Chemerin reveals its chimeric nature

Teizo Yoshimura and Joost J. Oppenheim

Chemerin is a proinflammatory plasma protein that binds to the GPCR ChemR23/CMKLR1 on macrophages and plasmacytoid dendritic cells, and promotes chemotaxis. An orphan GPCR, CCRL2, has now been identified as an additional receptor for chemerin, providing a unique mechanism by which chemerin enhances inflammation. Furthermore, because recent data shows that chemerin-derived peptides possess antiinflammatory properties, chemerin may be involved in both the initiation and resolution of inflammation.

Chemerin, like many other plasma proteins, is secreted as a 143-aa precursor protein (pro-chemerin [or TIG-2] [1, 2]) and is activated when a 6-aa peptide is cleaved at its C terminus by serine proteases of the coagulation, fibrinolytic, and inflammatory cascades (Fig. 1) (3). Active chemerin (137-aa protein) binds to the G protein–coupled receptor (GPCR) ChemR23(CMKLR1), which is expressed on macrophages and pDCs, and induces cell migration (1, 4). A nonapeptide (chemerin-9) corresponding to the C terminus of the processed form of human chemerin was identified as a potent agonist for ChemR23, indicating the importance of the protein's C terminus for receptor binding and chemotactic activity (5).

Proteolysis of precursor proteins generates a wide variety of ligands that play important roles during the inflammatory responses. The classical leukocyte chemoattractants C5a and C3a, for example, which are enzymatically released from circulating C5 and C3 during activation of the complement pathway, regulate the recruitment and activation of leukocytes (6–8). Protease activation of precursor proteins is not limited to plasma proteins. CXCL7 is a chemoattractant generated from precursor protein stored in the α-granules of platelets, which induces chemotaxis of neutrophils via the GPCR CXCR2 (9). Human cathelicidin (hCAP18) is also stored in secondary granules of neutrophils. Cleavage of hCAP18 liberates its C terminus, yielding an antimicrobial peptide called LL-37. In addition to its antimicrobial effects, LL-37 induces chemotaxis and Ca^{2+} flux in monocytes via the GPCR FPRL1 (10).

Although chemerin was first described as a chemoattractant, a recent study by Cash et al. shows that chemerin cleavage generates a potent antiinflammatory peptide that also signals through ChemR23 and suppresses inflammation in the picomolar range (11). On page 2207 of this issue, Zabel et al. identify a GPCR, CCRL2 (also known as HCR, CRAM, or CKRX) (12, 13), as an additional receptor for chemerin that functions unlike any other known chemokine receptor (14).

Chemerin's suppressive side

In a recent study, Cash et al. made the surprising observation that pretreatment of mouse peritoneal macrophages with chemerin inhibited the production of inflammatory mediators in response to LPS and IFN-γ (11). This inhibitory effect required processing of chemerin by cysteine proteases and was in marked contrast to the proinflammatory properties of active chemerin produced by serine protease cleavage (3). Because the biological activity of chemerin depends on C-terminal processing, the authors hypothesized that peptides released upon C-terminal processing might be responsible for the inhibition.

To test this hypothesis, Cash et al. synthesized several peptides from the C-terminal end of mouse chemerin and tested them for inhibitory effects. One peptide, chemerin 15, possessed potent antiinflammatory effects at surprisingly low picomolar concentrations. Intraperitoneal administration of chemerin 15 to mice before zymosan challenge suppressed the recruitment of neutrophils and monocytes with a concomitant reduction in the expression of proinflammatory mediators. Chemerin 15 also appeared to signal through ChemR23, as it had no inhibitory effect in ChemR23-deficient mice.

Administration of neutralizing antibody against chemerin to mice before zymosan challenge markedly enhanced intraperitoneal infiltration by inflammatory cells. Because zymosan normally activates resident macrophages, chemerin-derived inhibitory peptides are presumably generated at the site of inflammation and appear to play an important role in down-regulating inflammatory responses. Thus, depending on the class of protease that processes pro-chemerin or chemerin, ChemR23 binding peptides with either pro- or antiinflammatory effects are produced.

