Ultrafiltration to Reject Human Interleukin-1-Inducing Substances Derived from Bacterial Cultures

CHARLES A. DINARELLO,^{1*} GERHARD LONNEMANN,¹ REDENTOR MAXWELL,¹ AND STANLEY SHALDON²

Department of Medicine, Tufts University School of Medicine and the New England Medical Center Hospital, Boston, Massachusetts 02111,¹ Department of Nephrology, University Hospital, Nimes, France²

Received 15 December 1986/Accepted 23 March 1987

Interleukin-1 (IL-1), a polypeptide cytokine, is an important mediator of host responses to infection and injury. Picogram per milliliter concentrations of bacterial products (endo- or exotoxins) stimulate human monocytes to produce IL-1 in vitro. The design of this study was based on the clinical model of bacterial contamination of fluid intended to be directly injected into humans. Physiologic saline contaminated with bacterial toxins was passed through a hollow fiber ultrafilter, and the ultrafiltrates were tested for their ability to induce human IL-1 production. The ultrafiltrates were added directly to freshly obtained human blood mononuclear cells, and after 24 h of incubation the supernatant media were assayed for the presence of IL-1. The results indicate that (i) the IL-1-inducing material(s) present in bacterial cultures of gram-negative organisms is rejected by a factor of 100 to 100,000 by molecular size exclusion and by absorption; (ii) rejection is sustained for at least 32 liters of fluid; (iii) the rejection of *Limulus*-reactive material by the ultrafilter is greater for purified endotoxin than for native endotoxins derived from live bacterial cultures; and (iv) nonendotoxin IL-1-inducing toxins (molecular weight, 24,000) from *Staphylococcus aureus* are not rejected or absorbed. These results demonstrate that there is a considerable margin of safety with the ultrafiltration method and that it can be applied to clinical situations.

Host responses to microbial invasion or inflammatory processes involve several organ systems. Fever, generalized malaise, headache, and increased sleep are common clinical signs and symptoms. Laboratory measurements reveal changes in many parameters. These include increases in neutrophil and other circulating leukocytes, decreases in iron and zinc, increases in copper, increases in hepatic acute-phase proteins, decreases in hepatic albumin synthesis, negative nitrogen balance, decreased hemoglobin synthesis, and other metabolic changes. Taken together, these responses are often called acute-phase responses.

Investigation over the past 30 years has resulted in the concept that many of the components of the acute-phase response are mediated by a molecule now called interleukin-1 (IL-1) (6, 7). Although the term IL-1 is new, molecules with acute-phase response-inducing properties were described as endogenous pyrogen, leukocytic endogenous mediator, mononuclear cell factor, catabolin, and lymphocyte-activating factor (for a review, see reference 6). Two IL-1 forms have been cloned (1, 21); and the multiple biological activities of IL-1, including fever and acute-phase responses, have been confirmed by using recombinant IL-1 (for a review, see reference 8).

The production of IL-1 becomes an important consideration in many disease processes. The induction of IL-1, particularly in humans, is so sensitive that elevated levels in plasma can be measured following host perturbations such as strenuous exercise (5) and ovulation (4). However, microbial products, particularly exo- and endotoxins from gram-positive and -negative bacteria respectively, are potent inducers of IL-1 (3, 9, 11, 12, 18). Human blood monocytes are particularly sensitive to these microbial agents, and in clinical studies in which endotoxins have been injected into human subjects the exquisite sensitivity of humans to these exogenous pyrogenic substances has been demonstrated.

For example, 1 to 2 ng of *Escherichia coli* endotoxin per kg produces fever and leukocytosis in humans (26). In vitro, picogram per milliliter concentrations of endotoxins or staphylococcal exotoxins induce human monocytes to produce large amounts of IL-1 (9, 12, 18).

The ability of human blood mononuclear cells (MNCs) to produce IL-1 in response to exogenous bacterial products is highly reliable, sensitive, and most importantly, clinically relevant. A test system for detecting substances that induce human IL-1 has been designed and has been used prospectively to detect contaminating pyrogens (10). In the test system, human MNCs obtained from normal human donors and cultured in vitro for 12 to 24 h did not release significant amounts of IL-1 into the supernatant. In the presence of substances which cause fever or other acute-phase responses, however, IL-1 production from these cells into the supernatant was observed. Unlike the *Limulus* amoebocyte lysate test (LAL), the IL-1 test is not restricted to endotoxins and, unlike the LAL, has no known false-positive results. For example, there are several substances that cause gelation in the LAL but that are not endotoxin (14). These include poly(I):polycytosinic acid [poly(I:C)], which induces IL-1 and is positive in the LAL, but its IL-1-inducing ability is not due to endotoxin. Another example is a cellulose material derived from cuprophane dialysis membranes (23) which has been reported to cause gelation in the LAL. The latter material is nonpyrogenic (in humans or rabbits) and does not induce human IL-1 in vitro (23).

