

Chemical Characterization of an Interleukin-1-Inducing Substance Derived from Human Mixed Leukocyte Reactions: IL-1-Inducing Substance Is Not Gamma Interferon

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Lymphocyte products released during the human mixed reaction were studied for their ability to stimulate human monocytes to produce endogenous pyrogen and lymphocyte activating factor. These two biological activities are considered properties of the same molecule, called interleukin-1 (IL-1). In these experiments, physical characteristics such as molecular weight, isoelectric point, and binding to concanavalin A (Con A) sepharose were studied under conditions which excluded bacterial endotoxins. Gel filtration revealed molecular weights of approximately 60 and 25 kD with IL-1-inducing activity. Isoelectric points ranged from 5.9 to 6.3. The IL-1-inducing properties of mixed leukocyte reaction supernates did not bind to Con A sepharose. Recombinant human gamma interferon did not induce IL-1 production under various conditions but rather augmented IL-1 induced by endotoxin. In contrast, the mixed leukocyte reaction results in production of lymphokines which directly stimulate IL-1 production in the absence of endotoxins.

INTRODUCTION

Host responses to various microbial challenges are characterized by a constellation of metabolic, endocrinologic, immunologic, and neurologic changes. Of the many responses which can be measured following the onset of microbial infection, fever ranks as one of the most dramatic. Despite the lack of temperature-measuring devices, fever had been described with remarkable detail for many centuries [1]. The mechanism by which microbial agents produce fever is now well established [2,3]. In brief, phagocytic mononuclear cells are stimulated by the microbe or its products and synthesize a polypeptide called endogenous pyrogen (EP). EP enters the circulation and causes fever by stimulating the synthesis of cyclo-oxygenase products of arachidonic acid metabolism in the hypothalamic area. The most likely product is prostaglandin E₂ (PGE₂) but other PGs may be involved. Although the precise location for EP's action in the hypothalamus remains unclear, the molecule also circulates in the peripheral circulation where it affects other tissues.

The extra-CNS activities of EP have been recently studied. These include the ability of EP to activate PGE₂ synthesis in skeletal muscle tissue, which contributes to the muscle wasting of febrile diseases, and in chondrocytes and synovial fibroblasts, which contribute to joint pain and enzymatic tissue destruction. In addition, EP augments

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lymphocyte responses to antigens and mitogens. This latter property of EP is often called lymphocyte activating factors (LAF). In several laboratories, LAF and EP have not been separated. Homogeneous preparations have both activities and hence it seems likely that these are properties of the same molecule. The term interleukin-1 (IL-1) is used to describe this molecule with EP, LAF, and other inflammatory properties [4].

In this report, studies are presented on the chemical nature of lymphocyte products produced during the mixed leukocyte reaction (MLR) which stimulate human monocytes to synthesize IL-1 [5]. These studies examine endogenously produced IL-1-stimulating factors rather than those of microbial origin. The production of these IL-1-inducing factors in the MLR is due to the antigenic stimulus provided by alloantigens. Thus, the clinical relevance of these experiments can be directly related to fever and other host responses associated with rejection of foreign tissue or graft-versus-host reactions. In a broader sense, these experiments are also related to mechanisms of fever in several immunologically mediated diseases in which the primary response is the stimulation of specifically sensitized T-cells. This model of using a product derived from sensitized T-cells to stimulate IL-1 production was first described by Atkins and co-workers in 1972 [6] and further expanded in subsequent reports [7-9]. In addition, concanavalin A (Con A) has been used to stimulate the production of EP-inducing factors from human lymphocytes [10].

The physical nature of these factors elaborated by T-cells has not been characterized. Of particular importance to these studies is the exquisite sensitivity of monocytes to endotoxin. Picogram/ml amounts of endotoxins stimulate IL-1 production *in vitro* [11]. Hence in characterizing the physical properties of the MLR supernate for its ability to induce IL-1 production, extraordinary care must be used to avoid spurious stimulation by endotoxins. Using specific procedures designed to eliminate the introduction of endotoxins and incorporating polymyxin B, which inhibits the IL-1-inducing property of endotoxin into the monocyte culture media [12], we report here the direct stimulation of human monocyte IL-1 production by factors present in the MLR supernate.

MATERIALS AND METHODS

Materials

All glassware, water, buffers, and gel-filtration media were pyrogen-free. Sand-filtered, reverse osmosis, and ion-exchange water was filtered through a polysulfon Nucleopore filter (exclusion of 300 Daltons) at 25 pounds per square inch (HydroServices, Durham, NC). The endotoxin content of this water was below that detectable in the limulus assay (see the following paragraph). Water used in these experiments for column buffers was autoclaved for 90 minutes. Dialysis tubing (Spectopor, Los Angeles, CA) was rinsed in pyrogen-free water, autoclaved for 30 minutes, and then rinsed again with sterile water. Alpha methyl D-mannoside (grade III) was purchased from Sigma (St. Louis, MO). Human recombinant gamma interferon (IFN) was a gift from Genentech, Inc., South San Francisco, CA. Concanavalin A (Con A) sepharose was purchased from Sigma; phytohemagglutinin (PHA) was obtained from Burroughs Wellcome (Research Triangle, NC).

