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Bacterial-Lipopolysaccharide-Induced Release of Lactoferrin from Human Polymorphonuclear Leukocytes: Role of Monocyte-Derived Tumor Necrosis Factor α

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We have examined the role played by human peripheral blood monocytes in mediating responses of human polymorphonuclear leukocytes (PMN) to bacterial lipopolysaccharide (LPS) in vitro. When incubated with *Salmonella typhimurium* LPS at 37°C, human PMN suspended in serum-free buffer released the specific granule constituent lactoferrin into the surrounding medium. Release of lactoferrin from PMN varied with the concentration of LPS (1 to 1,000 ng/ml) as well as with the duration of incubation (2 to 60 min) and was not accompanied by significant release of the cytoplasmic enzyme lactate dehydrogenase. LPS-induced release of lactoferrin from PMN was augmented significantly when cell suspensions were supplemented with additional monocytes and lymphocytes. Only monocytes, however, secreted significant amounts of lactoferrin-releasing activity (in a time- and concentration-dependent manner) when incubated separately with LPS. Lactoferrin-releasing activity was heat (80°C for 15 min) labile, eluted after chromatography on Sephadex G-100 with an apparent molecular weight of approximately 60,000, and was inhibited by antibodies to tumor necrosis factor α. Thus, LPS-induced noncytotoxic release of lactoferrin from human PMN suspended in serum-free buffer is mediated, at least in part, by tumor necrosis factor α derived from contaminating monocytes.

Effects of bacterial lipopolysaccharide (LPS) on polymorphonuclear leukocytes (PMN) have been studied extensively (reviewed in reference 25). LPS has been shown to inhibit PMN chemotaxis, as well as to augment PMN adhesiveness, hexose monophosphate shunt activity, oxygen radical production, and release of specific granule constituents (i.e., degranulation) (3, 5, 7, 13, 14, 24, 31, 33). Results of some studies suggest that these phenomena may not occur as a consequence of direct effects of LPS on PMN. Dahinden and Fehr (6), for example, were unable to demonstrate any augmentation of metabolic activity or release of granule constituents when LPS was added to human PMN in suspension. When LPS was added to PMN that were adherent to plastic petri dishes, however, the cells generated superoxide anion radicals, increased their hexose monophosphate shunt activity, and released granule enzymes. Guthrie et al. (12) also found no direct stimulatory effects of LPS on PMN oxidative metabolism. However, these investigators did demonstrate that pretreatment of PMN with LPS for at least 30 min augmented production of oxygen radicals when cells subsequently were exposed to other stimuli.

In contrast to what has been observed in experiments with peripheral blood PMN, there is ample evidence that LPS directly stimulates peripheral blood monocytes. Since LPS provokes synthesis and release by human monocytes of products (e.g., tumor necrosis factor α [TNF-α]) that can directly stimulate PMN (1, 9, 18, 21, 22, 28), we examined the role played by monocytes in mediating responses of PMN to LPS in vitro. We found that, when human PMN in suspension were exposed to LPS, the cells released significant amounts of the specific granule constituent lactoferrin. This response to LPS was augmented markedly, however, when the number of monocytes in the PMN suspensions was increased. Furthermore, isolated monocytes released significant amounts of lactoferrin-releasing activity (with properties similar to those of TNF-α) when incubated separately with LPS.

MATERIALS AND METHODS

Preparation of leukocyte suspensions. Human PMN were isolated from venous blood (anticoagulated with acid-citrate-dextrose) by centrifugation on Hypaque-Ficoll followed by dextran sedimentation (4). Contaminating erythrocytes were removed by hypotonic lysis. The leukocytes were then washed twice with phosphate (10 mM)-buffered 140 mM NaCl, pH 7.4 (PBS), and were suspended in the same buffer at a density of 10×10^6 PMN per ml. Final cell suspensions usually contained 98 to 99% PMN, <1.5% monocytes, <1.0% lymphocytes, and a platelet/leukocyte ratio of <2:1. Differential counts were determined by examining 200 cells in smears stained with Diff-Quik (American Scientific Products, Sunnyvale, Calif.).

