HHV8-encoded vMIP-I Selectively Engages Chemokine Receptor CCR8

AGONIST AND ANTAGONIST PROFILES OF VIRAL CHEMOKINES

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Daniel J. Dairaghi, Rong A. Fan, Brian E. McMaster, Michael R. Hanley, and Thomas J. Schall‡

From the Divisions of Discovery Biology and Molecular Pharmacology, ChemoCentryx, San Carlos, California 94070

Uncertainty regarding viral chemokine function is mirrored by an incomplete knowledge of host chemokine receptor usage by the virally encoded proteins. One such molecule is vMIP-I, a C-C type chemokine of undefined function and binding specificity, encoded by the Kaposi's sarcoma herpesvirus HHV-8. We report here that vMIP-I binds to and induces cytosolic [Ca\[^{2+}\] signals in human T cells selectively through CCR8, a CC chemokine receptor associated with Th2 lymphocytes. Furthermore, using a panel of 65 different human, viral, and rodent chemokines, we have established a comprehensive ligand binding “fingerprint” for CCR8. The receptor exhibits marked “high” affinity (K\(_d\) < 15 nM) only for four chemokines, three of them of viral origin: vMIP-I, vMIP-II, vMCC-I, and human I-309. A previously unreported second class of lower affinity ligands includes MCP-3 and possibly two other viral chemokines. vMIP-I and I-309 appear to act as CCR8 agonists: binding to and inducing cytosolic [Ca\[^{2+}\]] elevation through the receptor. By contrast, vMIP-II and vMCC-I act as potent antagonists: binding without inducing signaling, and blocking the effects of I-309 and vMIP-I. These results suggest a ligand hierarchy for CCR8, identifying vMIP-I as a selective viral chemokine agonist. CCR8 may thus engage a specific subset of chemokines with the potential to regulate each other during viral infection and immune regulation.

It has recently been appreciated that in addition to roles in regulating leukocyte trafficking, the chemokine system is intimately linked to the biology of infectious disease (1–3). This has prompted intensive investigation of viral chemokine analogues that have been identified in herpesvirus and poxvirus genomes. Human herpesviruses including CMV and HHV-8 (Kaposi’s sarcoma herpesvirus; Refs. 4 and 5), as well the poxvirus Molluscum contagiosum (MCV), encode versions of chemokines, chemokine receptors, and chemokine-binding proteins (6, 7). For example, HHV8 encodes three predicted CC-type chemokines (vMIP-I, vMIP-II, and vMIP-III) and one predicted chemokine receptor (ORF 74) (5, 8, 9). Similarly, human CMV has been long known to encode a chemokine receptor, a product of the US28 ORF (10, 11). More recently clinical isolates of CMV have been shown to encode two CXC chemokines, designated vCXC-1 and vCXC-2, which are products of the UL146 and UL147 ORFs (12, 13).

The selective advantage which these virally encoded chemokine elements confer to their pathogens is yet unclear. Although the multiple biological activities of viral chemokine elements are likely to be directly related to pathogenesis, including enhanced dissemination of viral particles (9, 14), the molecular mechanisms underlying the actions of most of the viral chemokines are largely unknown. In two cases, viral chemokines have been shown to interact with a spectrum of host chemokine receptors, such as evidenced by the actions of HHV8-encoded vMIP-II (15) and MCV-encoded vMCC-I (a product of MCV ORF148R; Refs. 7 and 16). These chemokines have been reported to have antagonist activities, but at least in the case of vMIP-II, this may be altered with N-terminal sequence variants (9, 15).

Apart from the promiscuous binding patterns of vMIP-II and vMCC-I, the receptor interactions of other virally derived chemokines have not been fully characterized. As such they remain “orphan” chemokine ligands, analogous to putative chemokine receptor-like molecules for which no ligands have yet been identified. One such molecule is the HHV8-encoded vMIP-I protein (5, 8), which was originally identified from genomic sequencing and subsequently reported to have some angiogenic and anti-HIV infectivity activities (5, 9, 17).