MLR Supernates

Human peripheral blood mononuclear cells were isolated from two unrelated donors, suspended in minimal essential medium (MEM; Microbiological Associates,

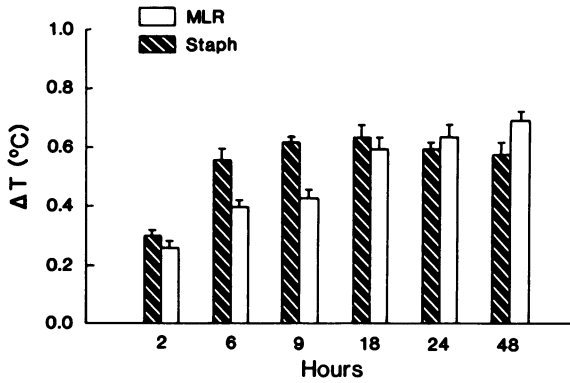


FIG. 1. Time course of EP production. The hatched bars represent EP from monocytes stimulated with heat-killed *S. albus* and the open bars represent the same donor's monocytes stimulated with a 1:10 dilution of a pool of MLR supernate. The results are mean peak fever \pm SEM.

Walkerville, MD), mixed, and incubated in upright polypropylene tubes as described previously [5]. Lots of MEM used in all cultures were screened for endotoxin using the limulus amoebocyte lysate (LAL) test from Associates of Cape Cod (Woods Hole, MA) with a sensitivity of 50 pg/ml. The lot of MEM used in these studies contained less than 50 pg/ml and did not stimulate IL-1 production from human mononuclear cells. MLR supernatant media were cultured for bacterial contamination, centrifuged, pooled, and concentrated tenfold in autoclaved dialysis bags by evaporation. The pools were aliquoted and kept frozen at -70°C until used.

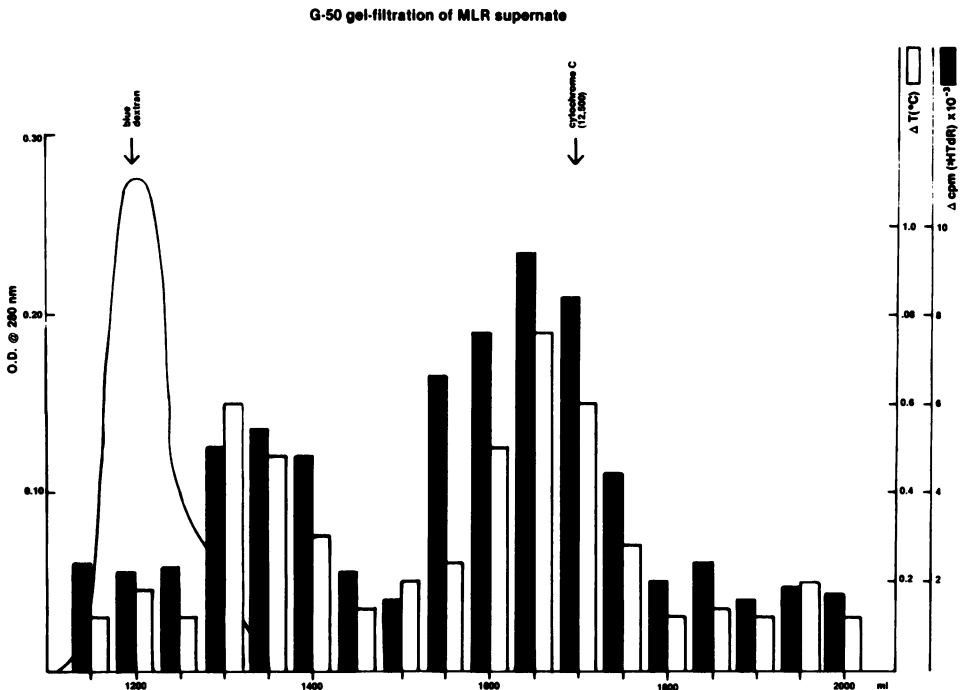


FIG. 2. Gel filtration of concentrated MLR supernates. LAF activity is shown in solid bars and EP activity is shown in open bars. Each fraction pool was assayed directly for intrinsic LAF (at 1:100 dilution) or EP by injecting 5.0 ml/rabbit. The results represent the mean fever peak \pm SEM in three rabbits.

