THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 269, No. 17, Issue of April 29, pp. 12403–12406, 1994
© 1994 by The American Society for Biochemistry and Molecular Biology, Inc.
Printed in U.S.A.

Communication

Interleukin-13 Induces Expression and Release of Interleukin-1 Decoy Receptor in Human Polymorphonuclear Cells*

(Received for publication, February 1, 1994, and in revised form, March 3, 1994)

Francesco Colotta‡§, Fabio Re‡¶, Marta Muzio‡, Nadia Polentarutti‡, Adrian Minty‖, Daniel Caput‖, Pascual Ferrara‖, and Alberto Mantovani‡

From the ‡Istituto di Ricerche Farmacologiche "Mario Negri," Centro Daniela e Catullo Borgomainerio, via Eritrea 62, 20157 Milano, Italy and ‖Sanofi-Elf Biorecherche, 31676 Labege, France

The aim of this study was to examine whether interleukin-13 (IL-13), a cytokine with anti-inflammatory activities, affected expression of interleukin-1 (IL-1) receptors (R) in human polymorphonuclear cells (PMN). Treatment with IL-13 augmented both type I and type II (decoy) R transcripts, with the latter being by far the most represented. The transcriptional inhibitor actinomycin D blocked the induction of IL-1 R mRNAs by IL-13. Nuclear run-off experiments demonstrated an augmented transcriptional rate of IL-1 decoy R in IL-13-treated B lymphoblastoid cells. The protein synthesis inhibitor cycloheximide blocked type I R expression but superinduced decoy R expression. IL-13 augmented the binding of radiolabeled IL-1β on the PMN surface with an increased number of IL-1 receptors and no change in K_d values. IL-13 induced the surface expression of IL-1 decoy R and the release by PMN of an IL-1-binding protein identified as a soluble version of the IL-1 decoy R. These results show that PMN is an important target for IL-13 and that induction of expression and release of the IL-1 decoy R, in concert with inhibition of cytokine synthesis, may represent an important mechanism by which IL-13 blocks IL-1, a central mediator of inflammatory reactions.

IL-13[1] is a recently identified cytokine active on different cell types including B cells (1–3), mononuclear phagocytes (1, 2, 4), large granular lymphocytes (1), and endothelial cells (4). The postulated murine homologue of IL-13 was originally cloned as the P600 gene (5, 6), expressed by the Th2 subset of T lymphocytes (6). The IL-13 gene is located on chromosome 5 q23–31 adjacent to IL-4 with which it shows about 25% homology (7). In certain respects, IL-13 strongly resembles IL-4 and IL-10 (see Refs. 8 and 9, respectively, for review). In particular, IL-13 shares with IL-4 and IL-10 the ability to suppress cytokine production in human mononuclear phagocytes (1). Recent results suggest that IL-4 and IL-13 may share a receptor component important for signal transduction (10).

IL-1α and IL-1β are potent pro-inflammatory cytokines, which induce a number of effects on different cell types (11). Two cellular receptors for IL-1 have been identified and molecularly cloned, the IL-1 receptor type I (IL-1R I) and IL-1 receptor type II (IL-1R II) (12, 13). In different cell types, the IL-1 signaling activity is mediated exclusively via the IL-1R I (14). In myelomonocytic cells, which express predominantly the IL-1R II, the biological activity of IL-1 is mediated by spare amounts of the IL-1R I (14, 15). In these cells, the IL-1R II has no signaling function and acts as a decoy for IL-1 (IL-1 decoy R), inhibiting its activities by preventing the IL-1 binding to the IL-1R I (15). IL-4 and glucocorticoids (GC) increased the surface expression of the IL-1 decoy R in human circulating PMN and induced the release of a soluble form of this molecule (15, 16).

The structural and functional relationship of IL-13 with IL-4, in particular its putative role in Th2 responses and anti-inflammatory activity, prompted us to study whether it affects expression and release of the type II decoy R in PMN. Our data indicate that IL-13 augments the surface expression of the IL-1 decoy R and the release of a soluble version of this molecule. The induction of a decoy R for IL-1 in PMN by IL-13 demonstrates that PMN also are targets of the biological activities of this recently described cytokine and are in keeping with the concept that IL-13 exerts anti-inflammatory activities on cells of the myelomonocytic lineage, a property shared with other cytokines produced by Th2 lymphocytes.

