Pyrogen Release *in Vitro* by Lymphoid Tissues from Patients with Hodgkin's Disease[1,2]

PHYLLIS BODEL

*Department of Internal Medicine, Yale University School of Medicine,
New Haven, Connecticut 06510*

The mechanism of fever in patients with Hodgkin's disease was investigated by examining endogenous pyrogen production by blood, spleen, and lymph node cells incubated *in vitro*. Blood leucocytes from febrile or afebrile patients with Hodgkin's disease did not produce pyrogen spontaneously. Spleen cells, however, frequently released pyrogen during initial incubations, unlike spleen cells from patients with non-malignant diseases. Pyrogen production occurred from spleens without observed pathologic infiltrates of Hodgkin's disease. Lymph nodes involved with Hodgkin's disease produced pyrogen more frequently than did nodes involved with other diseases. Pyrogen production by tissue cells was prolonged, required protein synthesis, and in some cases was due to mononuclear cells; it did not correlate with fever in the patient. These studies demonstrate spontaneous production of endogenous pyrogen *in vitro* by lymphoid tissue cells from patients with Hodgkin's disease.

Although fever occurs commonly in Hodgkin's disease (1, 2), its cause is unknown. In animal models, the pathogenesis of fever due to infection and hypersensitivity has been clearly related to production of endogenous pyrogen by blood or tissue leucocytes following stimulation by microbial or antigenic agents (3). Only phagocytic cells, including granulocytes, monocytes, and macrophages appear to produce pyrogen. After being released into the blood, this small protein apparently causes certain hypothalamic neurons to initiate mechanisms for heat production and conservation, resulting in fever. In man, although similar mechanisms have been postulated to explain clinical fevers, experimental support for such events is lacking.

This report describes the production of endogenous pyrogen *in vitro* by blood and lymphoid tissue cells from patients with Hodgkin's disease. The results demon-

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strate that lymphoid tissue cells, but not blood cells, from these patients often spontaneously produce pyrogen during *in vitro* incubations. In contrast, lymphoid tissues of patients with nonmalignant diseases produce little or no pyrogen under the same conditions.

MATERIALS AND METHODS

All glassware, solutions, and instruments were rendered sterile and pyrogen-free, using methods described previously (4). Samples of all specimens obtained from patients, as well as all supernatants to be injected into rabbits, were cultured in thioglycollate broth. Supernatants from experiments where bacterial contamination occurred were discarded.

Pyrogen Testing

Pyrogen testing was carried out in rabbits, as described previously (4–6). Automatic temperature recording equipment (Rustrak) was used in most experiments. Between 1 and 6 rabbits received samples of a single supernatant for testing, depending on the amount of material available. Whenever possible, the same rabbits received samples of supernatant from all experimental flasks in an experiment. A positive response was judged to be an average temperature elevation of $\geq 0.3°C$, when the maximum rise occurred between 30 and 75 min after injection of the test material. In this assay, the height of responses between 0.3 and 0.7°C ΔT were considered proportional to the quantity of pyrogen present in the sample (7, 8).

Blood Leucocytes

Blood leucocyte suspensions were prepared from heparinized venous blood (10 units/ml). Red blood cells were removed by natural sedimentation, or by addition of dextran as described previously (4), except that hypotonic lysis was omitted. After centrifugation of the leucocyte-rich supernatant at 600g for 15 min, the cells were suspended in modified Krebs–Ringer Phosphate (KRP) buffer (4), or tissue culture medium with heparin, penicillin, and streptomycin (Eagle's Minimum Essential Medium, Auto-POW, Flow Laboratories, Inc., Rockville, Maryland) (MEM). After determination of leucocyte count with a Coulter counter, cells were added to flasks with 10–15% autologous plasma or serum, to a concentration of $6{-}10 \times 10^6$ cells/ml. Heat-killed *Staphylococcus albus* (4), was added to some flasks as a phagocytic stimulus. After 18 hr incubation at 37°C, supernatants were recovered, stored, and tested for pyrogen. Usually each rabbit received supernatant from 3 to 5×10^7 leucocytes; in some experiments with blood leucocytes from febrile patients, doses were increased to 2×10^8.