Because the chemerin-derived inhibitory peptide acts via the same receptor as the proinflammatory chemerin, these structurally related agonistic and inhibitory peptides may compete at the level of receptor binding. In fact, picomolar levels of the inhibitory peptides are active, whereas nanomolar concentrations are required for agonistic effects by chemerin. Other GPCR ligands demonstrate similar competition. For example, an N-terminal deletion

T.Y. and J.J.O. are at Laboratory of Molecular Immunoregulation, Cancer and Inflammation Program, Center for Cancer Research, National Cancer Institute at Frederick, Frederick, MD 21702

CORRESPONDENCE
T.Y.: yoshimut@mail.nih.gov

The Rockefeller University Press $30.00
J. Exp. Med. Vol. 205 No. 10 2187-2190
www.jem.org/cgi/doi/10.1084/jem.20081736

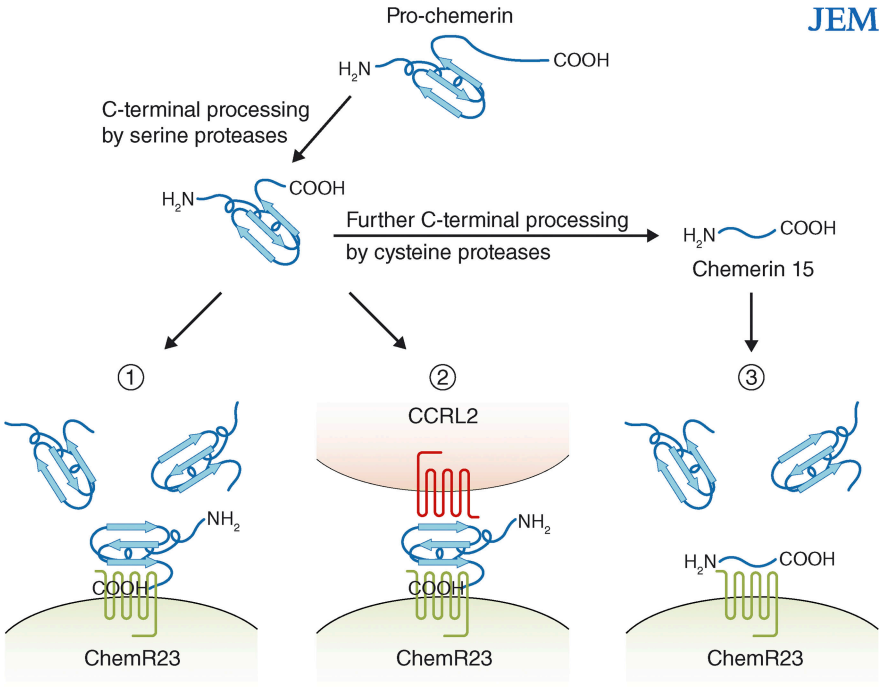

Figure 1. The regulation of the inflammatory responses by active, proinflammatory chemerin and inhibitory chemerin-derived peptides. Mature, active chemerin is generated from pro-chemerin via C-terminal processing by serine proteases. (1) Active chemerin directly activates cells by binding to ChemR23/CMKLR1, resulting in cell migration and calcium flux. (2) Active chemerin also binds to CCRL2 via its N-terminal domain and presents the C-terminal domain to ChemR23 expressed on neighboring cells. (3) Processing of chemerin by cystein proteases generates the inhibitory peptide chemerin 15, which binds to ChemR23 and inhibits the generation of proinflammatory mediators in response to LPS/IFN-γ.

variant of the chemokine MCP-1 (called 7ND) inhibits MCP-1–mediated monocyte chemotaxis (15). Extension of the human chemokine RANTES by a single residue at the N-terminus (Met-RANTES) creates a potent and selective RANTES antagonist (16). Met-RANTES also inhibits the effects of MIP-1α, a chemokine that shares its receptors with RANTES, raising the possibility that chemerin 15 may also inhibit the effect of other ChemR23 ligands, such as the antiinflammatory lipid resolvin E1. Like chemerin 15, resolvin E1 suppresses zymosan-induced peritonitis and sulfonic acid–induced colitis in mice (17, 18). It remains a mystery how the different Chem23R ligands can induce opposing effects through the same receptor. Future studies may identify other receptors that participate and are trans-activated by this GPCR.

Chemerin finds a new partner

Chemerin's complex functions have been amplified by two more reports. The first identified the orphan GPCR GPR1, which is closely related to ChemR23, as a second chemerin receptor (19). In this issue, Zabel et al. (14) unexpectedly identify a third chemerin-binding GPCR, CCRL2. The authors show that mouse CCRL2 (mCCRL2, the presumptive orthologue of human CCRL2 [20]) is constitutively expressed on mast cells. To examine whether CCRL2 plays a role in the inflammatory response, they used mice lacking the receptor. The absence of mCCRL2 did not affect basic mast cell functions in vitro or T cell–mediated contact hypersensitivity in vivo. The authors then examined the IgE-dependent passive cutaneous anaphylaxis (PCA) reaction, a mast cell–dependent model of atopic allergy. Although both wild-type and mCCRL2-deficient mice developed marked local inflammation when sensitized with a high dose of DNP-specific IgE (150 ng/ear) and challenged with antigen intravenously, the mCCRL2-deficient mice had significantly reduced PCA reactions when a lower sensitizing dose was used (50 ng/ear), suggesting that mCCRL2 ligation normally amplifies the inflammatory response. The amplified response was caused by mCCRL2 expression on mast cells, as mast cell–deficient mice engrafted with mCCRL2-deficient bone marrow progenitor cells had less ear swelling than did those engrafted with WT cells.