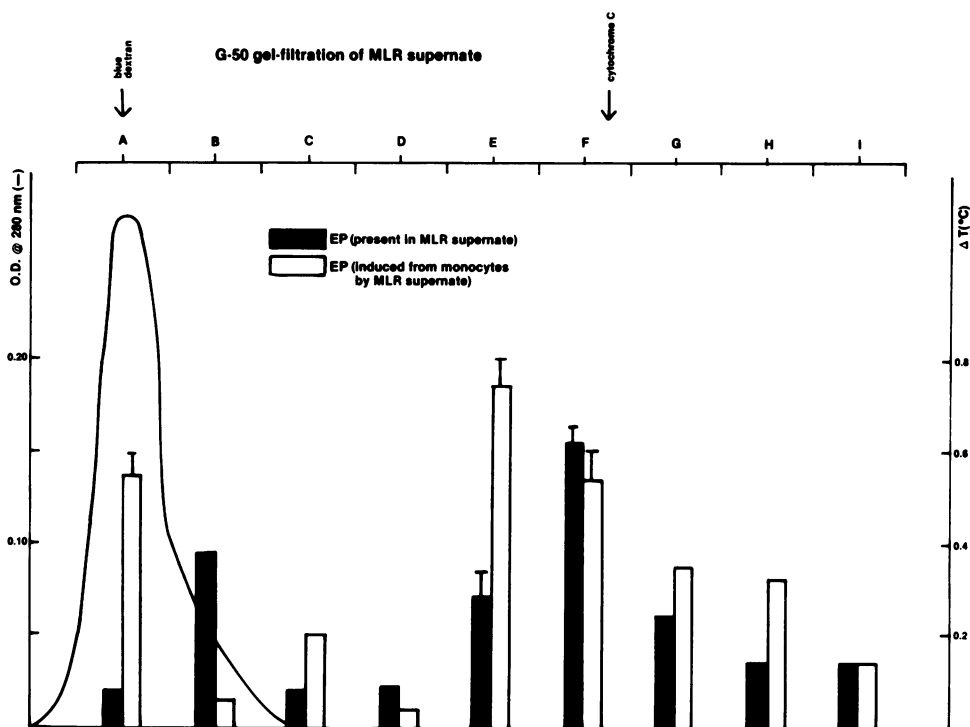


FIG. 3. Gel filtration of concentrated MLR supernates which induce EP. Gel filtration took place over G-50 (165 × 5.5 cm) in 0.15 M NaCl at 4°C. Each fraction pool was dialyzed against MEM, diluted 1:8, and assayed for EP (shown in solid bars). The open bars represent the same fractions incubated with human monocytes for 48 hours and the supernatant medium injected into rabbits.

Chromatographic Methods

Two types of chromatographic separations were employed: gel filtration and isoelectric focusing. All gel-filtration media and buffers were autoclaved and were non-reactive in the LAL. All columns were sterilized by either a 10 percent formaldehyde rinse or 15 minutes of autoclaving. Test tubes were either pyrogen-free polypropylene or siliconized and baked glass (180°C for four hours). Eluted fractions were not filtered but rather cultured to ascertain sterility. In addition, LAL assays were carried out on fractions to ascertain the endotoxin concentration.

All focusing materials were sterilized and washed with LAL-negative water. The isoelectric focusing gel (flatbed) was Sephadex G-75 superfine using an LKB apparatus. MLR supernatant pools were dialyzed in autoclaved standard dialysis bags (molecular weight cut-off = 8–10 kD) against 2,000 volumes of 1 mM NaCl and then mixed with LKB ampholines. The slurry was poured on to a glass plate, dried to a paste using a fan, and focused at 4°C with 8 watts constant power. After 24 hours, the paste was divided into fractions, each fraction was suspended in water (final ampholine concentration of 0.5 percent w/v), and pH was determined at 20°C. The fractions were then further diluted in MEM and the supernates cultured for bacteria.

Induction of IL-1

Crude or chromatographic fractions derived from MLR supernatants were assayed employing the mononuclear cells of an unrelated donor as described previously [5].

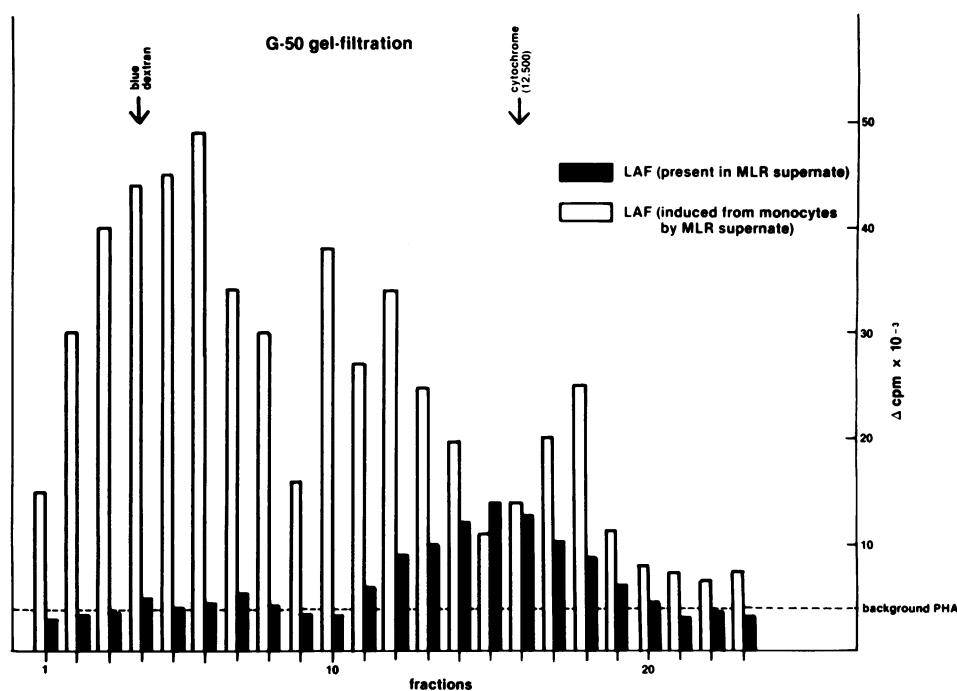


FIG. 4. Gel filtration of concentrated MLR supernates which induce LAF. Gel filtration took place over G-50 (80×1.5 cm) in MEM at 4°C . Each fraction was diluted 1:100 and assayed for intrinsic LAF activity (shown in solid bars). The open bars represent the same fractions diluted 1:10 and incubated with human monocytes for 48 hours. The supernatant medium was diluted 1:10 and assayed for LAF on the same set of thymocytes.