Mononuclear leukocytes from the Hypaque-Ficoll gradients were washed twice with PBS and, for most experiments, were suspended in PBS at a density sufficient to yield 10^6 monocytes per ml. Suspensions usually contained 20 to 30% monocytes, 70 to 80% lymphocytes, <1% PMN, and a platelet/leukocyte ratio of approximately 20:1. Monocytes were quantified by examining smears stained for nonspecific esterase activity, using α-naphthyl butyrate (Sigma Chemical Co., St. Louis, Mo.) as substrate (16). For some experiments, monocytes were purified further by allowing mononuclear leukocytes to adhere to sterile plastic petri dishes (35-mm diameter) (Falcon; Becton Dickinson Labware, Oxnard, Calif.) for 90 min at 37°C in buffer containing 10% (vol/vol) fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.). The dishes were then washed three times with warm (37°C) buffer to remove nonadherent cells (mostly

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lymphocytes). A final incubation at 4°C for 30 min with buffer containing 25 mM EDTA caused most adherent cells (primarily monocytes) to become detached. Monocytes were recovered after centrifugation (450 × *g* for 10 min), washed twice with PBS, and finally suspended in PBS. Suspensions of monocytes prepared in this manner usually contained <10% esterase-negative cells and no detectable platelets. Monocyte suspensions containing <20% esterase-negative cells also were prepared by the method of Recalde (26), using hypertonic NaCl.

LPS. LPS from *Salmonella typhimurium* Rbh2H, isolated and purified as described previously (8), was generously provided by Martti Vaara, National Public Health Institute, Helsinki, Finland. Lyophilized LPS was dissolved in distilled water at a concentration of 1.0 mg/ml. LPS subsequently was diluted 1:10 in PBS and then stored in aliquots at −20°C. To control for varying degrees of aggregation, stock solutions of LPS were thawed and then sonicated for 15 s before being diluted further.

TNF-α. Recombinant human TNF-α (2×10^7 U/mg) was obtained from Genentech, Inc., South San Francisco, Calif. The immunoglobulin G (IgG) fraction of neutralizing polyclonal rabbit antibodies to human TNF-α (10^4 neutralizing units per mg) as well as the IgG fraction of preimmune rabbit serum were purchased from Endogen, Inc., Boston, Mass. The activity of TNF-α was measured as described previously (1).

Measurements of lactoferrin release. PMN (10^6 cells per ml) were incubated at 37°C for 2 to 60 min with LPS, diluted supernatants of LPS-treated mononuclear cells, or recombinant TNF-α. All reagents were prewarmed, and incubations were terminated by placing tubes containing reaction mixtures in an ice-water bath. An equal volume of ice-cold PBS was then added, and reaction mixtures were centrifuged in the cold (4°C) at 450 × *g* for 10 min. Cell-free supernatants were either stored at −20°C for subsequent measurements of lactoferrin or assayed directly for lactoferrin and lactate dehydrogenase activity (see below). In some experiments, PMN were incubated at 37°C for 2 to 60 min with the synthetic chemotactic peptide *N*-formyl-methionyl-leucyl-phenylalanine (FMLP) (1.0 μM; Sigma Chemical Co.).

