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Immunology

# Induction of expression of monocyte interleukin 1 by Hageman factor (factor XII)

(cytokine/plasma contact system/mononuclear cell)

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ABSTRACT **The results reported here indicate that activated species of Hageman factor (HF, factor XII), a protein that mediates blood clotting, fibrinolysis, and activation of the complement cascade, induce elaboration of interleukin 1 (IL-1) by human monocytes. Augmentation of IL-1 production in mononuclear cell cultures was observed when HF was present along with lipopolysaccharide (LPS) but was not observed with HF alone. Furthermore, antiserum to HF abrogated the enhancement of IL-1 in cultures containing HF and LPS. Total IL-1 activity, which represents secreted and cell-associated IL-1, was enhanced in LPS-stimulated mononuclear cultures by HF. In the absence of LPS, the initial activation product of HF, $HF_a$, which contains the serine protease enzyme activity and the surface-binding domains of the protein, induced IL-1β protein and mRNA. In the presence of LPS, the enzymatic moiety ($HF_f$), which is also contained in HF and $HF_a$, amplified IL-1 production. Induction and amplification of monocyte IL-1 by HF provides further evidence for establishing a role for HF in the acute-phase reaction and the cellular immune response.**

A role for the plasma proenzyme Hageman factor (HF, factor XII) in the pathophysiology of inflammation is suggested by its apparent activation in acute infections, allograft rejection, and allergic reactions (1). Activation of HF is a first step not only in the initiation of the intrinsic clotting cascade but also in fibrinolysis, generation of kinins, and activation of the classical complement pathway (1, 2). More recently it has been shown that activated HF ($HF_a$) induces aggregation and degranulation of polymorphonuclear granulocytes (3). Furthermore, $HF_a$ decreases the functional expression of Fc receptors on blood monocytes, which are important in immunophagocytosis (4). The effect of HF on other functions of monocyte macrophages has not been reported.

A peptide product of activated monocytes, interleukin 1 (IL-1), is a key mediator of inflammatory responses whose diverse biological activities include induction of fever and the acute-phase proteins, stimulation of endothelial cell procoagulant activity, and enhancement of granulocyte adhesion to endothelial cells (5). Regulation of production of IL-1 has been shown to occur at multiple levels depending on the stimulus used (6, 7). However, many of the same agents that stimulate IL-1 production are also known to activate the plasma contact system through activation of HF (1, 8). For example, *in vitro* adherence of monocytes to surfaces is sufficient to induce IL-1 transcription. Similarly, adherence of HF to glass surfaces activates the molecule (9), and it has been speculated that exposure to damaged endothelium may serve as a physiologic stimulus *in vivo*. Other agents known to activate HF and induce IL-1 production by monocytes include such diverse molecules as urate crystals, sulfatides, dextran sulfate, bacterial lipopolysaccharides (LPSs), and silica particles. The present study was undertaken to assess whether activation of HF has a role in induction of IL-1 in human mononuclear cells. We have found that, upon its activation, HF is a potent inducer of IL-1 expression.

## MATERIALS AND METHODS

**Reagents.** HF (specific activity, 89 units/mg of protein) was purified from human plasma as described (10) and was devoid of other clotting factors and plasminogen. For some experiments, HF and dextran-activated $HF_a$ (11) (Enzyme Research Laboratories, South Bend, IN) were purchased. HF fragment ($HF_f$) was prepared by proteolysis as described by Goldsmith *et al.* (12). All protein preparations were subjected to polyacrylamide gel electrophoresis under reduced and unreduced conditions to ensure molecular integrity. Preparations were tested for endotoxin content using a chromogenic *Limulus* lysate assay (Whittaker Bioproducts). Endotoxin contamination of the preparations used in these experiments ranged from 30 to 100 pg/unit of HF. LPS (*Escherichia coli* F-583) was purchased (Sigma).

**Preparation of Cells.** Heparinized human blood (20 units/ml) was obtained from healthy volunteers. Peripheral blood mononuclear cells (PBMCs) were separated by Ficoll/Hypaque (Pharmacia) sedimentation (13). PBMCs were resuspended at $5 \times 10^6$ per ml in RPMI 1640 (M.A. Bioproducts) containing L-glutamine (2 mM), penicillin (50 units/ml), and streptomycin (50 μg/ml) (complete medium). For generation of cytokines cells were cultured without serum in 24-well plates (Falcon) (1 ml per well). In some experiments, after collection of culture supernatants, cell lysates were prepared by addition of 1 ml of complete medium to each well and three cycles of freeze-thawing of the plate. For analysis of RNA expression, PBMCs were cultured in 50-ml polypropylene conical tubes (10 ml per tube) to minimize adherence-induced IL-1 transcription.