Mononuclear cells were suspended in MEM at a concentration of $5 \times 10^6/\text{ml}$ containing 2 percent heat-inactivated human AB serum from a single donor. 1.0 ml of cells was aliquoted into each 2 cm diameter well (Costar, Hamden, CT) and the plate was gently rocked at 37°C for two hours. The non-adherent cells were then washed off with two rinses of warm MEM. The fractions to be tested were diluted in MEM containing 0.1 percent human AB serum and $12.5 \mu\text{g}/\text{ml}$ polymyxin B [12]. Each well was filled with 1.0 ml of diluted sample. After 48 hours, the supernatant medium from each well was centrifuged ($1,500 g$) and assayed for IL-1 activity. For induction of EP activity, larger amounts were required. To accomplish this, the same protocol was used except the 5 ml of cells were seeded on to 25 cm^2 flasks and 5.0 ml of diluted MLR fraction was added.

Assay for IL-1 Activity

Following stimulation, the supernatant was diluted and incubated with murine thymocytes as previously described [13] in the presence of $12.5 \mu\text{g}/\text{ml}$ of polymyxin B. Since some fractions eluting from either gel filtration or isoelectric focusing contained intrinsic LAF activity, each fraction was diluted and assayed for LAF activity directly, using the same thymocytes being employed to assay the LAF activity induced by these same fractions. Thus, the data are presented as LAF activity "present in the MLR supernate" as well as that "induced from monocytes by MLR supernate."

Fever production was assayed by the rabbit pyrogen test as described previously [14]. Each fraction or supernate was assayed in three rabbits. The amount of supernate

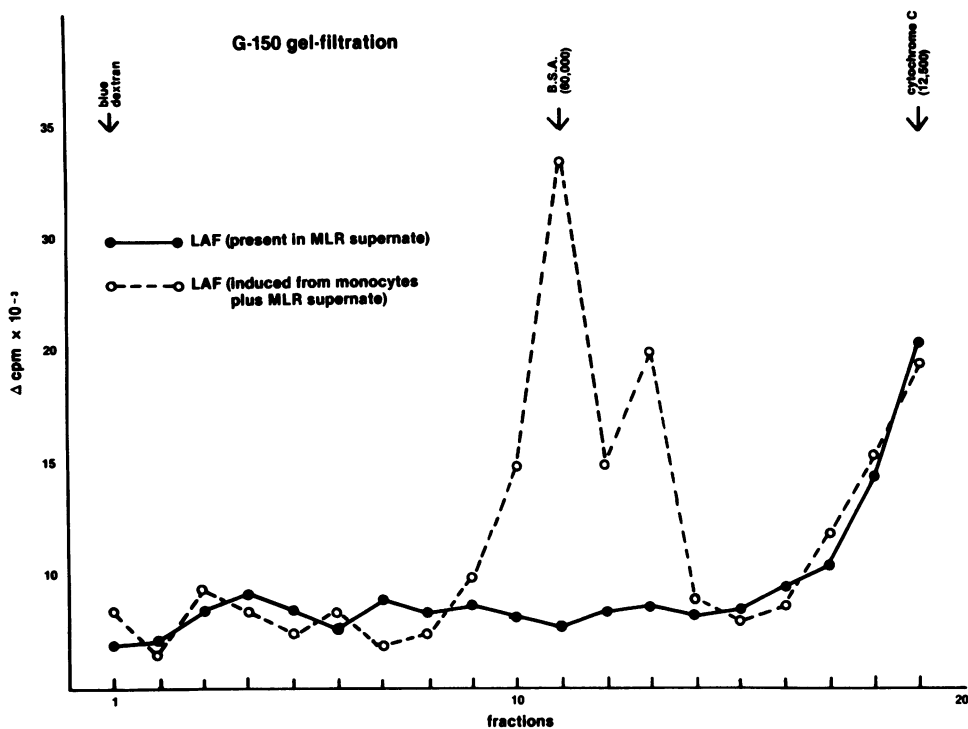


FIG. 5. Gel filtration of concentrated MLR supernates which induce LAF. Gel filtration took place over G-150 (80×1.5 cm) in MEM at 4°C . Each fraction was diluted 1:100 and assayed for intrinsic LAF activity (shown in solid circles). The open circles represent the same fractions diluted 1:10 and incubated with human monocytes for 48 hours. The supernatant medium was diluted 1:10 and assayed for LAF on the same set of thymocytes.

injected intravenously was equivalent to 1.2×10^6 monocytes/kg. Data are expressed at mean of peak increase over baseline line rectal temperature (\pm SEM). All fevers were monophasic, rapid in onset, and reached a peak elevation between 48–60 minutes following injection.

RESULTS

Generation Time of EP During the MLR

A pool of supernates from several two-way MLRs derived from the mononuclear cells of different donors was prepared as described in Methods. This MLR supernate pool was diluted 1:10 and mixed with the mononuclear cells of a single donor. The mixture was then divided into seven 5 ml aliquots in 25 cm^2 flasks. Mononuclear cells from the same donor were also mixed with heat-killed *S. albus* and similarly divided into flasks. All flasks were incubated at 37°C ; flasks were removed at various times, the cultures centrifuged, and supernates were stored at 4°C until tested for pyrogenicity. As shown in Fig. 1, EP was detected in the supernate of mononuclear cells stimulated with *S. albus* after six to nine hours, whereas the same amount of EP was not detected in MLR-stimulated cultures for 24–48 hours. Similar results have been reported for guinea pig monocyte EP induced by supernates derived from sensitized lymphocytes incubated with specific antigen [15].