This study was initiated to test the ability of a hollow fiber ultrafilter to reject human IL-1-inducing substances found in microbially contaminated fluids. No assumptions were made. The experiments were designed as if isotonic- and pH-correct fluid intended for use in humans by parenteral administration had become accidentally contaminated with common organisms. We used two water contaminants isolated from our laboratory, *Pseudomonas* sp. and *E. coli*.

* Corresponding author.

Bacterial cultures were first filtered to remove all bacteria and then used at various dilutions to challenge the filter.

MATERIALS AND METHODS

Materials. Hollow fiber ultrafilters (polyamide) were kindly provided by the Gambro Co., Hechingen, Federal Republic of Germany. They were sterile and were used only once (16). A routine blood pump was used at 200 ml/min, and pressure measurements did not exceed 50 mm of Hg. Disposable blood lines (Gambro) were used, and attachments were made under aseptic conditions. Tryptic soy broth, brain heart infusion broth, and Todd-Hewitt broth were obtained from Difco Laboratories, Detroit, Mich. RPMI 1640 medium and minimal essential medium (MEM) were purchased from Microbiological Associates, Walkersville, Md. The complete media contained 100 U of penicillin per ml, 100 µg of streptomycin per ml, and 0.01 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer. The LAL was obtained from Associates of Cape Cod, Woods Hole, Mass. The level of endotoxin in complete MEM was less than 50 pg/ml. Fetal calf serum was purchased from Hyclone. Phytohemagglutinin was obtained from Burroughs Wellcome Co., Research Triangle Park, N.C.; and tritiated thymidine was purchased from New England Nuclear Corp., Boston, Mass. Reference endotoxin (RE-5) was kindly provided by the Bureau of Biologics, Bethesda, Md. The *E. coli* endotoxin prepared by the hot phenol method was purchased from Sigma Chemical Co., St. Louis, Mo. Polymyxin B was obtained from Pfizer Inc., New York, N.Y. Human serum albumin (5%) was purchased from Abbott Laboratories, North Chicago, Ill.

Preparation of human MNCs. Blood was drawn into heparinized plastic syringes (50 U/ml), transferred into a sterile, pyrogen-free beaker, and diluted with two parts of sterile, pyrogen-free saline. A single lot of Ficoll-Hypaque was prepared, and it was established that it did not stimulate IL-1 production. After centrifugation for 40 min at $1,000 \times g$ at 22°C, the interphase MNC layer was removed with a pipette inserted through the plasma-saline layer. MNCs were washed in ample saline to remove the Ficoll-Hypaque. The MNCs were adjusted to a concentration of 5×10^6 cells per ml in complete MEM with 5% heat-inactivated (56°C for 60 min) human type AB serum.

Stimulation of MNCs. The MNC were suspended in MEM-serum and stored at 4°C until the ultrafiltrates were obtained. Ultrafiltration samples were collected in human serum albumin (final concentration, 1%) and stored at 4°C. When the experiment was over (2 h), the ultrafiltrates and MNCs were mixed as follows. A total of 100 µl of ultrafiltrates, other stimuli, or controls was added to wells (in duplicate); and then 100 µl of the MNC suspension was added to each well of 96-well flat-bottom microtiter plates (Nunc, Roskilde, Denmark). After 24 h at 37°C in 5% humidified CO₂, the supernatants from the duplicate wells were pooled (400 µl was pooled for each incubation condition). The supernatants were centrifuged in sterile Eppendorf tubes for 5 min, transferred to new Eppendorf tubes, and frozen at -70°C until they were assayed for IL-1 activity.

The ability of endotoxins to stimulate human MNC IL-1 production in vitro varies with the source of the endotoxin and its preparation (9). In this study we established that between 50 and 100 pg of the reference RE-5 endotoxin per ml stimulated small amounts of IL-1, whereas 50 pg/ml induced significant amounts of IL-1.

