

DIFFERENCES IN PYROGEN PRODUCTION BY
MONONUCLEAR PHAGOCYTES
AND BY FIBROBLASTS OR HeLa CELLS*

BY PHYLLIS BODEL AND HEATHER MILLER

*(From the Department of Medicine, Yale University School of Medicine, New Haven,
Connecticut 06510)*

Endogenous pyrogen (EP),¹ the mediator of fever, is a small protein which is synthesized and secreted by certain cells after stimulation by inflammatory agents (1). Phagocytosis of bacteria is a potent stimulus for EP production by blood neutrophilic (2, 3) and eosinophilic (4) granulocytes, as well as by cells of the mononuclear phagocyte system (MPS), including monocytes (5), peritoneal (6) and alveolar (7) macrophages, Kupffer cells of liver (8), and tissue macrophages in spleen and lymph nodes (7). None of these cell types contain or produce detectable pyrogen when first isolated in an unstimulated state. After phagocytosis, however, EP production begins within a few hours and continues for many hours or several days (9-11).

Granulocytes and cells of the MPS, the only cells known to produce EP, are phagocytes believed to be of bone marrow origin (12). Such cells, which rapidly ingest many different particles and have receptors for γ -globulin and complement on their cell membranes, have been termed "professional" phagocytes (13, 14), since phagocytosis appears to be a function for which they are specialized. Other cell types, which have a slower and more limited capacity for particle ingestion, and lack γ -globulin and complement receptors on their cell membranes, have been termed "nonprofessional" phagocytes. These include certain endothelial and epithelial cells, fibroblasts, and a number of cell lines maintained in vitro, such as HeLa cells (14).

The curious association of phagocytic capacity with the ability to produce pyrogen has been noted previously, but has remained unexplained (1). Since studies have not been made of the capacity of nonprofessional phagocytes, other than lymphoid cells (15), to produce EP, it is unclear whether the process of phagocytosis induces release of this protein, or whether EP production after phagocytosis is limited to certain cell types, specialized for both functions. Studies of EP production by nonprofessional phagocytes have been hampered by the requirement for large numbers of cells needed for the standard pyrogen assay in rabbits. Recently, we have reported that EP produced by mouse

* Supported by U. S. Public Health Service Grants No. AI 01564-20 and CA 14566-03.

¹ *Abbreviations used in this paper:* CE, chicken erythrocytes; EP, endogenous pyrogen; MEM, minimal Eagle's medium; MEM-Lact, minimal Eagle's medium with lactalbumin; MN, mononuclear; MPS, mononuclear phagocyte system; PMN, polymorphonuclear; SW/Yale, Swiss-Webster mice, Yale colony

macrophages, as well as human monocytes, causes fever in mice (16). This bioassay, which can detect EP produced by as few as 1×10^5 cells, makes it possible to study cells of mouse or human origin in tissue culture. The following studies were therefore done to investigate whether EP production would follow phagocytosis of particles by fibroblasts and HeLa cells, nonprofessional phagocytes.

Materials and Methods

All glassware, materials, and reagents were obtained or rendered sterile and pyrogen-free using methods described previously (11) or below. All supernates for pyrogen assay were cultured in thioglycollate broth before injection into mice, and contaminated samples were discarded.

Pyrogen Assay Supernates were tested for pyrogenicity by injection into mice. Swiss-Webster mice from the Yale University colony (SW/Yale) were used throughout. Techniques for injection and temperature monitoring were as described previously (16). Briefly, 8- to 10-wk-old female mice were placed in individual cages in an incubator maintained at 34–35°C and removed briefly every 10 min for rectal temperature readings. After a stable base-line temperature was established, each mouse was injected intravenously with 0.3 ml of a test solution, and its temperature monitored for 1 h. An occasional mouse in a group of 12 or 24 responded to injection with a fall in temperature greater than 0.4°C during the first 30 min after injection. Such a response was interpreted as an abnormal reaction to injection, and data from that individual mouse were excluded. All data from an experiment were discarded if injections of any test supernate produced significant numbers of hypothermic responses, a typical reaction of mice to endotoxin (16, 17), or if injection of "control" samples produced fevers.

The choice of strain of mice used in the bioassay proved to be important. Two other strains tested, AKR/J and A/J (The Jackson Laboratory, Bar Harbor, Maine), gave responses similar to those of SW/Yale and could be used for pyrogen assay. On the other hand, Swiss-Webster mice obtained from Charles River Breeding Laboratories, Wilmington, Mass., consistently gave hypothermic responses to injection of both control and EP-containing supernates and could not be used.