Tissue Cells

Sterile portions of spleens or lymph nodes obtained at operation were weighed, suspended in cold MEM, and then minced with fine scissors, or forced through wire gauze to prepare a tissue cell suspension. Leucocyte counts, differential smears for Wright's and Giemsa stain, and cultures were then taken, and the suspensions divided into aliquots for incubation according to wet weights. For spleens, approximately 1 g of tissue was suspended in a 5 ml volume, and for lymph nodes, 100–200 mg 10–15% autologous serum, or rarely, normal donor 0 negative serum was added to all incubation flasks. Heat-killed staphylococci were usually added to one flask.

The flasks were saturated with 5% CO_2 in air, sealed with parafilm over cotton plugs, and incubated with slow shaking on a Dubnoff or Egerbach incubator at 37°C overnight. The suspensions were then recovered by centrifugation at 600g for 15 min, and the cells resuspended in fresh medium for one or more additional incubations. Supernatants, after centrifugation at 2000g for 20 min, were stored at 4°C for 1–7 days, or at −18°C for 1–3 weeks, before injection.

Reagents

Pronase, B grade (Calbiochem, Los Angeles, California), was prepared for incubation with pyrogen-containing supernatants as described previously (4). Puromycin dihydrochloride 1×10^{-5} M, and Acti-Dione (cycloheximide) 2×10^{-5} M, (Nutritional Biochemicals Corp., Cleveland, Ohio) were added to lymph node and spleen cell suspensions in some experiments. Puromycin 5×10^{-5} M was shown to be inactive in the presence of frozen–thawed spleen tissue; cycloheximide was therefore substituted in all later experiments with spleen cells.

Clincal Data

All patients with Hodgkin's disease had unequivocal tissue diagnoses on biopsy. Diagnoses of other patients had been established by standard criteria. Chart review was used to obtain relevant clinical data for all patients. A reliable history, or documentation in hospital, of persistent unexplained fever within one month before operation was considered evidence for classification of the patient's disease as febrile.

Statistical Evaluation

Determination of significant differences was made using chi-square and Student's t test for small samples (9).

RESULTS

Blood Leucocytes

Studies were first made to determine whether blood leucocytes from patients with Hodgkin's disease produced pyrogen when the patients were febrile. Blood was obtained from 15 patients with Hodgkin's disease, 6 of whom were febrile. Blood was also obtained from febrile and afebrile patients with other diseases, including infections, leukemia, carcinoma, familial mediterranean fever, and drug reactions. All febrile patients had temperatures over 100°F at the time blood was drawn, and in most instances, the temperature was rising. Leucocytes were incubated overnight, and each supernatant was assayed for pyrogen in two or three rabbits. As shown in Table 1, circulating leucocytes from febrile donors never spon-

TABLE 1
PYROGEN RELEASE BY BLOOD LEUCOCYTES *in vitro*

	Hodgkin's disease	Non-lymphoma disease	Healthy subjects
Febrile donors	0/15*	0/19	—
Afebrile donors	2/18	2/30	3/37

* No. positive responses in rabbits (>0.3°C ΔT)/total.

TABLE 2

PYROGEN RELEASE BY SPLEEN CELLS *in vitro*

			Incubations					
			0–24 hr		24–48 hr		+ Staph (0–24 hr)	
	Average sample[a]		No. positive[b] total	$\Delta T(°C)$[c]	No. positive/ total	$\Delta T(°C)$	No. positive/ total	$\Delta T(°C)$
	g.	WBC×10⁸ %PMN						
Hodgkin's disease	1.08	2.7	18.2 ± 1.6	11/20	.36 ± .03 (.52 ± .03)[d]	16/20	.56 ± .04 (.60 ± .04)	20/20	.84 ± .04
Nonmalignant diseases	1.11	2.8	12.7 ± 1.9	2/17	.20 ± .03	8/15	.45 ± .05 (.60 ± .04)	16/16	.75 ± .07
				$P = <.01$	$P = <.001$		$P = <.05$		

