STUDIES ON THE MECHANISM OF ENDOGENOUS PYROGEN PRODUCTION

III. Human Blood Monocytes*

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Endogenous pyrogen (EP),[1] the product of stimulated cells which mediates experimental fever (1), was first believed to originate only from polymorphonuclear (PMN) leukocytes (2). The mechanisms by which these cells produce EP have been studied in several laboratories, first using cells from rabbit peritoneal exudates (3, 4) or leukocytes from rabbit blood (5). After it was discovered that human blood leukocyte pyrogen produces fever in rabbits (6), assays based on this activity were developed (7, 8), and have been used to examine in some detail the mechanism of pyrogen production by human blood leukocytes stimulated in vitro by phagocytosis (9, 10).

Recently, mononuclear cells of varying types have been shown to release pyrogen after stimulation. Such cell types include human blood monocytes (11), rabbit peritoneal macrophages (12), lung macrophages (13), and phagocytic cells of liver (14), spleen (13), and lymph node (13). Although monocytes and peritoneal macrophages produce larger amounts of pyrogen than do PMN leukocytes (11, 12), little is known about the processes by which mononuclear cells produce pyrogen. The following studies were therefore undertaken to examine the characteristics of cell activation, and production and release of pyrogen by human blood monocytes.

Materials and Methods

All materials, glassware, and reagents used in these studies were made sterile and pyrogen-free, usually by heating at 160°C for 2 h or autoclaving for 90 min. Materials which were not heat-stable were sterilized by passage through a Millipore filter (Swinnex-25, Millipore Corporation, Bedford, Mass.) and tested for pyrogenicity either by direct injection into rabbits, or by incubation with blood leukocytes and subsequent injection of incubation supernate.

Pyrogen Assay. Techniques for injection and temperature monitoring of rabbits were as described previously (8). Usually, supernates from 2–4 $\times$ 10^6 monocytes constituted a single assay dose. In most experiments, supernates from all samples were injected into the same group of rabbits.

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[1] *Abbreviations used in this paper:* EP, endogenous pyrogen; PMN, polymorphonuclear.

Quantification of results, and computation of derived ΔT were as described previously (8) (see also Results). Derived ΔT is expressed per 1×10^7 monocytes.

Preparation of Monocytes. Heparinized blood (10 U heparin/ml) was drawn by venipuncture from normal donors. 100–120 ml was added to 3 vol of saline, stirred slowly with a magnetic stirring bar, and the mixture layered by siphoning through polyethylene intravenous tubing over 8-ml Ficoll-Hypaque (15) in 40-ml centrifuge tubes. In some experiments, platelets were first removed by centrifuging the blood at 200 *g* for 20 min, separating platelets from the plasma supernate during another centrifugation at 2,000 *g* for 30 min, and then recombining plasma and blood cells before mixing with saline. After the rings of monocytes and lymphocytes had been obtained, the cells were washed once in heparinized Krebs-Ringer phosphate buffer, and the buttons resuspended in 6–8 ml of Eagle's Minimum Essential Medium (MEM; Auto-POW, Flow Laboratories, Inc., Rockville, Md.). Numbers of cells were determined with a Coulter particle counter, model Z_F (Coulter Electronics, Inc., Hialeah, Fla.), and differential smears were made.

Monocyte Incubation. Aliquots of 3×10^7 cells (20–30% monocytes, 70–80% lymphocytes) were suspended in 5 ml of 15% homologous serum-MEM, and placed in 30-ml tissue culture flasks (Falcon Plastics, Div. of BioQuest, Oxnard, Calif.). After brief exposure to CO_2 in air, the flasks were incubated in a humidified 5% CO_2 atmosphere at 37°C for varying times. At intervals, supernates were removed with a Pasteur pipette, and fresh medium placed in the flasks. Counts and differentials indicated that about 80% of the lymphocytes were removed at the time of the first medium change. When required, remaining nonadherent lymphocytes were removed by rinsing the monolayers with five changes of MEM. In some experiments, nonadherent cells were recovered by centrifugation at 150 *g* for 10 min, and either returned to the monocyte monolayers or incubated alone in separate flasks, at a concentration of 2×10^6 cells/ml. Nonadherent cells are referred to as lymphocytes. All supernates were prepared for injection by centrifugation at 1,000–2,000 *g* for 20–40 min. They were cultured to confirm sterility, and stored at 4°C for 1–7 days before assay (8).