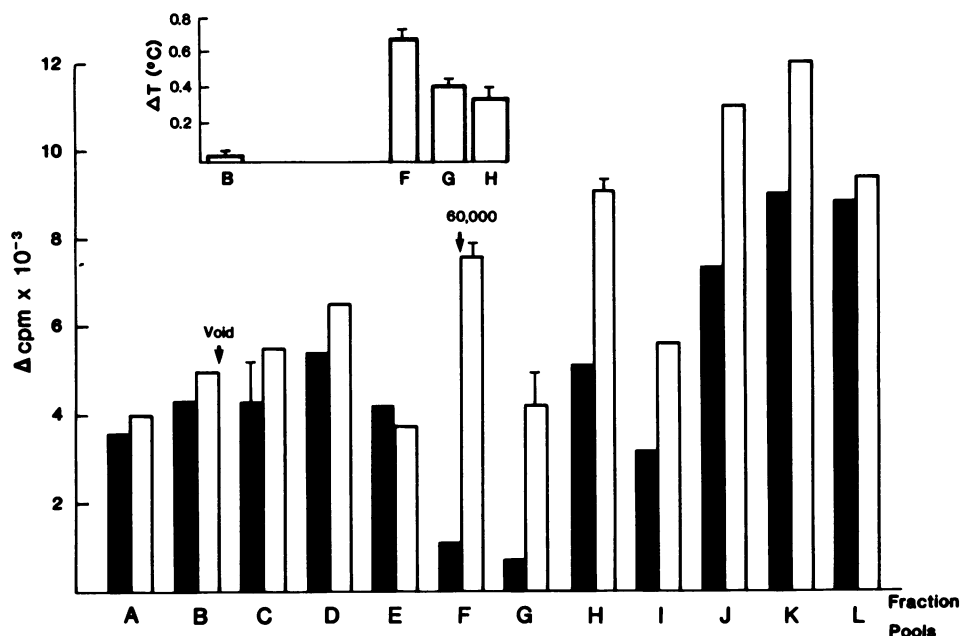


FIG. 6. Gel filtration of concentrated MLR supernates which induce LAF and EP. Gel filtration took place over G-150 (145 × 2.0 cm) in MEM at 4°C. Every five fractions were pooled, diluted 1:100, and assayed for intrinsic LAF activity (shown in solid bars). The open bars represent the same fractions diluted 1:10 and incubated with human monocytes for 48 hours. The supernatant medium was diluted 1:10 and assayed for LAF on the same set of thymocytes. Fraction pools B and F-H were also incubated with monocytes, and the supernates were injected into rabbits. The inset depicts the mean peak fever produced from these fractions.

Molecular Weight Determinations of EP and LAF Present in MLR Supernates

Initial experiments to characterize the IL-1-inducing property of MLR supernates involved concentration of a large (500 ml) pool of supernates prior to chromatographic separations. The concentrate contained EP and LAF activity. In order to identify the molecular weight species of EP and LAF activity in the concentrated pool, the pool was chromatographed over Sephadex G-50 and every five fractions were combined and assayed for EP and LAF activity. As shown in Fig. 2, EP and LAF co-elute in the same fraction pools at molecular weights of approximately 38 and 16 kD. Similar molecular weight determinations have been reported for human EP and LAF when the monocytes are stimulated with either heat-killed *S. albus* or bacterial endotoxin [4]. Thus, the MLR supernate pool contains EP and LAF with molecular weights indistinguishable from those observed using microbial inducers. Using these data, all subsequent experiments were carried out measuring both the intrinsic EP and LAF activity of each fraction and the EP and LAF induced by such fractions.

Molecular Weight Determination of IL-1-Inducing Substance(s) in MLR Supernates

The concentrated pool of MLR supernates was chromatographed over G-50 and every ten fractions were combined. Each pool was injected directly into rabbits to determine the intrinsic EP activity. Each pool was also incubated with the monocytes