In an attempt to identify the ligand of mCCRL2, the authors screened known chemokines, but none of them stimulated chemotaxis of mCCRL2-transfected cells. To the authors' surprise, however, chemerin blocked the binding of anti-mCCRL2 antibody to mouse peritoneal mast cells. Despite binding to mCCRL2 with high affinity, chemerin elicited no functional response from mCCRL2-expressing cells. Binding failed to trigger intracellular calcium mobilization, chemotaxis, or mCCRL2 internalization. Instead, incubating mCCRL2-transfected cells with chemerin resulted in a time-dependent increase in surface-bound chemerin. These chemerin-loaded cells then triggered calcium flux in responder cells expressing ChemR23. Thus, CCRL2 seems to concentrate bioactive chemerin and facilitate its presentation to ChemR23 on adjacent cells.

A wide variety of soluble proinflammatory mediators are produced and released at inflammatory sites, and mechanisms have been developed to retain or concentrate those mediators by preventing their diffusion. Chemokines, for example, bind to glycosaminoglycans, resulting in the formation of leukocyte-attracting chemokine gradients in tissues (21). Bacterial LPS binds to LPS-binding protein, which enhances the subsequent binding of LPS to MD-2 in the TLR4–MD-2 receptor complex, thus initiating the TLR4 intracellular signaling cascade (22). Some ligands, such as TNF, are present in both soluble and cell membrane–bound form. In its membrane-bound form, the receptor binding domain of TNF is exposed, which may explain why DC activation by neutrophil-derived TNF requires cell–cell contact (23). Because neutrophils generally produce cytokines at much

lower levels than other cell types, such as macrophages, the use of cell membrane–bound ligands may allow neutrophils to activate receptors on other cells more efficiently. Like TNF, chemerin appears to bind to CCRL2 using its N-terminal region, which exposes its critical cell signaling C terminus. Thus, CCRL2 may not only concentrate chemerin in a tissue microenvironment but may also effectively present it to nearby cells expressing the ChemR23 receptor. It is therefore likely that cell-bound chemerin is a more stimulatory ligand than its soluble form.

A GPCR unlike all others

The paper by Zabel et al. identifies mCCRL2 as having a unique GPCR activity (14). The human CCRL2 gene resides on chromosome 2, in close proximity to the genes encoding the chemokine receptor CCR2 and CCR5 genes. The sequence similarity between CCRL2 and CCR2/5 strongly suggests that CCRL2 belongs to the family of signal transducing chemokine receptors. Previous studies have reported that CCRL2 is activated by MCP-1, -2, -3, RANTES, and joint fluid from rheumatoid arthritis patients (24–26). However, it remains unclear whether human CCRL2 is, in fact, a functional chemokine receptor. Human CCRL2 lacks the conserved DRYLAIV motif in the second intracellular loop that is required for functional chemokine receptors to signal. Other related GPCRs, such as DARC (Duffy antigen), D6, and CCX-CKR (chemokine receptor; Chemocentrix), also lack this motif. Interestingly, these GPCRs are bound by chemokines, but do not transduce signals for cell migration or intracellular calcium mobilization, likely because they lack the DRYLAIV motif. Instead, these nonfunctional ("silent" or "decoy") receptors act as sponges to absorb, internalize, and clear chemokines, helping to dampen inflammation. Indeed, mice deficient in DARC or D6 displayed exacerbated inflammatory responses (27, 28). mCCRL2 is similar to the silent GPCRs in its lack of the DRYLAIV motif, but dissimilar in its failure to internalize ligands upon binding, putting it (for now) in a GPCR class of its own.

There are now three known types of functionally distinct receptors in the chemokine GPCR family. The first are functional, signal-transducing chemokine receptors. The second are so called decoy receptors that bind to and clear chemokines from the environment, but do not transduce signals or activate cells. Finally, the newly identified type of receptor reported by Zabel et al. neither internalizes its ligands nor transduces signals. Instead, it plays a proinflammatory role by presenting bound ligands to functional signaling receptors expressed on neighboring cells.