To prepare a pool of monocyte pyrogen, cells from three donors were obtained (total 9.1×10^7 monocytes), incubated overnight with heat-killed staphylococci, and supernates tested for pyrogen content and sterility. All supernates were then pooled, divided into appropriate doses in separate tubes, and frozen at −20°C until use. An average minimal pyrogenic dose for a rabbit ($\Delta T \geq 0.3$°C) was calculated to be derived from 8×10^5 monocytes.

Cells in tissue culture flasks were observed daily by light microscopy. In some experiments cells were incubated on cover slips in small Petri dishes, and at various time intervals cover slips were rinsed, fixed in buffered formalin or glutaraldehyde for 30 min, or mounted without fixing, and examined by phase microscopy.

Cell-Asociated Pyrogen. For assay of cell-associated (intracellular) pyrogen content, after appropriate incubation, cell monolayers were rinsed three times with MEM, a small volume of fresh medium added, and the flasks placed at −20°C. Subsequently the cells were disrupted by freeze-thawing 5 times by alternating placement of the flasks at −20° or 37°C. Supernatant medium was then removed and the flask surface was rinsed vigorously with an additional volume of fresh medium. All fluids obtained were centrifuged at 2,000 *g* for 20 min and the supernates assayed for pyrogen content, as above.

Reagents. A particle-free preparation of endotoxin was prepared by centrifugation of typhoid vaccine (Monovalent Reference Std. NR V-LS no. 1) at 20,000 *g* for 1 h. Clear supernate contained about 10 pyrogenic doses per ml (average ΔT 1.0°C per dose). Heat-killed *Staphylococcus albus* was prepared as described previously (9); 20–30 bacteria per monocyte were added to flasks. Puromycin HCl was prepared as described previously (9), and added to final concentrations of 0.8, or 5×10^{-6} M.

Results

Assay of Monocyte Endogenous Pyrogen. In order to determine the dose-response characteristics of human monocyte pyrogen assayed in rabbits, a pool of pyrogen-containing supernate was prepared from monocytes stimulated with heat-killed staphylococci (see Methods). Average temperature responses in seven rabbits to injection of two doses of this pooled pyrogen are presented in Fig. 1.

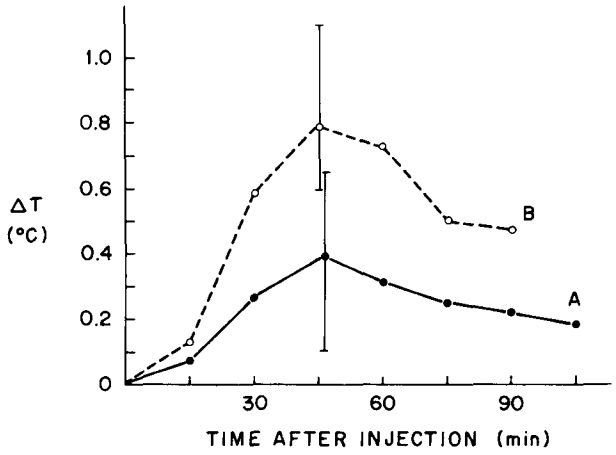

FIG. 1. Average temperature responses in the same seven rabbits after injection of monocyte pyrogen at two doses. Total range of peak responses at 45 min is shown. Supernate per rabbit was derived from 1.2×10^6 monocytes (A) or 3×10^7 monocytes (B).

The response is monophasic, with the peak temperature elevation occurring between 45 and 60 min after injection; these characteristics are the same as those previously reported for human blood leukocyte (PMN) pyrogen (6).

The dose-response data are presented in Fig. 2 *a* and *b*. Maximum temperature responses are plotted against increasing doses of pyrogenic supernate, on a linear (*a*) and semilogarithmic (*b*) scale. The results again closely resemble those obtained previously with human PMN leukocyte pyrogen (8), except that equivalent pyrogenic responses (average ΔT 0.6°C) were obtained with supernate from 2×10^6 monocytes compared to 3×10^7 PMN leukocytes. Although the temperature responses to 10-fold increased doses of monocyte pyrogen follow a logarithmic curve, as determined by statistical tests for trend,[2] the lower portion of the curve (average ΔT from 0.3°–0.9°C) is linear (see Fig. 2 *a*). This range, therefore, was used routinely to estimate pyrogenic content of experimental supernates.