Lactoferrin was measured in cell-free supernatants of reaction mixtures, using minor modifications of a previously described enzyme-linked immunoassay (23). Briefly, microtiter plates (Immulon I; Dynatech Laboratories, Inc., Alexandria, Va.) were coated overnight at 4°C with rabbit anti-human lactoferrin (Cooper Biomedical, Inc., Malvern, Pa.) and then washed four times with 1.0% (vol/vol) Tween 20 (Sigma Chemical Co.) in PBS (PBS-Tween). Plates were then incubated at 37°C for 90 min with lactoferrin standards (Calbiochem-Behring, La Jolla, Calif.) and with supernatants of reaction mixtures that had been diluted either 1:10 or 1:30 in PBS containing 1.0% (wt/vol) bovine serum albumin (Sigma Chemical Co.) (PBS-bovine serum albumin). After the plates were washed again (six times) with PBS-Tween, peroxidase-conjugated rabbit anti-human lactoferrin (diluted 1:400 in PBS-bovine serum albumin) (Cooper Biomedical, Inc.) was added. Plates were incubated at 37°C for an additional 90 min and then washed six times with PBS-Tween. Peroxidase substrate (2,2-azino-di[3-ethyl-benzthiazoline] sulfonic acid; Zymed Laboratories, Inc., South San Francisco, Calif.) in citrate buffer, pH 4.2, was then added and allowed to incubate for 10 min at room temperature before the reactions were stopped with 2.0 mM sodium azide. A_{414} was measured with a Titertek Multiskan MCC enzyme-linked immunosorbent assay reader (Flow Laboratories, Inc., McLean, Va.). Results are expressed as nanograms of lactoferrin released per 10^6 PMN. All values were corrected for background levels of lactoferrin in the medium surrounding suspended PMN prior to incubation with either buffer alone or LPS.

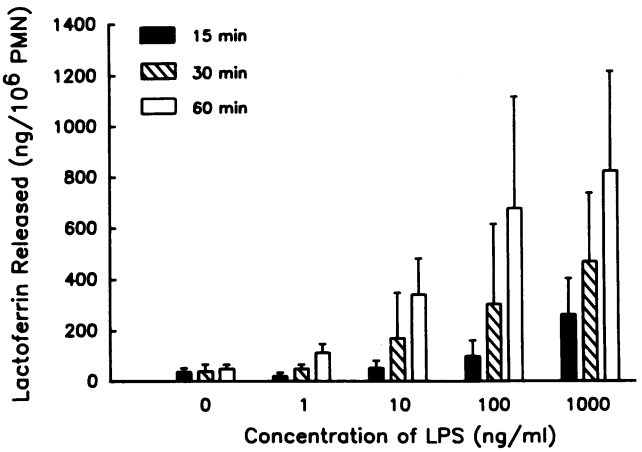

FIG. 1. Release of lactoferrin from human PMN (10^6 cells per ml) incubated with LPS (0 to 1,000 ng/ml) for 15 (solid bars), 30 (hatched bars), or 60 (open bars) min. Results represent mean values (± standard deviation) obtained in five experiments performed in duplicate.

Release of the cytoplasmic enzyme lactate dehydrogenase was measured as described previously (32) and used as an indicator of cell viability (11). Lactate dehydrogenase activity released into supernatants of reaction mixtures is expressed as a percentage of the total activity recoverable from simultaneously run duplicate reaction mixtures to which the detergent Triton X-100 (0.2%, vol/vol) (Sigma Chemical Co.) had been added. All values were corrected for background enzyme activity in medium blanks.

Molecular sieve chromatography. Aliquots (1.0 ml) of supernatants of LPS-treated monocytes were applied to a calibrated column (1.0 by 40 cm) of Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, N.J.) and were eluted with PBS, pH 7.4, at at flow rate of 3.0 ml/h. Lactoferrin-releasing activity was measured in undiluted aliquots of 1.0-ml fractions directly, after heating at 80°C for 15 min, or after treatment at room temperature for 3 min with antibodies to human TNF-α (10 to 40 μg/ml).

RESULTS

Release of lactoferrin from LPS-treated PMN. When incubated with *S. typhimurium* LPS at 37°C, human PMN in suspension released the specific granule constituent lactoferrin into the surrounding medium. Release of lactoferrin from PMN varied with the concentration of LPS as well as with the duration of incubation (Fig. 1) and was not accompanied by significant release of the cytoplasmic enzyme lactate dehydrogenase. For example, the percentages of total lactate dehydrogenase released by PMN that had been incubated for 60 min with buffer alone, with 100 ng of LPS per ml, or with 1,000 ng of LPS per ml were nearly identical (1.8 ± 0.2, 2.1 ± 0.3, and 2.5 ± 0.5, respectively; $n = 5$). Thus, release of lactoferrin from PMN exposed to LPS occurred as a consequence of granule exocytosis rather than as a result of cell death.