[a] Suspension from which supernatant was derived per rabbit dose.
[b] Numbers of spleens giving average $\Delta T \geq 0.3°C$.
[c] Average $\Delta T \pm$ SEM of all supernatants, assayed in 36–82 rabbits.
[d] Average $\Delta T \pm$ SEM of positive ([b]) supernatants, assayed in 18–44 rabbits.

taneously released pyrogen during *in vitro* incubations. As shown in the lower half of the table, blood cells obtained from afebrile patients with Hodgkin's disease, other diseases, or from healthy donors, also rarely released pyrogen. All preparations tested except one were capable of pyrogen release when heat-killed staphylococci were added to provide a phagocytic stimulus (not shown). These studies indicate that circulating leucocytes obtained from febrile patients with Hodgkin's disease, or other disorders, do not spontaneously release pyrogen *in vitro*.

Spleen Cells

In order to look for pyrogen production by tissue cells of patients with Hodgkins's disease, portions of spleens were obtained at the time of splenectomy from 20 patients with biopsy-proven Hodgkin's disease, and from 17 patients with nonmalignant diseases. The diagnoses in this latter group included idiopathic thrombocytopenic purpura (6 cases), hereditary spherocytosis (4 cases), cirrhosis (2 cases), and one case each of splenic artery aneurysm, thalassemia, traumatic rupture, thrombotic thrombocytopenic purpura, and lupus erythematosus. Spleen cells were suspended for successive 24-hour incubations, as described in methods, and pyrogen release determined. The results of these studies are presented in Table 2. Significant release of pyrogen occurred spontaneously during the first 24 hr of incubation in 11 of 20 preparations of spleen cells from patients with Hodgkin's disease, but in only 2 of 17 from patients with other diseases. This difference is significant ($p = <.01$). During a second incubation, detectable pyrogen was released from 16 of 20 suspensions in the first group, and from 8 of 15 in the second. In almost all instances, tissues which released pyrogen during a first incubation released even more during a second incubation. All preparations contained cells which were capable of pyrogen release, since they all released pyrogen when heat-killed staphylococci were added.

In addition to the difference in frequency of spontaneous pyrogen production, the quantity of pyrogen released by spleen cells in the malignant and nonmalignant groups was also different. The average fevers in rabbits following injection of supernatants from all tissues in each of the two groups are shown in separate columns in Table 2. Larger pyrogenic responses were produced by supernatants from tissues in the first group; the differences are significant for results of both incubations
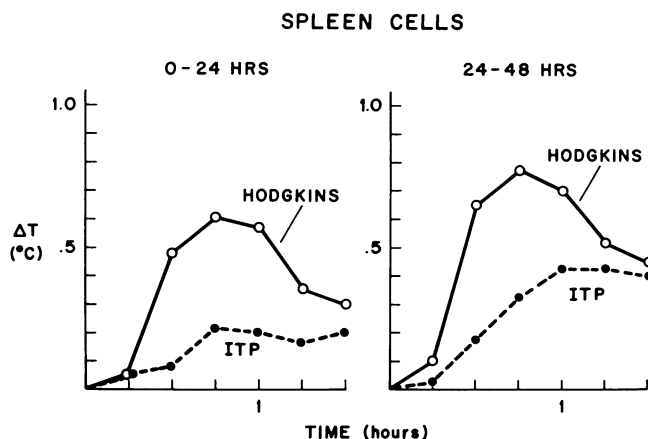
SPLEEN CELLS

FIG. 1. Pyrogen in incubation supernatants of spleen cell suspensions from two patients. Average febrile responses in 2 or 4 rabbits after injection at time 0 of supernatants from incubated spleen cells, obtained from one patient with Hodgkin's disease (Hodgkins) and one with idiopathic thrombocytopenic purpura (ITP). Each rabbit received supernatant corresponding to the following wet weights and white cell counts of tissue suspension: 1.3 g, $2.7 \times 10^{\cdot}$ (Hodgkins); 1.0 g, $4.0 \times 10^{\mathfrak{s}}$ (ITP).

($p = <.001$, $<.05$). Average fevers produced by supernatants of "positive" tissues only are also included for some groups in Table 2, as indicated. Pyrogen release after addition of staphylococci was slightly greater by tissues in the first group.