EXPERIMENTAL PROCEDURES

Cells—Human PMN were separated from the peripheral blood of human healthy donors by Percoll gradient centrifugation (15). Briefly, whole blood was fractionated by Ficoll gradient centrifugation (Seromed-Biochem KG, Berlin, FRG), and PMN, collected from the pellet, were layered on top of 62% Percoll (Pharmacia, Uppsala, Sweden). PMN (≥98% pure as assessed by morphology) were resuspended at 5–10 × 10^6/ml in RPMI 1640, 10% FCS (Hyclone Laboratories, Logan, UT).

Reagents—Human recombinant IL-13 (batches Mo and Mo2) was from Sanofi-Elf Recherches, Labège, France. Human recombinant IL-1β was obtained through the courtesy of Dr. J. E. Sims (Immunex Corp., Seattle, WA). Actinomycin D (ActD) and cycloheximide (CH) were from Sigma.

IL-1 Binding Assay—After treatment with 20 ng/ml IL-13 for 12–14 h at 37 °C, 1–2 × 10^6 PMN were incubated with decreasing concentrations of ^{125}I-IL-1β (180 μCi/μg, NEN, Bad Homburg, FRG) in the presence or absence of a 200-fold molar excess of cold cytokine in 0.1 ml of binding buffer (phosphate-buffered saline, 0.1% bovine serum albumin, 0.02% sodium azide, Sigma) at 4 °C for 4 h. Preliminary experiments showed that binding reached the plateau under these conditions. To separate bound from free radiolabeled IL-1, cells were centrifuged over a cushion of 20% sucrose, 1% bovine serum albumin. Scatchard analysis was performed by the LIGAND program.

Northern Blot Analysis—RNA isolation and analysis were as described (15). Probes were an *Eco*RI-*Hin*dIII fragment of 477 base pairs and a *Eco*RI-*Sal*I 750-base pair fragment from IL-1R I and IL-1R II cDNAs, respectively. Membranes were washed twice with 2 × SSC, 1%

* This work was supported by Consiglio Nazionale delle Ricerche, p.f. ACRO, by the Mario Negri-Weizmann Fund, and by Istituto Superiore di Sanità, V° AIDS Project. The generous contribution of the Associazione Italiana per la Ricerca sul Cancro (AIRC) is gratefully acknowledged. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ To whom correspondence and reprint requests should be addressed. Tel.: 39-2-39014530; Fax: 39-2-3546277.

¶ Recipient of an AIRC fellowship.

[1] The abbreviations used are: IL, interleukin; R, receptor; IL-1R, IL-1 receptor; IL-1R I, IL-1 R type I; IL-1R II, IL-1 R type II; GC, glucocorticoids; PMN, polymorphonuclear cells; ActD, actinomycin D; CH, cycloheximide; FCS, fetal calf serum; DSS, disuccinimidyl suberate; PAGE, polyacrylamide gel electrophoresis; mAb, monoclonal antibody.

This is an Open Access article under the CC BY license.

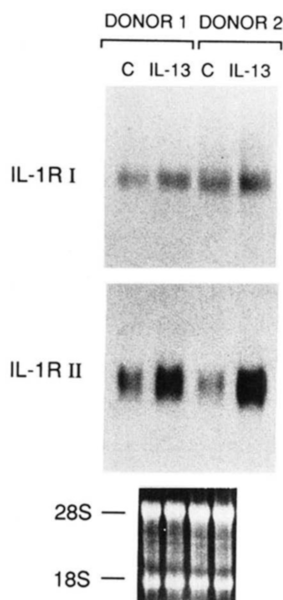

FIG. 1. **Induction of IL-1R I and IL-1R II transcripts in IL-13-treated PMN.** Cells were incubated with 20 ng/ml IL-13 for 4 h. Total RNA was extracted and analyzed by Northern blotting. The same membrane was first hybridized with the IL-1R I probe and then with the IL-1 decoy R probe. The *lower part* of the figure shows the ethidium bromide-stained membrane. The membrane was exposed to autoradiography for 25 and 5 h for type I R and decoy R transcripts, respectively. The results are from two representative donors out of five tested.

