Chemoattractants Induce Rapid Release of the Interleukin 1 Type II Decoy Receptor in Human Polymorphonuclear Cells

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Summary

Molecules representative of different classes of chemotactic agents, including formyl-Met-Leu-Phe (FMLP), C5a, leukotriene B_4, platelet-activating factor, and interleukin (IL)-8, caused a rapid reduction in the IL-1 binding capacity by human polymorphonuclear leukocytes (PMN), a cell type expressing predominantly the IL-1 type II decoy receptor (IL-1 decoy RII). N-t-Boc-Met-Leu-Phe, an antagonist for the FMLP receptor, inhibited the loss of IL-1 binding capacity induced by FMLP. Monocyte chemotactic protein 1, a chemokine related to IL-8 but inactive on PMN, had no effect on IL-1 binding in this cell type. FMLP was selected for further detailed analysis of chemoattractant-induced loss of IL-1 binding by PMN. The action of FMLP was rapid, reaching 50% of its maximum (80%) at 30 s, the earliest measurable time point, and plateauing between 10 and 30 min. Dose–response analysis revealed that maximal reduction of IL-1 binding was reached at FMLP concentrations that were also optimal for chemotaxis (50% effective dose = 5×10^{-9} M). The loss of IL-1 binding capacity caused by FMLP was determined by a reduction in receptor number with no change in their affinity. The effect of FMLP on IL-1 receptor (IL-1R) was selective in that the PMN surface structures IL-8R, CD16, CD18, and major histocompatibility complex class I antigens were unaffected under these conditions. Loss of surface IL-1R was not due to an augumented rate of internalization. FMLP caused rapid release of a 45-kD IL-1–binding molecule identified as the IL-1 decoy RII. After FMLP-induced release, PMN reexpressed newly synthesized receptors, reaching basal levels by 4 h. FMLP-induced release of the IL-1 decoy RII did not impair the responsiveness of PMN to IL-1 in terms of promotion of survival and cytokine production. FMLP-induced release of the IL-1 decoy RII was unaffected by protein synthesis inhibitors, was blocked by certain protease inhibitors, and was mimicked by agents (the Ca^{++} ionophore A23187 and phorbol myristate acetate) that recapitulate elements in the signal transduction pathway of chemoattractant receptors. The time frame and concentration range of chemoattractant-induced rapid release of the IL-1 decoy RII are consistent with the view that IL-1 decoy RII release is an early event in the multistep process of leukocyte recruitment. Rapid chemoattractant-induced IL-1 decoy RII release in the circulation may counteract IL-1 leaking into the systemic circulation from sites of inflammation while preserving the capacity of leukocytes to respond to IL-1 in tissues. This phenomenon may contribute as well to the antiinflammatory effect of systemic administration of chemotactic agents.

IL-1α and IL-1β are potent mediators of immune and inflammatory reactions whose spectrum of activity encompasses a number of different cell types (1). Two receptors for IL-1 termed type I and type II (IL-1RI and IL-1RII), usually coexpressed in different cell types, have been identified and cloned

(1, 2). IL-1 signaling activity appears to be mediated exclusively via the IL-1RI, whereas the IL-1RII has no signaling property and acts in myelomonocytic cells as a decoy for IL-1, inhibiting its activity by preventing IL-1 from binding to the IL-1RI (3, 4).

A soluble version of IL-1 decoy RII has been identified in the supernatants from B lymphoblastoid cells (5–7) and PHA-activated human mononuclear cells (8), in human plasma (9), and in inflammatory synovial fluid (8). Recently, we found

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that IL-4 (4), IL-13 (10), and the antiinflammatory gluco-corticoid, dexamethasone (11), were potent inducers of IL-1 decoy RII expression and release in human myelomonocytic cells. Up-regulation of IL-1 decoy RII by IL-4, IL-13, and dexamethasone was gene and protein synthesis dependent. The released soluble IL-1 decoy RII retains its ability to bind IL-1α, IL-1β, and the IL-1R antagonist, although with different relative affinities compared to the membrane-bound receptor (11). The ability of certain cytokines and glucocorticoids to induce expression and release of a decoy target for IL-1 may contribute to the antiinflammatory activities of these agents (4, 11).

Various agents, including chemotactic signals, cause rapid shedding of TNFR (12). These previous observations prompted us to examine the regulation of IL-1 decoy RII release from human PMN exposed to chemotactic stimuli. We found that treatment of PMN with chemoattractants results in massive (up to 90%), rapid (evident within 30 s), and protein synthesis–independent release from the PMN surface of a soluble form of IL-1 decoy RII, followed by reexpression of newly synthesized receptor. This event is mediated by a preformed proteolytic enzyme. Rapid release of the IL-1 decoy RII is an early event in the multistep process of PMN recruitment, which likely plays a role in the regulation of local versus systemic inflammatory reactions.