Tissue Culture Cell Lines. Human fibroblasts of a normal adolescent male were obtained from Dr. J. Mahoney; LMTK⁻ and L-W cells (L fibroblasts) from the Department of Molecular Biophysics and Biochemistry; and S₃ HeLa cells from Dr. Lon Hodge, Department of Human Genetics, all of Yale University. Cells were maintained as monolayers in 75 cm² plastic flasks (Falcon Plastics, Division of BioQuest, Oxnard, Calif.) with Eagle's minimum essential medium (MEM, Auto-POW, Flow Laboratories, Inc., Rockville, Md.), 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 50 U penicillin/ml, and 50 µg streptomycin/ml ("complete medium"), and incubated at 37°C in 95% air, 5% CO₂. Medium for HeLa and L cells was changed every 2–3 days. Once a week cells were removed from flasks by treatment with Viokase (18) (Grand Island Biological Co., Grand Island, N. Y.), and 1.5×10^6 cells were transferred to new flasks with 20 ml complete medium.

Phagocytic Particles Heat-killed *Staphylococcus albus*, prepared as described previously (5), was added to flasks at a ratio of 20 bacteria per cell. Latex particles (Dow Diagnostics, Indianapolis, Ind., 0.822 µm diameter, 10% solids) were diluted by adding three drops to 1 ml saline, and autoclaved for 90 min. 0.5 ml of this suspension, containing about 2×10^{10} particles, was added per flask for phagocytosis experiments. Chicken erythrocytes (CE) (Gibco Diagnostics, Madison, Wisc.) were washed, incubated for 24 h in 3% glutaraldehyde, washed repeatedly, cultured for sterility, and stored at 4°C (19). Aliquots were added to flasks to provide a ratio of four CE per cell.

Assays for Endotoxin on Latex Particles. To test for endotoxin contamination of the latex particle suspension, prepared as described above, heparinized rabbit blood was obtained by cardiac puncture, centrifuged at 500 g, supernatant plasma was removed, and the sedimented blood cells resuspended in MEM to about 1×10^7 leukocytes/ml. Portions were incubated for 18 h in Erlenmeyer flasks with or without 1 or 2 ml of latex particles, or with heat-killed staphylococci ("positive" control). Supernates were then removed, centrifuged at 1,000 g, in some experiments filtered through Millipore filters (Swinnex 25) to remove remaining latex particles, and assayed for EP in rabbits using standard techniques (7). Supernate from $4-6 \times 10^7$ leukocytes constituted

one rabbit dose. In other experiments, 0.5 ml of latex solution was injected intravenously into each of two rabbits, and their temperatures monitored for 1¹/₄ and 1¹/₂ h afterwards.

Incubations of Tissue Culture Cells. Flasks of HeLa cells, L cells, and human fibroblasts were selected for experiments 4–6 days after seeding. All supernate was removed from the flasks, and 5 ml fresh complete medium were added, with or without phagocytic particles. After 6-h incubation, supernates were discarded, and the flasks were rinsed three times with warm MEM containing no serum. 5 ml MEM with 0.25% (wt/vol) lactalbumin, 2 mM L-glutamine, 50 U penicillin, and 50 μ g streptomycin/ml (MEM-Lact) were then added, and the flasks were incubated for 18 or 48 h. For experiments with CE, one flask contained only MEM-Lact and CE. For experiments with latex, one flask of cells and latex particles, after being rinsed and provided with fresh medium at 6 h, was then placed at 4°C instead of 37°C. Supernate was removed from all flasks at 18 or 48 h, cultured, centrifuged at 1,200 g for 10 min, and after dilution when necessary, samples were injected for pyrogen assay. Cells from flasks incubated without particles were removed with Viokase, and the cell numbers determined with a Coulter counter (Model ZF, Coulter Electronics Inc., Hialeah, Fla.). Cells from flasks to which particles had been added were similarly removed and percent phagocytosis estimated from Wright's stained cover slip smears.