**Cytokine Assays.** The murine thymocyte proliferation assay was used for assessment of IL-1 activity of samples as described (14). Briefly, 6- to 8-wk-old $C_3H/HeJ$ mice were sacrificed, and their thymuses were minced and pressed through a wire sieve. The cells were washed twice in complete medium and suspended at $15 \times 10^6$ per ml in RPMI 1640 supplemented with 20% (vol/vol) fetal calf serum (HyClone) and 0.05 mM 2-mercaptoethanol. The samples to be tested were diluted serially in complete medium. Thymocytes (0.1 ml) and 0.1 ml of serially diluted samples were placed in

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Abbreviations: HF, Hageman factor (factor XII); LPS, lipopolysaccharide; IL, interleukin; PBMC, peripheral blood mononuclear cell.

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triplicate wells in 96-well flat-bottom culture plates and received phytohemagglutinin (1 μg/ml). Cultures were incubated for 3 days at 37°C in a humidified atmosphere with 5% $CO_2$. One microcurie of [$^3$H]thymidine (6.7 Ci/mmol; 1 Ci = 37 GBq) (ICN) was added to each well 8 hr before harvesting the cell cultures. $^3$H incorporation was determined by spectrophotometry. Results were analyzed by the probit method using recombinant IL-1β (Cistron, Boston) as a standard (15). In some experiments IL-1 and IL-6 activities of PBMC supernatants were assessed by ELISA using commercial kits (R & D Systems, Minneapolis).

**Preparation of RNA.** RNA was prepared by the guanidinium cesium method as described (16). After quantification, samples were separated electrophoretically on a 1% agarose/2.2 M formaldehyde gel and transferred to nylon membranes (GIBCO). RNA then was cross-linked to the membranes using UV light (Stratagene). Prehybridization was carried out for 4 hr at 42°C in a solution containing 50% deionized formamide, 1× Denhardt's solution, 2× standard saline citrate (SSC; 1× SSC = 0.15 M NaCl/15 mM sodium citrate), and 0.5% SDS with heat-denatured salmon sperm DNA (100 μg/ml). Hybridization was performed in the same solution to which dextran sulfate was added (15% final concentration) and it contained the *Nde* I–*Eco*RI fragment of the full-length human IL-1β cDNA sequence (gift of P. Lomedico, Hoffman–LaRoche), which was $^{32}$P-labeled by random priming. Filters were washed (1× SSC/0.01% SDS at 55°C for 15 min) and exposed to XAR-5 film (Kodak) at −70°C with an intensifying screen. In some experiments the membranes were stripped and reprobed with a fragment of the cDNA for β-actin (American Type Culture Collection).

## RESULTS

**Effect of HF on IL-1 Production by PBMCs.** LPS has been shown to activate HF directly and in proportion to its lipid A content (17). In initial experiments, PBMCs were cultured with HF at 0.3 unit/ml alone or along with LPS over a concentration range (0.1–10 μg/ml). Control cultures did not receive HF. Augmentation of IL-1 activity was seen in 24-hr culture supernatants that contained HF and LPS, as compared to LPS alone, and was significant with 1 and 10 μg of LPS per ml ($P < 0.05$) (Fig. 1). No IL-1 activity was detected in supernatants prepared in the presence of HF alone, indicating the inability of the proenzyme to induce IL-1 synthesis on its own. Since very small amounts of LPS induce IL-1 activity, these data also confirmed a lack of significant LPS contamination of the HF preparation used in these experiments. To ascertain that the increase in IL-1 activity seen was specifically related to HF and not due to a contaminating factor present in the preparation, in some experiments HF was incubated with a polyclonal antiserum to HF (rabbit, IgG), or control rabbit IgG, for 15 min at 21°C before addition to PBMC cultures. Pretreatment with anti-HF antibody, but not control antibody, reduced the LPS-induced cytokine activity of PBMC supernatants (Table 1).