Materials and Methods

Cells. Human PMN were separated from the peripheral blood of human healthy donors by Percoll gradient centrifugation (13). Briefly, whole blood was fractionated by Ficoll gradient centrifugation (Seromed-Biochem KG, Berlin, Germany), and PMN, collected from the pellet, were layered on top of 62% Percoll (Pharmacia LKB, Uppsala, Sweden) after a centrifugation at 1,500 rpm for 20 min at room temperature. PMN pellets (≥98% pure as assessed by morphology) were resuspended at 10^7/ml in RPMI 1640 (Seromed-Biochem KG) with 2 mM glutamine (Seromed-Biochem KG) and 20 mM Hepes (Merck, Darmstadt, Germany). All reagents contained <0.125 EU/ml of endotoxin as checked by limulus amebocyte lysate assay (Microbiological Associates, Inc., Rockville, MD).

Reagents. FMLP was purchased from Sigma Chemical Co. (St. Louis, MO), dissolved in ethanol at 10^{-3} M, and stored at $-20°C$ until use. Human recombinant C5a was a kind gift from Dr. H. S. Showell (Pfizer Central Research, Groton, CT). Human recombinant IL-8 was from Dainippon (Osaka, Japan). Platelet-activating factor (PAF),[1] leukotriene B₄ (LTB₄), A23187, PMA, and cycloheximide (CH) were purchased from Sigma Chemical Co. Human recombinant monocyte chemotactic protein (MCP)-1 was from Pepro Tech Inc. (Rocky Hill, NJ).

Human recombinant IL-1β was obtained through the courtesy of Dr. J. E. Sims (Immunex Corp., Seattle, WA). Protease inhibitors used in this study (PMSF, N-tosyl-lysyl-chloromethyl ketone

[TLCK], N-tosyl-phenylalanyl-chloromethyl ketone [TPCK], and EGTA) were from Sigma Chemical Co. EDTA was from Merck. α₁-antitrypsin was from Calbiochem Corp. (La Jolla, CA).

IL-1–Binding Assay (4, 10, 11). Routinely, Percoll-purified PMN (10^7/ml) were incubated with or without the indicated stimuli in serum-free medium RPMI 1640 at 37°C in 5% CO_2 for 30 min in polypropylene 50-ml conical tubes (2070; Falcon Labware, Becton Dickinson and Co., Oxnard, CA). Next, cells were washed with binding buffer (RPMI 1640, 0.2% BSA, pH 7.4 [Sigma Chemical Co.]), and 4 × 10^6 PMN were incubated with 600 pM ^{125}I-IL-1β (sp act 180 μCi/μg; New England Nuclear, Bad Homburg, Germany) in the presence or absence of a 100-fold molar excess of cold cytokine in 50 μl binding buffer at 4°C for 18 h in polystyrene 96 round-bottomed well microplates (3077; Falcon Labware) on a shaking platform. Preliminary experiments showed that binding reached the plateau (8 h) under these conditions. To separate bound from free ^{125}I-IL-1β, cells were resuspended, transferred to Eppendorf tubes, washed in binding buffer, resuspended in 70 μl of binding buffer, and finally layered on the top of a 200-μl cushion of 20% sucrose (Merck) and 1% BSA in 400-μl polypropylene tubes (342867; Beckman Instruments, Palo Alto, CA) and centrifuged at 10,000 rpm for 30 s at room temperature. The cellular pellets were counted in a gamma counter. When IL-8 binding was studied, ^{125}I-IL-8 (Amersham International, Little Chalfont, UK) (sp act 2,000 Ci/mmol) was used under the same experimental conditions described above. To obtain a saturation curve, untreated or FMLP-treated PMN were incubated with decreasing amounts of ^{125}I-IL-1β, in the presence or absence of a 100-fold molar excess of cold cytokine. Next, Scatchard analysis was performed by the LIGAND (4.1; National Institutes of Health, Bethesda, MD) program to determine the affinity and numbers of receptors for IL-1β. To investigate the kinetics of loss of IL-1 binding, PMN were incubated at 10^7/ml in RPMI 1640 at 37°C for different periods of time (30 s–30 min) in the presence or absence of FMLP. Subsequently, cells were washed and resuspended in binding buffer to evaluate IL-1 binding as previously described. When reexpression of IL-1 decoy RII was studied, PMN were pretreated in medium or with FMLP for 30 min to induce release of IL-1R from PMN. PMN were then washed and incubated in 10% FCS (Hyclone Laboratories, Inc., Logan, UT) RPMI 1640 medium (recovery period) for various periods of time and tested for IL-1β binding.

