

The Search for an Endogenous Activator

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Certain febrile diseases are unaccompanied by infection or apparent hypersensitivity. In myocardial infarction or pulmonary embolism, for example, fever has been attributed to inflammation and/or tissue necrosis. Exogenous (microbial) pyrogens stimulate both human and animal monocytes/macrophages to produce endogenous pyrogen (EP) *in vitro*. To determine if plasma and cellular endogenous mediators (EMs) of inflammation induced EP production, human mononuclear cells (M/L) were incubated for 18 hours with varying amounts of EM and the supernates assayed for EP in rabbits. Neutrophils (PMNs), which do not generate EP and yet are a feature of acute inflammation, were tested. Neither viable, phorbol myristic acetate-stimulated PMNs nor sonicated PMNs, red blood cells, or M/L stimulated human monocytes to produce EP. Human C_{3b} and C_{5a}, which mediate phagocytosis and chemotaxis, respectively, were also inactive. Despite its chemoattractant properties, the synthetic peptide FMLP failed to induce EP release. Since Poly I:Poly C (PIC: a synthetic, double-stranded RNA) is a potent pyrogen in rabbits, we investigated PIC, as well as a native, single-stranded RNA (from *E. coli*) and DNA (from calf thymus). None was active *in vitro*, and only PIC caused fever when given to rabbits intravenously. In summary, we have been unable to find an endogenous activator of EP from human monocytes to explain fevers associated with inflammation alone.

INTRODUCTION

Fever is a characteristic sign of inflammation whether it be due to infection, tissue damage, or a hypersensitivity reaction. The pathogenesis of fever in infectious diseases has been attributed to certain microbial factors (such as LPS-endotoxin) that induce monocytes or macrophages of the host to release endogenous pyrogen/interleukin-1 (EP/IL-1). Specialized cells in the anterior hypothalamus are acted upon by EP/IL-1 to initiate the peripheral changes (decreased heat loss and increased heat production) that elevate body temperature [1]. Other exogenous pyrogens, including antigen-antibody complexes or antigens and lectins (which appear to act indirectly via the release of lymphokines), are also recognized as inducers of EP production. However, the sequence of events by which inflammation causes fever, in the apparent absence of infection or allergy, remains unclear. Recently, urate crystals have been reported to release EP *in vitro*, suggesting one mechanism whereby an endogenous activator may cause fever in gouty inflammation [2].

There are, however, other diseases often associated with fever such as myocardial or pulmonary infarction, thrombophlebitis, and dissecting aneurysm for which there is no known pyrogenic stimulus. Fever in these illnesses has generally been attributed to

associated inflammation—a response to tissue injury that involves the coordinated release of multiple endogenous agents [8].

In an attempt to identify the probable cause of fever in such diseases, components of the inflammatory process and associated by-products of tissue disruption were evaluated for their capacity to activate human monocytes to release EP *in vitro*.

METHODS

General

Previously employed methods were utilized to insure that all procedures, glassware, and reagents were sterile and pyrogen-free [3].

Pyrogen Assay

Techniques for assay of EP in trained rabbits were the same as reported previously [3]. Since rabbits become sensitized and react with fever when given foreign antigens after repeated intravenous inoculations, new groups of animals were used at weekly intervals. The fever response was expressed as the maximum temperature increase from baseline occurring within 60 minutes following injection. Significance of mean responses was calculated by the appropriate *t*-test.

Preparation of Cells and Serum

Human venous blood (100–200 ml) was collected from normal volunteers with heparinized (10 μ /ml) syringes (heparin sodium injection [10,000 μ /ml]; Eli Lilly and Company, Indianapolis, IN). Each 25 ml of blood was diluted with 15 ml Eagle minimal essential media ([MEM] Auto-Pow; Flow Laboratories, Inc., Rockville, MD) containing penicillin/streptomycin ([5,000 μ /ml/5,000 μ /ml] Gibco Laboratories, Grand Island, NY) and 1 ml of heparin (1,000 μ /ml). Leukocytes (enriched for monocytes and lymphocytes) were collected by centrifugation through Ficoll-Hypaque (Ficoll; Pharmacia Fine Chemicals, Piscataway, NJ; Hypaque sodium [50 percent]; Winthrop Laboratories, New York, NY) density gradients [4].

Dextran (100,000–200,000 molecular weight; ICN Nutritional Biochemicals, Cleveland, OH) sedimentation with hypotonic lysis of erythrocytes (RBCs) was used to obtain neutrophils (PMNs) [5].