Shown in Fig. 2 are the results of a representative exper-

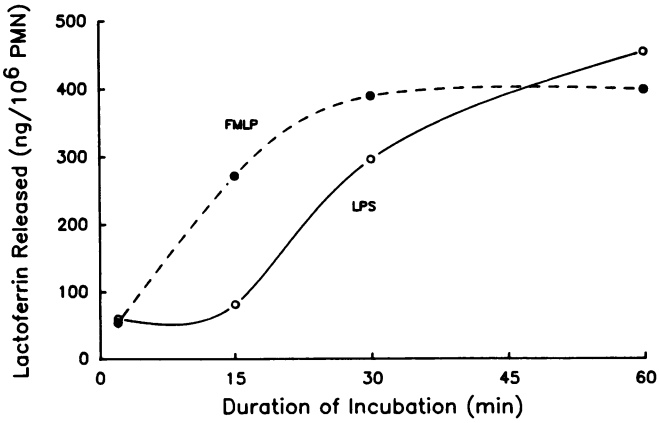

FIG. 2. Kinetics of lactoferrin release from human PMN (10^6 cells per ml) incubated for 2 to 60 min with either 1.0 μM FMLP (●) or 100 ng of LPS per ml (○). Results are average values obtained in one experiment performed in duplicate and are representative of results obtained in two additional experiments.

iment demonstrating that the kinetics of lactoferrin release from PMN exposed to LPS differ from those of lactoferrin release from PMN exposed to the chemotactic peptide FMLP. Whereas 1.0 μM FMLP provoked maximal release of lactoferrin during the first 30 min of incubation, PMN exposed to 100 ng of LPS per ml continued to release lactoferrin even after 60 min of incubation (not shown). The total amount of lactoferrin released from PMN after 60 min of incubation with 100 ng of LPS per ml was comparable to, or even exceeded, the amount released from PMN exposed to 1.0 μM FMLP. Nevertheless, significant release of lactoferrin from PMN exposed to 100 ng of LPS per ml was not detected during the first 15 min of incubation.

Effects of mononuclear leukocytes on LPS-induced release of lactoferrin from human PMN. The lag period that we observed, as well as the relatively slow and prolonged release of lactoferrin from human PMN incubated with LPS (Fig. 2), suggested the possibility that the effect of LPS on PMN is indirect and is mediated by the small number of monocytes that routinely contaminate suspensions of PMN prepared by centrifugation on Hypaque-Ficoll. To test this possibility, we examined effects on LPS-induced lactoferrin release of supplementing PMN suspensions with mononuclear leukocytes. The addition of even small numbers of monocytes (e.g., 0.02×10^6 cells per ml) to PMN (10^6 cells per ml) in suspension enhanced release of lactoferrin when the cells subsequently were incubated with 100 ng of LPS per ml (Table 1). In experiments not shown, we documented

TABLE 1. Effects of added monocytes on LPS-induced release of lactoferrin from PMN[a]

Monocytes added to PMN suspensions (10^6/ml)	Lactoferrin (ng/10^6 PMN) released by:	
	PMN alone	PMN + 100 ng of LPS per ml
None	55 ± 18	280 ± 26
0.02	74 ± 12	340 ± 31
0.06	61 ± 10	401 ± 27
0.2	66 ± 15	462 ± 44

[a] Suspensions of PMN (10^6 cells per ml) were incubated for 60 min at 37°C with or without LPS (100 ng/ml) or added monocytes. Results represent mean values (± standard deviation) obtained in three experiments performed in duplicate.