IL-1R Internalization. PMN (4 × 10^6/50 μl) were incubated in binding buffer with 1 nM ^{125}I-IL-1β for 4 h at 4°C on a shaking platform, in the presence or absence of a 100-fold molar excess of unlabeled IL-1β. PMN were washed twice in binding buffer to remove unbound cytokine and then resuspended in serum-free medium RPMI 1640 (4 × 10^6/100 μl) and incubated for various periods of time at 37°C, with or without 10^{-7} M FMLP, to induce receptor internalization. At each time point, 4 × 10^6 cells were washed, acid washed for 5 min (50 mM glycine; 0.8% NaCl, pH 3) to remove surface-bound cytokine, and washed again, and pellets were counted in a gamma counter to determine the amount of internalized cytokine.

Chemotaxis Assay. The chemotactic response of human circulating PMN to FMLP was tested as described (14, 15). Briefly, 25 ± 1 μl of FMLP, diluted in 1% FCS RPMI 1640, was seeded in the lower compartment of the chemotaxis chamber, and 50 μl of cell suspension (1.5 × 10^6/ml) was seeded in the upper compartment.

The two compartments were separated by a 5-μm pore size polyvinylpyrrolidone-free polycarbonate filter (Nuclepore Corp., Pleasanton, CA). Chambers were incubated at 37°C in air with 5% CO_2 for 90 min. At the end of the incubation, filters were

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[1] *Abbreviations used in this paper:* CH, cycloheximide; LTB₄, leukotriene B₄; MCP, monocyte chemotactic protein; PAF, platelet-activating factor; PKC, protein kinase C; TLCK, N-tosyl-lysyl-chloromethyl ketone; TPCK, N-tosyl-phenylalanyl-choromethyl ketone.

removed, fixed, and stained with Diff-Quik (Harleco, Gibbstown, NJ), and five high power oil immersion fields were counted.

Flow Cytometry. The following antibodies were used for flow cytometry: The anti-CD16 mAb KD1 was a kind gift from Dr. E. Ciccone (Istituto Tumori, Genova, Italy), and anti-MHC class I molecule mAb W6/32 and anti-CD18 mAb Ts1/18 were from American Type Culture Collection (Rockville, MD). After treatment with FMLP for 30 min as detailed above, an aliquot of cells was examined for IL-1β binding, and the remaining PMN were resuspended in 0.1 ml saline (Bieffe, Bergamo, Italy) with 2% human serum (pool from different healthy donors) and mAb. After 30 min of incubation on ice, cells were washed twice and resuspended in saline containing 2% human serum and a fluoresceinated antibody against mouse IgG (Becton Dickinson Immunocytometry Systems, Mountain View, CA). After 30 min on ice, cells were washed twice and analyzed by flow cytofluorometry with a FACSTAR® plus apparatus (Becton Dickinson and Co.).

Affinity Cross-linking. Cross-linking experiments were described in detail (11). 30 × 10^6 PMN were stimulated with 10^{-7} M FMLP in 1 ml of RPMI 1640 at 37°C for 30 min. Medium was recovered and concentrated 10 times by membrane filtration (Amicon, Beverly, MA; cut-off 10,000). When studied, 200 μl was added to 1 nM ^{125}I-IL-1β, with or without a 200-M excess of cold IL-1β or 10 μg/ml M1 (blocking mAb anti–IL-1RI) or M22 (blocking mAb anti–IL-1RII) (16, 17) kindly provided by Dr. J. E. Sims, and incubated at 4°C for 4 h. After addition of 1 mM disuccinimidyl suberate from Pierce Chemical Co. (Rockford, IL) at 4°C for 30 min, samples were analyzed by 8% SDS-PAGE under reducing conditions, and dried gels were exposed to autoradiography for 1–3 d.

IL-6 and IL-8 Production. PMN were exposed for 30 min to FMLP or medium. After washing, the cells were pulsed with 20 ng/ml of IL-1β for 4 h. Finally, cells were washed and incubated for an additional 24 h. The supernatants were collected and tested for IL-8 production by ELISA assays (the antibodies were from Dr. M. Ceska, Sandoz Forschung Institut, Vienna, Austria) and for IL-6 production by bioassay, using the 7TD1 hybridoma cell line as previously described (18).

Statistical Analysis. The differences among experimental groups were tested by the Student's *t* test.