Both monocyte-lymphocyte (M/L) fractions and PMNs were washed twice in MEM and the pellets resuspended in MEM with either 10 percent fetal bovine sera ([FBS], Flow Laboratories, Inc.) or 10 percent autologous normal human sera (NHS) except for the Poly I:Poly C experiments where aliquots were incubated with MEM alone. Human blood for sera was obtained by venipuncture, allowed to clot for two hours at 37°C, and centrifuged at 1,800 rpm \times 15 minutes. To abolish complement activity, sera was then placed in a 56°C stationary water bath for 15 minutes.

Cell counts were determined with a Coulter Particle Counter (model Z_i; Coulter Electronics, Inc., Rockville, MD). Viability was \geq 90 percent by eosin red exclusion. M/L fractions contained an average of 30 percent M, 68 percent L, and 1–2 percent PMNs. Each aliquot contained two or more doses of $3\text{--}5 \times 10^6$ M with $6\text{--}10 \times 10^6$ L each in a volume of 4 ml/dose unless otherwise stated.

Polymyxin B

Polymyxin B (PMB) (50,000 μ /ml) was diluted in physiologic saline to 2,000 μ /ml and added to each dose containing a non-cellular experimental activator (i.e., FMLP, PMA, nucleic acids, C_{5a}, and C_{3b} to prevent false positive results secondary to

endotoxin [6]). When added to either complement or FMLP, one batch of PMB regularly activated cells to produce EP. Since further tests with other batches did not confirm these findings, they have been omitted from the results.

Incubation

Aliquots of M/L suspended in MEM alone or MEM/NHS were placed in 30 ml tissue culture flasks (Falcon Labware, Div. Becton, Dickinson and Co., Oxnard, CA) and various activators/EMs added. Each experiment had a "negative" control (M/L in MEM with sera) and a "positive" control of either heat-killed staphylococci (20:1, bacteria:phagocytes) or 1 ng lipopolysaccharide (LPS) [7]. Exogenous pyrogens (staphylococci and LPS) were usually added with each EM to confirm that these M/L cultures were capable of EP release. All preparations were incubated for 18 hours at 37°C in 5 percent CO₂; the aliquots were then centrifuged, and the supernates assayed for endogenous pyrogen (EP). Cell viability after 18 hours incubation was >85 percent. The samples were cultured for 48 hours in thioglycolate broth, and, in the rare instance of contamination, the results were discarded.

Endogenous Materials

Neutrophils (PMNs) PMNs were separated from human blood by dextran sedimentation and hypotonic lysis of erythrocytes. PMN fractions contained an average of 86 percent PMNs, 4 percent L, 0.2 percent M, and 10 percent eosinophils. 3×10^7 PMNs were suspended in 2 ml MEM/NHS. Sonication (Sonifer, Model 200, Branson Sonic Power Co., Danbury, CT) was used for PMN disruption. Before each experiment the sonicator probe was wrapped in foil and baked in an oven at 240°C to insure that it was pyrogen-free. After attachment, the probe was rinsed with 300 ml sterile sodium chloride irrigation solution containing 10 ml penicillin-streptomycin ([5,000 μ /ml/5,000 μ /ml]). As a control, 2 ml MEM/NHS alone was sonicated first (10 seconds \times 3 at 30 watts) and incubated with M/L. The experimental cells (viable or sonicated) were incubated with aliquots of M/L cells and assayed for EP production.

Phorbol Myristic Acetate (PMA) PMA (Consolidated Midland Co., Brewster, NY) was reconstituted as 2.5 mg PMA per 1 ml of DMSO, diluted to 200 mg/ml in DMSO, and stored at -70°C. Prior to use, it was prepared as 100 ng/ml sodium chloride solution. As a control, 100 ng PMA was incubated with $3.0 \pm 0.3 \times 10^6$ M and $8.87 \pm 1.6 \times 10^6$ L in 4 ml MEM/NHS per dose and assayed for EP-inducing activity.

To stimulate the respiratory pathway and release the metabolites and primary granules of PMNs, PMA was added (in dosages of 200 ng/ 2.5×10^7 cells) and incubated in a stationary water bath for ten minutes at 37°C. To remove excess PMA, the cells were centrifuged and resuspended in fresh MEM/NHS. Hydrogen peroxide production by PMA-stimulated PMNs was measured by extinction of scopoletin fluorescence to verify that cell stimulation had occurred [8].