IL-1 assay of MNC supernatants. The frozen supernatants were thawed and assayed on either D10.G4.1 (D10) cells or

thymocytes from C3H/HeJ mice (age, 6 to 9 weeks) as described elsewhere (15, 19). D10 cells were used between days 8 and 14 following a four-way split. The IL-1-containing supernatants were diluted in RPMI 1640 medium and assayed in triplicate. Polymyxin B (12.5 µg/ml) was added to the RPMI 1640 medium to bind any endotoxin. In the IL-1 assay, the first six wells contained phytohemagglutinin controls (no IL-1 or MNC supernatants); the next wells contained the IL-1 standards (1 and 10 U/ml). The diluted samples filled the rest of the plate in the order that the ultrafiltrates were collected, and then the last three wells contained phytohemagglutinin only (another control). The phytohemagglutinin was added to the T-cell suspension just before the cells were pipetted into the wells. Cells were incubated for 48 h at 37°C in 5% CO₂ and pulsed with [³H]thymidine. After 24 h the DNA was trapped on glass filters, and radioactivity was determined by liquid scintillation. The results shown represent the mean of triplicate cultures. Standard deviations of the triplicate cultures were <15% and are not depicted.

Preparation of bacterial culture filtrates. Three organisms were selected for these studies: a laboratory water contaminant of the genus *Pseudomonas*, a clinical isolate of *E. coli*, and *Staphylococcus aureus* isolated from a patient with toxic shock syndrome (strain FRI-1169 from the Centers for Disease Controls, Atlanta, Ga.) (18). The *Pseudomonas* culture was grown in tryptic soy broth, *E. coli* was grown in brain heart infusion broth, and *S. aureus* was grown in Todd-Hewitt broth with a low glucose concentration (<0.05%) (18). All cultures were grown in a shaking water bath at 37°C for 48 h. They were centrifuged and filtered (pore size, 0.22 µm), and sterility was confirmed by culturing. They were stored at 4°C and diluted just before testing. The number of organism was estimated by determining the A₆₅₀ and was found to be approximately 5×10^8 /ml. The LAL reactivities of both gram-negative culture filtrates were positive at a 1:10,000,000 dilution; therefore, our culture supernatants had approximately 100 µg of LAL-reactive material per ml. The LAL of the staphylococcal filtrate was negative at a 1:100 dilution. In addition, staphylococcal toxin materials were incubated with human MNCs in the presence of polymyxin B (12.5 µg/ml), which blocks any endotoxin contained in these filtrates (13, 18). Polymyxin B was not used in endotoxin-related experiments.

LAL. The lysate had a reported sensitivity of 20 pg/ml but, on occasion, it was sensitive to 10 pg/ml. All water used for these studies was LAL negative. The water was produced in our laboratory by reverse osmosis and ultrafiltration. The ultrafilter (Nuclepore Corp., Pleasanton, Calif.) had a molecular weight exclusion of 500. The water was pumped through the filter at 12 to 20 lb/in². All tubing was made of polyethylene and was cleaned every 6 months with hypochlorous acid.

Ultrafiltration. The ultrafilters were fixed on a stationary rack, and the inlet-outlet and ultrafiltration ports were connected aseptically to standard dialysis blood lines. The outlet port was clamped. Two sterile, pyrogen-free, 4-liter flasks were employed as the supply and drainage vessels. Sterile pipettes were attached to the blood lines to drain the 4-liter supply flask. A peristaltic blood pump was interposed between the supply vessel and the inlet port. Prior to ultrafiltration, 4 liters of saline was pumped through the device. All ultrafiltration took place at room temperature and a flow rate of 200 ml/min. Under these conditions, the inlet pressure, which was monitored continuously, did not exceed 50 mm of Hg. At the end of each 4-liter ultrafiltration, a sample was

FIG. 1. IL-1 activity from human MNCs after incubation with the various materials indicated under the horizontal axis. MNC supernatants were diluted 1:20 and assayed on D10 cells (see text).

taken directly from the ultrafilter outlet port. All samples intended for incubation were collected in 0.1% (final concentration) human serum albumin.