Incubations of Mouse Macrophages. Mouse peritoneal macrophages were obtained from normal 6-wk-old SW/Yale female mice, using standard techniques (20). After centrifugation, cells were suspended in MEM-Lact, at a concentration of 2.5×10^6 cells/ml, and 5 ml were pipetted into 25 cm² Falcon flasks. Flasks were incubated for 2 h at 37°C to allow cells to adhere, and phagocytic particles were then added to some flasks. For experiments with CE or staphylococci, incubations were then continued for 18 h. For experiments with latex, 3 h after particles were added, all flasks were rinsed three times with warm MEM, and then reincubated for 18 h with fresh MEM-Lact, some at 37°C and some at 4°C. For estimation of phagocytosis, 0.5 ml of the initial suspension of cells and particles was placed on a glass cover slip in a Petri dish, incubated for 3 h, rinsed, dried, and stained with Wright's stain. Control flasks were prepared and incubated, and all supernates processed and injected, as described above for tissue culture cells.

Assays of Mouse Macrophage Pyrogen In each experiment, samples of supernates derived from the same numbers of peritoneal cells in experimental and control flasks were compared in the pyrogen assay. Significant amounts of pyrogen were sometimes produced by macrophages incubated without phagocytic particles, presumably due to "activation" in vivo²; all data from these experiments was excluded. For evaluation of dose-response characteristics of pyrogens, supernates from cells incubated with either latex or CE were compared with those from cells incubated with staphylococci. Serial twofold dilutions of these supernates were first tested for pyrogenic activity in groups of mice. The dilution producing maximum average temperature elevation was assigned the value "X" for pyrogen content, and was compared to dilutions of the same supernate having values "2X" and "1/2X". The dilution "X" varied from 1:2 to 1:8 in different experiments, but was always the same for both pyrogen-containing supernates in any one experiment.

Results

Mouse Macrophages Incubated with Latex or Erythrocytes. Most studies of EP release in vitro by PMN or MN phagocytes have employed bacteria as phagocytic particles. Nonprofessional phagocytes, however, ingest bacteria poorly or not at all. Therefore, experiments were first carried out to determine whether nonbacterial particles could be used to stimulate pyrogen release from cells with proven pyrogen-producing capacity. Latex particles or glutaraldehyde-treated CE were added to flasks containing mouse macrophages, the cells were allowed to ingest the particles, and after 18 h supernates were removed and assayed for EP in mice. Control flasks contained macrophages with heat-killed staphylococci (positive control), macrophages without phagocytic particles, and

² Mouse and guinea pig resident (unelicited) peritoneal macrophages rarely produce EP spontaneously when incubated in vitro; rabbit resident macrophages, on the other hand, usually do (Bodel and Atkins, unpublished observations)

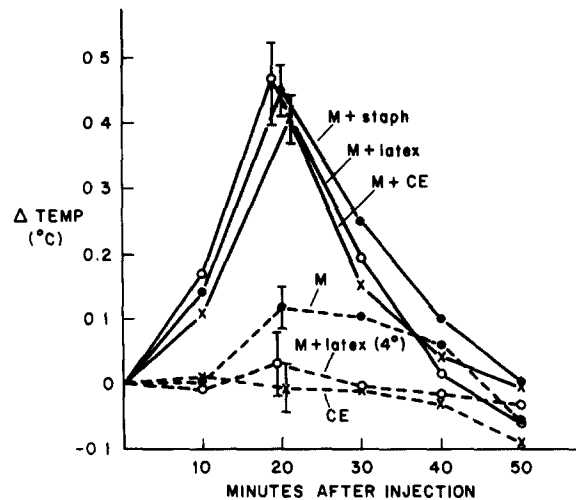


FIG. 1. Temperature responses in mice after intravenous injection of incubation media from mouse macrophages incubated alone (M), with heat-killed staphylococci (M + staph), latex particles (M + latex), or glutaraldehyde-treated chicken erythrocytes (M + CE). Responses are also shown to injection of media from erythrocytes (CE) or macrophages with ingested latex particles maintained at 4°C (M + latex 4°). Each point represents the average change in temperature from base line of 16-37 mice (four upper curves) or 7-11 mice (two lower curves). Supernate from $1-4 \times 10^8$ mouse peritoneal cells was injected into each mouse. SEM are shown at time of maximum febrile response.

CE without macrophages. In addition, one flask of macrophages to which latex had been added was incubated for 3 h at 37°C to allow phagocytosis to occur, but then placed at 4°C for the next 18 h.