Complement (C_{3b} and C_{5a}) A mixture of purified human complement fragments (C_{3b} and C_{5a}) were kindly supplied by Dr. Donald Kreutzer (University of Connecticut School of Medicine, Farmington, CT). The ED₅₀, defined as the concentration of the complement factor equivalent to one-half its maximum chemotactic response [9], was 16.7 microliters (μ l). Three concentrations/activities were used: $36 \times (600 \mu$ l), $48 \times (800 \mu$ l), and $72 \times (1,200 \mu$ l) the ED₅₀. Each complement concentration was added to 10^6 M and $4-6 \times 10^6$ L, incubated, and assayed.

N-Formyl-methionyl-leucyl-phenylalanine (FMLP) FMLP (Haemachem, Inc. Lot No. 81B1) was provided by Julia Metcalf (Yale University, New Haven, CT) at a concentration of 10^{-2} M in DMSO. The stock solution was diluted with physiologic saline to produce either a final concentration of: (a) 10^{-8} M, at which concentration Showell et al. have demonstrated the chemoattractant properties of FMLP [10]; or (b) 10^{-5} M, at which concentration Hatch et al. demonstrated that FMLP triggers a burst of oxidative metabolism leading to the generation of superoxide [11]. Each FMLP concentration was added to 2.6 ± 0.3 M and $5.65 \pm 3.0 \times 10^6$ L, incubated, and assayed.

Red Blood Cells (RBCs) As previously stated, PMNs were processed and the RBCs were removed from the remaining pellet and diluted into various concentrations (refer to Table 3) using sodium chloride irrigation solution in 1 ml aliquots. Disruption of RBCs was by sonication as described above. An aliquot of disrupted RBCs equivalent to 4×10^7 – 1×10^9 whole RBCs was added to 3×10^6 M, incubated, and assayed.

Mononuclear Cells (M/L) M/L were obtained by processing with Ficoll-Hypaque density gradients. Sonication was performed as described above and cell contents equivalent to 2×10^7 M and 2.7×10^7 lymphocytes in 2 ml per dose were incubated with viable M/L and assayed.

Nucleic Acids

1. Poly I: Poly C—Poly L-lysine (Poly I:C), a synthetic, double-stranded RNA, was kindly supplied by Dr. John Kirkwood (Yale University, New Haven, CT). Stock Solution (Flow Lot 3, 111379 CMC 0.5 percent, Lot cc-134) contained 2 mg/ml Poly I:C and 1.5 mg/ml Poly L-lysine. The Poly I:C stock solution was diluted to a dose equivalent to 40 μ g/ml sodium chloride solution. Each dose was added to 4×10^6 M and 6.6×10^6 Ly, incubated, and assayed.
2. Short-chain nucleic acids—Short-chain calf thymus DNA (1 mg/ml) and *E. coli*-derived RNA (10 mg/ml), kindly provided by Dr. Joan Steitz (Yale University School of Medicine, New Haven, CT), were supplied as stock solutions and diluted with physiologic sodium chloride solution to concentrations of 0.3 mg/ml RNA, 0.5 mg/ml RNA, and 0.2 mg/ml DNA. Each of these mediators was then added to make one or more doses containing 4 ml of MEM with 5.4×10^6 M and 1.04×10^7 L, incubated, and assayed.
3. Long-chain nucleic acids—100 μ g of calf thymus DNA (in 3 ml sodium chloride solution) and 100 μ g of *E. coli*-derived RNA (in 2 ml sodium chloride solution) were given intravenously to determine their pyrogenicity and their capacity to induce EP production *in vivo*.

RESULTS

One aliquot (two doses) of each substance to be evaluated was incubated with one or two known activators (staph and LPS) to insure that the monocytes were not inhibited from releasing EP.¹ In all cases these positive controls produced a mean febrile response of 0.3°C or greater.

¹Three additional agents, prostoglandin E₁, fibronectin (plasma fibronectin) kindly supplied by Dr. Philip J. Spagnuolo (Case Western Reserve University, OH), and platelet activating factor (PAF) were also tested, in doses which have pharmacologic effects, and none was capable of stimulating EP release.

TABLE 1
Release of Endogenous Pyrogen from Human Monocytes Incubated with Various Agents *in Vitro*
(Agents: PMNs and PMA)

Activator	T(°C) ^b	No. Trials
Sonicated medium (MEM)	0.01 ± 0.0	11
Sonicated PMNs (3 × 10 ⁷)	0.02 ± 0.0	13
Sonicated PMNs + LPS (1 ng)	0.34 ± 0.09	5
Sonicated PMNs + Staphylococci (20:1)	1.50 ± 0.30	2
Viable PMNs	0.0	2
LPS (1 ng)	0.88 ± 0.08	6
Staphylococci (20:1)	1.40 ± 0.20	2
PMA (100 ng) ^a	0.04 ± 0.0	10
PMA (100 ng) + LPS (1 ng)	0.88 ± 0.12	3
LPS (1 ng)	1.43 ± 0.09	3
PMA-stimulated PMNs (3 × 10 ⁷)	0.0	2
Viable PMNs	0.0	2

^aPolymyxin B (2,000 μ/dose) was added to the experimental aliquot prior to incubation to remove any contaminating endotoxin due to the agent's non-sterile preparation.