Endogenous Pyrogen Assay After Cell Stimulation by Endotoxin. Monocytes stimulated with endotoxin adhered well to plastic or glass, unlike cells stimulated by phagocytosis, and were therefore convenient cells to use for timed studies of pyrogen release. Tests were done to determine whether endotoxin would confuse the assay for EP produced during the incubation. When the maximum dose of endotoxin was mixed with medium and injected into rabbits moderate pyrogenicity was observed (average ΔT 0.58°C in eight rabbits). However, after the same dose was incubated with cells for 2–3 h, no significant pyrogen remained in the supernate (average ΔT 0.14°C in seven rabbits). Similar rapid disappearance of endotoxin has been observed with blood (16) and exudate (17) PMN leukocytes. In addition, pyrogen released by or present in monocytes after 8- or 18-h incubations with endotoxin was tested in rabbits before and after they were rendered tolerant to endotoxin by a single, large injection of typhoid

[2] Kindly performed by Dr. Grace Wyshak.

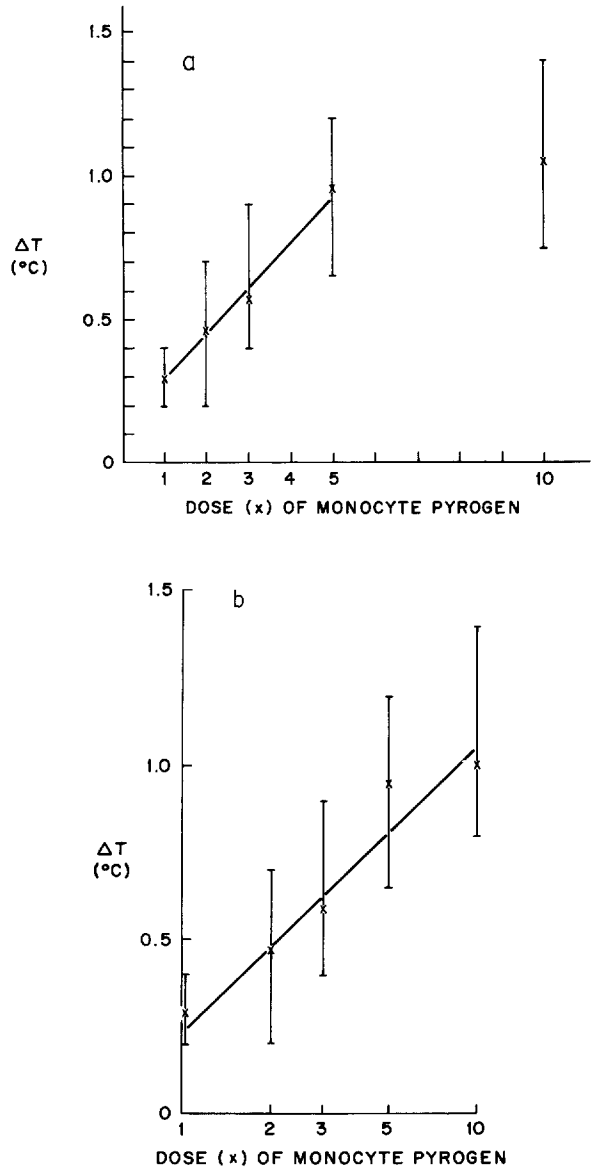

FIG. 2. (*a*) Average maximum height of fever in the same seven rabbits after injection of increasing doses of monocyte pyrogen, 1× – 10×, where 1× is a minimal pyrogenic dose derived from about 8×10^5 monocytes (see Methods). Total range of responses is enclosed in brackets. (*b*) The same data are plotted with log dose on the abscissa. The best line describing the curve was determined by the method of least squares.

vaccine (18). Pyrogenicity of the solutions was not changed in the tolerant animals. Under these experimental conditions, therefore, endotoxin apparently does not confuse the assay for EP.

Effect of Incubation Conditions on Pyrogen Release. In initial studies, monocytes were stimulated with endotoxin or phagocytosis, and incubated either

in shaking Erlenmeyer or stationary tissue culture flasks for 18 h. Cells to which staphylococci had been added produced two or three times as much pyrogen as did those stimulated with endotoxin. Monocytes fixed to plastic produced the same amounts of pyrogen as did cells suspended in a shaking system. Cells incubated without stimuli in either system did not release pyrogen, except on rare occasions. Monocytes maintained in tissue culture flasks without added stimuli for up to 5 days also did not contain detectable pyrogen, nor release it spontaneously, even though they were observed to enlarge, spread, and become increasingly vacuolated, as described by others (19).

Time Required for Cell Activation. Monocyte monolayers were exposed to endotoxin for 15, 45, or 90 min. Endotoxin-containing medium was then discarded, fresh medium added to flasks, and the incubation continued for 18 h. One set of flasks was incubated with the original endotoxin medium for 18 h. All supernates were then assayed for pyrogen. The results are shown in Table I.