In this manuscript, we have reported our investigation of the biochemical properties of vMIP-I. Specifically, we have examined the activities of vMIP-I on primary human T cells and compared these actions to those from a spectrum of other human and human virus-encoded chemokines. We have also introduced comprehensive receptor profiling, using an array of over five dozen recombinant chemokines to define comprehensively the molecular recognition properties of CCR8. In so doing, we have identified that CCR8, which is known to bind human I-309 and is associated with human Th2 cells (18–22), is also the molecular target of vMIP-I. Unlike vMIP-II, which is promiscuous in its binding profile, vMIP-I selectively engages CCR8. Moreover, whereas vMIP-II and vMCC-II are CCR8 antagonists, vMIP-I, like I-309, a CCR8 agonist. Last, our comprehensive binding profile shows that other chemokines previously not known to bind to CCR8, such as MCP-3 and possibly two other viral chemokines, may engage the receptor at moderate to low affinities. Thus, an expanded ligand binding fingerprint for human CCR8 has been defined, and most chemokines that engage this

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‡ To whom correspondence should be addressed: ChemoCentryx, 1539 Industrial Rd., San Carlos, CA 94070. Tel.: 650-632-2900; Fax: 650-632-2910; E-mail: tschall@chemocentryx.com.

1 The abbreviations used are: MCV, poxvirus Molluscum contagiosum; PBMC, peripheral blood mononuclear cell; FBS, fetal bovine serum; IL-2, interleukin 2; CMV, cytomegalovirus; ORF, open reading frame; HHV8, human herpesvirus 8.

2 For convenience, we denote the viral CC chemokine gene product encoded by the Molluscum contagiosum virus ORF MC148R as vMCC-I (viral, Molluscum CC chemokine-I). No standard nomenclature yet exists for the human chemokine superfamily, let alone chemokines encoded by viruses, but this designation follows the convention for designating chemokines simply as either “CXC” or “CC,” followed by a numerical designator of order of entry into publicly available gene sequence data banks. (A systematic nomenclature is under consideration and will be published by a chemokine nomenclature committee elsewhere.)
receptor are of viral origin. These results suggest that human Th2 function may be a prime target of viruses through the actions of virally encoded chemokines. In particular, Th2 cells may be controlled through CCR8 by the actions vMIP-I, and these effects may be cross-regulated by other viral chemokines.

**Experimental Procedures**

**Cells and Cell Culture**—Human peripheral blood mononuclear cells (PBMC) were obtained from buffy coats of healthy blood donors (Stanford blood bank) by density gradient on LSM (lymphocyte separation media) as described in the protocol of the manufacturer (ICN). Isolated PBMC were resuspended in RPMI 1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin. PBMC were cultured (37°C, 16 h) to allow monocytes to adhere. Nonadherent lymphocytes were transferred in suspension (initial density 10^6/ml), and subsequently cultured for 12–15 days (RPMI 1640 medium containing 10% FBS, 1 ng/ml human recombinant IL-2 (R&D Systems), fed weekly). In all cases, the phenotype of the cultured cells was >90% CD3-positive (T cells of CD20-negative (B cells), and CD45RA-negative (immature T cells). PBMC were resuspended in RPMI 1640 medium supplemented with 5% FBS, 1% penicillin-streptomycin. PBMC were cultured (37°C, 16 h) to allow monocytes to adhere. Nonadherent lymphocytes were transferred in suspension (initial density 10^6/ml), and subsequently cultured for 12–15 days (RPMI 1640 medium containing 10% FBS, 1 ng/ml human recombinant IL-2 (R&D Systems), fed weekly). In all cases, the phenotype of the cultured cells was >90% CD3-positive (T cells of CD20-negative (B cells), and CD45RA-negative (immature T cells).