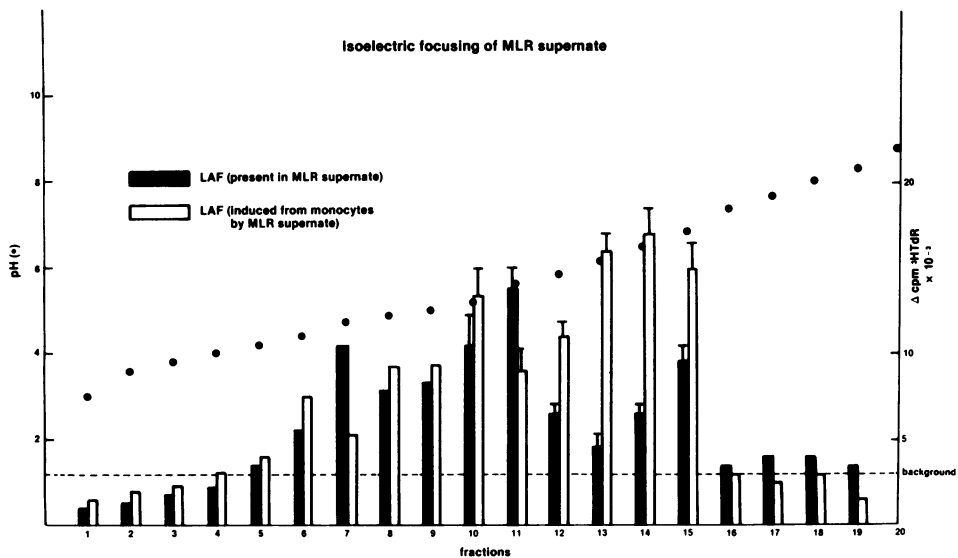


FIG. 7. Isoelectric focusing of MLR supernates. Each fraction was dialyzed against MEM, diluted (1:10), and assayed for intrinsic LAF activity (shown in solid bars). These fractions were also incubated with human monocytes at a dilution of 1:5 and the supernates assayed for LAF (open bars) at a 1:10 dilution.

from a single donor for 48 hours. The supernates from these latter incubations were injected into the same rabbits. As shown in Fig. 3, two pools contained EP-inducing activity. The approximate molecular weights of these pools are >50 and 20–30 kD. There was also a small amount of EP-inducing activity at 8–10 kD. Further experiments were designed using smaller columns and determination of LAF-inducing activity in the MLR supernate.

In the next experiment, the MLR pool was chromatographed over a small G-50 column; each fraction was diluted and assayed directly for intrinsic LAF activity as well as incubated with human mononuclear cells for LAF-inducing activity. As shown in Fig. 4, the same elution profile was obtained using LAF-inducing activity. The dilution of each fraction was below the detection of intrinsic LAF, whereas LAF-inducing activity was found in fractions >50, 20–30, and 8–10 kD, similar to the results shown in Fig. 3.

In further experiments, the MLR supernate pool was chromatographed over G-150 in order to determine the size of the IL-1-inducing peak eluting at >50 kD. Once again, each fraction was diluted and assayed for intrinsic LAF activity and also incubated with monocytes for LAF-inducing activity. As shown in Fig. 5, LAF-inducing activity was detected in fractions eluting at approximately 60 kD and 20–30 kD.

In order to test for EP-inducing activity in the 60 kD fractions, a large G-150 column was used. The eluted fractions were combined and evaluated for LAF-inducing activity. As shown in Fig. 6, a similar elution pattern was observed. Fraction pools were also incubated with monocytes for EP-inducing activity, using active pools F, G, and H as well as inactive pool B. EP-inducing activity was present in those pools which had induced LAF (inset to Fig. 6).

Isoelectric Focusing of MLR Supernates

Two isoelectric focusing experiments were done: one performed using a pool of unconcentrated MLR supernates and another using a pool of MLR from several

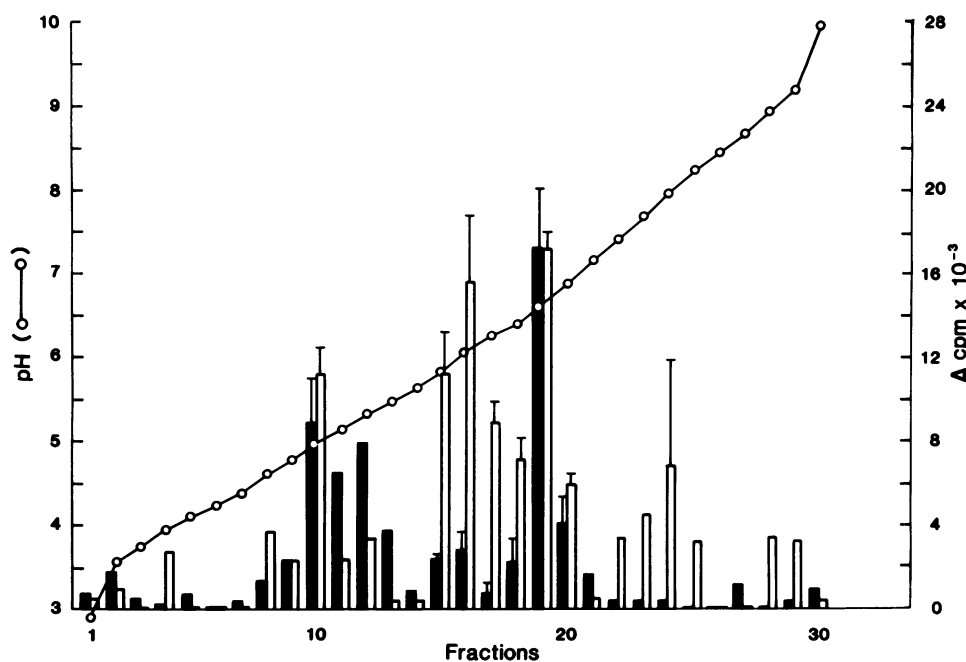


FIG. 8. Isoelectric focusing of concentrated MLR supernates. Each fraction was dialyzed, diluted (1:10), and assayed for intrinsic LAF activity (shown in solid bars). These fractions were also incubated with human monocytes at 1:10 and the supernates assayed for LAF (open bars) at a 1:10 dilution.

donors which was concentrated prior to being tested. In each experiment, the focused material was assayed for intrinsic LAF activity and was incubated with monocytes for the elaboration of LAF and EP.