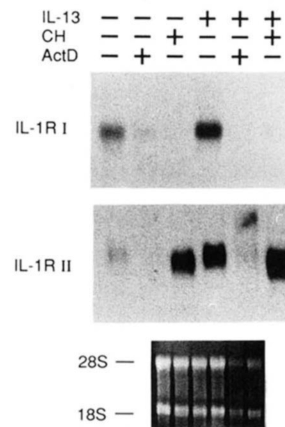

FIG. 2. **Effects of metabolic inhibitors on IL-13-induced expression of IL-1 R transcripts.** PMN were incubated with IL-13 (20 ng/ml), with or without ActD (1 µg/ml) or CH (10 µg/ml), for 4 h and then analyzed for IL-1R I and IL-1 decoy R transcripts. The same membrane (shown in the *lower part* of the figure) was hybridized with both probes and exposed to autoradiography as detailed in Fig. 1.

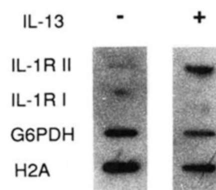

FIG. 3. **Nuclear run-off transcription from IL-13-treated Raji cells.** The B lymphoblastoid cell line Raji (20 × 10^6 ml) in RPMI 1640, 10% FCS was incubated with or without 20 ng/ml IL-13 for 3.5 h. The radiolabeled transcripts were isolated and hybridized to plasmids containing the cDNA sequences of IL-1R I, IL-1 decoy R, glucose-6-phosphate dehydrogenase (*G6PDH*) and histone H2A.

SDS (Merck) at 60 °C and exposed for 24–36 h for IL-1R I or 4–6 h for IL-1R II expression at −80 °C. RNA transfer to membranes was checked by UV irradiation.

Nuclear Run-off—B lymphoblastoid Raji cells in RPMI 1640, 10% FCS were incubated with 20 ng/ml IL-13 for 3.5 h. Then, radiolabeled nuclear transcripts were purified from isolated nuclei and hybridized to DNA immobilized to nitrocellulose membranes, as described in Ref. 17.

Affinity Cross-linking—Cross-linking experiments were described in detail (15). Briefly, for surface affinity cross-linking, 30 × 10^6 PMN treated with 20 ng/ml IL-13 for 14 h were incubated in binding buffer with 1 nM ^{125}I-IL-1β at 4 °C for 4 h. After the addition of 1 mM disuccinimidyl suberate (DSS, Pierce Chemical Co.), the cell pellet was lysed in 100 µl of lysis buffer (0.5% Triton X-100, 25 mM Hepes, 1 mM phenylmethylsulfonyl fluoride, 100 µg/ml aprotinin, and leupeptin, Sigma). The debris-free supernatant was analyzed by 8% SDS-PAGE under reducing conditions, and dried gels were exposed to autoradiography for 1–3 days. For soluble covalent cross-linking, 30 × 10^6 PMN were cultivated with 20 ng/ml IL-13 for 14 h in RPMI 1640 without serum at 37 °C. Medium was recovered and concentrated 10 times by membrane filtration (Amicon, Beverly, MA; cutoff, 10,000). 200 µl were added with 1 nM ^{125}I-IL-1β, with or without a 200 molar excess of cold IL-1β or 10 µg/ml M1 (anti-IL-1R I) or M22 (anti-IL-1R II) monoclonal antibody (13, 18) (kindly provided by Dr. J. E. Sims), and incubated at 4 °C for 4 h. After addition of 1 mM DSS at 4 °C for 30 min, samples were analyzed by gel electrophoresis as described above. Densitometric analysis of autoradiographic signals was performed with a scanning densitometer (GS 300, Hoefer Scientific Instruments, San Francisco, CA).

RESULTS

Expression of IL-1R was examined by Northern blotting. Since untreated PMN express predominantly the type II decoy R transcripts, membranes were exposed to autoradiography for 4–6 h for the IL-1 decoy receptor mRNA and 24–36 h for the type I transcripts. IL-13 augmented the mRNA expression of IL-1 decoy R in human circulating PMN (Fig. 1 shows two representative donors out of five). After 4 h of treatment with 10 ng/ml IL-13, IL-1 decoy R transcripts augmented 2–6 fold in this series of five donors. Also type I receptor transcripts were induced by IL-13 (1.2–4 fold). Given the difference in exposure times, the most represented transcripts induced by IL-13 were those coding for the IL-1 decoy R.

