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Pillars of Immunology

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Where the Confusion Began: Cloning the First Chemokine Receptors

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The articles highlighted in this issue of *The Journal of Immunology* as *Pillars of Immunology* describe the independent cloning of two distinct receptors for the chemokine IL-8, now known as CXCL8 (1, 2). These articles established the foundation for what has become a substantial scientific edifice housing both fundamental knowledge about leukocyte biology and practical insights from drug discovery efforts to develop agents that block the actions of this receptor class. To call these two articles "pillars" seems entirely appropriate.

These articles also introduced us to the incredible complexity that has come to characterize the chemokine system. That story began many years earlier with the recognition that low molecular weight materials released at sites of inflammation could stimulate leukocyte accumulation. Support for this idea came from the ability of bacterially derived peptides such as *N*-formyl-methionyl-leucyl-phenylalanine, designated fMLP (3), or complement fragments such as C5a (4) to attract leukocytes in vitro or in vivo. Another potent, locally produced leukocyte chemoattractant was the cytokine IL-1 (5, 6), which was released from cells in response to LPS and was presumed to be the mediator of the ability of LPS to elicit leukocyte infiltration in vivo, because that bacterial product itself did not have inherent chemoattractant activity in vitro (7).

The biological problem posed by these chemotactic factors was their lack of specificity: they attracted both neutrophils and monocytes with similar potency. How then could one explain the monomorphic infiltrates routinely associated with certain diseases? The answer would be leukocyte-specific chemoattractants, and the first one was discovered as a contaminant in IL-1 preparations. IL-1 had been isolated from the supernatants of LPS-treated monocytes, and this partially purified material attracted neutrophils in vitro and in vivo. After purification to homogeneity, IL-1 lost its chemotactic properties (8). However, the non-IL-1-containing components of the starting material retained neutrophil chemoattractant activity, and purification of that activity yielded a factor now known as IL-8 or CXCL8 in the systemic nomenclature (9–11). Its cardinal attribute was an ability to attract neutrophils but not monocytes.

The amino acid sequence of IL-8 showed that it was a member of a family of closely related proteins now known as chemokines. Some 45 nonallelic chemokine genes have been identified, and the proteins they encode are, to a large extent, involved in leukocyte recruitment. A reasonable understanding of the physiology of chemokine-mediated leukocyte attraction was gained quickly after the first proteins were purified, but a molecular understanding of this process lagged. Specific high affinity receptors for IL-8 could be demonstrated on neutrophils and myeloid cell lines (12, 13). Curiously, however, IL-8 binding could be efficiently competed in certain settings by other chemokines, including CXCL1, CXCL2, CXCL3, and CXCL5 (also known as Gro-α, -β, -γ, and ENA-78, respectively). The receptors for leukocyte attractants such as fMLP and C5a had been cloned and were known to be members of the seven transmembrane-spanning, G protein-coupled family of cell surface receptors (7-TMS GPCRs)[2] (15, 16). Neutrophil activation by IL-8 had been shown to be inhibited by pertussis toxin, suggesting that its receptor would also be G coupled (17). However, single transmembrane-spanning, tyrosine kinase growth factor receptors, such as those for platelet-derived growth factor, can mediate chemotactic responses by cells that express them, so no assumptions could safely be made about the structure of chemokine receptors.

Some of the molecular questions about chemokine activity were answered by the publication of these two "pillars." Both were technical tours de force but used very different approaches to clone receptors for IL-8. The group from Genentech (Holmes et al.) (1) used what had already become a classic expression cloning technique. They transfected pools of clones from a neutrophil cDNA library into COS cells, identified pools that imparted IL-8 binding ability, and isolated a unique clone by sib selection. Expressing this clone in COS cells generated IL-8 binding activity with a K_d of 3.6 nM and no binding activity for fMLP (binding to other chemokines was not reported in this article). The expressed receptor also produced a calcium flux in 293 cells in response to IL-8 but not fMLP.

DNA sequencing placed the predicted protein squarely in the 7-TMS GPCR family with some notable similarities to other chemoattractant receptors. In particular, the predicted protein was 79% identical to what had been cloned earlier that year as a nominal rabbit fMLP receptor (18). However, the sequence of the rabbit clone significantly diverged from that of the human fMLP receptor (only 28% identical), and so the National Institutes of Health (NIH) group (Murphy and Tiffany) (2) hypothesized that humans would have a homolog of this rabbit receptor that would be distinct from the human fMLP receptor. They screened a cDNA library from a myelomonocytic leukemia cell line using an oligonucleotide probe derived from the

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[2] Abbreviations used in this paper: 7-TMS-GPCR, seven transmembrane-spanning G protein-coupled family of cell surface receptors; ELR, glutamate-leucine-arginine.