^bMean (± SEM) febrile responses of rabbits to EP released by monocytes incubated 18 hours with each agent

Neutrophils (PMNs)

Neither intact, activated, or disrupted PMNs were capable of stimulating EP release from monocytes (Table 1).

Complement Fragments (C_{3b} and C_{5a})

The human complement fragments of C_{3b} and C_{5a} were not mediators of EP release *in vitro* (Table 2).

FMLP

Neither concentration of 10⁻⁵M nor 10⁻⁸M was capable of stimulating EP release from monocytes (Table 2). Similarly FMLP (10⁻⁸M) was nonpyrogenic when given intravenously.

Sonicated Cells

Neither sonicated RBCs nor M/L stimulated EP release (Table 3).

Nucleic Acids

Multiple trials revealed that mammalian DNA and bacterial RNA as well as Poly I:Poly C were incapable of stimulating human monocytes to produce EP *in vitro* (Table 4).

Long-chain RNA and DNA were also injected intravenously (one recipient each) but did not evoke any change in temperature suggesting *in vivo* EP production (Table 4).

DISCUSSION

The inflammatory reaction has been defined as a series of multiple interlocking components. Injury of any type induces an immediate acute inflammatory response with two main effects: (a) exudation of fluid containing plasma and tissue mediators of

TABLE 2
Release of Endogenous Pyrogen from Human Monocytes Incubated with Various Agents *in Vitro*
(Agents: Complement By-Products [C_{3b} and C_{5a}] and FMLP)

Activator	T(°C) ^b	No. Trials
C _{3b} and C _{5a} (600 μl) ^a	0.04 ± 0.03	6
C _{3b} and C _{5a} (800 μl) ^a	0.0	2
C _{3b} and C _{5a} (1,200 μl) ^a	0.15 ± 0.05	2
LPS (1 ng)	<u>0.63 ± 0.02</u>	3
FMLP (10 ⁻⁸ M) ^a	0.02	5
FMLP (10 ⁻⁸ M) + LPS (1.5 ng)	<u>0.78 ± 0.08</u>	2
LPS (1 ng)	<u>0.72 ± 0.13</u>	3
FMLP (10 ⁻⁵ M) ^a	0.0	3
FMLP (10 ⁻⁸ M) ^c	0.0	6

^aPolymyxin B (2,000 μ/dose) was added to the experimental aliquot prior to incubation to remove any contaminating endotoxin due to the agent's non-sterile preparation.

^bMean (± SEM) febrile responses of rabbits to EP released by monocytes incubated 18 hours with each agent

^cResponse of rabbits to injection of agent in 4 ml of saline

inflammation, and (b) exudation of white blood cells, initially polymorphonuclear leukocytes and later mononuclear forms with their associated release of cell products [12]. Subsequently, factors in the exudate induce healing. Myocardial and pulmonary infarction, which are both associated with fever, result in tissue death and necrosis, thereby activating the inflammatory response *in vivo*. Many of the pathways by which infection produces inflammation and fever have been defined. However, no component of the inflammatory response itself has been implicated as an endogenous inducer of EP release and hence of fever.

As the characteristic cell present in acute inflammation, the PMN, no longer recognized as producing EP, is an obvious choice to be tested as a possible EM of EP release [13,14]. PMNs contain cytoplasm, nucleus, and azurophilic (primary) and specific (secondary) granules and selectively release granule-associated (lysosomal) enzymes when appropriately stimulated. These lysosomal enzymes are capable of producing many of the features of the acute inflammatory response [15]. Therefore, both sonication of PMNs and PMA-induced primary granule release were utilized to produce breakdown of the PMN into its cellular and metabolic components. In our experiments with human blood cells, none of the PMN fractions obtained by sonication or induced by PMA was pyrogenic or an inducer of EP release. Although Herion et al. [16] reported that lysosomal fractions of rabbit (but not human) blood cells and rabbit exudate cells were pyrogenic, the findings in regard to rabbit cells were not confirmed in a later study by Hahn et al. [17]. Positive controls in which LPS or staphylococci were added to samples containing PMN fractions verified that stimulation of EP release from monocytes was possible. It is interesting to note that EP production by LPS but not staphylococci was reduced by about one-half in the presence of sonicated PMNs (Table 1). Collins and Wood have similarly shown that lysosomes and lysosomal basic proteins of leukocytes diminish *in vitro* the pyrogenic and lethal actions of endotoxin (LPS) [18].