Induction of IL-1R transcripts by IL-13 was completely blocked in the presence of the transcriptional inhibitor ActD (Fig. 2 shows one representative experiment out of three), thus suggesting that induction of IL-1R mRNAs could be at the transcriptional level. In order to confirm this possibility, we carried out nuclear run-off experiments. Since we encountered considerable difficulties in performing nuclear run-off experiments from blood PMN, in one experiment we used Raji cells, a B lymphoblastoid cell line that expresses high levels of IL-1 decoy R (13). Results, shown in Fig. 3, demonstrated that IL-13 augmented the transcriptional rate of the IL-1 decoy R (10-fold increase as assessed by densitometric analysis and normalization for the transcriptional rate of the two housekeeping genes shown in Fig. 3). IL-1R I transcripts were undetectable both in untreated and IL-13-treated Raji cells, thus confirming and extending a previous observation (13).

The protein synthesis inhibitor CH had opposite effects on the expression of the IL-1R I and IL-1 decoy R transcripts (Fig. 2). The base-line and IL-13-induced transcripts of IL-1R I were blocked by CH. By contrast, CH superinduced expression of decoy R mRNAs both in untreated and IL-13-treated PMN. These data indicate that an intact protein synthesis is required for type I expression, whereas the decoy R transcripts are augmented if protein synthesis is inhibited. Thus, different mechanisms may underlie both constitutive and IL-13-inducible expression of IL-1R I and IL-1 decoy R transcripts. These results were confirmed in three different donors.

Next we examined IL-1R expression on PMN surface by binding experiments with radiolabeled IL-1β. As shown in the representative experiment of Fig. 4, IL-13 (20 ng/ml, 14 h of treatment) augmented the specific binding of ^{125}I-IL-1β on PMN. Scatchard analysis demonstrated that the number of

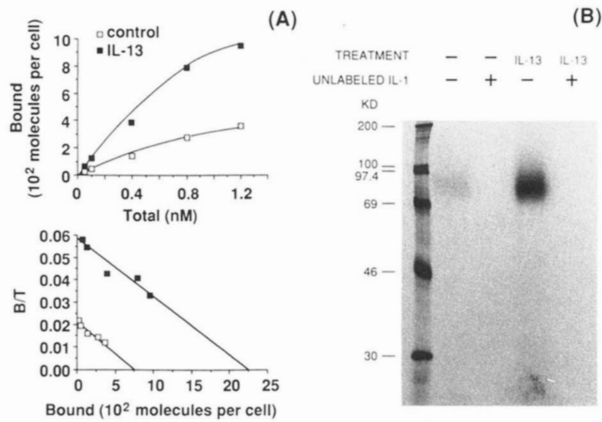

FIG. 4. **Surface expression of IL-1R in IL-13-treated PMN.** *A*, saturation curve and Scatchard analysis of radiolabeled IL-1β binding to IL-13-treated PMN. After treatment with IL-13 (20 ng/ml) for 18 h at 37 °C, 2×10^6 cells were incubated with various concentrations of ^{125}I-labeled IL-1β in the presence or absence of 200 times as much of unlabeled cytokine. *B/T*, bound/total. *B*, surface affinity cross-linking of radiolabeled IL-1β to IL-13-total PMN. PMN were incubated with IL-13 (20 ng/ml) for 14 h. Then radiolabeled IL-1β (with or without a 200 molar excess of unlabeled IL-1β) was added and, after treatment with DSS, cross-linked products were analyzed by SDS-PAGE under reducing conditions. The *first lane* on the *left* shows molecular weight markers.

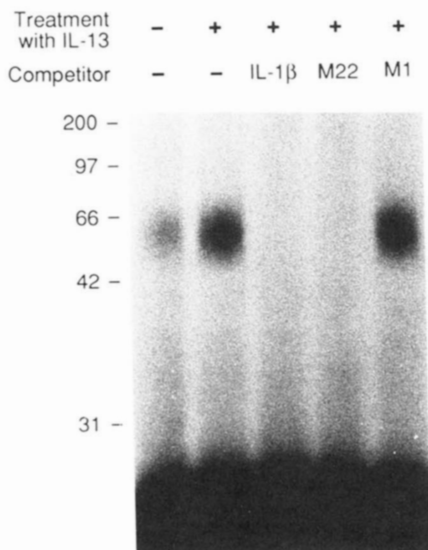

FIG. 5. **Affinity cross-linking of radiolabeled IL-1β to conditioned supernatants from PMN treated with IL-13.** Cells were incubated for 14 h with 20 ng/ml IL-13. Conditioned media were then concentrated and incubated with radiolabeled IL-1β, in the presence of a 200 molar excess of unlabeled IL-1β or with 10 µg/ml M22 (anti-IL-1R II) or M1 (anti-IL-1R I) mAbs. After treatment with DSS, cross-linked products were analyzed by SDS-PAGE under reducing conditions. Molecular weight standards are shown on the *left*.