**Systems**—were cultured in Iscove’s modified Dulbecco’s medium (supplemented with 5% FBS, 1% penicillin-streptomycin). Other T cell population markers, CD4, CD8, CD56, and HLA-DR, demonstrated donor variability. Human CCR8 NSO transfected cells (R&D Systems) were cultured in Iscove’s modified Dulbecco’s medium (supplemented with 5% FBS, 1% penicillin-streptomycin).

**Reagents—**Human, viral, and murine recombinant chemokines were obtained from R&D Systems. I-labeled I-309 was obtained from NEN Life Science Products. The monoclonal antibodies used in flow cytometry were from R&D Systems (MAB155 (CCR1); MAB150 (CCR2); MAB330 (CCR1); MAB331 (CCR2); MAB160 (CCR3); MAB173 (CCR4)), and from the National Institutes of Health AIDS Research and Reference Reagent Program (7B11 (CCR3), and 2D7 (CCR5)).

**Flow Cytometry**—The flow cytometric analyses were performed using standard protocols. Briefly, cells were washed in phosphate-buffered saline containing 1% bovine serum albumin, resuspended at 2 x 10^6 cells per well in 96-well V-bottom plates (Costar) and incubated with the appropriate monoclonal antibody. They were washed three times and stained with the secondary antibody. The labeled cells were analyzed on a FACScan (Becton Dickinson), and results presented have been gated for viable cells using light scattering.

**Global Displacement Profile of I-309 from CCR8 Using 65**

**Chemokine vMIP-I Interaction with Chemokine Receptor CCR8**

**RESULTS**

**Chemokine Receptor Expression and [Ca^{2+}]~i~ Signaling of IL2-cultured Primary Human T Cells—**To assess whether primary human immune cells were capable of being acted upon by virally encoded chemokines, we established individual cultures of IL2-treated lymphocytes, comprising primarily T cells (>90% by anti-CD3 staining using flow cytometry). The cell surface expression of functional chemokine receptors was assessed in these cell populations, using chemokine receptor antibodies where available and by establishing intracellular [Ca^{2+}]~i~ signaling profiles in response to a panel of chemokines of characterized binding and signaling specificities.

Direct measurement of the cell surface expression of chemokine receptors (as detected by specific antibodies and fluorescence-activated cell sorter) in cell cultures from multiple donors exhibited a consistent pattern. There was high expression of CCR5, CXCR3, and CXCR4, with mixed expression of CCR2; other chemokine receptors were consistently negative (Fig. 1A). Because the available panel of anti-chemokine receptor antibodies is sparse, however, we further characterized the presence and function of all known chemokine receptors on these cells by measuring intracellular [Ca^{2+}]~i~ fluxes after chemokine challenge (Fig. 1B). These signaling patterns are completely consistent with the expression pattern seen by direct antibody detection, e.g. [Ca^{2+}]~i~ signals are seen in response to MCP-1, -2, -3, and -4 (via CCR2) and CCR5, CXCR3, and CXCR4, with their respective ligands. In addition, the presence of CCR4, CCR6, CCR7, and CCR8 were inferred from the signaling profile (Fig. 1B).

We challenged the cells with viral chemokines, two of which, vMIP-II and vMCC-I, have been reported to bind to multiple chemokine receptors. Despite their reported binding properties, neither induced an intracellular [Ca^{2+}]~i~ response. Indeed, notably the only viral chemokine stimulating a response was the HHV8-encoded vMIP-I. This result indicated that vMIP-I had agonist activity for signaling; likely through one or more of the chemokine receptors expressed on T cells.