The results of these experiments are shown in Fig. 1. Whereas macrophages incubated without phagocytic particles produced little or no pyrogen, macrophages that had ingested latex or CE produced as much pyrogen as those that had ingested staphylococci. Injection of each of these pyrogen-containing supernates caused identical prompt, monophasic fevers, with peak temperature elevations occurring 20 min later. These febrile responses are characteristic of macrophage EP, produced after phagocytosis of staphylococci in previous studies (16); endotoxin, by contrast, produces hypothermia in mice (16, 17). As also shown in Fig. 1, no temperature change occurred after injection of supernate from flasks of macrophages and latex maintained at 4°C. This result indicates that amounts of endotoxin which could be detected in our bioassay were absent from such supernates. It also indicates that the process of pyrogen release from macrophages after ingestion of latex does not occur in the cold. Additional control experiments concerning the pyrogen released by macrophages after ingestion of latex and CE are described in a later section.

Fibroblasts and HeLa Cells Incubated with Latex or Erythrocytes. Experiments were next carried out to investigate whether phagocytosis of latex particles by nonprofessional phagocytes would also result in production and release of EP. Mouse L fibroblasts, human fibroblasts, and HeLa cells were selected for study. Supernates from overnight incubations of these cell lines in pyrogen-free media were initially tested and found to contain no detectable pyrogen. There-

TABLE I
Pyrogen Release from Different Cell Types after Phagocytosis of Latex

Cell type	Latex	No phagocytes × 10 ⁵ /dose*					Latex control (4°C)‡
		0 5-1 0	1 1-5 0	5 1-10	11-20	>21	
		$\Delta T - 20 \text{ min } (^{\circ}\text{C})\parallel$					
L cell	-	<u>-0 01 ± 0 06 (7)§</u>	0 05 ± 0 04 (12)	<u>-0 02 ± 0 04 (12)</u>	0 00 ± 0 03 (30)	0 05 ± 0 05 (11)	0 00 ± 0 02 (47)
	+	0 03 ± 0 03 (8)	-0 00 ± 0 04 (22)	0 01 ± 0 03 (18)	0 03 ± 0 05 (21)	0 00 ± 0 03 (11)	
Human fibroblast	-	<u>-0 08 ± 0 05 (4)</u>	0 04 ± 0 03 (14)				0 00 ± 0 04 (20)
	+	-0 10 ± 0 04 (4)	-0 01 ± 0 03 (16)				
HeLa	-	0 00 ± 0 06 (9)	0 03 ± 0 03 (19)	0 02 ± 0 03 (19)	<u>-0 02 ± 0 04 (12)</u>	<u>-0 04 ± 0 03 (10)</u>	-0 02 ± 0 02 (42)
	+	0 02 ± 0 03 (11)	-0 02 ± 0 03 (14)	0 00 ± 0 03 (19)	0 00 ± 0 04 (12)	0 02 ± 0 04 (11)	
Mouse macrophage	-	0 05 ± 0 03 (12)	0 04 ± 0 05 (16)	0 08 ± 0 05 (4)			0 03 ± 0 03 (27)
	+	<u>0 33 ± 0 06 (20)</u>	<u>0 36 ± 0 06 (29)</u>	0 08 ± 0 08 (4)			

Positive assays are underlined
 * Cells containing ingested particles from which supernate was derived
 ‡ See text for details
 § Number of injections
 || Average change in temperature from base line 20 min after injection, ± SEM

fore, latex particles were added to some flasks, and after a 6-h incubation to allow phagocytosis to occur, cell layers were rinsed repeatedly and incubated overnight, or for an additional 24-48 h in some experiments. Supernates were tested for pyrogen at different dilutions to detect either small or large amounts of pyrogen (16).

The results of these experiments are shown in Table I. No pyrogen was ever detected in supernates from nonprofessional phagocytes, either in the presence or absence of latex particles. All supernates from longer incubations were also nonpyrogenic (not shown). The results of the pyrogen assays are grouped according to the numbers of observed phagocytic cells from which the injected supernate was derived. Estimates of per cent of fibroblasts containing ingested latex (illustrated in Fig. 2a) varied in different experiments from 31 to 100%, and of HeLa cells from 65 to 73%. Data from assays of EP produced by macrophages after phagocytosis of latex are shown for comparison (Table I, bottom line); the positive assay results are underlined. These data are similarly grouped by numbers of phagocytic cells, calculated by assuming that 60% of mouse peritoneal cells were macrophages (20), and that all macrophages ingested latex.