RESULTS

Ability of the intact ultrafilter challenged with *Pseudomonas* culture filtrate to reject IL-1-inducing substances. A standard experimental procedure was used throughout this study. The filter was first challenged with 4 liters of saline and then with successive 4-liter portions of bacterial filtrates. The bacterial filtrate used to challenge the filter induced IL-1 from MNCs at dilutions of 10^{-2} and 10^{-3} (Fig. 1). The ultrafiltrates from 4 through 32 liters did not induce IL-1, however. The filter was challenged with two concentrations of bacterial filtrate: 4 and 8 liters were a 10^{-3} dilution, 12 and 16 liters were a 10^{-4} dilution, and 20 and 24 liters were a 10^{-3} dilution (Fig. 2). The prefilter dilution of 10^{-4} of the *Pseudomonas* filtrate induced large amounts of IL-1. IL-1-inducing material in the ultrafiltrate was detected when the ultrafilter was challenged with a 10^{-2} dilution (Fig. 2). Thus, the rejection factor was

FIG. 2. IL-1 activity from human MNCs incubated with the various materials indicated under the horizontal axis.

TABLE 1. Rejection of IL-1-inducing substances in *Pseudomonas* culture filtrate by ultrafiltration

Expt	Minimal concn of prefilter IL-1 induction ^a	Reduction after 32 liters (fold)
1	10^{-3}	10
2	10^{-4}	100
3	10^{-4}	100
4	10^{-4}	100
5	10^{-4}	100

^a Dilution of *Pseudomonas* bacterial culture filtrate.

100. The results of five separate ultrafiltration experiments in which a rejection factor was calculated by using this experimental design are given in Table 1. The data in Table 1 represent 40 determinations. With the exception of experiment 1, results of the remaining four experiments showed that a challenge dilution of the bacterial filtrate of 10^{-4} resulted in no IL-1-inducing substances until a dilution of 10^{-2} (rejection factor of 100, similar to that depicted in Fig. 2).

IL-1-inducing substances in ultrafiltrates of *E. coli*. We next challenged the ultrafilters with a culture filtrate of *E. coli*. The *E. coli* culture filtrate was very potent in inducing IL-1 from MNCs. The data shown in Fig. 3 are the results of ultrafiltrates after 8 liters of bacterial culture challenge. A dilution of 10^{-9} of the prefilter material induced IL-1 production (Fig. 3). When challenged with a 10^{-5} dilution of this filtrate, however, the ultrafiltrate contained no IL-1-inducing substances. At a challenge concentration of 10^{-4} dilution, the IL-1-inducing activity of the ultrafiltrate was only slightly above that of the control level of saline. Thus, the ultrafilter was capable of rejection by a factor of 100,000.

Inability of ultrafiltration to reject *S. aureus* toxic shock syndrome toxin. There was no apparent rejection of the *S. aureus* bacterial filtrate by the filtration device (Fig. 4). This was ascertained by comparing the amount of IL-1-inducing material contained in the ultrafiltrate when the filter was challenged with dilutions of the bacterial culture filtrate to the amount before ultrafiltration. These results are not surprising, since the toxic shock toxin is a neutral polypeptide with a molecular weight of 24,000.

Rejection of purified *E. coli* endotoxin. To test whether our

FIG. 3. IL-1 activity from human MNCs after incubation with the materials indicated under the horizontal axis. The pre- and postultrafiltration samples were taken after 8 liters of challenge fluid had passed through the filter.

FIG. 4. IL-1 activity from human MNCs after incubation with the materials indicated under the horizontal axis. MNCs were incubated with sample in the presence of polymyxin B. TSS, toxic shock syndrome toxin.

filtration procedures were correct, we challenged the ultrafilters with a commercially available phenol extract of endotoxin. The ultrafilter was challenged with various concentrations of purified endotoxin (10 µg/ml at 4 and 8 liters; 100 µg/ml at 12 and 16 liters; 10 µg/ml at 20 and 24 liters; Table 2). The ultrafiltrate was then diluted for the endpoint LAL reaction. The challenge endotoxin was also diluted for an endpoint analysis. The rejection was in the range of 10^6 (Table 2). Thus, we conclude that our procedures are correct and that the differences in rejection between the *Pseudomonas* and *Staphylococcus* culture filtrates are valid.