**Cytoplasmic Ca^{2+} Signaling in Response to vMIP-I and Human I-309 Are Specifically Cross-desensitized—**Cross-desensitization of functional responses by chemokines has been routinely used to assess action at a shared receptor (10). Thus, vMIP-I responses were systematically evaluated for cross-desensitization of [Ca^{2+}]~i~ signals induced by the chemokines which were previously shown to stimulate the T cell responses represented in Fig. 1B. With a single exception, vMIP-I-induced [Ca^{2+}]~i~ signaling failed to be desensitized by any of the chemokine-induced [Ca^{2+}]~i~ responses, and vice versa. The one exception was with the CCR8-selective chemokine, I-309, where near total cross-desensitization of calcium responses was observed (Fig. 2). Control responses to SDF-1α (Fig. 2) and other chemokines (not shown) confirmed that the cross-desensitization was at the level of the CCR8 receptor only and not because of interactions with other chemokine receptors or post-receptor depletion of calcium stores.

**Global Displacement Profile of I-309 from CCR8 Using 65 Different Chemokines—**To rapidly and thoroughly define the ligand binding fingerprint of a given chemokine receptor, we have established an approach to comprehensively “interrogate” chemokine receptors using a large array of purified chemokines. We used this approach independently to confirm the interaction of vMIP-I with CCR8. Employing radioligand binding of 125l-labeled I-309 to intact CCR8 stable transfectants, chemokine specificity for CCR8 was exhaustively determined. All known chemokines which can be obtained in purified form were used as cold competitors (initially at a saturating final concentration of 200 nM), against 125l-labeled I-309 in binding experiments. The displacement data, expressed in Fig. 3, showed intriguingly that the only potent chemokine interactions for human CCR8 other than human I-309 were the viral chemokines vMIP-I, vMIP-II, and vMCC-I. Moreover, the power of this screening approach was highlighted by the emergence of a potential second class of lower affinity chemokine
competitors, including MCP-3 and possibly vMIP-III and vCXC-I (Fig. 3).

Determination of Binding Constants—The binding interactions identified in the primary screening were examined quantitatively by radioligand binding competition to CCR8 stable transfectants and Scatchard transformation of the displacement data (Fig. 4A, and inset). The results confirmed the high affinity binding (of greater affinity than apparent $K_i \sim 15 \text{ nM}$) of I-309 and the viral chemokines in the rank order I-309, vMIP-I, vMIP-II, vMCC-I. The results were very similar in human lymphocytes (Fig. 4B), with IC50 values that were closely aligned between the two cell populations (Fig. 4C). The class of potential moderate to low affinity ligands exhibited affinities roughly between $K_i; 50$ to $250 \text{ nM}$. These included chemokines not previously reported to bind to CCR8: MCP-3, $K_i; 80 \text{ nM}$; and two viral chemokines, vCXC1 and vMIP-III, each of $K_i; 250 \text{ nM}$ (data not shown). The physiologic significance of these lower affinity interactions has not been investigated.

vMIP-I- and I-309-mediated CCR8 Signaling Is Competitively Antagonized by vMIP-II and vMCC-I—The HHV8-encoded chemokine vMIP-II has been previously reported as having either agonist or antagonist activities on specific chemokine receptors (9, 15, 25), whereas vMCC-I has been previously reported as a broadly acting chemokine receptor antagonist (16). To assess the interactions of the high affinity CCR8 ligands I-309, vMIP-I, vMIP-II, and vMCC-I, we performed a series of signaling cross-desensitization experiments using various combinations of these chemokines. While vMIP-I and I-309 consistently showed agonist activity, triggering $[\text{Ca}^{2+}] \text{ response}$ in T cells, neither vMIP-II nor vMCC-I induced a $[\text{Ca}^{2+}] \text{ response}$. In fact, challenging the T cells first with 100 nM of either vMIP-II and vMCC-I revealed that these viral chemokines exhibited direct CCR8 antagonist activity, such that the subsequent responses to both I-309 and vMIP-I were blocked (Fig. 5A). Thorough dose responses were obtained, defining to what extent this inhibition could be overcome by

![Chemokine receptor and signaling profiling of IL-2-cultured T lymphocytes.](image1)