receptors was 750 ± 105/cell in untreated cells and 2,250 ± 325/cell in IL-13-treated PMN, with no change in K_d values (1.1 ± 0.6 and 1.12 ± 0.7 × 10^{-10} M, respectively). Similar results were obtained in three different donors. In order to identify the receptor induced on PMN surface by IL-13, we performed surface affinity cross-linking experiments (Fig. 4). After subtraction of the ligand mass, the molecular size of the cross-linked product was consistent with the IL-1R II (68 kDa). Identical results were found in three different experiments. Thus the predominant IL-1R induced in PMN by IL-13 is, both at the mRNA and the protein levels, the IL-1 decoy R.

We recently found that PMN release a soluble version of the IL-1 decoy R in response to IL-4 and GC (15, 16). Cross-linking experiments performed on supernatants from IL-13-treated PMN revealed the presence of an IL-1-binding protein of approximately 45 kDa (Fig. 5 shows one representative experiment out of five). The specific nature of this IL-1 binding protein was demonstrated by competition with a 200-fold excess of unlabeled IL-1. The cross-linked product was completely inhibited by the presence of a blocking mAb directed against the IL-1R II (M22) but not by an anti-IL-1R I mAb (M1), thus confirming that the protein released by IL-13-treated PMN is a soluble version of the IL-1 decoy R.

Having found that IL-13 up-regulates expression and release of the IL-1 decoy R, we tested, along the line of our previous studies with IL-4 (15), whether IL-13 inhibited IL-1-induced survival of PMN. In 10 different donors (data not shown), IL-13 inhibited IL-1-induced survival by 70–84%. Consistently with a decoy function of IL-1R II, the inhibitory effect of IL-13 was overridden by high (1 µg/ml) concentrations of IL-1.

CONCLUDING REMARKS

In this paper we describe the regulation by IL-13 of IL-1 receptors in human circulating PMN. Exposure to IL-13 increased surface expression and release of IL-1 decoy R (IL-1R II). The induction of type I and decoy R transcripts by IL-13 required gene transcription, whereas inhibition of protein synthesis had contrasting effects. In the presence of CH, induction of type I transcripts was inhibited, whereas decoy R mRNA was superinduced. Thus, different molecular mechanisms may underlie the induction by IL-13 of IL-1R transcripts in PMN. These findings demonstrate that also PMN, in addition to B cells (1–3), monocytes (1, 2, 4), large granular leukocytes (1), and endothelial cells (4)[2] are responsive to IL-13.

IL-13 is a pleiotropic cytokine that elicits a complex set of responses in different cell types (1–4). Its activities are in keeping with a role of this cytokine in Th2-type responses and in inhibition of inflammation. The results reported here are consistent with the latter function of IL-13. Expression and release by IL-13 of the IL-1 decoy R, in concert with inhibition of IL-1 production (1) and induction of the IL-1 receptor antagonist (19), would block IL-1, a central mediator of inflammation.

It is interesting in this context that IL-13 (1), IL-4 (8), and IL-10 (9), three polypeptide mediators of Th2 responses, share the capacity to inhibit the production and function of inflammatory cytokines. By and large, the observation that diverse molecules with anti-inflammatory activity, such as GC (15, 16), IL-4 (15), and IL-13 (this paper), share the capacity to induce expression, and release of the decoy R is consistent with the view that this IL-1-buffering molecule represents an important pathway for down-regulation of IL-1 activity and a target for immunological intervention.

Acknowledgment—We thank Dr. J. E. Sims for the generous gift of cytokines and antibodies.