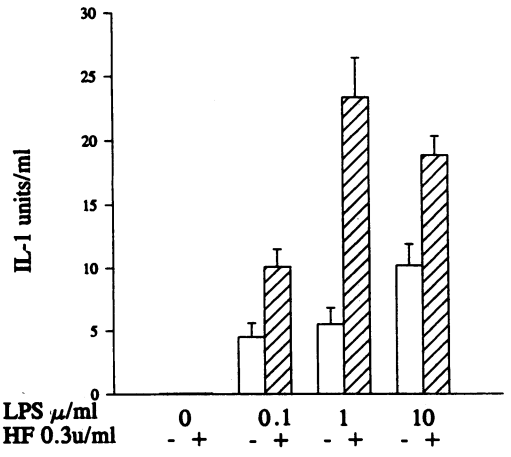


FIG. 1. Augmentation of IL-1 production by HF. PBMCs were cultured in the presence (▨) or absence (□) of HF (0.3 unit/ml) and LPS at the indicated concentration (0–10 μg/ml). After 24 hr IL-1 activity of the cell-free supernatants was assessed in the mouse thymocyte proliferation assay and is expressed as units of IL-1 per ml (mean + SEM of four experiments).

Table 1. Abrogation of HF-induced IL-1 activity in PBMC cultures by antibody to HF

| Exp. | Culture | IL-1 activity, units/ml: Medium | LPS |
|---|---|---|---|
| 1 | Medium | 0.0 | 2.0 |
| | HF | 0.0 | 7.0 |
| | HF + HF Ab | 0.0 | 3.2 |
| | HF + control Ab | 0.0 | 9.1 |
| 2 | Medium | 0.0 | 1.5 |
| | HF | 0.0 | 6.0 |
| | HF + HF Ab | 0.0 | 2.0 |
| | HF + control Ab | 0.0 | 7.5 |

PBMCs were cultured with medium, medium containing HF (0.3 unit/ml), HF pretreated with rabbit antibody to HF (HF Ab), or HF pretreated with rabbit IgG (control Ab). Duplicate cultures received medium or LPS (0.1 μg/ml). IL-1 activity of cell-free supernatants at 24 hr was measured in the mouse thymocyte proliferation assay.

We postulated that the increased IL-1 activity in PBMC supernatants cultured in the presence of HF and LPS might be due to LPS-associated conformational changes in HF that lead to its activation. However, activated HF is a serine protease and bears 40% amino acid homology with plasmin (18), a molecule that has been implicated in the cleavage of membrane-associated IL-1 from IL-1-producing monocytes (19). Thus the observed increase in IL-1 activity of PBMC culture supernatants, prepared in the presence of LPS and HF, might have been due to the more efficient cleavage of IL-1 from cells rather than to increased production of the cytokine. To examine this possibility, PBMCs were cultured in the presence and absence of HF and stimulated with LPS (1 μg/ml) overnight. IL-1 activity was determined in supernatants (secreted) and cell lysates (cell-associated). Fig. 2 shows the results for three donors. In each experiment, total IL-1 (secreted plus cell-associated) activity was greater in

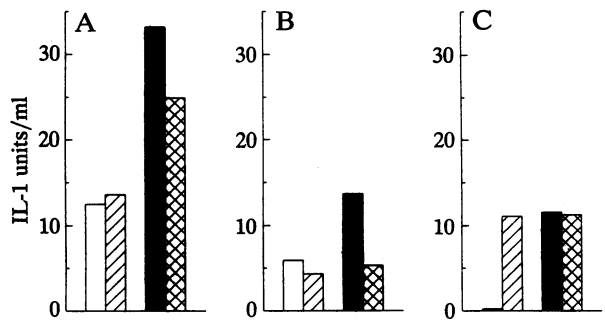


FIG. 2. Effects of HF on secreted and cell-associated IL-1 activity in PBMC cultures. Cells were cultured in the presence of LPS (1 μg/ml) alone or LPS and HF (0.3 unit/ml) for 24 hr. Supernatants were harvested and cell lysates were prepared. Supernatant (secreted) and lysate (cell-associated) IL-1 activity is expressed in units/ml as determined in the mouse thymocyte proliferation assay and is shown for three separate donors (*A*, *B*, and *C*): secreted (□) and cell-associated (▨) IL-1 in cultures with LPS and secreted (■) and cell-associated (▨) IL-1 in cultures with LPS plus HF.

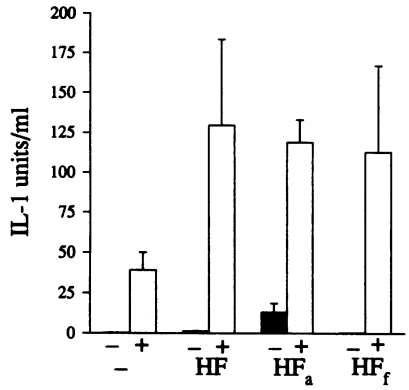


FIG. 3. Induction of IL-1 activity by HF and its activated forms in PBMCs. Cells were cultured with medium, HF, $HF_a$, or $HF_f$ in the absence (■) or presence (□) of LPS at 1 μg/ml. IL-1 activity (units/ml; mean + SEM of three experiments) was assessed using the thymocyte proliferation assay.

cultures in the presence of HF and LPS, as compared to LPS alone. Cell-associated IL-1 was detected at equal or greater concentrations, whereas secreted IL-1 activity was always greater in the presence of HF and LPS. Thus, HF increases total IL-1 production in PBMC cultures in the presence of LPS. The kinetics of the appearance of IL-1 activity was examined in PBMC cultures stimulated with LPS in the presence and absence of HF. The appearance and accumulation of IL-1 under both conditions over time were similar (data not shown), indicating that the rate of processing of IL-1 was not affected by HF.