TABLE 2. Effects of supernatants from LPS-treated mononuclear leukocytes on release of lactoferrin from PMN[a]

PMN incubated with:	Lactoferrin released (ng/10^6 PMN)
Buffer alone	45 ± 11
LPS (50 ng/ml)	117 ± 21
Supernatants of mononuclear leukocytes incubated with 100 ng of LPS per ml (diluted 1:1)	201 ± 28

[a] Suspensions of PMN (10^6 cells per ml) were incubated for 60 min at 37°C with buffer alone, LPS (50 ng/ml), or supernatants (diluted 1:1) of mononuclear leukocytes (10^6 monocytes per ml) that had been incubated for 120 min at 37°C with LPS (100 ng/ml). Results represent mean values (± standard deviation) obtained in three experiments performed in duplicate.

that lactoferrin was not released by mononuclear leukocytes that were incubated either alone or with LPS.

Effects of supernatants from LPS-treated mononuclear leukocytes on release of lactoferrin from PMN. Considering the observations described above, we next examined whether the enhancing effect of mononuclear leukocytes on LPS-induced lactoferrin release from PMN was mediated by a factor (or factors) derived from monocytes. In initial experiments, suspensions of mononuclear leukocytes (containing 10^6 monocytes per ml) were incubated with 100 ng of LPS per ml for 120 min. Cell-free supernatants of these reaction mixtures (diluted 1:1 with buffer) were then added to suspensions of PMN (10^6 cells per ml). After incubation at 37°C for an additional 60 min, lactoferrin release was quantified. Supernatants of mononuclear leukocytes that had been incubated with 100 ng of LPS per ml provoked significantly greater release of lactoferrin from PMN than did LPS alone (Table 2). As was the case with PMN exposed to LPS alone, lactoferrin release in response to supernatants of LPS-treated mononuclear leukocytes was not accompanied by significant release of the cytoplasmic enzyme lactate dehydrogenase (data not shown).

Lactoferrin-releasing activity in supernatants of mononuclear leukocytes that had been incubated with LPS varied with the concentration of LPS and with the duration of incubation (Fig. 3 and 4). Lactoferrin-releasing activity was

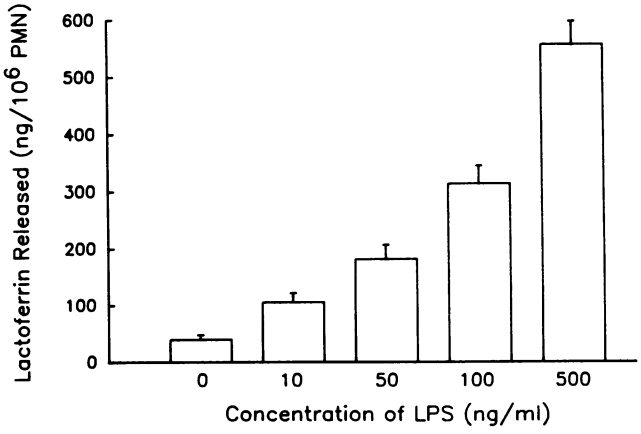

FIG. 3. Generation of lactoferrin-releasing activity by mononuclear leukocytes (5×10^6 monocytes and 20×10^6 lymphocytes per ml) incubated for 60 min with LPS (0 to 500 ng/ml). Aliquots (0.5 ml) of supernatants of LPS-treated mononuclear leukocytes were incubated with an equal volume of PMN (2×10^6 cells per ml) for 60 min at 37°C. Results are mean values (± standard deviation) obtained in three experiments.