The results of the unconcentrated MLR pool are shown in Fig. 7. There is a single peak of LAF-inducing activity in fractions 13–15 with a pI 6–6.3. In the second experiment using the concentrated MLR pool, fractions 15–17 induced the most LAF activity and had a pI of 5.9–6.2 (see Fig. 8). There was also another peak of LAF-inducing activity with a pI of 7.5. Fractions 15–17 were pooled and incubated with human monocytes from a single donor in order to stimulate EP production. Control fractions were also obtained (fractions 12–14 and 26–28). After 48 hours of incubation, these supernates were injected into rabbits. Control fractions 13 and 14 did not stimulate EP ($T = 0.22 \pm 0.06^\circ\text{C}$). However, fractions 15–17 induced typical EP fevers ($T = 0.56 \pm 0.08^\circ\text{C}$).

IL-1-Inducing Activity of MLR Supernatants Eluted From Con A Sepharose

Concentrated MLR supernatants were dialyzed against phosphate-buffered saline (pH 7.4) and applied to a Con A sepharose column. The column was washed with one volume of buffer and these were combined for assay on monocytes. The column was then washed with two column volumes of 0.5 M alpha methyl D-mannoside. These were pooled, diluted, and assayed for IL-1-inducing activity. The results are shown in Table 1. As depicted, the IL-1-inducing activity does not bind to Con A sepharose but rather is recovered in the non-binding material. Washing the column with 0.5 M alpha methyl D-mannoside did not result in the elution of an IL-1-inducing activity. These experiments were repeated with similar results. During the repeat experiment, the column was washed with 20 percent ethylene glycol following the alpha methyl

TABLE 1
IL-1-Inducing Activity of MLR Supernatants

Materials Tested	T °C ^a	CPM ^b
<i>Experiment 1</i>		
MLR (1:8) + MO	0.80 ± 0.1	—
MLR (1:80) + MO	—	6,135 ^c
MLR (1:800) + MO	—	2,122
Non-binding (1:8) + MO	0.80 ± 0.02	—
Non-binding (1:10) + MO	—	6,886
Non-binding (1:100) + MO	—	4,965
AMM elution (1:8) + MO ^d	0.05 ± 0.03	—
AMM elution (1:10) + MO	—	2,511
AMM elution (1:100) + MO	—	2,201
<i>Experiment 2</i>		
MLR (1:80) + MO	—	7,643
Non-binding (1:80) + MO	—	5,121
AMM elution (1:10) + MO	—	1,429
20% ethylene glycol ^e (1:10) + MO	—	5,968

^aPeak fever in three rabbits (± SEM)

^bCPM subtracted from CPM of thymocytes incubated without PHA

^cThe PHA response = 2,372 after subtraction of thymocytes CPM without PHA

^dAlpha methyl mannoside 0.5 M; controls containing 0.05 and 0.005 M AMM with unfractionated MLR supernates were unaffected by the saccharide.

^eDialyzed against MEM prior to assay on MO

D-mannoside elution. The 20 percent ethylene glycol wash contained significant LAF-inducing activity, as shown in Table 1.

Effect of Recombinant Human Gamma IFN on IL-1 Production

Of the many lymphokines which are produced during a MLR, gamma IFN has recently received attention as an important modulator of monocyte/macrophage function [16–20]. Therefore, experiments were designed to test the ability of homogeneous human gamma IFN on monocyte IL-1 production. Despite several trials using concentrations of gamma IFN from 10^{-1} to 10^5 units/ml, mononuclear cells from four different donors did not produce IL-1. Cell lysates as well as cell supernatant media contained no LAF activity after 24 or 48 hours of incubation. Indomethacin (1 µg/ml) was added to cultures to block any suppressive effects of PGE₂ but there was no evidence that gamma IFN was inducing IL-1. These supernatants similarly did not contain EP activity when injected into rabbits.

However, when small amounts of endotoxin were deliberately added to the culture medium in the presence of various amounts of gamma IFN, the 24 supernatant media contained increased LAF activity. The results are shown in Figs. 9A and 9B. In these studies, polymyxin B was not added to the mononuclear cell cultures. As shown, the amount of LAF produced was small and we could not demonstrate significant increased EP activity in these supernatants. Thus, we conclude from these experiments that gamma IFN induces IL-1 production by augmenting the stimulatory effects of endotoxin. In fact, this finding may explain the findings of other investigators employing gamma IFN in their experiments [21] since many tissue culture media