Results

Effects of PMN Chemoattractants on IL-1 Binding. Table 1 summarizes the effects of various agents representative of different classes of chemoattractants (formylated peptides, complement components, lipids, chemokines) on the capacity of PMN to bind ^{125}I-IL-1β. The different concentrations of the stimuli were chosen according to their optimal activity in the chemotactic assay. Results in Table 1 are a compound of different experiments and are presented as the percentage of binding relative to control at the 30-min time point. FMLP, C5a, LTB$_4$, PAF, and IL-8 caused reductions of IL-1 binding to PMN of 80% (ranging from 73 to 94%), 50% (from 43 to 54%), 52% (from 47 to 66%), 37% (from 11 to 30%), and 25% (from 21 to 37%), respectively. The formyl peptide N-t-Boc-Met-Leu-Phe, an inactive FMLP receptor antagonist, caused no reduction at high concentrations (10^{-4} M) and partially inhibited the activity of FMLP. MCP-1, a chemokine that is inactive on PMN as a chemoattractant, caused no loss of IL-1 binding capacity. The signal transduction pathways of chemotactic factor serpentine receptors in-

**Table 1.** *Loss of IL-1 Binding Capacity in PMN Exposed to Different Stimuli*

Treatment (dose or dilution)	IL-1 binding (percentage of control)
FMLP (10^{-7} M)	19.8 ± 7.2 (13)
BOCMLP (10^{-4} M)	95.3 ± 4.6 (2)
BOCMLP (10^{-4} M) + FMLP (10^{-7} M)	55.5 ± 6.3 (2)
C5a (50 ng/ml)	50.8 ± 4.9 (3)
PAF (10 nM)	63.8 ± 11.6 (2)
LTB$_4$ (100 nM)	48.8 ± 4.9 (2)
IL-8 (100 ng/ml)	75.0 ± 8.2 (3)
MCP-1 (100 ng/ml)	97.5 ± 2.7 (2)
A23187 (10^{-7} M)	8.4 ± 8.1 (3)
PMA (10 ng/ml)	41.3 ± 3.6 (2)
Ethanol (1:1,000)	98.0 (1)
DMSO (1:1,000)	99.6 (1)

Human peripheral blood PMN were incubated with the indicated stimuli for 30 min at 37°C and then examined for surface binding of ^{125}I-IL-1β. Data are expressed as percentage of IL-1 binding over the same PMN population cultured in parallel with medium alone. Data are shown as mean ± SD from the number of independent experiments indicated in parentheses.

clude activation of PKC and a rapid increase in intracellular free Ca^{++}. The protein kinase C (PKC) activator PMA and the Ca^{++} ionophore A23187 mimicked chemoattractants by causing rapid loss of IL-1 binding capacity. Subsequent analysis was conducted, mostly with the prototypic chemoattractant FMLP.

Decreased IL-1 Binding Is Determined by Loss of Surface IL-1R. Having found that FMLP drastically reduced IL-1 binding to PMN, we examined whether this effect was due to a change in receptor affinity and/or a reduction in receptor number. Fig. 1 shows a saturation curve of IL-1 binding on untreated and FMLP-treated PMN. Scatchard analysis (Fig. 1) demonstrated that FMLP reduced the number of IL-1R on PMN surface. In this particular experiment, the number of IL-1R per cell was 517 in untreated and 79 in FMLP-treated PMN. No significant change in receptor affinity was detected (kD values: 5.5 × 10^{-10} M and 4 × 10^{-10} M in untreated and treated cells, respectively).

Kinetics and Dose–Response of IL-1β Binding Loss Induced by FMLP. PMN were cultured alone or with FMLP (10^{-7} M) for various periods of time. Next, cells were recovered and examined for ^{125}I-IL-1 binding. Culture of PMN in medium alone caused a modest and slow reduction of IL-1β binding (Fig. 2). However, reduction of IL-1β binding induced by FMLP in PMN was rapid and pronounced. As shown in Fig. 2, IL-1β binding was reduced by 50% at 30 s, the earliest measurable time point. Maximal reduction was reached after 30 min. Thereafter, no further consistent reduction of IL-1 binding on treated PMN was evident (not shown).
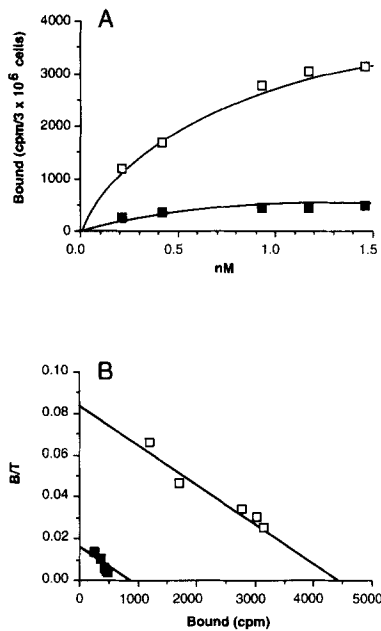
Figure 1. Saturation curve and Scatchard analysis of ^{125}I-IL-1 binding to PMN treated with FMLP. (*A*) PMN were either untreated or treated with 10^{-7} M FMLP for 30 min. Cells were then incubated with 1.5–0.2 nM ^{125}I-IL-1β, in the presence or absence of a 100-M excess of unlabeled IL-1β. Data are expressed as cpm specific bound to 3×10^6 cells. Similar results were obtained in two experiments. (*B*) Scatchard analysis of the saturation curve shown in *A*. ($\square$), control; ($\blacksquare$), FMLP.