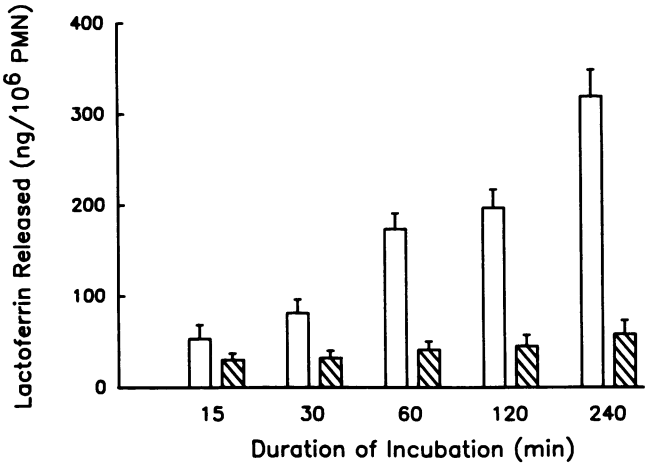

FIG. 4. Generation of lactoferrin-releasing activity by mononuclear leukocytes (5×10^6 monocytes and 20×10^6 lymphocytes per ml) incubated either with (open bars) or without (hatched bars) 100 ng of LPS per ml for 15 to 240 min. Aliquots (0.5 ml) of supernatants of LPS-treated mononuclear leukocytes were incubated with an equal volume of PMN (2×10^6 cells per ml) for 60 min at 37°C. Results represent mean values (± standard deviation) obtained in three experiments.

detectable in supernatants collected after incubating mononuclear leukocytes (5×10^6 monocytes per ml) with 100 ng of LPS per ml for only 15 min and increased progressively as the duration of incubation was increased. Supernatants of mononuclear leukocytes that had been incubated in the absence of LPS caused only negligible release of lactoferrin from PMN (Fig. 4) and did not significantly augment release of lactoferrin provoked by LPS (data not shown).

Having established that supernatants of LPS-treated mononuclear leukocytes stimulate human PMN to release lactoferrin, we next sought to determine whether the lactoferrin-releasing activity was derived from monocytes, lymphocytes, or both. Shown in Table 3 are the results of representative experiments in which we measured lactoferrin-releasing activity in supernatants prepared by incubating suspensions of either mixed mononuclear leukocytes (from Hypaque-Ficoll gradients) or purified monocytes with 100 ng of LPS per ml for 120 min. When suspensions were adjusted to contain equivalent numbers of esterase-positive cells, incubation of purified monocytes and mixed mononuclear leukocytes with LPS yielded nearly identical amounts of lactoferrin-releasing activity. Furthermore, only when the number of contaminating monocytes was increased did supernatants of LPS-treated lymphocyte suspensions exhibit significant lactoferrin-releasing activity (data not shown).

Characterization of lactoferrin-releasing activity. Chromatography of supernatants of LPS-treated monocytes on Sephadex G-100 yielded two major peaks of lactoferrin-releasing activity, which eluted with apparent molecular weights of approximately 60,000 and <3,000 (Fig. 5). The <3,000-molecular-weight peak of activity also was observed after chromatography of LPS alone, but neither peak was observed after chromatography of supernatants of untreated monocytes (not shown).

Since the <3,000 peak of lactoferrin-releasing activity observed after chromatography of supernatants of LPS-treated monocytes most likely contained residual LPS, attempts were made to further characterize the activity in the higher-molecular-weight peak. Lactoferrin-releasing activity in fractions from the 60,000-molecular-weight peak was completely destroyed by heating at 80°C for 15 min (Table 4). In contrast, lactoferrin-releasing activity in fractions from the <3,000-molecular-weight peak was unaffected by heating at 80°C for 15 min (not shown).

Lactoferrin-releasing activity in the 60,000-molecular-weight peak was inhibited by treatment with the IgG fraction of polyclonal rabbit antibodies to human TNF-α (but not by treatment with an equivalent amount of preimmune IgG) (Table 4). Finally, recombinant human TNF-α was found to be capable of directly provoking release of lactoferrin from human PMN (Table 4).