TABLE I

Effect of Time of Incubation with Endotoxin on Monocyte Pyrogen Production

	Incubation with endotoxin				
	0 min	15 min	45 min	90 min	18 h
	$\Delta T(°C) + SEM$*				
Pyrogen in 18-h supernate	0.10 ± 0.02	0.12 ± 0.04	0.50 ± 0.08	0.58 ± 0.08	0.67 ± 0.07

* Average of eight assays each.

Although a 15-min exposure to endotoxin was not sufficient to initiate pyrogen production, 45-min and 90-min exposures were almost as effective as incubation for 18 h with endotoxin. These results indicate that monocytes require only 45 min of contact with medium containing endotoxin in order to produce pyrogen subsequently. Similar results were obtained when phagocytosis of staphylococci was used as the stimulus.

Effects of Lymphocytes on Pyrogen Production by Monocytes. Since lymphocytes can influence the function of monocytes or macrophages in other systems (20–23), the role of lymphocytes in the initiation or continuation of pyrogen production was examined. Initial experiments showed that flasks from which lymphocytes had been removed by repeated washings produced as much pyrogen following addition of either endotoxin or staphylococci as did flasks containing 10–20% lymphocytes. To examine this question further, two types of experiments were done.

In the first, lymphocytes were incubated with endotoxin for 1–2 h before being added to monocyte monolayers, to determine whether such lymphocytes could initiate pyrogen production by monocytes. After exposure to endotoxin, the lymphocytes were washed twice and suspended in fresh medium to remove traces of endotoxin. Other lymphocytes were treated similarly, but endotoxin was not added. To some flasks of monocytes no lymphocytes were returned but endotoxin was added directly. After 16 h, pyrogen content of all supernates was measured.

The results are shown in Table II. Preincubation of lymphocytes with endotoxin did not result in significant pyrogen production by monocytes when the two cell types were subsequently incubated together. Thus no evidence was obtained that lymphocytes mediate activation of monocytes by endotoxin.

To examine the influence of lymphocytes on continuing pyrogen production by monocytes, flasks containing both cell types were incubated with staphylococci or endotoxin for 2–3 h. Supernatant fluid and nonadherent cells were then removed from all flasks, and fresh incubation medium returned; to some flasks, the nonadherent cells were returned. Other aliquots of lymphocytes were resuspended in the original or fresh medium and incubated separately. After 16 h, pyrogen content of all supernates was measured. The results of four such experiments with endotoxin, shown in Table III, indicate that pyrogen release by monocytes was not altered when lymphocytes were present. Thus, there was no apparent effect of lymphocytes on the continuing production of pyrogen by monocytes.

Stability of Monocyte Pyrogen During Incubation. EP from PMN leukocytes retains most of its activity during overnight incubation at 37°C, pH 7.4 (8). EP from monocytes was similarly tested. Pyrogen-containing supernate, collected from cells stimulated by phagocytosis, was assayed for pyrogen content. Aliquots were then reincubated for 18 h, either alone or with cells which had been stimulated by endotoxin or phagocytosis during the preceding 24 h. The results of

TABLE II

Failure of Endotoxin-Exposed Lymphocytes to Stimulate Monocyte Pyrogen Production

	Monocytes + normal lymphocytes	Monocytes + endotoxin-treated lymphocytes	Monocytes + endotoxin (lymphocytes removed)	Endotoxin-treated lymphocytes
		$\Delta T(°C) \pm SEM$*		
Pyrogen in 18-h supernates	0.12 ± 0.03	0.24 ± 0.08	0.89 ± 0.12	0.17 ± 0.07

* Average of eight assays each, using the same eight rabbits.

TABLE III

Effect of Lymphocyte Removal on Pyrogen Production by Monocytes Stimulated with Endotoxin

	Monocytes†	Monocytes with lymphocytes returned	Lymphocytes
		$\Delta T(°C) \pm SEM$*	
Pyrogen in 18-h supernate	0.59 ± 0.07	0.58 ± 0.07	0.09 ± 0.03

* Average of 9–17 assays each.

† Nonadherent cells and medium removed 2–3 h after addition of endotoxin. See text for details.

three such experiments are presented in Table IV. No significant loss of activity was observed when pyrogen-containing supernate was incubated at 37° for 18 h, compared to aliquots stored at 4°C. When supernate was reincubated with monocytes, little pyrogenic activity was lost. Previously stimulated monocytes, incubated alone in fresh medium, produced insignificant amounts of pyrogen when tested at this dosage (right-hand column, lower row). Thus, most of the pyrogenic activity of monocyte pyrogen appears to be retained during 18-h incubation both with previously stimulated cells and with products initially released by these cells, which are present in the pyrogen-containing supernate itself. Consequently, investigation of the dynamics of pyrogen production and release from monocytes appeared feasible.