A dose–response curve of FMLP is shown in Fig. 3, in which PMN from the same preparation were tested in parallel for IL-1β binding and chemotaxis. The half-maximal response was reached for both IL-1β binding loss and chemotaxis at $\sim 5 \times 10^{-9}$ M. The two activities considered, IL-1 binding loss and chemotaxis, peaked at the same FMLP concentration (10^{-7} M). At higher doses (10^{-5}–10^{-6} M), reduction of IL-1 binding was similar to the optimal concentration, whereas the chemotactic response at 10^{-5} M was $\sim 70\%$ of the maximal response. On the basis of these results, PMN were routinely incubated with 10^{-7} M FMLP for 30 min at 37°C.
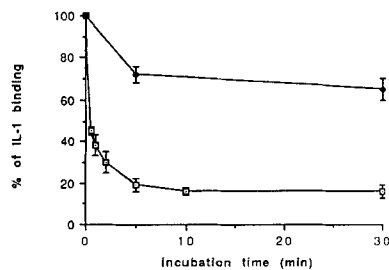
Figure 2. Kinetics of IL-1 binding loss induced by FMLP on PMN. PMN were incubated with 10^{-7} FMLP for the indicated periods of time and then examined for IL-1 binding. Data are expressed as the percentage of specific IL-1 binding with respect to PMN at time 0. Data are expressed as the mean ± SD of three experiments. ($\blacklozenge$), control; ($\square$), FMLP.
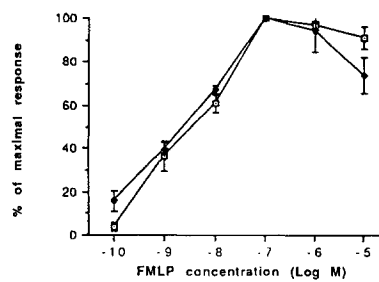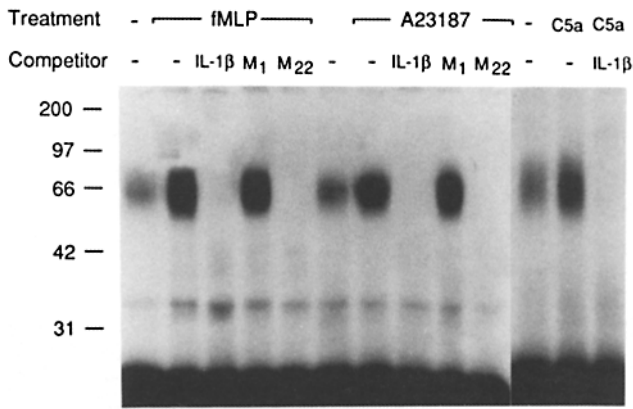

Figure 3. Chemotaxis and loss of IL-1 binding capacity of PMN exposed to different concentrations of FMLP. PMN were exposed to different concentrations of FMLP for 30 min and examined for IL-1 binding. The same PMN preparation was assayed for chemotactic responsiveness to different concentrations of FMLP. Results, expressed as the percentage of the maximal response obtained in each assay, are the mean, with range, of two experiments. ($\square$), IL-1 binding loss; ($\blacklozenge$), chemotaxis.

Release of the IL-1 Decoy RII. IL-1 binding sites could disappear from cell surface by internalization or release. As shown in Table 2, the internalization process of receptor-bound IL-1 was not affected by FMLP, in that the percentage of internalized IL-1R was not different at all the time points studied, in untreated versus FMLP-treated cells. To examine the possibility that FMLP induced the release of IL-1R from treated PMN, cells were incubated with FMLP, and the cell-free supernatants were recovered and examined by cross-linking with ^{125}I-IL-1β. As shown in Fig. 4, FMLP-treated PMN released an IL-1–binding molecule of ~ 45 kD (after subtraction of the IL-1β molecular weight). The specific nature of the cross-linking was demonstrated by competition with an excess of unlabeled IL-1. IL-1 binding product was totally inhibited by the presence of a blocking mAb (M22) directed against the IL-1 decoy RII but not by an anti–IL-1RI mAb (M1). These observations were confirmed and extended to other stimuli that induced reduction of IL-1 binding on human PMN. As shown in Fig. 4, C5a and A23187 also induced the release by PMN of an IL-1–binding molecule identified

Table 2. *Effect of FMLP on Internalization of IL-1R in Human PMN*

Time of incubation	Percent IL-1 internalized	
	Medium	FMLP
min		*10^{-7} M*
0	4 ± 1.0	9 ± 5.0
15	17 ± 0.1	21 ± 1.6
30	25 ± 0.8	27 ± 2.0
40	34 ± 3.0	31 ± 1.5

IL-1R internalization in the presence or absence of FMLP was assessed as detailed in Materials and Methods. The numbers in the table are the percentage of pellet-associated (internalized) specific radioactivity over the radioactivity present on cell surface at the end of the binding at 4°C (mean ± SD of three independent experiments).