Similar attempts to demonstrate pyrogen production were then carried out in another series of experiments using L fibroblasts to which CE were added. The results of these studies are presented in Table II. No pyrogen production by fibroblasts was ever detected, whether the cells were incubated with or without CE. Assays of supernates collected after 48-h incubations were also uniformly negative (not shown). Estimates of numbers of phagocytic fibroblasts (illustrated in Fig. 2b) varied in these experiments from 17 to 30%. Data from mouse macrophages incubated with CE are included for comparison on the bottom line, grouped as described above by numbers of phagocytic cells, assuming that all macrophages ingested erythrocytes; positive assays are underlined. In the experiments using larger doses of supernate, a small pyrogen release by macrophages incubated without CE is present.

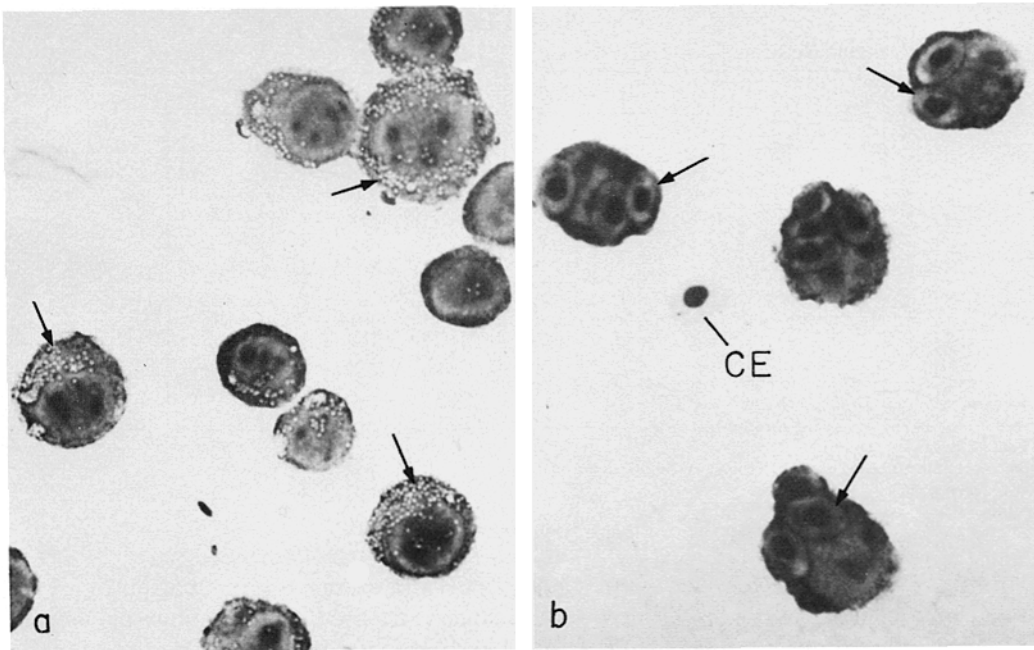


FIG. 2. *L* fibroblasts after phagocytosis of (a) latex and (b) glutaraldehyde-treated chicken erythrocytes. Latex particles 0.8 μ m diameter, seen as white spheres, are intracellular in most cells (arrows, a); nucleated erythrocytes are intracellular (arrows, b) and extracellular (CE). Fibroblasts were suspended with Viokase, and cover slip smears stained with Wright's stain. (a) \times 640, (b) \times 800

TABLE II
Pyrogen Release from Mouse L Fibroblasts and Macrophages after Phagocytosis of CE

Cell type	CE	No phagocytes $\times 10^5$ /dose*			CE control‡
		0.5-1.0	1.1-5.0	5.1-10	
$\Delta T-20 \text{ min } (^{\circ}\text{C})\ \$					
L fibroblast	-	0.01 \pm 0.05 (16)§	0.01 \pm 0.02 (36)	0.08 \pm 0.07 (6)	0.01 \pm 0.02 (36)
	+	0.02 \pm 0.07 (15)	0.04 \pm 0.02 (35)	0.07 \pm 0.07 (6)	
Macrophage	-	-0.02 \pm 0.05 (6)	0.16 \pm 0.04 (16)		0.02 \pm 0.02 (18)
	+	0.17 \pm 0.03 (15)	0.37 \pm 0.03 (32)		

*. ‡. §. || For explanation of symbols see Table I.