Phorbol myristic acetate (PMA) has been reported to be a potent inducer of lymphocyte-activating factor (LAF) [19]. Through the work of several investigators, it now appears that LAF and EP are similar, if not the same, molecule [20,21].

TABLE 3
Release of Endogenous Pyrogen from Human Monocytes Incubated with Various Agents *in Vitro*
(Agent: Sonicated RBCs and M/L)

Activator	T(°C) ^a	No. Trials
Sonicated RBCs (4×10^7)	0.0	7
Sonicated RBCs (1×10^8)	0.01	7
Sonicated RBCs (1×10^8) + LPS (1 ng)	0.52 ± 0.06	3
Sonicated RBCs (1×10^9)	0.05 ± 0.05	2
Sonicated RBCs (1×10^9) + LPS (1 ng)	0.70 ± 0.23	3
Sonicated M/L	0.0	2
Sonicated M/L + LPS (1 ng)	1.0	1
LPS (1 ng)	1.0 ± 0.0	2

^aMean (\pm SEM) febrile responses of rabbits to EP released by monocytes incubated 18 hours with each agent

Therefore, it is necessary to discuss the lack of EP release in our data when cells were incubated with PMA. The addition of polymyxin-B (PMB) in our experiments may have inactivated contaminating LPS and thus removed the true stimulus (LPS) to EP (and the previously reported LAF) release [6]. However, a more likely explanation is provided by the recent observation that PMA binds to unknown serum, cellular, and other proteins to produce its mitogenic effects (by directly stimulating IL-2 rather than IL-1 (LAF) release as previously suggested [22,23]. This PMA-protein complex is nonpyrogenic in rabbits [23] and, as we have found here, is not an inducer of EP (LAF).

C_{3b} and C_{5a} , activated by-products of the complement cascade, are important in promoting certain aspects of inflammation such as chemotaxis, phagocytosis, increased vascular permeability, and histamine release. The significance of testing the complement cascade by-products (C_{3b} and C_{5a}) for their ability to induce EP production as a side effect of inflammation rests on the following: (1) chemotactic C_3 fragments have been found in recently infarcted heart muscle [24] and in synovial fluids of inflammatory non-rheumatoid joint disease [25]; (2) chemotactic C_5 fragments are present in synovial fluids of rheumatoid arthritis [25]; and (3) chemotactic factors and substances enhancing phagocytosis are regularly generated in any type of inflammatory process.

Although Goodman et al. have reported that C_5 is an inducer of LAF production [26], human fragments C_{3b} and C_{5a} failed to produce EP in the presence of PMB in our experiment. Again it is clear that the validity of EP/LAF production may require reinvestigation of earlier reports where the possibility of endotoxin (LPS) contamination has not been excluded [27]. In our work, both C_{5a} and C_{3b} were potent inducers of EP *in vitro* unless PMB was added.

The stimulation of PMN random locomotion, chemotaxis, lysosomal enzyme release, and the generation of superoxide (O_2^-) are some of the activities of synthetic N-formylmethionyl peptides including N-formyl-methionyl-leucyl-phenylalanine (FMLP) [10,11]. These activities are responses of phagocytic cells (both PMNs and M) to multiple stimuli and are commonly present during the inflammatory process. FMLP combines its chemotactic properties with the ability to trigger a burst of oxidative metabolism, thereby simulating the natural activities of C_{5a} . As Table 2 demonstrates, the natural chemoattractant C_{5a} and FMLP were incapable of provok-

TABLE 4
Release of Endogenous Pyrogen from Human Monocytes Incubated with Various Agents *in Vitro*
(Agents: Synthetic and Natural Nucleic Acids)

Activator	T(°C) ^a	No. Trials
Poly I: Poly C (40 μg)	0.0	3
LPS (1 ng)	1.17 ± 0.16	3
Short-Chain Nucleic Acids:		
<i>E. coli</i> -derived RNA (0.3 mg)	0.03 ± 0.0	4
<i>E. coli</i> -derived RNA (0.5 mg)	0.11 ± 0.04	4
Calf thymus DNA (0.2 mg) ^b	0.10 ± 0.0	2
Long-Chain Nucleic Acids:		
<i>E. coli</i> -derived RNA (100 μg) ^b	0.0	1
Calf thymus DNA (100 μg) ^b	0.0	1

^aPolymyxin B (2,000 μ/dose) was added to the experimental aliquot prior to incubation to remove any contaminating endotoxin due to the agent's non-sterile preparation.