**Fig. 1.** Chemokine receptor and signaling profiling of IL-2-cultured T lymphocytes. A, cell surface immunostaining of IL-2-treated lymphocytes as analyzed by flow cytometry using monoclonal anti-receptor antibodies against CCR1, CCR2, CCR3, CCR5, CXCR1, CXCR2, CXCR3, and CXCR4. The y axis indicates relative cell number (5000 events collected), and the x axis is relative fluorescent intensity. The results are representative of results from at least three independent experiments using different human buffy coat preparations. B, intracellular $[\text{Ca}^{2+}]$ signaling profiles induced by chemokine ligands. Cells were tested for intracellular calcium mobilization using chemokines at a standard test concentration $\sim 100 \text{ nM}$. The calibration bars indicate the relative fluorescence ratio (vertical bar) and time (horizontal bar). The results are representative of results from at least three independent experiments using different human buffy coat preparations. Agonists acting on a common chemokine receptor are clustered to emphasize that, in all cases, the predicted receptor selectivity of chemokines is consistent with the flow cytometry analysis. In addition, the presence of receptors for which detection antibodies are not available (e.g., CCR4, CCR6, CCR7, and CCR8) can be inferred by the $[\text{Ca}^{2+}]$ response to the cognate ligands for these receptors.

![I-309 and vMIP-I signaling cross-desensitization.](image2)

**Fig. 2.** I-309 and vMIP-I signaling cross-desensitization. IL-2-treated lymphocytes were stimulated sequentially with $[100 \text{ nM}]$, I-309, vMIP-I, and SDF-1α (lower trace), or vMIP-I, I-309, and SDF-1α (upper trace) as indicated, revealing specific cross-desensitization between I-309 and vMIP-I. The calibration bars indicate the relative fluorescence ratio (vertical bar) and time (horizontal bar). The results are representative of results from at least three independent experiments using different human buffy coat preparations.
increased concentrations of I-309 or vMIP-I (Fig. 5B). The profiles strongly suggest competitive antagonism by vMCC-I and vMIP-II of the actions of I-309 and vMIP-I.

Discussion

Details regarding the molecular recognition of host chemokine receptors by the HHV8-encoded chemokine vMIP-I have not previously been elucidated. This study investigated the ability of vMIP-I and other viral chemokines to induce intracellular Ca²⁺ signaling in human T cells, and sought to define the ligand binding specificity of vMIP-I among endogenous chemokine receptors. Through comprehensive chemokine signaling and binding “profiling,” we have shown on both human T cells and chemokine receptor transfectants that vMIP-I selectively engages the Th2-associated chemokine receptor CCR8 with high affinity. The interaction of vMIP-I with CCR8 appears very selective in that signaling-desensitization experiments demonstrated cross-desensitization only with I-309.

Thus, like I-309 (18–20), vMIP-I appears to be a signal-inducing CCR8 agonist, while the only other high affinity ligands, the viral chemokines vMIP-II and vMCC-I (15, 16), act as nonsignaling antagonists of CCR8.

Data presented here support concepts in the literature regarding the action of I-309 and vMCC-I on CCR8, but do not substantiate the notion that CCR8 also engages MIP-1b and TARC as functional ligands (26). It should be noted that the assignment of those ligands was based not on direct biochem-
ical binding and signaling analyses of CCR8 and its putative ligands but rather on a gain-of-function assay in Jurkat cells. In that study, Jurkat cells appeared to become chemotactically responsive to MIP-1β and TARC after CCR8 transfection (26). The data presented here suggest the possibility that the gain of migratory function may have been connected to, or perhaps coincident with, transfection or CCR8 introduction but are not directly a consequence of CCR8 binding functions. It is also notable that despite a report that binds multiple chemokine receptors (16), we observe significant selectivity of vMCC-I for CCR8. In addition to excluding some ligands as potentially acting directly through CCR8, the definition of a comprehensive ligand binding fingerprint for this receptor suggests that it may interact also with MCP3 and possibly two additional viral chemokines, vMIP-III and vCXC-I. These interactions are apt to occur at much more modest affinity constants than those of the high affinity CCR8 ligands vMIP-I, I-309, vMIP-II, and vMCC-I. Additional studies are necessary to determine whether this class of lower affinity interactions is of physiological relevance.