$HF_a$ is the initial activation product of HF ($M_r$ 80,000) that results either from a conformational change of the zymogen upon binding to negatively charged surfaces or by limited proteolysis. $HF_a$ retains the surface-binding domain ($M_r$ 52,000) and the enzymatic moiety ($M_r$ 28,000) of HF. Further proteolysis of $HF_a$ culminates in release of the enzymatic domain, $HF_f$ ($M_r$ 28,000). To examine the relative ability of the different forms of HF to induce IL-1 activity, we cultured PBMCs with HF, $HF_a$, or $HF_f$ in the presence and absence of LPS at 1 μg/ml. After 24 hr total IL-1 activity was assessed. In the presence of LPS, all three molecules induced IL-1 activity (Fig. 3). Neither HF nor $HF_f$ induced IL-1 in the absence of LPS. $HF_a$ on its own induced some IL-1 production that was about 30% of LPS-stimulated cytokine activity. This activity, however, was about 10% of that of the cultures containing $HF_a$ with LPS.

The thymocyte proliferation assay used in the experiments described reflects the activity of other cytokines, such as monocyte IL-6 and T-cell IL-2. We therefore prepared PBMC supernatants in the presence of $HF_a$ and examined the IL-1 and IL-6 contents of the samples by ELISA for these molecules. The absence of IL-2 activity was confirmed in a bioassay for IL-2 (data not shown). IL-1β and IL-6 were

Table 2. Induction of IL-1 and IL-6 in PBMC culture supernatants by HF

| Culture | IL-1β, ng/ml | IL-6, ng/ml |
|---|---|---|
| Medium | 0 | 0 |
| $HF_a$ | 10.6 | 41.8 |
| $HF_a$ (60°C, 1 hr) | 0 | 0 |
| LPS | 1.2 | 22.7 |
| LPS (60°C, 1 hr) | 1.1 | ND |

PBMCs were cultured in the presence of $HF_a$, $HF_a$ that was heated to 60°C for 1 hr, LPS, and LPS that was heat treated. IL-1β and IL-6 activities were measured by ELISA. The concentration of $HF_a$ was 2.5 μg/ml and LPS was used at 1 μg/ml. ND, not determined.

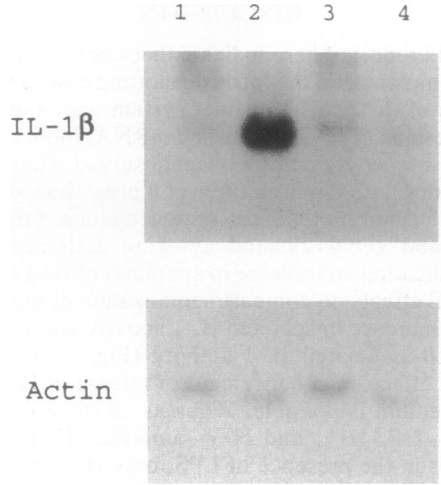


FIG. 4. Induction of IL-1β mRNA by $HF_a$. PBMCs ($10 \times 10^7$) were cultured in polypropylene tubes with medium (lane 1), $HF_a$ (2.5 μg/ml) (lane 2), $HF_a$ heated at 60°C for 1 hr (lane 3), or HF (5 μg/ml) for 3 hr (lane 4). After extraction, the Northern blot was analyzed for IL-1β message and, after stripping, for β-actin message.

induced by $HF_a$ in PBMC cultures (Table 2). Heat denaturation of $HF_a$ at 60°C for 1 hr before addition to cultures abrogated cytokine induction. Heat treatment of LPS did not affect its capability to stimulate cytokine activity in PBMC cultures.