Control Experiments. In previous studies, phagocytosis of latex by rabbit granulocytes has been reported not to induce significant EP release either in vivo or in vitro (2). It was therefore possible that contamination of our latex preparation with small amounts of endotoxin could be responsible for the observed EP production by macrophages, rather than the act of phagocytosis of these inert particles. Further studies were done to exclude this possibility. Intravenous injection of 0.5 ml of latex suspension containing about 2×10^{10} particles did not cause fever in two rabbits. Also, incubation of rabbit blood leukocytes with latex failed to induce EP release, unlike incubation of the cells

with staphylococci (average maximum ΔT 0.17°C in 10 rabbits and 0.66°C in 7 rabbits, respectively). Since as little as 0.001 μg of endotoxin will induce EP release in such an in vitro system (21), no evidence was obtained for endotoxin contamination of the latex preparation.

In previous studies (16), using EP from macrophages that had ingested staphylococci, we showed that the height of the febrile response in mice was dose related. Highly concentrated solutions containing EP, as well as dilute solutions of the same material, produced smaller peak temperature elevations than did samples of intermediate concentration. To further investigate the characteristics of the pyrogen produced by macrophages after phagocytosis of latex and CE, and to confirm the absence of pyrogen in control samples from the same experiments, supernates from stimulated and control macrophages were injected into mice in different dilutions.

The results of these experiments, presented in Fig. 3, show that injection of all three pyrogen-containing supernates produced similar dose-response curves. Samples with pyrogen content 2X, as well as $1/2$ X, consistently produced lower average temperature elevations than did intermediate dilutions (pyrogen content X) of the same supernate. Media from control flasks in these experiments (cells or particles incubated alone, or cells with latex maintained at 4°C) were injected at these same dilutions, and neither febrile nor hypothermic responses occurred. Results from macrophages incubated alone are shown on the right in Fig. 2. The similarities in the pyrogens produced after phagocytosis of these different particles were further established by tests of heat and pronase sensitivity. Results were the same as those obtained previously with pyrogen from macrophages stimulated by phagocytosis of staphylococci (16). Thus, these findings support the conclusion that phagocytosis of latex and CE, as well as staphylococci, induces release of an endogenous pyrogen from mouse macrophages in vitro, and that these particles are therefore appropriate for tests of pyrogen production by other cell types.

Discussion

The present studies were carried out to determine whether phagocytosis, a potent stimulus to EP production in vitro by polymorphonuclear (PMN) and mononuclear (MN) phagocytes, would similarly induce pyrogen release from mouse or human fibroblasts and HeLa cells, nonprofessional phagocytes (14). Assays for pyrogen content of supernates from tissue culture lines of these cells were performed in mice, a bioassay that can detect EP produced by as few as 1×10^5 mouse or human MN phagocytes (16). There was no evidence of pyrogen production by fibroblasts or HeLa cells after phagocytosis of latex or erythrocytes, particles which they ingest readily, as documented in our experiments and reported previously (19, 22, 23). Mouse macrophages, professional phagocytes also studied for comparison, produced easily detectable EP under the same conditions.

It is possible that unfavorable conditions or inadequate duration of the incubations resulted in these negative findings. However, under similar experimental conditions, fibroblasts and HeLa cells synthesize and release other proteins (24, 25). Our incubations were not continued longer than 48 h before assay of any

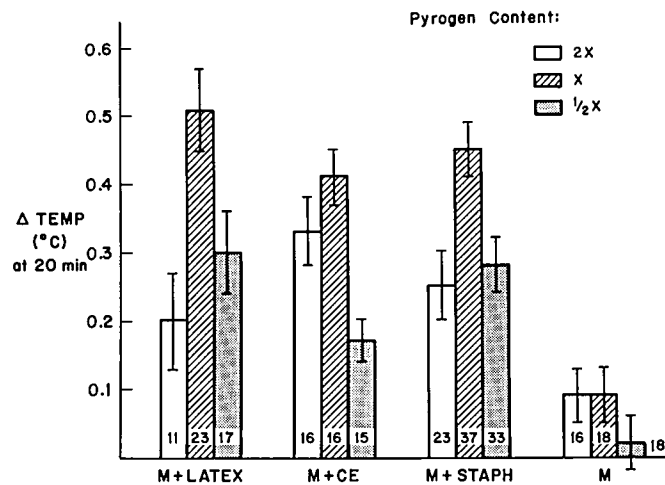


FIG. 3. Average temperature responses in mice to intravenous injection of twofold dilutions of supernates from mouse macrophages incubated alone (M), with latex (M + latex), glutaraldehyde-treated chicken erythrocytes (M + CE), or heat-killed staphylococci (M + staph). Average change in temperature from base line at 20 min after injection \pm SEM is shown by the height of the bars, and numbers of mice injected in parentheses within the bar. In each experiment, the dilution giving maximum average temperature elevation is labeled (see Materials and Methods).

supernate because endogenous pyrogens lose activity during incubation at 37°C (26). Since we examined supernates from up to 3.1×10^7 phagocytic fibroblasts and 4.2×10^7 HeLa cells, we should have detected pyrogen production even if these cells had only 1/300 of the capacity of mouse macrophages to synthesize and release EP during a 24- or 48-h incubation.

