

Blocking Chemokine Receptors

Marco Baggiolini and Bernhard Moser

From the Theodor Kocher Institute, University of Bern, CH-3000 Bern 9, Switzerland

In our first review on chemokines (1), we suggested that blockade of the IL-8 receptor or inhibition of IL-8 gene expression could be a new principle for designing anti-inflammatory agents. The unexpected growth of the chemokine family and consequent redundancy of the system eventually made it clear that acting at the level of chemokine gene expression was rather hopeless, but the idea of influencing inflammation with chemokine receptor antagonists has remained valid and inhibitors were recently put to the test. A new literature, including several articles in this issue of *The Journal of Experimental Medicine*, indicate that the idea was worth trying.

How to Make a Chemokine Receptor Antagonist. By scanning mutagenesis (2) and selective deletion or substitution of NH₂-terminal residues (3, 4) it was shown that the Glu-Leu-Arg (ELR) motif preceding the first cysteine is essential for IL-8 activity. The ELR motif is conserved in all CXC chemokines that act on neutrophils (ELR chemokines) and is recognized by both IL-8 receptors, CXCR1 and CXCR2. It contributes to high-affinity binding and is the receptor triggering part of the molecule (5). The ELR motif alone, however, is not sufficient since linear and cyclic ELR-containing oligopeptides do not bind to the IL-8 receptors nor stimulate neutrophils (4). ELR chemokines have additional, selective binding sites, and it was not possible to confer activity on neutrophils to IP10 or monocyte chemoattractant protein (MCP)-1 by introducing the ELR motif at the NH₂ terminus (4). The three-dimensional nuclear magnetic resonance structure of IL-8 (6) shows that the conformationally disordered NH₂-terminal domain is anchored by the disulfide bonds to the well-ordered core of the protein. It is believed that interactions with domains of the core determine receptor selectivity and facilitate the access of the ELR motif to the receptor triggering site.

The first chemokine antagonist was obtained by truncation of IL-8. The analogue with an NH₂-terminal arginine followed by the first cysteine, (R)IL-8, is inactive on neutrophils, but still has considerable receptor affinity and efficiently competes for binding with IL-8 and other ELR chemokines inhibiting their biological activities. Other antagonists were obtained by substitution within the ELR motif, and one of the most potent is (AAR)IL-8 (7). Antagonists are also generated by truncation of other ELR chemokines, which have high affinity for CXCR2, but only low affinity for CXCR1. Although (R)IL-8 blocks equally well, CXCR1 and CXCR2, the corresponding analogues of GRO α and platelet factor (PF) 4, (R) growth regulated protein (GRO) α and (R)PF4, block only CXCR2

(8). These observations indicate that receptor selectivity of ELR chemokines is determined by binding domains beyond the NH₂-terminal region. It is interesting, however, that the arginine preceding the first cysteine is an absolute requirement for recognition by both IL-8 receptors and that the Arg-Cys¹ arrangement also occurs in the chemokines that act via CXCR3 (IP10 and Mig) and CXCR4 (stromal cell-derived factor [SDF]-1).

NH₂ terminally truncated CC chemokines are also potent antagonists. MCP-1 derivatives obtained by deletion of eight or nine NH₂-terminal residues, MCP-1(9-76) and MCP-1(10-76) block CCR2 and prevent the responses elicited by MCP-1, MCP-2, and MCP-3, but not by RANTES, macrophage inflammatory protein (MIP)-1 α , or MIP-1 β (9). By contrast the corresponding truncation analogues of RANTES, RANTES(9-68), and MCP-3, MCP-3(10-76), block more than one CC chemokine receptor and inhibit the responses induced by MCP-1, MCP-3, and RANTES (10). The loss of selectivity suggests that determinants within the NH₂-terminal domain are important for receptor recognition by these chemokines. In addition, antagonists were obtained by NH₂-terminal extension of MCP-3 with Arg-Glu-Phe (11) or RANTES with a methionine (12), and by chemical modification of the NH₂ terminus of RANTES (13).