^bResponse of rabbits to agent injected intravenously

ing EP release. Thus it appears from these data that neither of these agents, despite having receptors on monocytes, is responsible for producing fever with inflammation alone.

The synthetic, double-stranded RNA, Poly I:Poly C, was reported to be a potent pyrogen in rabbits [28]. Natural DNA and RNA are nuclear contents released with cellular disruption, but they have never been investigated for their ability to produce EP. In general, the nucleotide sequence of DNA is both the source of information for the synthesis of proteins and the chemical basis of heredity. RNA functions as a template for genetic information and protein synthesis.

In 1970 Nordland et al. [28] reported that human sera (and plasma) and, to a lesser extent, calf sera had an inhibitory effect (due, presumably, to an RNAase) on the pyrogenicity of Poly I:Poly C. Therefore, all our experiments with nucleic acids were performed in MEM alone. The results (Table 4) suggest that neither synthetic nor natural nucleic acids stimulate EP release from human monocytes *in vitro* even in the absence of these serum inhibitors.

In a review of 24 cases of acute hemolytic anemia of various etiologies, Wallenstein and Aggeler describe 13 patients with temperatures of 38°C or higher for which no cause could be found [29]. In similar fashion, Hirst et al. reviewed 505 cases of dissecting aneurysm and found fever higher than 38°C in 23.6 percent of the patients and higher than 39°C in 7.9 percent [30]. Despite features common to both, for example fever and red blood cell (RBC) disruption and hemolysis, the mechanisms by which acute nonimmune hemolytic anemia and dissecting aneurysms produce fever are unknown. These characteristics suggested that sonicated/disrupted RBCs might contain endogenous activators of EP release. As Table 3 demonstrates, dosages up to 1×10^9 sonicated RBCs failed to generate EP from monocytes, making it unlikely that this stimulus alone is responsible for producing fever in clinical situations where there is nonimmune hemolysis or sequestration of blood in tissues or cavities.

CONCLUSION

In 1979, Atkins and Bodel discussed several disease categories and the frequency with which they were associated with fever [31]. They concluded that "clinical fever

seems clearly associated with those diseases in which inflammation, in one form or another, plays a dominant role." In infectious diseases, for example, the role of phagocytosis and/or endotoxin is well established. The endogenous agents and synthetic materials studied in this report were chosen for their known involvement in the inflammatory process and/or therefore possible role as EP-producing stimuli.

By 1968 Bodel and Hollingsworth [32] had described a pyrogen (resembling EP) in the joint fluid of patients with rheumatoid arthritis. In 1983 Wood et al. [33] reported small quantities of a molecule similar to IL-1 from the joint fluids of patients with rheumatoid arthritis, psoriatic arthritis, Reiter's syndrome, osteoarthritis, gout, and traumatic arthritis. In certain systemic collagen vascular diseases (systemic lupus erythematosus, Still's disease—juvenile rheumatoid arthritis) fever is commonly prominent and autoimmunity with associated antigen (Ag)-antibody (Ab)-complement complexes may play a significant role in the pathogenesis of fever as well as other features of these illnesses.

Ag-Ab complexes alone do not stimulate EP release *in vitro*; however, when complement is added, the total complex can induce EP production [34]. Our work suggests that complement factors will not evoke EP release. Complement may thus act as an essential cofactor for endogenous Ag-Ab complexes which then are capable of inducing EP production. Consistent with this hypothesis, injected complexes (in Ag-excess) were markedly less pyrogenic in rabbits which had been decomplemented by cobra venom [35].

Cellular debris, including nucleic acids, is also a major component of the inflammation process. Lysosomal and bactericidal components of phagocytic cells as well as the by-products of activated lymphocytes are all present at the site of tissue injury. Febrile clinical conditions associated with tissue injury and often sterile inflammation include multiple pulmonary emboli, thrombophlebitis, pancreatitis, myocardial infarction, and dissecting aneurysms. The cellular subfractions of disrupted PMNs, M/L, and RBCs that were studied suggest that none of these components alone is the cause of fever associated with sterile inflammation and/or tissue necrosis. Whether multiple factors are required together to stimulate EP production remains to be determined.