Rate of Pyrogen Production and Release. Studies were carried out to determine the rate at which pyrogen is produced, released, or retained in monocytes after stimulation by nonparticulate endotoxin. The results of these

TABLE IV

Stability of Monocyte Pyrogen During Incubation

	4°C	18-h incubation (37°C)	
		Alone	With monocytes
		$\Delta T(°C) + SEM$*	
Pyrogen-containing supernate	0.83 ± 0.08	0.81 ± 0.07	0.68 ± 0.09
Fresh medium	—	—	0.17 ± 0.05

* Average of 8–12 assays each.

studies are shown in Fig. 3. The cumulative pyrogen content of the incubation medium, removed at different times during incubation, is shown on the left, and cell-associated pyrogen content at the specified times on the right. Significant pyrogen is not detectable within or without the cell for up to 3 h after stimulation. After this, increasing amounts appear in the incubation medium; significant pyrogen release was sometimes observed up to 36–48 h after the start of incubation, but rarely thereafter. Levels of detectable cell-associated pyrogen are absent initially, but rise to moderate levels during the 18-h incubation.

Requirement for Protein Synthesis. To investigate the importance of protein synthesis on continuing production and release of pyrogen by monocytes, puromycin 0.8, 2, or 5 $\times$ 10^{-6} M was added to cells at varying times after endotoxin, and supernatant and cell-associated pyrogen were assayed after further incubations. The results of studies with 2 $\times$ 10^{-6} M puromycin are presented in Fig. 4. Addition of puromycin at the beginning of incubation, as well as 3 h afterwards, significantly inhibited pyrogen release. This later effect could not be due to interference with cell activation, since, as noted above, the presence of endotoxin in the medium after 1 h of incubation is not needed for normal pyrogen production to occur subsequently. Experiments were also done to measure levels of cell-associated pyrogen in the presence of puromycin. When tested after 8 h incubation with endotoxin, normal cells contained significant

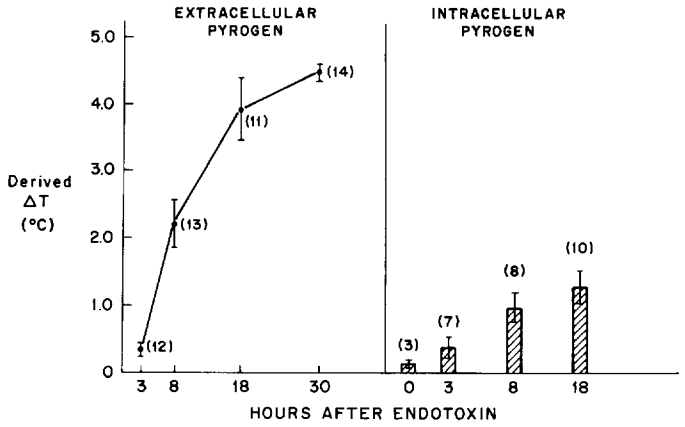

FIG. 3. Average maximum height of fever, ± SEM, corrected for dose and cell number (see Methods) following injection of incubation medium (extracellular pyrogen) or supernates from disrupted cells (intracellular pyrogen). Cumulative pyrogen content is plotted on the left panel; individual determinations at various time intervals on the right panel. Numbers of assays are shown in parentheses.

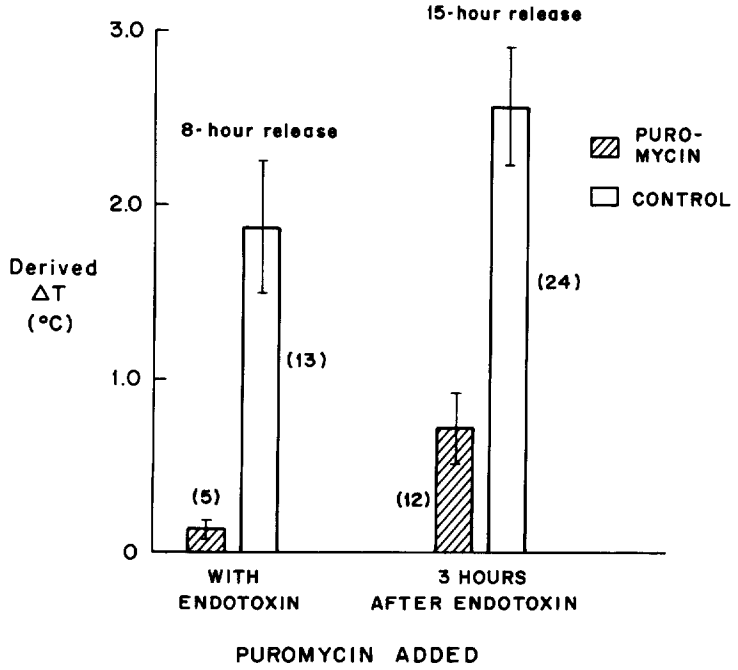

FIG. 4. Average maximum height of fever ± SEM, corrected as described in Fig. 5, following injection of supernates from monocytes incubated with endotoxin with or without puromycin (2×10^{-6} M). On the left, puromycin was added with endotoxin; on the right, 3 h later. Numbers in parentheses indicate numbers of assays.