TABLE 3. Effects of supernatants from LPS-treated monocytes on release of lactoferrin from PMN[a]

Supernatants prepared from:			Lactoferrin released (ng/10⁶ PMN)
Monocytes (10^6)	Lymphocytes (10^6)	LPS (ng)	
		100	131
0.9	6.2[b]		55
0.9	6.2[b]	100	242
1.0	0.01[c]		53
1.0	0.01[c]	100	268
		100	180
3.5	8.5[b]		78
3.5	8.5[b]	100	412
3.5	0.8[d]		66
3.5	0.8[d]	100	558

[a] Suspensions of PMN (10^6 cells per ml) were incubated for 60 min at 37°C with supernatants of 1.0-ml reaction mixtures containing the indicated numbers of monocytes and lymphocytes that had been incubated for 120 min at 37°C with and without LPS (100 ng/ml). Results represent average values obtained in two separate experiments performed in duplicate.

[b] Mononuclear cells recovered from Hypaque-Ficoll gradients.

[c] Monocytes purified adherence.

[d] Monocytes purified after treatment with hypertonic NaCl (26).

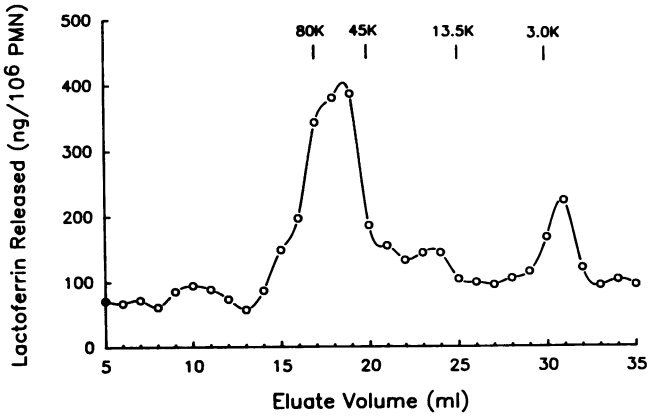

FIG. 5. Elution profile of lactoferrin-releasing activity after chromatography on Sephadex G-100 of a supernatant prepared by incubating mononuclear leukocytes (2.0×10^6 monocytes per ml) with 100 ng of LPS per ml for 120 min at 37°C. Aliquots (0.5 ml) of 1.0-ml fractions were incubated with an equal volume of PMN (2×10^6 cells per ml) for 60 min at 37°C. Molecular weights of markers used to calibrate the column are shown. Results are from a single experiment and are representative of results obtained in two additional experiments.

TABLE 4. Properties of monocyte-derived lactoferrin-releasing activity[a]

PMN incubated with:	Lactoferrin released (ng/10^6 PMN)
Buffer alone	49
Sephadex G-100 fractions[b]	230
Heated at 80°C for 15 min	55
+ Anti-TNF-α IgG (10 μg/ml)[c]	117
+ Anti-TNF-α IgG (40 μg/ml)[c]	81
+ Preimmune IgG (40 μg/ml)[c]	239
Recombinant TNF-α (25 U)	157
50 U	186
50 U + anti-TNF-α IgG (10 μg/ml)[c]	83
50 U + preimmune IgG (10 μg/ml)[c]	194

[a] Suspensions of PMN (10^6 cells per ml) were incubated for 60 min at 37°C with buffer alone, Sephadex G-100 fractions (diluted 1:1), or recombinant TNF-α. Results represent values obtained in one experiment performed in duplicate and are representative of results obtained in two other experiments.

[b] Pooled fractions from the peak of lactoferrin-releasing activity which eluted with an apparent molecular weight of approximately 60,000.

[c] Sephadex G-100 fractions and TNF-α were mixed with either anti-TNF-α IgG or an equivalent amount of preimmune IgG for 3 min before being added to PMN.