Ability of damaged ultrafilters challenged with *E. coli* culture filtrate to reject IL-1-inducing substances. In the experiments used to determine the ability of damaged ultrafilters challenged with *E. coli* culture filtrate to reject IL-1-inducing substances, the design described above was used, except that the filters were damaged on purpose by tearing a hole in a single hollow fiber (Fig. 5). These ultrafilters failed the so-called bubble test for intact ultrafilters. Six of these filters were studied. The filters were challenged with a 10^{-3} dilution of the *E. coli* culture filtrate, and IL-1-inducing material was observed only after 8 liters (Fig. 6). The 8-liter sample had significantly less IL-1-inducing material than the challenge culture filtrate (10^{-4}). The results of a similar experiment, but with another filter, are shown in Fig. 7. Challenge of the filter with a 10^{-2} dilution of the bacterial culture filtrate resulted in the passage of IL-1-inducing material, but considerable rejection was still apparent at lower dilutions. In other studies, these

TABLE 2. Purified endotoxin challenge of ultrafilters

Challenge ^a	LAL of ultrafiltrate (pg/ml)
Saline	<10
Rinse	<10
4, 10	50
8, 10	50
12, 100	50
16, 100	50
20, 10	50
24, 10	<10

^a The first value is in liters, the second is in micrograms per milliliter.

FIG. 5. Single damaged hollow fiber of an ultrafilter.

damaged filters also rejected purified endotoxin by a factor of 1,000 (data not shown).

DISCUSSION

This study was based on the concept that incubation of materials with human MNCs is more relevant for the detection of pyrogens in materials intended for parenteral use than the LAL or the rabbit pyrogen test. The ability of substances to stimulate IL-1 production in vitro correlates with the ability to induce acute-phase changes in vivo. For example, recombinant human growth hormone, which had passed pyrogen and LAL tests, still produced fever and the entire spectrum of the acute-phase response in human subjects. When incubated in vitro with human monocytes, however, the supernatant contains large amounts of IL-1 (10). In a prospective study, various lots of growth hormone that did not induce IL-1 in vitro were found to be nonpyrogenic and nontoxic and had no acute-phase-inducing properties when injected into human subjects. Thus, the concept of a prospective IL-1 test system seems to be supported by these findings.

The conclusions that can be drawn from the results of this study are relevant to the production, use, and safety of

FIG. 6. IL-1 activity from human MNCs incubated with the materials indicated under the horizontal axis. The ultrafilters used in these experiments were damaged (see Fig. 5).

FIG. 7. IL-1 activity from human MNCs incubated under conditions similar to those shown in Fig. 6.

intravenous fluids. The results establish that a 4-liter rinse of these ultrafilters consistently did not contain any human IL-1-inducing substances and that the use of these filters would not induce fever or acute-phase changes. Although this might have been expected based on the results of the LAL and rabbit pyrogen tests of these filters, the lack of IL-1-inducing substances eluting from these filters provides additional evidence. We have used these filters to remove IL-1-inducing substances from tissue culture media (2) containing amino acids and glucose.

The results of this study demonstrate that the rejection of the IL-1-inducing material that is present in native bacterial culture filtrates is significantly less than that for purified endotoxin. The difference between rejection of the purified endotoxin and that of the bacterial culture filtrate is understandable because the former consists of a mixture of endotoxins with different molecular weights, and the latter forms large ($>1,000,000$ molecular weight) micelles. It is thought that various enzymes in the bacterial cultures contribute to degradative steps, whereas the extraction and purification of endotoxins from protein enzymes stabilizes the material. Purified endotoxin is rejected by U-2000 ultrafilters by a factor of 10^6 . This is not surprising considering the molecular weight exclusion of these filters (30,000) and the molecular size of purified endotoxins (25). The extraction of endotoxins by phenol or other methods hardly represents the natural state of lipopolysaccharides. There is now ample evidence that the lipid A moiety (25), some monosaccharide precursors (24), and low-molecular-weight synthetic lipid A preparations (20) are small enough to pass through a 30,000-molecular-weight exclusion filter. Moreover, these low-molecular-weight endotoxin fragments or lipid A precursors are biologically relevant and induce IL-1 (20, 22). Although most endotoxins produced by gram-negative bacteria are confined to the outer layer of the organism, sufficient amounts of material can be found in whole organisms suspended in water. The amount of endotoxin that leaches off varies with the type of organism, the culture conditions of the microenvironment, and the viability of the organisms. It is this type of endotoxin which is clinically relevant to the contamination of fluid for intravenous use.