The type of fever produced by injection of supernatant from successive incubations of spleen cells, from patients with malignant and nonmalignant disease, is illustrated in Fig. 1. The shape of the fever curves resembles that previously reported for human, as well as rabbit, leucocyte pyrogen, and does not resemble a typical response to endotoxin (9). Some rabbits received 12–15 pyrogenic injections during 5 days, without diminution in their febrile responses, indicating lack of development of tolerance. The protein nature of the pyrogen was established by incubation of a portion of spleen cell supernatant with pronase. Pyrogenicity was abolished, whereas the control portion incubated in Tris buffer alone retained activity (average ΔT 0.0°C and 0.50°C, respectively).

Although the numbers of total cells present in the tissue preparations in each group appeared comparable, differential counts of each spleen cell suspension were done to determine the types of cells present. The results of these studies indicated only small differences between the two groups of tissues in average numbers of polymorphonuclear leucocytes (see Table 2), or of large mononuclear cells. Moreover, there was no correlation between the numbers of either of these types of cells with the presence or absence of early, spontaneous pyrogen production in individual preparations. In Fig. 2, the percent of neutrophils in each spleen suspension is plotted against the average height of the fever response to supernatant in that experiment. A similar lack of correlation was observed when numbers of eosinophils were plotted in the same way. It was not possible to accurately distinguish by morphology in these preparations between monocytes, macrophages, and large cells of the lymphocytic series. In some experiments, heat-killed staphylococci were added to one portion of spleen cells, and after 2 hr incubation, cover slip smears of washed cells were examined to identify phagocytic cells. Most neutrophils contained bacteria, and between 5 and 10% of mononuclear cells.
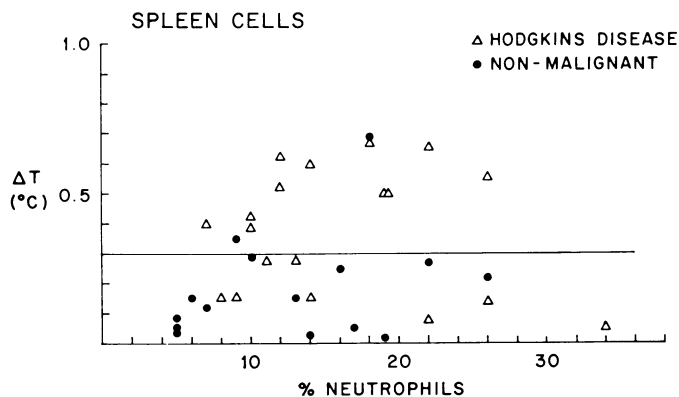
FIG. 2. Relation between per cent neutrophils and pyrogen production in spleen cell suspensions. Average height of fevers (ΔT) in rabbits to injection of supernatant from first incubations of spleen cell suspensions, plotted against percent neutrophils. Tissues from patients with Hodgkin's disease and nonmalignant diseases are included.

Experiments were next carried out to determine whether pyrogen production by spleen cell suspensions was an active metabolic process. Aliquots of three preparations held at 0°C for 24 hr failed to produce detectable pyrogen, unlike the aliquots incubated normally. In two other experiments, supernatant was removed from active spleen cell suspensions after one or two hours of incubation, and the cells disrupted by 5 cycles of freeze–thawing in dry ice and acetone. Neither supernatant nor cells contained detectable pyrogen. These results indicate that little or no pyrogen was present in active form in cells or incubation medium at the start of the incubation.

To determine whether protein synthesis was essential for pyrogen release by tissue cells, as it is in the case of blood PMN and mononuclear cells (10), cycloheximide (2×10^{-5} M) was added to some flasks, either during a first or second 24-hr incubation. Control flasks were incubated as usual without inhibitor. The results of these experiments are presented in Fig. 3. When cycloheximide was added at the start of the first incubation, it completely suppressed production of pyrogen by spontaneously active spleen cell suspensions. When cycloheximide was added during a second incubation, however, only partial suppression of production by these tissues occurred. Addition of inhibitor at 24 hr to flasks containing initially inactive cells (Fig. 3, right) effectively prevented late pyrogen release. These results indicate that initial inhibition of protein synthesis prevents pyrogen production by all spleen cell suspensions, but that only partial inhibition occurs when the inhibitor is added after significant pyrogen release has begun.

