition, β-chemokine production is clearly of importance in regulating the pathogenesis of AIDS (6, 12–16). Thus, given the ease with which MIP-1αP is transcribed and translated (21, 26, 28) MIP-1αP gene dosage may alter an individuals’ response in these pathological situations. In par-

---

4 G. J. Graham, unpublished observations.

5 G. J. Graham and R. J. B. Nibbs, unpublished data.
ticular, in light of the potent HIV entry inhibition by MIP-1αP described here, it would be of interest to test whether gene copy number affects the rate of progression to AIDS in HIV-infected individuals.

The demonstration of the potent HIV suppressive action of MIP-1αP also indicates that it is possible that, as has been demonstrated with the chemokine RANTES (33, 39), amino-terminal variants of this protein, such as AOP-linked MIP-1αP, may exhibit enhanced HIV1 entry inhibition and/or receptor antagonism and have potential as HIV1 therapeutics.

Acknowledgments—We thank Prof. John Wyke and Dr. Paul Clapham for critical reading of the manuscript. R. J. B. N. thanks Dr. Amanda Wilson for manuscript review and support services.

REFERENCES

1. Baggiolini, M. (1998) Nature 392, 565–568
2. Rollins, B. J. (1997) Blood 90, 909–928
3. Murphy, P. M. (1996) Cytokine Growth Factor Rev. 7, 47–64
4. Clapham, P. R. (1997) Trends Cell Biol. 7, 264–268
5. Cairns, J. S., and D’Souza, M. P. (1998) Nature Med. 4, 563–568
6. Cecchi, F., DeVico, A. L., Garzino-Demo, A., Arya, S. K., Gallo, R. C., and Lusso, P. (1995) Science 270, 1811–1815
7. Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J-L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J-M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggiolini, M., and Moser, B. (1996) Nature 382, 833–835
8. Bleul, C. C., Farzan, M., Choe, H., Parolin, C., Clark-Lewis, I., Sodroski, J., and Springer, T. A. (1996) Nature 382, 829–833
9. Samson, M., Liberti, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C.-M., Saragosti, S., Lapoumeroulie, C., Cognaxx, J., Forceille, J., Maydlermans, G., Verhofstede, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R. J., Collum, R. G., Doms, R. W., Vassart, G., and Parmentier, M. (1996) Nature 382, 722–725
10. Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Smith, M. W., Allikmets, R., Paxton, W. A., Martin, S. R., Tse, D., O’Brien, T. R., Skurnick, J., and DiMarzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R., and Landau, N. R. (1996) Nature 381, 661–666
11. Nakao, M., Noyama, H., and Shimada, K. (1990) Mol. Cell. Biol. 10, 3646–3658
12. Paxton, W. A., Monard, S. P., Hoxie, J. A., Sianani, M. A., Thompson, D. A., Wu, L., Mackay, C. R., Horuk, R., and Moore, J. P. (1998) J. Virol. 72, 396–404
13. Furci, L., Scarlatti, G., Burastero, S., Tambussi, G., Colognessi, C., Quillent, A., Quaglino, D., Saggioro, D., Chieco-Bianchi, L., Proost, P., VanDamme, J., and Mantovani, A. (1995) AIDS Res. & Hum. Ret. 11, 155–160
14. Simonna, G., Clapham, P. R., Picard, L., Offord, R. R., Rosenkilde, M. M., Schwartz, T. W., Buser, R., Wells, T. C. N., and Proudfoot, A. E. I. (1997) Science 276, 257–259
15. Bonini, J. A., and Steiner, D. F. (1997) DNA Cell Biol. 16, 1023–1030
16. Golig, L., Gosling, J., Montecarlo, F. S., Luisa, A. J., Teao, C-L., and Charo, I. F. (1996) J. Biol. Chem. 271, 7551–7558
17. Meyer, A., Coyle, A. J., Proudfoot, A. E. I., Wells, T. C. N., and Power, C. A. (1996) J. Biol. Chem. 271, 14445–14451
18. Wolpe, S. D., Davatelis, G., Sherry, B., Beutler, B., Hesse, D. G., Nguyen, H. T., Lyle Middawer, L. J., Nathan, C. F., Lewry, S. F., and Cerami, A. (1988) J. Exp. Med. 167, 570–581
19. Weber, M., Ugucioni, M., Baggioioli, M., Clark-Lewis, I., and Dahinden, C. A. (1996) J. Exp. Med. 183, 681–689
20. Proost, A. E. I., Power, C. A., Hoogewerf, A. J., Montjivet, M. O., Borlat, F., Offord, R. E., and Wells, T. C. N. (1996) J. Biol. Chem. 271, 2599–2603
21. Oguchi, J., Ugucioni, M., Dwedal, B., Baggioioli, M., and Clark-Lewis, I. (1996) J. Biol. Chem. 271, 10521–10527
22. Oravec, T., Pall, M., Rodriguez, G., Gorrell, M. D., Ditto, M., Nguyen, N. Y., Boykins, R., Unsworth, E., and Norcross, M. A. (1997) J. Exp. Med. 186, 1855–1872
23. Mack, M., Luckow, B., Nelson, P. J., Chak, J., Simmons, G., Clapham, P. R., Signoret, N., Marsh, M., Stangasser, M., Borlat, F., Wells, T. N., Schlondorff, D., and Proudfoot, A. E. (1997) J. Exp. Med. 187, 1215–1224
24. Shioda, T., Kato, A., Ohnishi, Y., Tashiro, K., Ikegawa, M., Nakayama, E. E., Hu, H., Kato, A., Sakai, Y., Liu, H., Honjo, T., Nomoto, A., Iwamoto, A., Morimoto, C., and Nagai, Y. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 6321–6326
25. Proost, P., DeMeester, I., Schols, D., Struyf, S., Lambeir, A-M., Wuyts, A., Opdeacker, G., De Clercq, E., Scharpe, S., and Van Damme, J. (1998) J. Biol. Chem. 273, 7222–7227
26. Crump, M. P., Gong, J-H., Loetscher, P., Rajaratnam, K., Amara, A., Azzenza-Seisdedos, F., Virelizier, J-L., Baggioioli, M., Sykes, B. D., and Clark-Lewis, I. (1997) EMBO J. 16, 6996–7007