REFERENCES

1. Minty, A., Chalon, P., Derocq, J. M., Dumont, X., Guillemot, J. C., Khagat, M., Labit, C., Leplatois, P., Liauzun, P., Miloux, B., Minty, C., Casellas, P., Loison, G., Lupker, J., Shire, D., Ferrara, P., and Caput, D. (1993) *Nature* **362,** 248–250
2. McKenzie, A. N. J., Culpepper, J. A., de Waal Malefyt, R., Briere, F., Punnonen, J., Aversa, G., Sato, A., Dang, W., Cokcs, B. G., Menon, S., de Vries, J. E., Bancherau, J., and Zurawski, G. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 3735–3739
3. Punnonen, J., Aversa, G., Cocks, B. G., McKenzie, A. N. J., Menon, S., Zurawski, G., de Waal Malefty, R., and de Vries, J. E. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 3730–3734
4. Herbert, J. M., Savi, P., Laplace, M. C., Lalè, A., Dol, F., Dumas, A., Labit, C., and Minty, A. (1993) *FEBS Lett.* **328,** 268–270
5. Cherwinski, H. M., Schumacher, J. H., Brown, K. D., and Mosmann, T. R.

[2] M. Sironi, unpublished data.

(1987) *J. Exp. Med.* **166**, 1229–1244
6. Brown, K. D., Zurawski, S. M., Mosmann, T. R., and Zurawski G. (1989) *J. Immunol.* **142**, 679–687
7. McKenzie, A. N. J., Li, X., Largaespada, D. A., Sato, A., Kaneda, A., Zurawski, S. M., Doyle, E. L., Milatovich, A., Francke, U., Copeland, N. G., Jenkis, N. A., and Zurawski, G. (1993) *J. Immunol.* **150**, 5436–5444
8. Paul, W. E. (1991 *Blood* **77**, 1859–1870
9. Mossman, T. R., Schumacher, J. H., Street, N. F., Budd, R., O'Garra, A., Fong, T. A. T., Bond, M. W., Moore, K. W. M., Sher, A., and Fiorentino, D. F. (1991) *Immunol. Rev.* **123**, 209–230
10. Zurawski, S. M., Vega, F., Jr., Huyghe, B., and Zurawski, G. (1993) *EMBO J.* **12**, 2663–2670
11. Dinarello, C. A. (1991) *Blood* **77**, 1627–1652
12. Sims, J. E., March, C. J., Cosman, D., Widmer, M. B., McDonald, H. R., McMahan, C. J., Grubin, C. E., Wignall, J. M., Jackson, J. L., Call, S. M., Friend, D., Alpert, A. R., Gillis, S., Urdal, D. L., and Dower S. K. (1988) *Science* **241**, 585–589
13. McMahan, C. J., Slack, J. L., Mosley, B., Cosman, D., Lupton, S. D., Brunton, L. L., Grubin, C. E., Wignall, J. M., Jenkins, N. A., Brannan, C. I., Copeland, N. G., Huebner, K., Croce, C. M., Cannizzaro, L. A., Benjamin, D., Dower, S. K., Spriggs, M. K., and Sims, J. E. (1991) *EMBO J.* **10**, 2821–2832
14. Sims, J. E., Gayle, M. A., Slack, J. L., Alderson, M. R., Bird, T. A., Giri, J. G., Colotta, F., Re, F., Mantovani, A., Shanebeck, K., Grabstein, K. H., and Dower, S. K. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 6155–6159
15. Colotta, F., Re, F., Muzio, M., Bertini, R., Polentarutti, N., Sironi, M., Giri, J. G., Dower, S. K., Sims, J. E., and Mantovani, A. (1993) *Science* **261**, 472–475
16. Re, F., Muzio, M., De Rossi, M., Polentarutti, N., Giri, J. G., Mantovani, A., and Colotta, F. (1994) *J. Exp. Med.* **179**, 739–743
17. Colotta, F., Polentarutti, N., Sironi, M., and Mantovani, A. (1992) *J. Biol. Chem.* **267**, 18278–18283
18. Spriggs, M. K., Lioubin, P. J., Slack, J., Dower, S. K., Jonas, U., Cosman, D., Sims, J. E., and Bauer J. (1990) *J. Biol. Chem.* **265**, 22499–22505
19. Muzio, M., Re, F., Sironi, M., Polentarutti, N., Minty, A., Caput, D., Ferrara, D., Mantovani, A., and Colotta, F. (1994) *Blood* **83**, 1738–1743