amounts of pyrogen (average derived ΔT 0.96°C, as shown in Fig. 3), whereas cells incubated with endotoxin and puromycin contained no detectable pyrogen (average derived ΔT 0.16°C, six assays). These results, then, indicate that monocytes require late protein synthesis, after activation and early pyrogen production has begun, in order for production of pyrogen to continue normally.

Discussion

Although PMN leukocytes were originally believed to be the sole source of EP, recent work has shown that several mononuclear cell types can produce pyrogen. Human blood monocytes (11), lung macrophages (13), and tissue mononuclear cells of liver (14), spleen, and lymph nodes (13) released pyrogen in vitro after stimulation by endotoxin, phagocytosis, or an antigen such as old tuberculin. When incubated in saline, rabbit peritoneal exudate monocytes released pyrogen even without a specific stimulus (12). Presumably these cells, like exudate PMNs (24), have been activated in vivo. Both blood (11) and exudate (12) monocytes produced larger amounts of pyrogen than did blood or exudate PMNs.

The results reported here define certain characteristics of the process of pyrogen production by human blood monocytes. Like PMN leukocytes (9), these cells contain no detectable pyrogen at the time of harvest from blood, and if the cells are not stimulated, pyrogen production does not occur. Also, like PMN leukocytes (7, 9, 10), pyrogen production from monocytes begins within several hours after stimulation, requires only a short interval of contact with activator, and is prevented if puromycin is added initially. The pyrogen produced, like that from PMN leukocytes (8), loses little activity during incubation at 37°C, pH 7.4, even in the presence of activated cells or their products.

Several differences, however, were noted between production of pyrogen by monocytes and by PMN leukocytes. Monocytes produced 10–15 times as much pyrogen as equal numbers of PMN leukocytes. Furthermore, production continued for up to 36 h, unlike pyrogen production by PMN leukocytes, which continued for only 12–16 h (9, 10). Finally, continuing production of pyrogen by monocytes was inhibited by low concentrations of puromycin (≤ 1 μg/ml), whereas fivefold and 20-fold higher concentrations of this inhibitor had no effect on production of pyrogen by PMN leukocytes, once initial activation had occurred (9, 10). Although puromycin at 1 μg/ml is eventually lethal for macrophages in tissue culture (25), and caused rounding and detachment of monocytes after overnight incubation in these experiments, such effects are delayed, whereas inhibition of pyrogen production occurred within hours. In addition, at a sublethal concentration of 0.4 μg/ml (25), puromycin also partially inhibited late pyrogen synthesis by monocytes. These results suggest that the observed effects of puromycin are probably not due to nonspecific toxicity, but represent a requirement for continued protein synthesis by pyrogen-producing monocytes. Unlike stimulated PMN leukocytes, therefore, which have only an early requirement for protein synthesis, stimulated monocytes may continuously synthesize pyrogen molecules during incubation. Such a process would be consistent with morphologic (19, 26) and biochemical (19) evidence that monocytes synthesize many proteins, including lysosomal enzymes. Mononu-

clear phagocytes in vitro both synthesize and release complement components, interferon, and transferrin (27).

Monocytes (19), as well as macrophages (28), mature during culture in vitro to cells which have been called "activated," since they contain increased concentrations of enzymes, and exhibit heightened activity, including pinocytosis, phagocytosis, and various metabolic reactions. It was expected that such cells might also begin to synthesize pyrogen, since this is a normal response of stimulated phagocytic cells. However, monocytes maintained in culture for as long as 5 days never spontaneously produced pyrogen, nor did they contain detectable amounts. This finding suggests that stimuli leading to pyrogen production can be differentiated from those leading to cell maturation, or "activation."