DISCUSSION

Results of the studies described in this report indicate that LPS-induced noncytotoxic release of the specific granule constituent lactoferrin from human PMN in suspension is mediated largely, or perhaps entirely, by one or more factors derived from monocytes. Whereas LPS acted in a concentration- and time-dependent manner to provoke release of lactoferrin from PMN (Fig. 1 and 2), degranulation was augmented significantly when cell suspensions were supplemented with additional monocytes and lymphocytes (Table 1). Only monocytes, however, secreted significant amounts of lactoferrin-releasing activity when incubated separately with LPS (Fig. 3 and 4; Tables 2 and 3).

All of the experiments described in this report were performed with PMN suspended in buffer without added serum. Under these conditions, LPS reportedly provokes changes in PMN shape (13) and "primes" PMN for subsequent responses to other stimuli (12), but does not cause PMN to degranulate or to generate reactive oxygen metabolites (6, 7, 19, 33). However, in experiments with PMN that were adherent to plastic surfaces or with PMN suspended in media containing fresh serum, LPS has been found to be capable of provoking release of granule constituents and of enhancing PMN oxidative metabolism (3, 5–7, 31, 33). To explain these observations, it probably is significant that human PMN secrete contents of their specific granules and produce superoxide anion radicals when they either adhere to plastic surfaces in the absence of other stimuli (15, 34) or are exposed to complement (C5)-derived peptides generated when fresh serum is incubated with LPS (10, 11, 33).

LPS-induced release of lactoferrin from PMN suspended in serum-free buffer appears to be dependent upon the presence of monocytes. Even the small number of monocytes that routinely contaminate suspensions of "purified" PMN appear to be sufficient to mediate LPS-induced secretion of lactoferrin. It also is possible that LPS-induced changes in PMN shape or LPS-induced "priming" of PMN are mediated, at least in part, by products of monocytes.

Although we did not determine precisely the mechanism(s) by which monocytes mediate lactoferrin release from PMN, our data suggest a role for TNF-α. First, chromatography of supernatants of LPS-treated monocytes on Sephadex G-100 yielded a major peak of lactoferrin-releasing activity which eluted with an apparent molecular weight of approximately 60,000 (Fig. 5). It should be noted that whereas TNF-α has a molecular weight of approximately 17,000, it spontaneously forms higher-molecular-weight biologically active oligomers (1). Indeed, a recent report suggests that the most active form of human TNF-α is a trimer with an apparent molecular weight of approximately 55,000 (29). Although chromatography of supernatants of LPS-treated monocytes also yielded a peak of lactoferrin-releasing activity which eluted with an apparent molecular weight of <3,000, an identical peak was observed following chromatography of LPS alone (not shown). Furthermore, whereas the activity in the 60,000-molecular-weight peak was destroyed by heating at 80°C for 15 min (a property of TNF-α) (27), the activity in the <3,000-molecular-weight peak resisted such treatment (a property of LPS). Second, lactoferrin-releasing activity in the 60,000-molecular-weight peak was inhibited by treatment with the IgG fraction of polyclonal rabbit antibodies to human TNF-α (Table 4). Finally, recombinant human TNF-α directly provoked release of lactoferrin from PMN (Table 4).

TNF-α is synthesized and released into the surrounding medium when human monocytes as incubated with LPS (1, 21) and has been reported previously to be capable of provoking selective release from normal human PMN of specific granule contents (18). It should be emphasized, however, that our results do not exclude the possibility that products of LPS-stimulated monocytes other than TNF-α provoke release of lactoferrin from human PMN. For example, when incubated with LPS, human monocytes also synthesize and release interleukin-1 (2), as well as a factor (distinct from interleukin-1) that stimulates human PMN to enhance their oxidative metabolism (17). It is noteworthy that, although interleukin-1 isolated from supernatants of activated monocytes has been reported to be capable of causing human PMN to degranulate (19, 30), recombinant interleukin-1 apparently does not exhibit this activity (20).

Whatever the precise mediator may be, results of the studies described in this report document that monocytes contribute significantly to some, if not all, responses of human PMN to LPS. Consequently, contamination of PMN suspensions with even very small numbers of monocytes cannot be ignored.

ACKNOWLEDGMENTS

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