Treatment   - ┌──── fMLP ────┐   ┌──── A23187 ───┐ - C5a C5a

Competitor   -   - IL-1β M₁ M₂₂ -   - IL-1β M₁ M₂₂ -   - IL-1β

200 —

97 —

66 —

42 —

31 —

Figure 4. Affinity cross-linking of ¹²⁵I-IL-1β to soluble IL-1 decoy RII from PMN. PMN were cultured with or without the indicated stimuli for 30 min in serum-free RPMI 1640. Supernatants were then recovered, concentrated, combined with ¹²⁵I-IL-1β, and examined by chemical cross-linking with disuccinimidyl suberate and SDS-PAGE. Competitors added before cross-linking were a 100-fold excess of cold (unlabeled) cytokine or 10 μg/ml anti–IL-1RI (mAb M1) or anti–IL-1RII (mAb M22).

Table 4. *Effect of Protein Synthesis Inhibition on FMLP-induced Loss of IL-1 Binding in PMN*

| Treatment | CH | IL-1 binding | |
		Specific cpm bound	Percentage of control
	10 μg/ml		
Medium	−	920 ± 171	100.0
	+	712 ± 69	77.3
FMLP (10⁻⁷ M)	−	189 ± 58	20.5
	+	131 ± 97	14.2

PMN were incubated with the indicated agents for 30 min at 37°C and then examined for binding of ¹²⁵I-IL-1. Data are shown as specific binding of labeled IL-1 ± SD of triplicates. Similar results were obtained in two experiments.

as a soluble form of the IL-1 decoy RII. Thus, the loss of IL-1 binding induced by chemoattractants is largely, if not exclusively, accounted for by release in a soluble form of IL-1 decoy RII from the PMN surface.

Specificity of IL-1 Binding Loss Induced by FMLP. To study the specificity of FMLP-induced reduction of IL-1 binding, PMN treated with FMLP 10⁻⁷ M were examined for the expression of other surface molecules. As shown in Table 3, FMLP, while reducing IL-1 binding, did not affect the expression of CD18 and MHC class I molecules. Expression of CD16 was not reduced by FMLP treatment. Furthermore, binding of ¹²⁵I-IL-8 was not significantly affected by treatment with FMLP. These results indicate that the decrease of IL-1 binding induced by FMLP in human PMN is considerably selective.

Role of Protein Synthesis and Proteolytic Enzymes. IL-1 binding loss on PMN treated with FMLP did not require de novo protein synthesis. As shown in Table 4, the presence of the protein synthesis inhibitor CH did not affect the reduc-

tion of IL-1 binding induced by FMLP. CH alone slightly reduced IL-1 binding (23% reduction) on PMN.

To test whether proteolytic cleavage could be responsible for FMLP-induced release of IL-1 decoy RII, PMN were treated with FMLP in the presence or absence of different protease inhibitors (Table 5). All the inhibitors were tested at the highest nontoxic dose (trypan blue dye exclusion) as assessed in preliminary experiments (data not shown). PMSF at 1 mM only partially blocked the effect of FMLP (44–56% inhibition of FMLP activity). TLCK inhibited FMLP effect by 36% at 0.5 mM. α₁-Antitrypsin strongly inhibited FMLP activity (77% inhibition). By contrast, TPCK, another serine-threonine protease inhibitor, as well as chelators of Mg⁺⁺ and Ca⁺⁺ cations (EGTA and EDTA), failed to affect FMLP-induced IL-1 binding loss on PMN.

IL-1R Reexpression. Next, we examined whether FMLP-induced release of IL-1R was followed by reexpression of IL-1R on PMN surface. Results are shown as percentage of IL-1 binding in the FMLP-treated population versus the untreated cells at each time point. As shown in Fig. 5, FMLP induced

Table 3. *Specificity of Release of IL-1R in FMLP-treated PMN*

| Treatment | Expression of | | | | |
	IL-1R*	IL-8R*	CD16‡	MHC class I‡	CD18‡
Medium	5,200 ± 604	40,398 ± 4,768	291	548	433
FMLP	1,300 ± 150§	32,875 ± 825‖	324	537	430

PMN were incubated with or without FMLP for 30 min. Cells were then washed and examined for binding of radiolabeled IL-1 and IL-8, or for expression of CD16, MHC class I, and CD18 antigens by flow cytometry. Results are a compound of three different experiments.
* Specific cpm bound ± SD from triplicates.
‡ Mean channel of fluorescence.
§ P <0.01.
‖ Not significant.