Both histamine and serotonin (5-hydroxytryptamine) are vasoactive amines released from mast cells and platelets during acute allergic inflammation. We did not investigate these agents which, like the kinins, may produce hypotension and dramatic changes in vascular tone and permeability when given in large doses. With the exception of systemic mastocytosis, where fever is occasionally present as a prominent finding [36], most diseases in which these agents are released are not characteristically febrile.

Ryan and Majno [12] have defined inflammation as a response of living tissue to local injury which leads to accumulation of blood cells and fluids, a process presumably selected in evolution as a defense against infection. In those febrile diseases associated with sterile, nonallergic inflammation, the "invader" may be some cellular by-product of tissue damage which the body does not recognize as "self." Production of Ag-Ab complexes with fixation of complement may then stimulate monocytes to produce EP and hence fever. One argument against autosensitization as the mechanism of fever production in such states, however, is the rapid onset of fever that occurs, often within hours, after trauma, thrombophlebitis, or infarction.

Although the endogenous stimuli to EP production in acute sterile inflammation thus remain unidentified, physicians have long been aware that fever and inflamma-

tion are closely associated [37]. Studies in ferrets [38] and, more dramatically, in poikilothermic lizards [39] indicate that fever is not only a product of inflammation, but, in turn, directs and accelerates the inflammatory response to the site of the infection (presumably by the generation of various chemotactic stimuli) and thereby limits its spread. Only now are we beginning to discover the number and diversity of interrelated phenomena that are controlled by IL-1 and that have presumably contributed to evolutionary aspects of survival [40].

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REFERENCES

1. Dinarello CA, Wolff SM: Pathogenesis of fever in man. *New Eng J Med* 298:607-612, 1978
2. Duff GW, Atkins E, Malawista SE: The fever of gout: Urate crystals activate endogenous pyrogen production from human and rabbit mononuclear phagocytes. *Trans Assoc Am Physn* 96:234-245, 1983
3. Atkins E, Feldman JD, Francis L, Hursh E: Studies on the mechanisms of fever accompanying delayed hypersensitivity. The role of the sensitized lymphocyte. *J Exp Med* 135:1113-1132, 1972
4. Bodel P: Studies on the mechanism of endogenous pyrogen production. III. Human blood monocytes. *J Exp Med* 140:954-965, 1974
5. Skoog WA, Beck WS: Studies on the fibrinogen, dextran, and phytohemagglutinin methods of isolating leukocytes. *Blood* 11:436-454, 1956
6. Duff GW, Atkins E: The inhibitory effect of PMB on endotoxin-induced endogenous pyrogen production. *J Immunol Meth* 52:333-340, 1982
7. Duff GW, Atkins E: The detection of endotoxin by *in vitro* production of endogenous pyrogen: Comparison with limulus amebocyte lysate gelation. *J Immunol Meth* 52:323-331, 1982
8. Root RK, Metcalf J, Oskino N, Chance B: H₂O₂ Release from human granulocytes during phagocytosis. I. Documentation, quantitation, and some regulating factors. *J Clin Invest* 55:945-955, 1975
9. Kreutzer DL, O'Flaherty JT, Orr W, Showell HJ, Word PA, Beck EL: Quantitative comparisons of various biological responses of neutrophils to different active and inactive chemotactic factors. *Immunopharmacology* 1:39-47, 1978
10. Showell HJ, Freer RJ, Zigmond SH, Schiffman E, Aswanikumar S, Corcordn B, Becker EL: The structure-activity relations of synthetic peptides as chemotactic factors and inducers of lysosomal enzyme secretion for neutrophils. *J Exp Med* 143:1154-1169, 1976
11. Hatch GE, Gardner DE, Menzel DB: Chemiluminescence of phagocytic cells caused by N-formylmethionyl peptides. *J Exp Med* 147:182-195, 1978
12. Ryan G, Majno G: Acute Inflammation. *Am J of Path* 86:185-276, 1977
13. Hanson DF, Murphy PA, Windle BE: Failure of rabbit neutrophils to secrete endogenous pyrogen when stimulated with staphylococci. *J Exp Med* 151:1360-1371, 1980
14. Dinarello CA, Scott D, Rosenwasser LJ, Wolff SM: Purified human neutrophils do not produce leukocytic pyrogen nor lymphocyte activating factor (Abstract). *Clin Res* 30:364A, 1982
15. Goldstein IM: Polymorphonuclear leukocyte lysosomes and immune tissue injury. *Prog Allergy* 20:301-340, 1976
16. Herion JC, Spitznagel JK, Walker RI, Zeya HI: Pyrogenicity of granulocyte lysosomes. *Am J Physiol* 211:693-698, 1966
17. Hahn HH, Chenk SF, Elfenbein CD, Wood WB Jr: Studies on the pathogenesis of fever. XIX. Localization of pyrogen in granulocytes. *J Exp Med* 131:701-710, 1970
18. Collins RD, Wood WB Jr: Studies on the pathogenesis of fever. VI. The interaction of leukocytes and endotoxin *in vitro*. *J Exp Med* 110:1005-1016, 1959
19. Mizel SB, Rosenstreich DL, Oppenheim JJ: Phorbol myristic acetate stimulates LAF production by the macrophage cell line, P388D₁. *Cell Immunol* 40:230-235, 1978
20. Rosenwasser LJ, Dinarello CA, Rosenthal AS: Adherent cell function in murine T-lymphocyte antigen