The downstream consequences of activation of CCR8 by vMIP-I are not yet established, but to date we have found little or no chemotactic activity of either I-309 or vMIP-I in IL2-treated T cells (data not shown). This is despite the fact that these ligands induce robust cytoplasmic [Ca²⁺] responses in functional T cells from multiple donors and despite the fact that the same cells respond robustly to SDF-1 in both [Ca²⁺] signals and in migration. One intriguing possibility is suggested from the action of I-309 on murine T cell lymphomas, where the human chemokine seems to protect the cells from dexamethasone-induced apoptosis (27). We are currently testing whether vMIP-I may regulate the survival of T lymphocytes through CCR8 as inhibition of apoptosis may be a highly desirable function for a herpesvirus such as HHV8, which typically achieves long-lasting latency in infected cells.

It is also important to note that the ability of I-309 or vMIP-I to act as chemoattractants in vivo may be very different. For example, in T cells CCR8 expression is biased toward selective, if not exclusive, expression in anti-inflammatory Th2 subpopulation (21, 22). Indeed, accumulation of Th2 T cells has been reported in Kaposi’s sarcoma lesions (25) although it has been interpreted to proceed through an action of vMIP-II, rather than vMIP-I, on the CCR8 receptor. Consequently, the in vivo effects of vMIP-I, or other viral chemokines, may not be predicted completely from receptor interactions only. Nevertheless, the chemokine binding fingerprint of CCR8 elucidated here raises intriguing questions regarding the seeming predilection on the part of viral chemokines for targeting this receptor. It is interesting to speculate that some special advantage is conferred to human viruses via manipulation of CCR8, possibly through skewing of Th1/Th2 responses in a virally infected host.

In short, comprehensive chemokine profiling provides a novel tool in defining the recognition specificity and functional cross-talk of ligands for chemokine receptors such as CCR8. The definition of vMIP-I as a functional agonist for CCR8, and one which is potentially cross-regulated by other viral chemokines, may
provide new insight into the function of viral chemokines and of Th2 cells during infection and immune regulation.