A Lot of Impetus from HIV. The recent discovery that chemokine receptors, together with CD4, act as recognition sites for HIV infection and that some chemokines have HIV-suppressive activity (14) boosted the search for therapeutic strategies targeting chemokine receptors. A basic question had to be answered, however, before going to work. Is the inhibition of viral entry dependent on chemokine activity and/or chemokine receptor signaling, or are the chemokines simply competing for the virus binding site? It was shown that the entry of M-tropic strains is prevented by RANTES antagonists (13, 15). This was an important observation since the use of natural chemokines to block infection was considered potentially dangerous because of side effects due to leukocyte activation.

In this issue, Wu et al. (16) show that CCR5 can be blocked by mAbs. Although chemokines and viral gp120 compete for the same receptor, their binding sites are partly different. To map the sites, Wu et al. (16) generated a panel of mAbs and studied their effects on the interaction of M-tropic HIV-1 strains and chemokines with chimeric receptors consisting of different portions of CCR5 and CCR2b. An antibody, 2D7, recognizing the second extracellular loop of CCR5 blocks the binding and the biological activ-

ity of all three chemokine ligands, RANTES, MIP-1 α , and MIP-1 β , as well as infection by M-tropic and dual-tropic HIV-1 strains indicating that this domain is shared by the chemokines and gp120. By contrast, antibodies binding to the NH₂-terminal region of CCR5 block infection, but have no effect on chemokine activity. Wu et al. consider the second extracellular loop as the most promising target for blocking agents because this domain appears to be essential for chemokine as well as virus binding.

Handy Inhibitors Coming. Chemokines and chemokine mutants with antagonist properties are far from being ideal drugs. As proteins, they pose galenic problems and are unlikely to be orally active. This issue of *The Journal of Experimental Medicine* brings news from Europe, Japan, and the United States indicating that HIV-coreceptor interactions can be inhibited with chemokine-unrelated, low-molecular weight compounds. Three compounds are presented that were previously known for their inhibitory effects on HIV replication. They block the entry of T-, but not M-tropic strains by interacting with CXCR4. In cells expressing only CXCR4 and CD4, inhibition of dual-tropic strains is also observed.

Schols et al. (17) describe the effect of AMD3100, which belongs to a class of heterocyclic compounds called bicyclams. AMD3100 inhibits the entry of T-tropic viruses, competes for the binding of an mAb that is specific for CXCR4, and blocks SDF-1 dependent Ca²⁺ mobilization and chemotaxis in receptor-expressing cells. Together with the lack of effects on CCR5-, CCR1-, and CCR2b-dependent activities, these data demonstrate that AMD3100 is selective for CXCR4. AMD3100 appears to be effective in vivo and, as suggested by in vitro data, to be more potent as inhibitor of HIV entry than of SDF-1-mediated functions. This dissociation may be important because blockade of SDF-1 activity could be dangerous, as suggested by the defects observed in mice lacking the SDF-1 gene (18).

Murakami et al. (19) present an 18-residue peptide, T22, an amusing derivative of polyphemusin II, that specifically inhibits Env-dependent fusion and infection by T-tropic strains of cells transfected with CXCR4 and CD4, as well as PBMC. Since T22 also inhibits Ca²⁺ mobilization induced by SDF-1, the antiviral activity is likely to depend on competition for coreceptor binding by the virus. As for the bicyclam, the in vitro data suggest that significant antiviral activity is obtained at concentrations of T22 that only partially block the responses to SDF-1. More thorough studies, however, must be performed to clarify this point. Interestingly, Murakami et al. have compared T22 with an inactive analogue of similar size and physicochemical properties. This control strengthens the evidence for the selective mode of action of T22.

Doranz et al. (20) describe similar effects of a highly cationic oligopeptide containing nine arginines, ALX40-4C, that inhibits Env-dependent fusion and entry of T-tropic

HIV strains by interacting with CXCR4. ALX40-4C also prevents Ca²⁺ mobilization in response to SDF-1 and the binding of Hoxie's mAb, 12G5, which is known to recognize the first and second extracellular loop of CXCR4 and to inhibit virus entry. The interaction between ALX40-4C and the receptor loops is likely to depend on charge since the loops contain several anionic residues. Such an interaction cannot occur with CCR5, explaining why infection by M-tropic viruses is not affected by ALX40-4C. The authors point out that their antiviral compound also inhibits infection by type 1 herpes simplex suggesting that interactions with other receptor proteins may occur.