The ultrafilters consistently rejected IL-1-inducing substances by a factor of 100, but in the case of *E. coli* ultrafilters rejected IL-1-inducing substances by a factor of 100,000. The results indicate that there is a wide margin of safety, however, since the amount of LAL-positive material in the two gram-negative bacterial filtrates that were used was purposefully high to challenge the filter. Critical to the

interpretation of these studies is the level of contamination of the bacterial filtrate. The dilutions of the bacterial filtrate were 10^{-2} to 10^{-4} . The 10^{-2} dilution represents a bacterial count of approximately 5×10^6 organisms per ml. This is a rather high number by most clinical standards, but this level of contamination is hardly visible on casual inspection.

Does the ultrafilter reject bacterial products by molecular size exclusion, adsorption, or both? The data obtained with the damaged filter suggest that absorption is taking place. Purified endotoxin was absorbed similarly. It is likely that membranes with positive or multiple charges (as is the case for the latter with the polyamide U-2000 ultrafilter) can absorb the negatively charged endotoxins.

Although the ultrafilter rejected IL-1-inducing substances found in gram-negative bacterial culture filtrates, the staphylococcal culture supernatant containing the toxic shock toxin seemingly was unaffected. We have shown previously (18) for the *S. aureus* toxin associated with toxic shock syndrome that the purified toxin has a molecular weight of about 24,000 and that the ability of this polypeptide to induce human IL-1 is, on a weight basis, equal or more potent than endotoxin. Thus, results of this study support the conclusion that a small peptide with a neutral pI is not absorbed or excluded. It should be noted, however, that growth of toxic shock-associated strains and toxin production are not like those in gram-negative organisms. In fact, the bacteria need a relatively rich culture medium and special pH and low glucose concentrations to produce the toxin. Thus, it is highly unlikely that contamination of water by toxic shock-associated strains of *S. aureus* would result in significant toxin production.

The relevance of these observations to the quality of dialysate used in routine hemodialysis has become important since the demonstration that regenerated cellulose capillary dialysis allows IL-1-inducing substances in the dialysate compartment to activate human blood monocytes in the blood compartment (2). Recent data indicate that under the conditions of in vitro hemodialysis, IL-1-inducing substances pass from the dialysate into the blood compartment (22). This was demonstrated by use of the same methods that were used in this study. Results of this study also support the original observation made by Henderson and Bears (17) that on-line ultrafiltration is a safe procedure for use in hemofiltration.

ACKNOWLEDGMENTS

This study was supported by Public Health Service grant AI15614 from the National Institutes of Health and by the Gambro Co., Hechingen, Federal Republic of Germany.

We thank Hermann Goehl and Peter Konstantin for assistance in these experiments.

LITERATURE CITED

1. Auron, P. E., A. C. Webb, L. J. Rosenwasser, S. F. Mucci, A. Rich, S. M. Wolff, and C. A. Dinarello. 1984. Nucleotide sequence of human monocyte interleukin-1 precursor cDNA. *Proc. Natl. Acad. Sci. USA* 81:7907-7911.
2. Bingel, M., G. Lonnemann, K. M. Koch, C. A. Dinarello, and S. Shaldon. 1987. Enhancement of in vitro human interleukin-1 production by sodium acetate. *Lancet* i:14-16.
3. Bodel, P., and E. Atkins. 1967. Release of endogenous pyrogen by human monocytes. *N. Engl. J. Med.* 276:1002-1006.
4. Cannon, J. G., and C. A. Dinarello. 1985. Increased plasma interleukin-1 activity in women after ovulation. *Science* 227:1247-1249.
5. Cannon, J. G., W. J. Evans, C. N. Meredith, V. A. Hughes, and