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**REFERENCES**

1. Weiss, R. (1996) *Nat. Med.* 2, 277–278
2. Bacon, K. B., Greaves, K. B., Dairaghi, D. J., and Schall, T. J. (1998) in The Cytokine Handbook (Thompson, A., ed), 3rd Ed., pp. 753–775, Academic Press, London
3. Baggiolini, M. (1998) *Nature* 392, 565–568
4. Dittmer, D., and Kedes, D. (1998) *BioEssays* 20, 367–370
5. Moore, P., Boshoff, C., Weiss, R., and Chang, Y. (1996) *Science* 274, 1739–1744
6. McFadden, G., Lalani, A., Everett, H., Nash, P., and Xu, X. (1998) *Semin. Cell Dev. Biol.* 9, 359–368
7. Senkevich, T. G., Bugert, J., J., Sisler, J., R., Koonin, E. V., Darai, G., and Moss, B. (1996) *Science* 273, 813–816
8. Nicholas, J., Ruvolo, V., Zong, J., Ciudo, D., Guo, H., Reitz, M., and Hayward, G. (1997) *J. Virol.* 71, 1894–1900
9. Dairaghi, D. J., Oldham, E., Bacon, K., and Schall, T. J. (1997) *J. Biol. Chem.* 272, 28206–28209
10. Sozzani, S., Luini, W., Bianchi, P., Allavena, P., Wells, T., Napolitano, M., Bernardini, G., Veechi, A., D’Ambrosio, D., Mazzeo, D., Sinigaglia, F., Santoni, A., Magi, E., Romagnani, S., and Mantovani, A. (1998) *Blood* 92, 4036–4039
11. Bernadini, G., Hedrick, J., Sozzani, S., Luini, W., Spineti, G., Weiss, M., Menon, S., Zlotnick, A., Montovani, A., Santoni, A., and Napolitano, M. (1998) *Eur. J. Immunol.* 28, 582–588
12. Cha, T., Tom, E., Kemble, G. W., Duke, G. M., Mocarski, E. S., and Spaete, R. R. (1996) *J. Virol.* 70, 78–83
13. Penfold, M. E. T., Dairaghi, D. J., Duke, G. M., Saederup N, Mocarski, E. S., Kemble, G. W., and Schall, T. J. (1999) *Proc. Natl. Acad. Sci. U. S. A.*, in press
14. Saederup, N., Dairaghi, D. J., Schall, T. J., and Mocarski, E. S. (1999) *Proc. Natl. Acad. Sci. U. S. A.*, in press
15. Kledal, T., Rosenkilde, M., Celma, F., Simmons, G., Johnsen, A., Alouani, S., Power, C., Luttichau, H., Gerstoft, J., Clapham, P., Clark-Lewis, I., Wells, T., and Schwartz, T. (1997) *Science* 277, 1656–1659
16. Damon, I., Murphy, P., and Moss, B. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 6403–6407
17. Nicholas, J., Ruvolo, V., Zong, J., Ciudo, D., Guo, H., Reitz, M., and Hayward, G. (1997) *J. Virol.* 71, 1663–1974
18. Roos, R., Leetscher, M., Legler, D., Clark-Lewis, I., Baggiolini, M., and Moser, B. (1997) *J. Biol. Chem.* 272, 17251–17254
19. Goya, I., Gutierrez, J., Varona, R., Kremer, L., Zaballos, A., and Marquez, G. (1998) *J. Immunol.* 160, 1975–1981
20. Tiffany, H., Laurent, L., Gao, J., Pease, J., Locati, M., Combadiere, C., Modi, W., Bonner, T., and Murphy, P. M. (1997) *J. Exp. Med.* 186, 165–170
21. D’Ambrosio, D., Iellem, A., Bencechi, R., Mazzeo, D., Sozzani, S., Montovani, A., and Sinigaglia, F. (1998) *J. Immunol.* 161, 5111–5115
22. Zingoni, A., Soto, H., Hedrick, A., Stoppacciaro, A., Storlazzi, C., Sinigaglia, F., D’Ambrosio, D., O’Garra, A., Robinson, D., Rocchi, M., Santon, A., Zlotnick, A., and Napolitano, M. (1998) *J. Immunol.* 161, 547–551
23. Bacon, K., Schall, T. J., and Dairaghi, D. J. (1998) *J. Immunol.* 160, 1894–1900
24. Dairaghi, D. J., Oldham, E., Bacon, K., and Schall, T. J. (1997) *J. Biol. Chem.* 272, 28206–28209
25. Sozzani, S., Luini, W., Bianchi, G., Allavena, P., Wells, T., Napolitano, M., Bernardini, G., Veechi, A., D’Ambrosio, D., Mazzeo, D., Sinigaglia, F., Santoni, A., Magi, E., Romagnani, S., and Mantovani, A. (1998) *Blood* 92, 4036–4039
26. Bernadini, G., Hedrick, J., Sozzani, S., Luini, W., Spineti, G., Weiss, M., Menon, S., Zlotnick, A., Montovani, A., Santoni, A., and Napolitano, M. (1998) *Eur. J. Immunol.* 28, 582–588
27. Van Snick, J., Houssiau, F., Proost, P., Van Damme, J., and Renaud, J. (1996) *J. Immunol.* 157, 2570–2576