- recognition. IV. Enhancement of murine T-lymphocyte antigen recognition by human leukocytic pyrogen. *J Exp Med* 150:709–714, 1979
21. Murphy PA, Simon PL, Willoughby MF: Endogenous pyrogens made by rabbit peritoneal exudate cells are identical with lymphocyte-activating factors made by rabbit alveolar macrophages. *J Immunol* 124:2498–2501, 1980
 22. Orosz CG, Roopernian DC, Bach FH: Phorbol myristic acetate and in vitro T-lymphocyte function. I. PMA may contaminate lymphokine preparations and can interfere with interleukin bioassays. *J Immunol* 130:1764–1769, 1983
 23. Rosenwasser LJ, Dinarello CA, Chalef B, Reddish M, Strom PB: Phorbol myristate acetate-protein complex mimics bioactivity of human IL-1. Direct evidence that PMA temporarily enters and is released from cellular compartments during superinduction protocol. *Cell Immunol*, in press
 24. Hill JH, Ward PA: The phlogistic role of C₃ leukotactic fragments in myocardial infarcts of rats. *J Exp Med* 133:885–900, 1971
 25. Ward PA, Zraifler NJ: Complement derived leukotactic factors in inflammatory synovial fluids of humans. *J Clin Invest* 50:606–616, 1971
 26. Goodman MG, Chenoweth DE, Weigle WO: Detection of interleukin-1 secretion and enhancement of humoral immunity by binding of human C_{5a} receptors. *J Exp Med* 156:912–917, 1982
 27. Fumarola D: Contaminating Endotoxin: A serious problem in immunologic research. *Cell Immunol* 58:216–217, 1981
 28. Nordland JJ, Wolf SM, Levy HB: Inhibition of biologic activity of Poly I:Poly C by human plasma. *Proc Soc Exp Biol Med* 133:439–444, 1970
 29. Wallenstein RO, Aggeler PM: Acute Hemolytic Anemia. *Am J Med* 37:92–104, 1969
 30. Hirst AE, Johns VJ Jr, Kime SW: Dissecting aneurysm of the aorta: a review of 505 cases. *Medicine* 37:217–279, 1958
 31. Atkins E, Bodel P: Clinical fever: its history, manifestations and pathogenesis. *Fed Proc* 38:57–63, 1979
 32. Bodel PT, Hollingsworth JW: Pyrogen release from human synovial exudate cells. *Br J Exp Pathol* 49:11–19, 1968
 33. Wood DD, Ihrie EJ, Dinarello CA, Cohen PL: Isolation of an interleukin-1-like factor from human joint effusions. *Arthritis Rheum* 26:975–983, 1983
 34. Arend WP, Joslin FG, Massoni RJ: Stimulation of production of IL-1 and an IL-1 inhibitor in human monocytes. *Br J Rheum*, in press
 35. Mickenberg ID, Snyderman R, Root RK, Mergenhagen SE, Wolff SM: The relation of complement consumption to immune fever. *J Immunol* 107:1466–1476, 1971
 36. Clinicopathologic Conference: Systemic Mastocytosis. *Am J Med* 61:671–679, 1976
 37. Atkins E: Fever: historical perspectives and evolution of modern views. *Br J Rheum*, in press
 38. Toms GL, Davies JA, Woodward CG, Sweet C, Smith H: The relation of pyrexia and nasal inflammatory response to virus levels in nasal washings of ferrets infected with influenza viruses of differing virulence. *Br J Exp Path* 58:444–458, 1977
 39. Bernherin HA, Bodel PT, Askenase PW, Atkins E: Effects of fever on host defence mechanisms after infection in the lizard *Dipsosaurus dorsalis*. *Br J Exp Path* 59:76–84, 1978
 40. Dinarello CA: Interleukin-1. *J Infect Dis* 6:51–95, 1984