It is somewhat surprising that all three low molecular weight coreceptor inhibitors described in this issue interact with CXCR4 and not with other coreceptors. Since the compounds were all known for their antiviral properties, it is possible that the screening criteria adopted for their selection were biased in favor of inhibition of CXCR4-dependent viruses. On the other hand, inhibitors of CXCR4 may simply be easier to find. The present finding of three structurally different compounds with similar biological effects indicates that modeling of the interactions with the receptor could help to design compounds that bind to CCR5, or preferably to more than one coreceptor.

The Next Steps. The evidence for effective chemokine receptor blockade by small compounds, some of which have a good chance to be bioavailable after oral application, is a promising starting point. The current developments should not be restricted to antiviral therapy, since chemokine antagonists can be potentially useful as antiinflammatory, antiallergic, and immunoregulatory agents. A paper that appeared in the July 7th issue of *The Journal of Experimental Medicine* demonstrates that a selective antagonist of CCR2, the MCP receptor, has antiinflammatory properties in vivo. Gong et al. (21) show that repeated injections of a truncated analogue of MCP-1, MCP-1(9-76), prevents the chronic inflammatory arthritis that spontaneously develops in MRL-*lpr* mice. In contrast, controls that were treated with wild-type MCP-1 had more marked arthritis symptoms. Given the selectivity of the antagonist the results of this study highlight the validity of chemokine receptor blockade as antiinflammatory principle.

Although the present papers clearly show that chemokine receptor inhibitors are promising as potential drugs, considerable work still has to be done to gain information about specificities and the mode of action of the inhibitors. It is also important to define shared and selective recognition sites for chemokines and the V3 loop of gp120, and to determine their affinities and their binding kinetics. With this background it will be possible to optimize low molecular weight compounds as inhibitors of the binding of gp120 or chemokines, and to design antagonists that act on multiple receptors.

This work was supported by grant 31-039744.93 to M. Baggiolini and B. Moser, and by grant 438+-050291 to B. Moser from the Swiss National Science Foundation.

Received for publication 29 August 1997.