Attempts were made to correlate the behavior of spleen cell preparations *in vitro* with clinical findings in patients with Hodgkin' disease. These results are presented in Table 3. It is clear that initial spontaneous pyrogen release *in vitro* did not correlate with fever *in vivo*. Tissues from some markedly febrile patients released little or no pyrogen *in vitro,* whereas tissues from afebrile patients frequently produced large amounts of pyrogen. Similarly, pathologic infiltrates of Hodgkin's disease in the spleen were not often associated with spontaneously pyrogen-producing cells. For example, as shown in Table 3, although 11 spleens from patients with biopsy-proven Hodgkin's disease at other sites released significant amounts of pyrogen
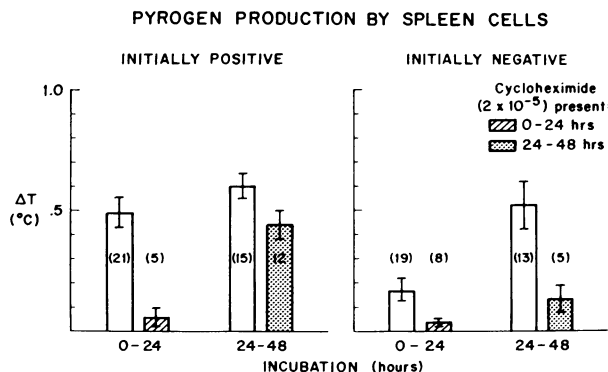
PYROGEN PRODUCTION BY SPLEEN CELLS

INITIALLY POSITIVE   INITIALLY NEGATIVE

Cyclcheximide
(2 x 10⁻⁵) present:
0-24 hrs
24-48 hrs

ΔT (°C)

(21) (5)   (15) (12)   (19) (8)   (13) (5)

0-24   24-48   0-24   24-48
INCUBATION (hours)

FIG. 3. Effect of cycloheximide on pyrogen production by spleen cells. Average height of fevers (ΔT) ± SEM in rabbits to injection of supernatants from successive incubations of spleen cells. Cycloheximide was added to samples at 0 or 24 hr, as indicated. Numbers of rabbits are shown in parentheses. Results of experiments in which pyrogen release (average ΔT) during a first incubation was $\gtrless 0.3\,°C$ are presented on the left, "initially positive;" others, "initially negative," are on the right.

TABLE 3
SPLEEN CELLS FROM PATIENTS WITH HODGKIN'S DISEASE

Initial pyrogen release	Hodgkin's Disease in tissue		Unexplained fever[a]		Stage[b]	
	+	−	+	−	I, II	III, IV
Positive[c] (11)	1	10	4	7	8	3
Negative (9)	4	5	3	6	3	6

[a] Present in hospital or by history within one month of admission.
[b] Assigned after diagnostic procedures completed.
[c] Average $\Delta T \gtrless 0.3°C$ (see text).

initially, only one of these spleens contained characteristic infiltrates of the disease on pathologic examination. Correlations were also not evident with the classification of type of Hodgkin's disease, age of the patients, or diagnostic or therapeutic procedures preceding operation. An apparent relationship was noted, however, between spontaneous pyrogen release *in vitro* and stage of disease. Most spleens from patients with Stage I or II disease were initially positive, whereas most from patients with Stage III or IV disease were initially negative. This was probably not related to differences in therapy, since only 1 patient of 6 in this latter category had received treatment within three years of surgery. Data on the presence or absence of anergy in the patients with Hodgkin's disease was insufficient for analysis.

Lymph Node Cells

Pyrogen production by lymph node cells, obtained at operation from febrile and afebrile patients with malignant and nonmalignant diseases, was also examined.