Table 5. *Effects of Protease Inhibitors on FMLP-induced Loss of IL-1 Binding in Human PMN*

Protease inhibitor	Experiment number	IL-1 binding		Percent inhibition of FMLP activity*
		FMLP	FMLP + inhibitor	
		Percentage of control		
α_1-antitrypsin (100 μg/ml)	1	43	90	
	2	34	98	
	3	30	69	77 ± 20
PMSF (1 mM)	1	30	62	
	2	26	62	
	3	43	75	
	4	20	55	49 ± 5
TLCK (0.5 mM)	1	30	50	
	2	40	63	
	3	34	62	36 ± 7
TPCK (100 μM)	1	27	24	-4
EDTA (5 mM)	1	20	23	
	2	30	28	
	3	27	31	2 ± 4
EGTA (1 mM)	1	20	17	
	2	30	28	
	3	33	35	-1 ± 4

Cells were incubated with FMLP (10^{-7} M) alone or with the indicated inhibitor for 30 min and subsequently tested for IL-1 binding.
* Percentage of inhibition was calculated as $\{[(FMLP + inhibitor) - (FMLP)]/[100 - (FMLP)]\} \times 100$.

a 65% reduction of IL-1 binding tested after 30 min (time 0 of recovery) of incubation. After a recovery period of 4 h, PMN showed an IL-1 binding level comparable to that of the untreated population. To define whether IL-1R reexpression required protein synthesis, CH was added during the recovery period. As shown in Fig. 5, recovery of IL-1 binding after pretreatment with FMLP was inhibited by the presence of CH during the recovery period.

Responsiveness to IL-1 after IL-1 Decoy RII Stripping. It was important to assay whether chemoattractant-induced stripping of a substantial fraction of membrane IL-1 decoy receptor was associated with a loss of IL-1 responsiveness. PMN were exposed to FMLP (10^{-7} M) for 30 min, carefully washed, and pulsed for 3 h with IL-1 (2–20 ng/ml). After removal of IL-1 by washing, cells were incubated for 24–72 h, and the responsiveness of PMN to IL-1 was observed in terms of cytokine production (IL-6 and IL-8) and prolongation of survival. IL-1 was a poor inducer of cytokine production in PMN compared to LPS used as positive control, with weak but significant responses in only three out of seven donors tested. FMLP did not reduce IL-1-induced cytokine production (not shown). FMLP treatment slightly, but not significantly, augmented the IL-1-induced prolongation of PMN
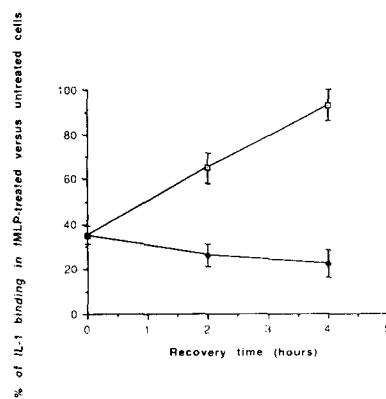
Figure 5. Reexpression of IL-1R on FMLP-treated PMN. PMN were treated with or without FMLP (10^{-7} M, 30 min). After washing (time of recovery = 0), cells were incubated for various periods of time in RPMI 1640 with 10% FCS medium in the presence or absence of 10 μg/ml CH. Next, cells were examined for IL-1 binding. Data are expressed as the percentage of IL-1 binding with respect to medium-treated cells at each time point (mean, with range, of two experiments). (□), medium; (◆), cycloheximide.

survival (4), with 59 ± 12% surviving cells at 48 h compared to 49 ± 3% of cells exposed to IL-1 alone (mean ± SD of three experiments).

Discussion

The results presented here show that molecules representative of different classes of chemotactic agents, including formyl peptides, complement components, lipid metabolites, and chemokines, cause rapid loss of IL-1R from human PMN. Receptors for the chemoattractants considered in the present study are seven transmembrane-spanning proteins coupled to G proteins. By and large, increases in intracellular free Ca^{++} and PKC activation are early events associated with chemoattractant activation of PMN (19). The PKC activator PMA and the Ca^{++} ionophore A23187 mimicked the action of chemoattractants in causing rapid and extensive loss of IL-1 binding in PMN.