No evidence was found to implicate blood lymphocytes in either activation or continuing production of pyrogen by monocytes. In other systems, lymphocytes alter both the morphology and function of monocytes or macrophages (20–23). In an in vitro model of delayed hypersensitivity fever, pyrogen release from blood cells was related to specific interactions between antigen, sensitized lymphocytes, and some pyrogen-producing cells in the blood (29). Similar interactions, however, were not observed when usual doses of endotoxin were used as the activator (30). Our data suggest that lymphocytes are not necessary intermediates in the processes of monocyte activation or pyrogen production, either by a nonparticulate preparation of endotoxin, or by phagocytosis of heat-killed bacteria. However, a few lymphocytes remain with monocytes under the conditions of our experiments (31), and the possibility that these few cells play an important role cannot be excluded.

Several lines of evidence suggest that pyrogen from mononuclear cells is important in both experimental and clinical fevers. Studies of endotoxin tolerance in rabbits, both in vitro (14) and in vivo (32), implicated Kupffer cells, rather than blood cells, in the failure of tolerant animals to develop fever after i.v. injection of endotoxin. In man, high fevers are characteristic of granulocytopenic states, such as acute monocytic leukemia and agranulocytosis, and of granulomatous diseases in which inflammatory lesions contain predominantly mononuclear cells (1). Spleen and lymph node tissues from patients with Hodgkin's disease frequently produce pyrogen in vitro; experimental results suggest that tissue mononuclear cells are the source of this pyrogen in many instances (33). Human mononuclear cell pyrogen has a larger molecular weight than PMN leukocyte pyrogen (34), and is produced for a longer time (33, and results above). Thus, the sustained fevers characteristic of many diseases in which malignant or inflammatory mononuclear cells are predominant may be due to prolonged release of this pyrogenic protein by activated monocytes or macrophages in situ.

Summary

The characteristics of pyrogen production and release by human blood monocytes were investigated. A dose-response assay of monocyte pyrogen in rabbits indicated a linear relationship of temperature elevation to dose of pyrogen at lower doses. Monocytes did not contain pyrogen when first obtained, nor did they

release it spontaneously even after 5 days of incubation in vitro. Pyrogen production was apparent 4 h after stimulation by endotoxin or phagocytosis, and continued for 24 h or more. Puromycin, an inhibitor of protein synthesis, prevented both initiation and continuation of pyrogen production and release. Pyrogen-containing supernates retained most pyrogenic activity during overnight incubation even in the presence of activated cells. Lymphocytes appeared to play no role in either initiation or continuation of pyrogen production in these studies.

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References

1. Atkins, E., and P. Bodel. 1973. *In* The Inflammatory Process. B. Zweifach, L. Grant, and R. T. McCluskey, editors. Academic Press, Inc., New York. 467.
2. Bennett, I. L., Jr., and P. B. Beeson. 1953. Studies on the pathogenesis of fever. I. The effect of injection of extracts and suspensions of uninfected rabbit tissues upon the body temperature of normal rabbits. *J. Exp. Med.* **98**:477.
3. Kaiser, H. K., and W. B. Wood, Jr. 1962. Studies on the pathogenesis of fever. IX. The production of endogenous pyrogen by polymorphonuclear leucocytes. *J. Exp. Med.* **115**:27.
4. Berlin, R. D., and W. B. Wood, Jr. 1964. Studies on the pathogenesis of fever. XII. Electrolytic factors influencing the release of endogenous pyrogen from polymorphonuclear leucocytes. *J. Exp. Med.* **119**:697.
5. Berlin, R. D., and W. B. Wood, Jr. 1964. Studies on the pathogenesis of fever. XIII. The effect of phagocytosis on the release of endogenous pyrogen by polymorphonuclear leucocytes. *J. Exp. Med.* **119**:715.
6. Bodel, P., and E. Atkins. 1966. Human leukocyte pyrogen producing fever in rabbits. *Proc. Soc. Exp. Biol. Med.* **121**:943.
7. Root, R. K., J. J. Nordlund, and S. M. Wolff. 1970. Factors affecting the quantitative production and assay of human leukocytic pyrogen. *J. Lab. Clin. Med.* **75**:679.
8. Bodel, P. 1974. Studies on the mechanism of endogenous pyrogen production. II. Role of cell products in the regulation of pyrogen release from blood leukocytes. *Infect. Immun.* In press.
9. Bodel, P. 1970. Studies on the mechanism of endogenous pyrogen production. I. Investigation of new protein synthesis in stimulated human blood leucocytes. *Yale J. Biol. Med.* **43**:145.
10. Nordlund, J. J., R. K. Root, and S. M. Wolff. 1970. Studies on the origin of human leukocytic pyrogen. *J. Exp. Med.* **131**:727.
11. Bodel, P., and E. Atkins. 1967. Release of endogenous pyrogen by human monocytes. *N. Engl. J. Med.* **276**:1002.
12. Hahn, H. H., D. C. Char, W. B. Postel, and W. B. Wood, Jr. 1967. Studies on the pathogenesis of fever. XV. The production of endogenous pyrogen by peritoneal macrophages. *J. Exp. Med.* **126**:385.
13. Atkins, E., P. Bodel, and L. Francis. 1967. Release of an endogenous pyrogen in vitro from rabbit mononuclear cells. *J. Exp. Med.* **126**:357.