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sequence of the so-called rabbit fMLP receptor and isolated a single clone. However, its pattern of tissue-specific expression seemed much more consistent with that of an IL-8 receptor rather than an fMLP receptor. Expression in frog oocytes generated IL-8 binding activity and a calcium flux in response to IL-8. The receptor neither bound nor transduced a signal in response to fMLP, confirming its specificity. In fact, the rabbit cDNA was later shown to encode an IL-8 receptor (19).

Despite these molecular triumphs, a few years of confusion ensued. The receptors cloned by the Genentech and NIH groups were quite similar, sharing 77% amino acid identity, but were clearly distinct. The Genentech group had measured a K_d of 2 nM for IL-8 binding to the receptor they had cloned. The NIH group had attempted to derive a K_d for IL-8 binding to their protein in the oocyte expression system, but they were unable to saturate binding sites and so inferred that theirs was a low affinity receptor. Articles from that era refer to the Genentech group's molecule as the high affinity IL-8 receptor and to the NIH group's molecule as the low affinity IL-8 receptor, but this distinction turned out to be a technical artifact. When both receptors were expressed in mammalian cells, they were found to have identically high affinities for IL-8 (2 nM) and so were named IL-8RA and IL-8RB (20). In the systematic nomenclature, they are CXCR1 and CXCR2.

But one surprising finding remained. In an attempt to understand how some chemokines could compete with IL-8 for binding to neutrophils, the two receptors were ectopically expressed in mammalian cells and tested for ligand binding specificity (20). Although their affinities for IL-8 and CXCL6 (GCP-2) were again identical, IL-8RA (CXCR1) bound CXCL1, -2, -3, -5, and -7 with very low affinities (≈450 nM), whereas IL-8RB (CXCR2) bound them with high affinities (≈2 nM). Thus, IL-8RA (CXCR1) is a highly specific receptor for IL-8 and CXCL6, whereas IL-8RB (CXCR2) is a high affinity receptor for many chemokines of the CXC family that have a glutamate-leucine-arginine (ELR) motif in their N-terminal domains. Both receptors are expressed by neutrophils.

The field is still trying to figure out what this means. Mouse modeling has limited relevance because mice have only one receptor for these so-called ELR-CXC chemokines, a homolog of CXCR2, and no clear-cut functional homolog of IL-8. Chemical/biological approaches to dissecting the human system are made much more complex by the presence of two high affinity receptors for IL-8, necessitating highly specific antagonists to tease apart the functions of these receptors. RNA interference approaches may help target each receptor individually, but in vivo modeling will still present a challenge.

Because of cross-competition by ELR-CXC chemokines for neutrophil binding, the field was not terribly surprised by the observation that a single chemokine receptor could bind several ligands with comparably high affinities. Less expected was the discovery that two distinct chemokine receptors could have overlapping ligand specificities. That this was an attribute of the very first chemokine receptors to be cloned was a signal that chemokine physiology would be incredibly complex. The reasons for and the consequences of this profound and rather widespread redundancy in ligand/receptor pairing are still not understood. But these two papers are the "pillars" upon which nearly two decades of important work in this area of immunology rests.