We next examined whether activated HF increased mononuclear cell expression of IL-1β at the transcriptional level. PBMCs were cultured in polypropylene tubes in the presence of HF, $HF_a$, or heat-inactivated (60°C, 1 hr) $HF_a$ for 3 hr at 37°C with occasional agitation. Total RNA was extracted and subjected to Northern blot analysis. $HF_a$ significantly enhanced IL-1β steady-state mRNA expression; this effect was inhibited by heat denaturation of $HF_a$ and was not seen with HF itself (Fig. 4). In a second experiment, HF was activated by culture on glass Petri dishes for 1 hr. In control experiments Petri dishes were pretreated with hexadimethrine bromide (Polybrene), which inhibits surface activation of HF, and then HF or medium was incubated in these dishes for 1 hr. PBMCs were then added and cultures were continued for 3 hr on a rocking platform at 21°C to avoid excessive adherence. An increase in IL-1β expression was seen in cells cultured in the presence of glass-activated HF as compared to cells cultured in glass Petri dishes but without HF (Fig. 5). Polybrene treatment of glass abrogated activation of HF and reduced expression of IL-1β mRNA.

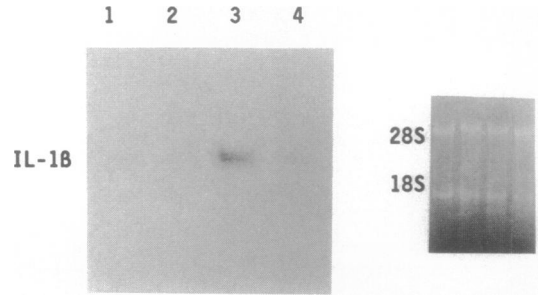


FIG. 5. Induction of IL-1β mRNA by glass-activated HF. PBMCs ($10 \times 10^7$) were cultured on glass Petri dishes that had been pretreated (1 hr, 21°C) with Polybrene (1 μg/ml) (lane 1), HF (25 μg/ml) (lane 3), Polybrene and HF (lane 2), or medium alone (lane 4). After 3 hr at 21°C, RNA was extracted and analyzed by Northern blotting for IL-1β message. Equal loading was assessed on the gel by ethidium bromide staining of the gel.

## DISCUSSION

The results reported here indicate that upon activation, HF, the initial molecule of the intrinsic clotting cascade and other responses of the plasma contact system, can induce monocyte expression of IL-1 protein and mRNA. Induction of IL-1 protein in monocyte cultures was observed when LPS was present along with HF in cultures or if preactivated HF, $HF_a$, was used but not by the inert zymogen alone. Furthermore, secreted and cell-associated cytokine activities were induced, indicating an increase in synthesis of IL-1 rather than differential effects on compartmentalization of the molecule. Since the increase in secreted IL-1 activity was greater than that of cell-associated IL-1 activity (Fig. 2), however, an additional effect on enhancement of release of IL-1 into the culture medium is possible. Whereas all three forms of the molecule—HF, $HF_a$, and $HF_f$—amplified IL-1 activity of monocytes in the presence of LPS, only $HF_a$ could activate IL-1 production independent of LPS. Furthermore, induction of IL-1 mRNA was observed with $HF_a$ and glass-activated HF but not with HF itself. Heat denaturation of $HF_a$ and prevention of surface activation of HF by Polybrene-treated glass reduced IL-1 mRNA expression. Using immunoassays for cytokines, we found IL-1$\beta$ and IL-6 in $HF_a$-induced monocyte culture supernatants. Therefore, the cytokine activity detected in the bioassay (mouse thymocyte proliferation assay) in part represents the presence of IL-6. Since IL-1 induces IL-6 in monocytes (20), however, this finding is not surprising. We have not found direct activation of IL-6 transcription in $HF_a$-stimulated monocyte cultures (data not shown).

Similar to induction of monocyte IL-1, induction of aggregation of neutrophils and modulation of Fc receptors on blood monocytes have been shown with $HF_a$ and not with HF or $HF_f$ (3, 4). Accordingly, one possibility is that subsequent to activation of HF the heavy chain of the molecule, which contains the surface-binding and epidermal growth factor (EGF)-like domain(s) (21), may interact with leukocytes to alter their functions. It is possible that $HF_a$-induced cellular activation is mediated through an interaction of the EGF-like domains, unmasked after a single proteolytic cleavage of HF subsequent to activation, with a putative EGF receptor on the surface of monocytes. On the other hand, the enzymatic moiety contained in all three forms of the molecule—HF, $HF_a$, and $HF_f$—may function as an amplifying factor for generation of IL-1 in LPS-stimulated cell cultures.

Induction of monocyte IL-1 by HF may be important in the pathophysiology of inflammatory and infectious diseases. Activation of HF may initiate or potentiate mononuclear phagocytic production of proinflammatory cytokines. The beneficial or detrimental effect of cytokine amplification may contribute to containment or progression of the inflammatory process.

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