14. Dinarello, C. A., P. Bodel, and E. Atkins. 1968. The role of the liver in the production of fever and in pyrogenic tolerance. *Trans. Assoc. Amer. Phys.* **81**:334.
15. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21**(Suppl. 97):77.
16. Snell, E. S., and E. Atkins. 1967. Interactions of Gram-negative bacterial endotoxin with rabbit blood *in vitro*. *Am. J. Physiol.* **212**:1103.
17. Gimber, P. E., and G. W. Rafter. 1969. The interaction of *Escherichia coli* endotoxin with leukocytes. *Arch. Biochem. Biophys.* **135**:14.
18. Atkins, E., and E. S. Snell. 1964. A comparison of the biological properties of Gram-negative bacterial endotoxin with leukocyte and tissue pyrogens. *In* Bacterial Endotoxins. M. Landy and W. Braun, editors. Institute of Microbiology, Rutgers University, New Brunswick, N.J.
19. Cohn, Z. A. 1968. The structure and function of monocytes and macrophages. *Adv. Immunol.* **9**:163.
20. Mackaness, G. B. 1969. The influence of immunologically committed lymphoid cells on macrophage activity in vivo. *J. Exp. Med.* **129**:973.
21. Nathan, C. F., M. L. Karnovsky, and J. R. David. 1971. Alterations of macrophage functions by mediators from lymphocytes. *J. Exp. Med.* **133**:1356.
22. Mooney, J. J., and B. H. Waksman. 1970. Activation of normal rabbit macrophage monolayers by supernatants of antigen-stimulated lymphocytes. *J. Immunol.* **105**: 1138.
23. Schmidt, M. E., S. D. Douglas, and A. D. Rubin. 1973. Human monocyte activation by supernatants from Concanavalin A (Con A) stimulated lymphocytes. *Cell. Immunol.* **9**:45.
24. Moore, D. M., S. F. Cheuk, J. D. Morton, R. D. Berlin, and W. B. Wood, Jr. 1970. Studies on the pathogenesis of fever. XVIII. Activation of leukocytes for pyrogen production. *J. Exp. Med.* **131**:179.
25. Cohn, Z. A., and B. Benson. 1965. The in vitro differentiation of mononuclear phagocytes. I. The influence of inhibitors and the results of autoradiography. *J. Exp. Med.* **121**:279.
26. Nichols, B. A., D. F. Bainton, and M. G. Farquhar. 1971. Differentiation of monocytes: origin, nature, and fate of their azurophil granules. *J. Cell Biol.* **50**:498.
27. Stecher, V. J. 1970. Synthesis of proteins by mononuclear phagocytes. *In* Mononuclear Phagocytes. R. van Furth, editor. F. A. Davis Co., Philadelphia. 133.
28. Cohn, Z. A. 1970. Lysosomes in mononuclear phagocytes. *In* Mononuclear Phagocytes. R. van Furth, editor. F. A. Davis Co., Philadelphia. 50.
29. Atkins, E., J. D. Feldman, L. Francis, and E. Hursh. 1972. Studies on the mechanism of fever accompanying delayed hypersensitivity. The role of the sensitized lymphocyte. *J. Exp. Med.* **135**:1113.
30. Atkins, E., and L. Francis. 1973. Role of lymphocytes in the pyrogenic response: comparison of endotoxin with specific antigen and the nonspecific mitogen, Concanavalin A. *J. Infec. Dis.* **128**(Suppl.):277.
31. Koller, C. A., G. W. King, P. E. Hurtubise, A. L. Sagone, and A. F. LoBuglio. 1973. Characterization of glass adherent human mononuclear cells. *J. Immunol.* **111**:1610.
32. Greisman, S. E., and C. L. Woodward. 1970. Mechanisms of endotoxin tolerance. VII. The role of the liver. *J. Immunol.* **105**:1468.
33. Bodel, P. 1974. Pyrogen release by lymphoid tissues in Hodgkin's disease. *Yale J. Biol. Med.* **47**: in press.
34. Dinarello, C. A., and S. M. Wolff. 1974. A new human endogenous pyrogen. *J. Exp. Med.* **139**:1369.