TABLE 4

PYROGEN RELEASE BY LYMPH NODE CELLS *in vitro*

| | | | Incubations | | | | | |
| | | | 0–24 hr | | 24–48 hr | | + Staph | |
	No. nodes	Average wet wt (mg)[a]	No. positive[b]/ total	$\Delta T(°C)$[c]	No. positive/ total	$\Delta T(°C)$	No. positive/ total	$\Delta T(°C)$
Hodgkin's disease	10	154	6/10	.33 ± .06 (.43 ± .07)[d]	5/10	.35 ± .08 (.49. ± .12)	6/7	.52 ± .05
Lymphoma	6	197	1/5	.22 ± .06	1/6	.32 ± .10	3/5	.41 ± .05
Metastatic carcinoma	6	161	1/6	.26 ± .06	2/6	.28 ± .06	2/2	.57 ± .06
Benign hyperplasia	6	203	0/6	.16 ± .04	2/6	.31 ± .09	4/5	.48 ± .06

[a] Suspension from which supernatant was derived per rabbit dose.
[b] Number of nodes giving average $\Delta T > 0.3°C$.
[c] Average $\Delta T \pm$ SEM of all supernatants, assayed in 7–16 rabbits.
[d] Average $\Delta T \pm$ SEM of positive (b) supernatants, assayed in 7–9 rabbits.

Patients with malignant diseases included 10 with Hodgkin's disease, 6 with other lymphomas, and 6 with metastatic squamous cell or adenocarcinoma. Lymph nodes obtained from an additional 6 patients were classified as showing benign hyperplasia. Six nodes were paraaortic or perisplenic, one was inguinal, and others were cervical, supraclavicular, or axillary. Tissue suspensions were prepared and incubated as described in Methods.

The results of these studies are presented in Table 4. Significant pyrogen was released spontaneously during a first incubation by 6 of 10 lymph node cell preparations from patients with Hodgkin's disease, 1 of 5 from patients with non-Hodgkin's lymphomas, and only 1 of 12 preparations from patients with other disorders. The difference between results with Hodgkin's disease tissues and all others combined is significant($p = <.01$). During a second incubation, pyrogen was released from about half of lymph node cell suspensions from patients with Hodgkin's disease, and about one-third of those in the other groups. Only small amounts of pyrogen were usually released by any of the lymph node suspensions, when tested at this dosage. Fifteen of 19 of the preparations produced detectable pyrogen when stimulated by addition of heat-killed staphylococci (see Table 4). Differential counts of free lymph node cells in all experiments showed >95% mononuclear cells, mostly small lymphocytes.

To investigate the effect of inhibitors of protein synthesis on pyrogen production by lymph node cells, puromycin was added in some experiments to flasks containing pyrogen-producing cells. Pyrogen production was either spontaneous or induced by addition of heat-killed staphylococci; since results of both types of experiments were the same, they have been combined. As shown in Fig. 4, lymph node cells, like spleen cells, usually continued to produce pyrogen for 2–3 days. Unlike the results with cycloheximide and spleen cells, however (see Fig. 3), addition of puromycin to lymph node cells either during a first or second incubation suppressed pyrogen release. Cycloheximide was equally effective as an inhibitor for lymph node cells (not shown). These results suggest that continuing protein synthesis is required for pyrogen release from lymph node cells *in vitro*.
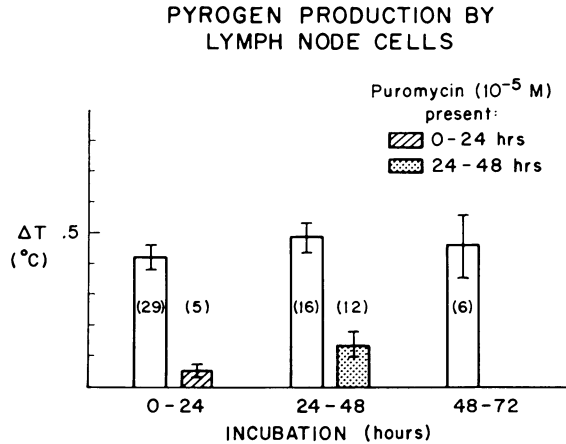
FIG. 4. Effect of puromycin on pyrogen production by lymph node cells. Average height of fevers (ΔT) $\pm$ SEM in rabbits to injection of supernatants from successive incubations of lymph node cell suspensions. In all but one experiment, lymph nodes were from patients with Hodgkin's disease. Puromycin was added at 0 or 24 hr, as indicated. Numbers of rabbits are shown in parentheses.