References

1. Baggiolini, M., A. Walz, and S.L. Kunkel. 1989. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. *J. Clin. Invest.* 84:1045-1049.
2. Hébert, C.A., R.V. Vitangcol, and J.B. Baker. 1991. Scanning mutagenesis of interleukin-8 identifies a cluster of residues required for receptor binding. *J. Biol. Chem.* 266: 18989-18994.
3. Clark-Lewis, I., C. Schumacher, M. Baggiolini, and B. Moser. 1991. Structure-activity relationships of interleukin-8 determined using chemically synthesized analogs. Critical role of NH₂-terminal residues and evidence for uncoupling of neutrophil chemotaxis, exocytosis, and receptor binding activities. *J. Biol. Chem.* 266:23128-23134.
4. Clark-Lewis, I., B. Dewald, T. Geiser, B. Moser, and M. Baggiolini. 1993. Platelet factor 4 binds to interleukin 8 receptors and activates neutrophils when its N terminus is modified with Glu-Leu-Arg. *Proc. Natl. Acad. Sci. USA.* 90: 3574-3577.
5. Baggiolini, M., B. Dewald, and B. Moser. 1994. Interleukin-8 and related chemotactic cytokines-CXC and CC chemokines. *Adv. Immunol.* 55:97-179.
6. Rajarathnam, K., I. Clark-Lewis, and B.D. Sykes. 1994. ¹H NMR studies of interleukin 8 analogs: characterization of the domains essential for function. *Biochemistry.* 33:6623-6630.
7. Moser, B., B. Dewald, L. Barella, C. Schumacher, M. Baggiolini, and I. Clark-Lewis. 1993. Interleukin-8 antagonists generated by N-terminal modification. *J. Biol. Chem.* 268: 7125-7128.
8. Jones, S.A., B. Dewald, I. Clark-Lewis, and M. Baggiolini. 1997. Chemokine antagonists that discriminate between interleukin-8 receptors-selective blockers of CXCR2. *J. Biol. Chem.* 272:16166-16169.
9. Gong, J.-H., and I. Clark-Lewis. 1995. Antagonists of monocyte chemoattractant protein 1 identified by modification of functionally critical NH₂-terminal residues. *J. Exp. Med.* 181: 631-640.
10. Gong, J.H., M. Uguccioni, B. Dewald, M. Baggiolini, and I. Clark-Lewis. 1996. RANTES and MCP-3 antagonists bind multiple chemokine receptors. *J. Biol. Chem.* 271:10521-10527.
11. Masure, S., L. Paemen, P. Proost, J. Van Damme, and G. Opdenakker. 1995. Expression of a human mutant monocyte chemotactic protein 3 in *Pichia pastoris* and characterization as an MCP-3 receptor antagonist. *J. Interferon Cytokine Res.* 15: 955-963.
12. Proudfoot, A.E.I., C.A. Power, A.J. Hoogewerf, M.O. Montjovent, F. Borlat, R.E. Offord, and T.N.C. Wells. 1996. Extension of recombinant human RANTES by the retention of the initiating methionine produces a potent antagonist. *J. Biol. Chem.* 271:2599-2603.
13. Simmons, G., P.R. Clapham, L. Picard, R.E. Offord, M.M. Rosenkilde, T.W. Schwartz, R. Buser, T.N.C. Wells, and A.E.I. Proudfoot. 1997. Potent inhibition of HIV-1 infectivity in macrophages and lymphocytes by a novel CCR5 antagonist. *Science (Wash. DC).* 276:276-279.
14. D'Souza, M.P., and V.A. Harden. 1996. Chemokines and HIV-1 second receptors-confluence of two fields generates optimism in AIDS research. *Nat. Med.* 2:1293-1300.
15. Arenzana-Seisdedos, F., J.L. Virelizier, D. Rousset, I. Clark-Lewis, P. Loetscher, B. Moser, and M. Baggiolini. 1996. HIV blocked by chemokine antagonist. *Nature (Lond.).* 383:400.
16. Wu, L., G. LaRosa, N. Kassam, D.J. Gordon, H. Heath, N. Ruffing, H. Chen, J. Humblis, M. Samson, M. Parmentier, J.P. Moore, and C.R. Mackay. 1997. Interaction of chemokine receptor CCR5 with its ligands: multiple domains for HIV-1 gp120 binding and a single domain for chemokine binding. *J. Exp. Med.* 186:1373-1381.
17. Schols, D., S. Struyf, J. Van Damme, J.A. Esté, G. Henson, and E. De Clercq. 1997. Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J. Exp. Med.* 186:1383-1388.
18. Nagasawa, T., S. Hirota, K. Tachibana, N. Takakura, S. Nishikawa, Y. Kitamura, N. Yoshida, H. Kikutani, and T. Kishimoto. 1996. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature (Lond.).* 382:635-638.
19. Murakami, T., T. Nakajima, Y. Koyanagi, K. Tachibana, N. Fujii, H. Tamamura, N. Yoshida, M. Waki, A. Matsumoto, O. Yoshie, T. Kishimoto, N. Yamamoto, and T. Nagasawa. 1997. A small molecule CXCR4 inhibitor that blocks T cell-line tropic HIV-1 infection. *J. Exp. Med.* 186:1389-1393.
20. Doranz, B.J., K. Grovit-Ferbasi, M.P. Sharron, S.H. Mao, M.B. Goetz, E.S. Daar, R.W. Doms, and W.A. O'Brien. 1997. A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor. *J. Exp. Med.* 186:1395-1400.
21. Gong, J.H., L.G. Ratkay, J.D. Waterfield, and I. Clark-Lewis. 1997. An antagonist of monocyte chemoattractant protein 1 (MCP-1) inhibits arthritis in the MRL-*lpr* mouse model. *J. Exp. Med.* 186:131-137.