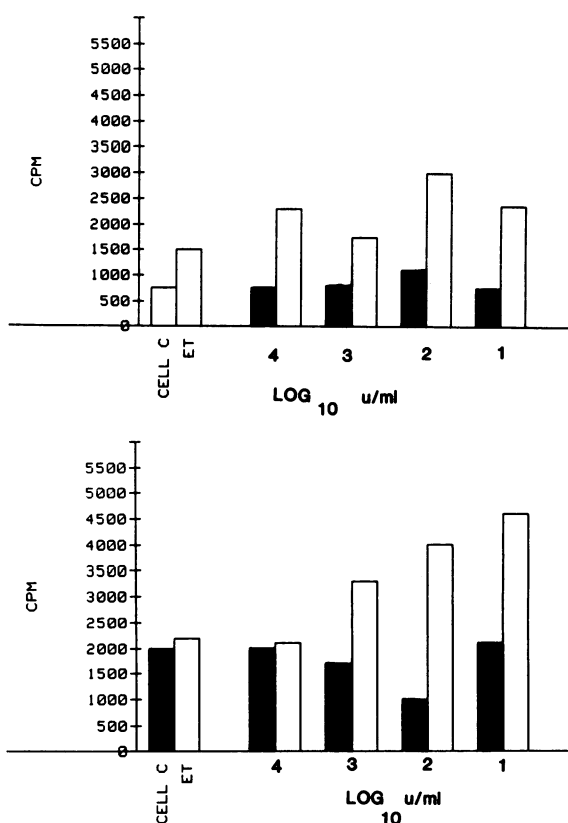


FIG. 9. A. LAF activity of human mononuclear cells incubated with gamma IFN. Control incubations are indicated. Endotoxin (ET) (0.5 ng/ml) was incubated with cells in the presence of increasing concentrations of IFN. Solid bars represent LAF activity from cells incubated with IFN only, whereas open bars depict cells incubated with both ET and IFN. Stimulated cell supernates were assayed at 1:100 dilutions. B. Similar to Fig. 9A, using the mononuclear cells from another donor. Cell control was incubated with 10^4 units/ml.

contain between 0.5 and 5 ng/ml endotoxin, as determined by the LAL assay (data not shown).

DISCUSSION

These studies were undertaken to define the chemical characteristics of lymphocyte products in the MLR which induce IL-1. There are many substances produced as a result of the allogeneic stimulation in the MLR; some are clearly candidates for IL-1 inducers by nature of their ability to activate monocytes and macrophages. These include migratory inhibitory factor (MIF), colony-stimulating factor (CSF), and macrophage-activating factor (MAF). Recently, there has been considerable evidence that some, although not all, MAF activity is due to gamma IFN [17,20]. The availability of homogeneous preparations of human recombinant gamma IFN has facilitated the testing of this substance as an inducer of IL-1 production. We were unable, however, to demonstrate IL-1 production from human mononuclear cells incubated with various concentrations of gamma IFN in the absence of endotoxins. On the contrary, gamma IFN augments endotoxin-induced IL-1 production and/or release. This finding is consistent with several reports on the ability of gamma IFN to increase macrophage expression of major histocompatibility complex antigens [18,19] and Fc receptor [16]. Others have also reported that alpha (leukocyte) IFN does not stimulate IL-1 production but rather augments IL-1 production induced by endotoxins [22]. Finally, we have shown that EP activity is augmented by small amounts of

endotoxin which contaminate human recombinant alpha IFN [14]. We speculate that some of the gamma IFN-mediated effects which have been reported, including increased IL-1 production [21], may, in fact, be partially due to an augmentation phenomenon of endotoxins present in commercially available tissue culture media and fetal calf serum.

The IL-1-inducing substance(s) reported in these studies is clearly not due to endotoxins. Incorporating polymyxin B in the IL-1-inducing culture inhibits endotoxin's ability to stimulate monocytes [12]. Furthermore, we have isolated IL-1-inducing activity at molecular weights of 60 and 30 kD, whereas endotoxins elute from gel-filtration chromatography at molecular weights >200 kD. In addition, the isoelectric point of the IL-1-inducing activity is 5.9–6.3, whereas endotoxins bind avidly to ion-exchange media at pH above neutrality, indicating a strongly negatively charged molecule. Finally, the heat lability of the IL-1-inducing activity would preclude endotoxin [5]. We conclude that the IL-1-inducing activity of MLR supernates is of endogenous origin (lymphocytes) and that the physical characteristics reported here are due to the direct stimulation of IL-1 production rather than an augmentation of endotoxin's effects.

There are several possibilities that the IL-1-inducing substance(s) in the MLR supernate is an identified lymphokine. MIF, which activates macrophages and prevents their migration, has two molecular weights (65 and 25 kD), which are similar to those we have reported in the present studies [23]. In addition, human MIF focuses at pIs of 4–6 [23]. For guinea pig MIF, the pIs are 3.5 and 5.5, with respective molecular weights of 65 and 25 [24]. Human MIF is resistant to neuraminidase treatment, and this is consistent with our findings that most of the IL-1-inducing activity does not bind to Con A sepharose. The fact that some of the activity was eluted with ethylene glycol suggests that there is a mixture of glycosylated proteins in the MLR which can stimulate IL-1 production.

Cerebrospinal fluid (CSF) derived from a murine L-cell line has been studied for its ability to induce IL-1 [25]. CSF-induced IL-1 production was clearly distinguishable from that of endotoxins since antibody to the L-cell CSF blocks CSF- but not endotoxin-induced IL-1 production. Chemical characteristics of the L-cell CSF are different from human CSF. Nevertheless, L-cell CSF seems to be one likely candidate for an IL-1-inducing lymphokine. Although the L-cell CSF was low in endotoxin concentration, it remains to be shown that CSF is functioning as a direct or augmenting IL-1 inducer.

With the molecular cloning of CSF, it seems likely that this issue will be resolved soon. The studies presented in this report establish the molecular heterogeneity of lymphocyte products in MLR supernates which induce IL-1 production in the absence of endotoxins. Future studies will focus on the identification of known lymphokines with this property, or perhaps there is a unique lymphokine responsible for initiating IL-1 production. The overall clinical importance of identifying such lymphocyte products will be related to immunologically mediated diseases such as drug fever, transplantation rejection, hypersensitivity reactions, and autoimmune phenomena.

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