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Disclosures

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References

1. Holmes, W. E., J. Lee, W. J. Kuang, G. C. Rice, and W. I. Wood. 1991. Structure and functional expression of a human interleukin-8 receptor. *Science* 253: 1278–1280.
2. Murphy, P. M., and H. L. Tiffany. 1991. Cloning of complementary DNA encoding a functional human interleukin-8 receptor. *Science* 253: 1280–1283.
3. Schiffmann, E., B. A. Corcoran, and S. M. Wahl. 1975. *N*-formylmethionyl peptides as chemoattractants for leukocytes. *Proc. Natl. Acad. Sci. USA* 72: 1059–1062.
4. Fernandez, H. N., P. M. Henson, A. Otani, and T. E. Hugli. 1978. Chemotactic response to human C3a and C5a anaphylatoxins. I. Evaluation of C3a and C5a leukotaxis in vitro and under stimulated in vivo conditions. *J. Immunol.* 120: 109–115.
5. Luger, T. A., J. A. Charon, M. Colot, M. Micksche, and J. J. Oppenheim. 1983. Chemotactic properties of partially purified human epidermal cell-derived thymocyte-activating factor (ETAF) for polymorphonuclear and mononuclear cells. *J. Immunol.* 131: 816–820.
6. Sauder, D. N., N. L. Mounessa, S. I. Katz, C. A. Dinarello, and J. I. Gallin. 1984. Chemotactic cytokines: the role of leukocytic pyrogen and epidermal cell thymocyte-activating factor in neutrophil chemotaxis. *J. Immunol.* 132: 828–832.
7. Colditz, I. G., and H. Z. Movat. 1984. Kinetics of neutrophil accumulation in acute inflammatory lesions induced by chemotaxins and chemotaxinigens. *J. Immunol.* 133: 2169–2173.
8. Yoshimura, T., K. Matsushima, J. J. Oppenheim, and E. J. Leonard. 1987. Neutrophil chemotactic factor produced by lipopolysaccharide (LPS)-stimulated human blood mononuclear leukocytes: partial characterization and separation from interleukin 1 (IL 1). *J. Immunol.* 139: 788–793.
9. Schroder, J.-M., U. Mrowietz, E. Morita, and E. Christophers. 1987. Purification and partial biochemical characterization of a human monocyte-driven, neutrophil-activating peptide that lacks interleukin 1 activity. *J. Immunol.* 139: 3474–3483.
10. Walz, A., P. Peveri, H. Aschauer, and M. Baggiolini. 1987. Purification and amino acid sequencing of NAF, a novel neutrophil activating factor produced by monocytes. *Biochem. Biophys. Res. Commun.* 149: 755–761.
11. Yoshimura, T., K. Matsushima, S. Tanaka, E. A. Robinson, E. Appella, J. J. Oppenheim, and E. J. Leonard. 1987. Purification of a human monocyte-derived neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *Proc. Natl. Acad. Sci. USA* 84: 9233–9237.
12. Leonard, E. J., A. Skeel, T. Yoshimura, K. Noer, S. Kutvirt, and D. Van Epps. 1990. Leukocyte specificity and binding of human neutrophil chemotactic factor that has peptide sequence similarity to other host defense cytokines. *J. Immunol.* 144: 1323–1330.
13. Samanta, A. K., J. J. Oppenheim, and K. Matsushima. 1989. Identification and characterization of specific receptors for monocyte-derived neutrophil chemotactic factor (MDNCF) on human neutrophils. *J. Exp. Med.* 169: 1185–1189.
14. Moser, B., C. Schumacher, V. von Tscharner, I. Clark-Lewis, and M. Baggiolini. 1991. Neutrophil-activating peptide 2 and *gro*/melanoma growth stimulatory activity interact with neutrophil-activating peptide 1/interleukin 8 receptors on human neutrophils. *J Biol. Chem.* 266: 10666–10671.
15. Boulay, F., M. Tardif, L. Brouchon, and P. Vignais. 1990. Synthesis and use of a novel *N*-formyl peptide derivative to isolate a human *N*-formyl peptide receptor cDNA. *Biochem. Biophys. Res. Commun.* 68: 1103–1109.
16. Gerard, N. P., and C. Gerard. 1991. The chemotactic receptor for human C5a anaphylatoxin. *Nature* 349: 614–617.
17. Thelen, M., P. Peveri, P. Kernen, V. von Tscharner, A. Walz, and M. Baggiolini. 1988. Mechanism of neutrophil activation by NAF, a novel monocyte-derived peptide agonist. *FASEB J.* 2: 2702–2706.
18. Thomas, K. M., H. Y. Pyun, and J. Navarro. 1990. Molecular cloning of the fMet-Leu-Phe receptor from neutrophils. *J. Biol. Chem.* 265: 20061–20064.
19. Thomas, K. M., L. Taylor, and J. Navarro. 1991. The interleukin-8 receptor is encoded by a neutrophil-specific cDNA clone, F3R. *J. Biol. Chem.* 266: 14839–14841.
20. Lee, J., R. Horuk, G. C. Rice, G. L. Bennett, T. Camerato, and W. I. Wood. 1992. Characterization of two high affinity human interleukin-8 receptors. *J. Biol. Chem.* 267: 16283–16287.