- C. A. Dinarello. 1986. Mechanisms contributing to increased plasma interleukin-1 activity. *J. Appl. Physiol.* **61**:1869-1874.
6. Dinarello, C. A. 1984. Interleukin-1. *Rev. Infect. Dis.* **6**:51-95.
7. Dinarello, C. A. 1984. Interleukin-1 and the pathogenesis of the acute phase response. *N. Engl. J. Med.* **311**:1413-1418.
8. Dinarello, C. A. 1986. Interleukin-1: amino acid sequences, multiple biological activities and comparison with tumor necrosis factor (cachectin). *Year Immunol.* 1985-86 **2**:68-89.
9. Dinarello, C. A., and J. M. Krueger. 1986. Induction of interleukin-1 by synthetic and naturally occurring muramyl peptides. *Fed. Proc.* **45**:2545-2548.
10. Dinarello, C. A., J. V. O'Connor, G. LoPreste, and R. L. Swift. 1984. Human leukocytic pyrogen test to detect pyrogenic material in growth hormone from recombinant *E. coli*. *J. Clin. Microbiol.* **20**:323-329.
11. Dinarello, C. A., and S. M. Wolff. 1982. Molecular basis of fever in humans. *Am. J. Med.* **72**:799-812.
12. Duff, G. W., and E. Atkins. 1982. The detection of endotoxin by in vitro production of endogenous pyrogen: comparison with *Limulus* amebocyte lysate gelation. *J. Immunol. Methods* **52**:323-332.
13. Duff, G. W., and E. Atkins. 1982. The inhibitory effect of polymyxin B on endotoxin-induced endogenous pyrogen production. *J. Immunol. Methods* **52**:333-340.
14. Elin, R. J., and S. M. Wolff. 1973. Non-specificity of the *Limulus* amebocyte lysate test: positive reactions with polynucleotides and proteins. *J. Infect. Dis.* **128**:349-352.
15. Gery, I., and B. H. Waksman. 1972. Potentiation of the T-lymphocyte response to mitogens. II. The cellular source of potentiating mediator(s). *J. Exp. Med.* **136**:143-155.
16. Goehl, H., P. Konstantin, and C. A. Gullberg. 1982. Hemofiltration membranes. *Contrib. Nephrol.* **32**:2030-2041.
17. Henderson, L. W., and F. Bears. 1978. Successful production of sterile, pyrogen-free electrolyte solution by ultrafiltration. *Kid. Internat.* **14**:522-525.
18. Ikejima, T., C. A. Dinarello, D. M. Gill, and S. M. Wolff. 1984. Induction of interleukin-1 by a product of *Staphylococcus aureus* associated with toxic shock syndrome. *J. Clin. Invest.* **73**:1312-1320.
19. Kaye, J., and C. A. Janeway, Jr. 1984. Induction of receptors for interleukin-2 requires T-cell antigen:Ia receptor cross-linking and interleukin-1. *Lymphokine Res.* **3**:175-182.
20. Kotani, S., H. Takada, I. Takahashi, M. Tsujimoto, T. Ogawa, T. Ikeda, K. Harada, H. Okamura, T. Tamura, and S. Tanaka. 1986. Low endotoxic activities of synthetic *Salmonella*-type lipid A with an additional acyloxyacyl group on the 2-amino group of beta (1-6) glucosamine disaccharide 1,4'-bisphosphate. *Infect. Immun.* **52**:872-874.
21. Lomedico, P. T., U. Gubler, C. P. Hellman, M. Dukovich, J. G. Giri, Y. E. Pan, K. Collier, R. Semionow, A. O. Chua, and S. B. Mizel. 1984. Cloning and expression of murine interleukin-1 in *Escherichia coli*. *Nature (London)* **312**:458-462.
22. Lonnemann, G., K. M. Koch, S. Shaldon, and C. A. Dinarello. 1986. Stimulation of monocyte interleukin-1 production by human plasma dialyzed against *Escherichia coli* microfiltrate in dialysate. *Blood Purification* **4**:207-208.
23. Pearson, F. C., J. Bohon, W. Lee, G. Bruzer, M. Sagona, G. Jakubowski, R. Dawe, D. Morrison, and C. A. Dinarello. 1984. Characterization of *Limulus* amebocyte lysate-reactive material from hollow-fiber dialysers. *Appl. Environ. Microbiol.* **48**:1189-1196.
24. Raetz, C. R. 1984. The enzymatic synthesis of lipid A: molecular structure and biological function of monosaccharide precursors. *Rev. Infect. Dis.* **6**:463-471.
25. Rietschel, E. T., H. W. Wollenweber, H. Brade, U. Zaehring, B. Linder, U. Seydel, H. Bradaczek, G. Barnickel, H. Labischinski, and P. Giesbrecht. 1984. In E. T. Rietschel (ed.), *Handbook of endotoxin*, p. 187-210. Elsevier Biomedical Press, Amsterdam.
26. Wolff, S. M. 1973. Biological effects of bacterial endotoxins in man. *J. Infect. Dis.* **128**:259-64.