Loss of IL-1 binding capacity in chemoattractant-treated PMN was due to the disappearance of the receptors on the cell surface, and it was associated with the release of a 45-kD IL-1–binding molecule. The rate of IL-1R internalization was not affected by FMLP. The 45-kD IL-1–binding molecule was identified as the IL-1 decoy RII, which represents the main IL-1R in myelomonocytic cells and an anti-IL-1 pathway (2, 4, 11).

Induction of IL-1 decoy RII release by chemoattractants was considerably selective, in that integral type I membrane (MHC class I, CD18, IL-8R) and phosphatidyl inositol–linked proteins (CD16) were unaffected under these conditions. Under very similar experimental conditions, various PMN activators, including chemoattractants, have been reported to cause rapid release of TNFR, and, under certain conditions, TNFR shedding is preferential for the p75 form (12, 20). In the present study, we could not identify induction of release of the signal-transducing IL-1RI, in that, by size and mAb blocking, only the IL-1 decoy RII was present in the supernatants of chemoattractant-treated PMN. Since the IL-1RI is present in minute amounts on the surface of PMN (1, 2, 4), some release cannot be completely excluded at present. However, it should be noted that FMLP-treated PMN retain responsiveness to IL-1 in terms of weak cytokine production and prolongation of survival. It was previously demonstrated that the response of myelomonocytic cells to IL-1 is mediated by the signal-transducing IL-1RI (3, 4). Thus, the maintenance of IL-1 responsiveness in FMLP-treated myelomonocytic cells suggests, at minimum, that sufficient numbers of the IL-1RI are retained in the face of loss of 70–80% IL-1 decoy RII. By and large, these data, showing IL-1 responsiveness after shedding of 70–80% IL-1 decoy RII, are consistent with the viewpoint that this molecule is not involved in signal transduction in myelomonocytic cells.

We previously found that certain cytokines (IL-4, IL-13) and glucocorticoid hormones augmented IL-1 decoy RII in PMN, in a transcription- and protein synthesis–dependent way (4, 10, 11). Up-regulation of newly synthesized receptor

on PMN surface was paralleled by the release of soluble IL-1 decoy RII from PMN cultivated for a relatively long period of time (>12 h) (4, 10, 11). Others found a soluble IL-1 decoy RII in biological fluids or supernatants from mitogen-activated mononuclear cells (5–9). Here we describe a different mechanism of release of the IL-1 decoy RII, in which shedding of preformed IL-1 decoy RII from the PMN surface is obtained in seconds and does not require protein synthesis. It is interesting to note that the above-mentioned stimuli were poorly effective in inducing the rapid release of IL-1 decoy RII (our unpublished data), suggesting that at least two different levels of control, activated by different classes of external signals, exist.

The biochemical process involved in rapid release of IL-1 decoy RII from activated PMN is still poorly understood. In the limited series of protease inhibitors tested here, certain molecules could, at least partially, prevent FMLP-induced release of IL-1 decoy RII. Interestingly, the protease inhibitor α_1-antitrypsin was the most effective one. Since we found that intact protein synthesis is not required for FMLP-induced release of IL-1 decoy RII, these data favor the possibility that a preformed proteolytic enzyme is involved. Rapid release of TNFR was not inhibited by PMSF or by other protease inhibitors (12), possibly suggesting that mechanisms involved in rapid release of IL-1R and TNFR from FMLP-activated PMN could be different.

In addition to migration, chemoattractants cause a complex spectrum of responses in PMN, including the oxidative burst and enzyme release. Typically, myelomonocytic cells exhibit different dose–response curves in terms of migration versus other functions. Chemotaxis dose–response curves are usually bell shaped, with ED_{50} considerably lower (usually 1 log) than those required, for instance, for oxidative burst. In the present study, we found that, with FMLP, the ED_{50} and peak concentrations for chemotaxis and for IL-1 decoy RII release were identical, though IL-1 decoy RII shedding did not decrease substantially at supraoptimal agonist concentrations. In the same vein, FMLP-induced release of IL-1 decoy RII was extremely rapid, reaching 50% of maximum at 30 s, the earliest measurable time point. The effective concentration range, similar to that required for chemotaxis, and the time frame (seconds) of chemoattractant-induced IL-1 decoy RII release are consistent with the view that, in the multistep process of PMN recruitment, IL-1 decoy RII shedding is an early event, already occurring in the blood compartment.

The in vivo pathophysiological significance of chemoattractant-induced IL-1 decoy RII and TNFR (12) release is a matter of speculation. Administration of chemoattractants in the circulation causes inhibition of local inflammatory reactions elicited by various agents, including IL-1 (21–23). Rapid release of IL-1 decoy RII and TNFR might contribute to the seemingly paradoxical antiinflammatory activity of systemically administered chemoattractants. Chemoattractant-induced release in the circulation of IL-1 decoy RII, an early event in recruitment, may provide a safeguard mechanism against IL-1 leaking from inflamed tissues.

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