The recent papers in JEM provide us with two novel insights. First, as reported by Cash et al., enzymatic proteolysis of precursor proteins, such as pro-chemerin, can result in the generation of both activating and inhibitory peptides. These opposing molecules with opposing activities can be generated by different classes of proteases, such as serine or cysteine proteases. Whereas serine proteases capable of producing activating peptides are released from neutrophils (29), cysteine proteases that generate inhibitory peptides are released from activated elicited macrophages (11). As neutrophils are typically the first cells to arrive at sites of inflammation, it is likely that the generation of proinflammatory peptides precedes the generation of antiinflammatory peptides, which may then help control the severity of inflammatory responses. Second, the findings of Zabel et al. reveal the existence of a new class of silent chemokine receptor–like GPCRs, which binds its ligand(s) and presents it to signaling receptors expressed on neighboring cells. Thus, soluble chemerin is a truly multifunctional protein with both stimulatory and inhibitory signaling capabilities, whereas cell-bound chemerin sends stimulatory signals by bridging cells that express the silent receptor with those expressing the ChemR23 receptor.

REFERENCES

1. Wittamer, V., J.D. Franssen, M. Vulcano, J.F. Mirjolet, E. Le Poul, I. Migeotte, S. Brézillon, R. Tyldesley, C. Blanpain, M. Detheux, et al. 2003. Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. *J. Exp. Med.* 198:977–985.
2. Meder, W., M. Wendland, A. Busmann, C. Kutzleb, N. Spodsberg, H. John, R. Richter, D. Schleuder, M. Meyer, and W.G. Forssmann. 2003. Characterization of human circulating TIG2 as a ligand for the orphan receptor ChemR23. *FEBS Lett.* 555:495–499.
3. Zabel, B.A., S.J. Allen, P. Kulig, J.A. Allen, J. Cichy, T.M. Handel, and E.C. Butcher. 2005. Chemerin activation by serine proteases of the coagulation, fibrinolytic, and inflammatory cascades. *J. Biol. Chem.* 280:34661–34666.
4. Zabel, B.A., A.M. Silverio, and E.C. Butcher. 2005. Chemokine-like receptor 1 expression and chemerin-directed chemotaxis distinguish plasmacytoid from myeloid dendritic cells in human blood. *J. Immunol.* 174:244–251.
5. Wittamer, V., F. Grégoire, P. Robberecht, G. Vassart, D. Communi, and M. Parmentier. 2004. The C-terminal nonapeptide of mature chemerin activates the chemerin receptor with low nanomolar potency. *J. Biol. Chem.* 279:9956–9962.
6. Goldstein, I.M. 1988. Complement: biologically active products. *In* Basic Principles and Clinical Correlates. J.I. Gallin and R. Snyderman, editors. Raven Press, New York. 55–74.
7. Gerard, N.P., and C. Gerard. 1991. The chemotactic receptor for human C5a anaphylatoxin. *Nature.* 349:614–617.
8. Ames, R.S., Y. Li, H.M. Sarau, P. Nuthulaganti, J.J. Foley, C. Ellis, Z. Zeng, K. Su, A.J. Jurewicz, R.P. Hertzberg, et al. 1996. Molecular cloning and characterization of the human anaphylatoxin C3a receptor. *J. Biol. Chem.* 271:20231–20234.
9. Walz, A., B. Dewald, V. von Tscharner, and M. Baggiolini. 1989. Effects of the neutrophil-activating peptide NAP-2, platelet basic protein, connective tissue-activating peptide III and platelet factor 4 on human neutrophils. *J. Exp. Med.* 170:1745–1750.
10. Yang, D., O. Chertov, and J.J. Oppenheim. 2001. Participation of mammalian defensins and cathelicidins in anti-microbial immunity: receptors and activities of human defensins and cathelicidin (LL-37). *J. Leukoc. Biol.* 69:691–697.
11. Cash, J.L., R. Hart, A. Russ, J.P. Dixon, W.H. Colledge, J. Doran, A.G. Hendrick, M.B. Carlton, and D.R. Greaves. 2008. Synthetic chemerin-derived peptides suppress inflammation through ChemR23. *J. Exp. Med.* 205:767–775.
12. Fan, P., H. Kyaw, K. Su, Z. Zeng, M. Augustus, K.C. Carter, and Y. Li. 1998. Cloning and characterization of a novel human chemokine receptor. *Biochem. Biophys. Res. Commun.* 243:264–268.
13. Migeotte, I., J.-D. Franssen, S. Goriely, F. Willems, and M. Parmentier. 2002. Distribution and regulation of expression of the putative human chemokine receptor HCR in leukocyte populations. *Eur. J. Immunol.* 32:494–501.
14. Zabel, B.A., S. Nakae, L. Zuniga, J.-Y. Kim, T. Ohyama, C. Alt, J. Pan, H. Suto,