Macrophages and fibroblasts are both cells of mesenchymal origin, and they resemble each other in a number of respects. For example, particle internalization, intracellular transport, phagosome-lysosome fusion, and initiation of digestion have been documented in fibroblasts incubated with macromolecules (27) and observed in experiments with erythrocyte ghosts (22) and latex particles (23). These processes appear virtually identical to those that occur in macrophages incubated with similar substances (28). In addition, both cell types synthesize and release various hydrolases, in some cases in increasing amounts after phagocytosis of particles. Thus, endocytosis stimulates synthesis and secretion of collagenase and certain neutral proteases by both cell types (24, 29). Plasminogen activator, one of these proteases, is secreted by viral-transformed fibroblasts (30) as well as by macrophages stimulated by thioglycollate or endotoxin and phagocytosis (29); the characteristics of its synthesis and secretion by macrophages (29) closely resemble those described for EP (11). We report elsewhere that supernates of 3T3 mouse fibroblasts transformed by SV-40 do not contain pyrogen.³ Thus, although fibroblasts resemble macrophages in many respects, their failure to produce EP in our experiments suggests that they differ from macrophages in their capacity to synthesize this particular protein.

³ Bodel, P., and H. Miller. Studies of pyrogen production by tumor cell lines in vitro. Manuscript in preparation.

In these experiments, phagocytosis of latex and erythrocytes has been shown for the first time to cause release of endogenous pyrogens from leukocytes in vitro. The characteristics of these pyrogens, both by bioassay and by heat and pronase sensitivity, were indistinguishable from those of EP produced after phagocytosis of bacteria, the stimulus used in previous work (16). This finding suggests that mouse macrophages respond to ingestion of particles of different size and characteristics with production and release of the same pyrogen. Although human blood leukocytes also release some pyrogen after phagocytosis of latex particles (Reynolds, C., P. Bodel, and E. Atkins. 1972. Unpublished observations), rabbit exudate leukocytes produce little or no pyrogen after in vitro incubation with latex as compared with pneumococci (2). The explanation for this species difference is unknown, but is not due to a failure of rabbit granulocytes and monocytes to phagocytize latex, since in our experiments both cell types contained many internalized particles when examined by electron microscopy. Of interest was the finding that only minimal neutrophil degranulation had occurred (Bainton, D. F., and P. Bodel. 1976. Unpublished observations).

At present, only professional phagocytes of bone marrow origin are known to have the capacity to produce EP, a small, biologically potent protein which seems to act specifically on hypothalamic neurons to elevate body temperature (1). Although the value of fever in host defense is still controversial (31, 32), it may be significant that EP production seems to be a unique characteristic of professional phagocytes, cells specialized for host defense.

Summary

Phagocytosis of bacteria stimulates "professional" phagocytes to produce and release endogenous pyrogen (EP), the protein that mediates fever. To determine whether "nonprofessional" phagocytes also have this capacity, mouse and human fibroblasts and HeLa cells were cultured after ingestion of latex or chicken erythrocytes (CE), and EP release into culture supernate measured by mouse assay. No detectable pyrogen was released by these cell types after phagocytosis, whereas both latex and CE stimulated EP production by cultured mouse macrophages. These studies support the hypothesis that only professional phagocytes of bone marrow origin synthesize EP and induce fever.

The authors thank Dr. Elisha Atkins for continued interest and support, Ms. Lorraine Francis for help with latex experiments, and Dr. Dorothy Bainton for assistance and advice.

Received for publication 4 November 1976.

References

1. Atkins, E., and P. Bodel. 1973. Fever. *In* The Inflammatory Process. B. W. Zweifach, L. Grant, and R. T. McCluskey, editors. Academic Press, Inc., New York. 3:467.
2. 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 leukocytes. *J. Exp. Med.* 119:715.
3. Bodel, P., and E. Atkins. 1966. Human leukocyte pyrogen producing fever in rabbits. *Proc. Soc. Exp. Biol. Med.* 121:943.

4. Meckenberg, I. D., R. K. Root, and S. M. Wolff. 1972. Bactericidal and metabolic properties of human eosinophils. *Blood*. 39:67.
5. Bodel, P., and E. Atkins. 1967. Release of endogenous pyrogen by human monocytes. *N. Engl. J. Med.* 276:1002.
6. 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.
7. Atkins, E., P. Bodel, and L. Francis. 1967. Release of an endogenous pyrogen in vitro from rabbit mononuclear cells. *J. Exp. Med.* 126:357.
8. 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. Am. Phys.* 81:334.
9. Bodel, P. 1970. Studies on the mechanism of endogenous pyrogen production. I. Investigation of new protein synthesis in stimulated human blood leukocytes. *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. 1974. Studies on the mechanism of endogenous pyrogen production III Human blood monocytes. *J. Exp. Med.* 140:954.
12. Van Furth, R., D. L. Langevoort, and A. Schaberg. 1975. Mononuclear phagocytes in human pathology – proposal for an approach to improved classification. *In Mononuclear Phagocytes in Immunity, Infection and Pathology*. Ralph van Furth, editor. Blackwell Scientific Publications, London. 1.
13. Rabinovitch, M. 1968. Phagocytosis: the engulfment stage. *Semin. Hematol.* 5:134.
14. Rabinovitch, M. 1970. Phagocytic recognition. *In Mononuclear Phagocytes*. R van-Furth, editor. Blackwell Scientific Publications, London. 299.
15. Kammermeyer, J. K., R. K. Root, D. P. Stites, P. R. Glade, and L. N. Chessin. 1968. The detection and characterization of phagocytic cells in established human cell lines synthesizing immunoglobins. *Proc. Soc. Exp. Biol. Med.* 129:522.
16. Bodel, P., and H. Miller. 1976. Pyrogen from mouse macrophages causes fever in mice. *Proc. Soc. Exp. Biol. Med.* 151:93.
17. Wardlaw, A. C., L. Boorman, and R. Reid. 1971. Assay of endotoxin by the hypothermic response of mice. *Br. J. Exp. Pathol.* 52:198.
18. Ruddle, N. H., and B. H. Waksman. 1968. Cytotoxicity mediated by soluble antigen and lymphocytes in delayed hypersensitivity. I. Characterization of the phenomenon. *J. Exp. Med.* 128:1237.
19. Rabinovitch, M. 1969. Uptake of aldehyde-treated erythrocytes by L-2 cells. *Exp. Cell Res.* 54:210.
20. 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.
21. Moore, D. M., S. F. Cheuk, L. 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.
22. Gropp, A., and K. Hupe. 1956. Phasenkontrastmikroskopische Beobachtungen bei der phagozytose an normalen fibroblasten, an zelleneines mause Sarkoms sowie an HeLa-zellen. *Zt. fur Krebsf.* 61:263.
23. Averman, L. R. 1961. Ingestion of latex particles by chicken fibroblasts in tissue culture. *Proc. Soc. Exp. Biol. Med.* 107:895.
24. Werb, Z., and J. J. Reynolds. 1974. Stimulation by endocytosis of the secretion of collagenase and neutral proteinase from rabbit synovial fibroblasts. *J. Exp. Med.* 140:1482.

25. Ghosh, N. K., and R. P. Cox. 1976. Production of human chorionic gonadotropin in HeLa cell cultures. *Nature (Lond.)*. 259:416.
26. 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.* 10:451.
27. Gordon, G. B., L. R. Miller, and K. G. Bensch. 1965. Studies on the intracellular digestive process in mammalian tissue culture cells. *J. Cell Biol* 25:41.
28. North, R. J. 1970. Endocytosis. *Semin. Hematol.* 7:161.
29. Gordon, S., L. C. Unkeless, and Z. A. Cohn. 1974. Induction of macrophage plasminogen activator by endotoxin stimulation and phagocytosis. *J. Exp. Med.* 140:995.
30. Unkeless, J. C., A. Tobia, L. Ossowski, L. P. Quigley, D. B. Rifkin, and E. Reich. 1973. An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. *J. Exp. Med.* 137:85.
31. Bennett, I. L., and A. Nicasri. 1960. Fever as a mechanism of resistance. *Bacteriol. Rev.* 24:16.
32. Kluger, M. J., D. H. Ringler, and M. R. Anver. 1975. Fever and survival. *Science (Wash. D C.)* 188:166.