D. Soler, S.J. Allen, et al. 2008. Mast cell-expressed orphan receptor CCRL2 binds chemerin and is required for optimal induction of IgE0mediated passive cutaneous anaphylaxis. *J. Exp. Med.* 205:2207–2220.
15. Zhang, Y., and B.J. Rollins. 1995. A dominant negative inhibitor indicates that monocyte chemoattractant protein 1 functions as a dimer. *Mol. Cell. Biol.* 15:4851–4855.
16. Proudfoot, A.E., C.A. Power, A.J. Hoogewerf, M.O. Montjovent, F. Borlat, R.E. Offord, and T.N. Wells. 1996. Extension of recombinant human RANTES by the retention of the initiating methionine produces a potent antagonist. *J. Biol. Chem.* 271:2599–2603.
17. Arita, M., F. Bianchini, J. Aliberti, A. Sher, N. Chiang, S. Hong, R. Yang, N.A. Petasis, and C.N. Serhan. 2005. Stereochemical assignment, antiinflammatory properties, and receptor for the omega-3 lipid mediator resolvin E1. *J. Exp. Med.* 201:713–722.
18. Arita, M., M. Yoshida, S. Hong, E. Tjonahen, J.N. Glickman, N.A. Petasis, R.S. Blumberg, and C.N. Serhan. 2005. Resolvin E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. *Proc. Natl. Acad. Sci. USA.* 102:7671–7676.
19. Barnea, G., W. Strapps, G. Herrada, Y. Berman, J. Ong, B. Kloss, R. Axel, and K.J. Lee. 2008. The genetic design of signaling cascades to record receptor activation. *Proc. Natl. Acad. Sci. USA.* 105:64–69.
20. Shimada, T., M. Matsumoto, Y. Tatsumi, A. Kanamaru, and S. Akira. 1998. A novel lipopolysaccharide inducible C-C chemokine receptor related gene in murine macrophages. *FEBS Lett.* 425:490–494.
21. Johnson, Z., A.E. Proudfoot, and T.M. Handel. 2005. Interaction of chemokines and glycosaminoglycans: a new twist in the regulation of chemokine function with opportunities for therapeutic intervention. *Cytokine Growth Factor Rev.* 16:625–636.
22. Kim, J.I., C.J. Lee, M.S. Jin, C.H. Lee, S.G. Paik, H. Lee, and J.O. Lee. 2005. Crystal structure of CD14 and its implications for lipopolysaccharide signaling. *J. Biol. Chem.* 280:11347–11351.
23. Bennouna, S., and E.Y. Denkers. 2005. Microbial antigen triggers rapid mobilization of TNF-α to the surface of mouse neutrophils transforming them into inducers of high level dendritic cell TNF-α production. *J. Immunol.* 174:4845–4851.
24. Zuurman, M.W., J. Heeroma, N. Brouwer, H.W. Boddeke, and K. Biber. 2003. LPS-induced expression of a novel chemokine receptor (L-CCR) in mouse glial cells in vitro and in vivo. *Glia.* 41:327–336.
25. Biber, K., M.W. Zuurman, H. Homan, and H.W. Boddeke. 2003. Expression of L-CCR in HEK 293 cells reveals functional responses to CCL2, CCL5, CCL7, and CCL8. *J. Leukoc. Biol.* 74:243–251.
26. Galligan, C., W. Matsuyama, A. Matsukawa, H. Mizuta, D.R. Hodge, O.M.Z. Howard, and T. Yoshimura. 2004. Up-regulated expression and activation of the orphan chemokine receptor, CCRL2, in rheumatoid arthritis. *Arthritis Rheum.* 50:1806–1814.
27. Dawson, T.C., A.B. Lentsch, Z. Wang, J.E. Cowhig, A. Rot, N. Maeda, and S.C. Peiper. 2000. Exaggerated response to endotoxin in mice lacking the Duffy antigen/receptor for chemokines (DARC). *Blood.* 96:1681–1684.
28. Jamieson, T., D.N. Cook, R.J. Nibbs, A. Rot, C. Nixon, P. McLean, A. Alcami, S.A. Lira, M. Wiekowski, and G.J. Graham. 2005. The chemokine receptor D6 limits the inflammatory response in vivo. *Nat. Immunol.* 6:403–411.
29. Wittamer, V., B. Bondue, A. Guillabert, G. Vassart, M. Parmentier, and D. Communi. 2005. Wittamer V, Bondue B, Guillabert A, Vassart G, Parmentier M, Communi D. *J. Immunol.* 175:487–493.