Correlations between pyrogen production by lymph node cells *in vitro* and clinical findings in patients with Hodgkin's disease were inconclusive because of the small number of tissues examined. All nodes but one (a non-pyrogen-producing tissue) had evidence of Hodgkin's disease on pathological examination. Four of 6 nodes from patients with unexplained fever produced spontaneous pyrogen *in vitro*, but the amounts of pyrogen were not greater than those produced by tissues from afebrile patients. No other patients, including those with non-Hodgkin's lymphomas, were febrile. No correlation was evident between pyrogen release and site of lymph node biopsy, stage or histologic type of Hodgkin's disease, or prior treatment or procedures. Lymphangiography preceded operation by 2 days to 2 weeks in 6 cases where intraabdominal nodes were studied; 4 of these (3 with Hodgkin's disease) produced significant early pyrogen.

DISCUSSION

Although pyrogen release from blood or tissue cells has been postulated in the etiology of fever associated with certain disease states (10), there is little experimental evidence for this in man. Snell (11) demonstrated a rapidly acting pyrogen in inflammatory effusions from patients with febrile diseases. The presence of this material, however, did not correlate with fever in the patient at the time the fluid was withdrawn. Endogenous pyrogen was present in joint fluids from afebrile patients with rheumatoid arthritis (12). Recently, extracts from two hypernephromas have been reported to contain endogenous pyrogen-like activity when tested in rabbits, whereas tumor tissue removed from one afebrile patient was not pyrogenic (13). Sokal and Shimaoka (14) reported a pyrogen of unknown nature in urine from febrile patients with Hodgkin's disease.

In the studies reported here, blood and lymphoid tissue cells from patients with Hodgkin's disease, as well as other conditions, were incubated *in vitro*, and pyrogen production assayed. Blood leucocytes from all febrile patients failed to release pyrogen during *in vitro* incubations, although they were capable of pyrogen release

when stimulated by heat-killed bacteria. These findings fail to implicate circulating blood leucocytes as an important source of endogenous pyrogen during fever.

When tissue cells were examined, however, prolonged "spontaneous" pyrogen production *in vitro* was noted by lymphoid tissue cells obtained from patients with Hodgkin's disease. Spleen and lymph node cells from patients with nonmalignant diseases produced significantly less pyrogen under the same conditions. The pyrogen caused rapid pyrogenic responses in rabbits, did not induce tolerance, and was inactivated by pronase; thus it resembled endogenous, or leucocyte, pyrogen, and not endotoxin (10).

The mechanism for the "spontaneous" and continuing production of pyrogen by these tissues is not clear. By analogy with results of studies of blood and exudate leucocytes (15–17), an activator, such as a microbial product or antigen, presumably initiates the process of pyrogen synthesis and release from tissue PMN or mononuclear cells. Spleen cell suspensions contained 6–35% PMN leucocytes, and variable numbers of large mononuclear cells, many of which were phagocytic. Such cells, like Kupffer cells, lung macrophages, and blood monocytes (10), are probably capable of pyrogen production. Active cell metabolism and protein synthesis was required for initiation of "spontaneous" pyrogen production by these tissues *in vitro,* as shown by the absence of pyrogen in cell extracts or supernatants obtained before incubation, after incubation at 4°C, or when cycloheximide was added during the first 24-hr period. Continuing pyrogen release by spleen cell suspensions, however, was only partially sensitive to inhibition by cycloheximide. Passive release of intracellular pyrogen, synthesized during a first incubation, could explain this finding. Alternatively, pyrogen production at this stage might be due mainly to PMN leucocytes, cells which, once activated, can produce pyrogen for a limited time in the presence of puromycin or cycloheximide (15, 16). Preliminary results provide some evidence for both mechanisms.

Pyrogen production by lymph node cells, which were >95% mononuclear, continued for 48–72 hr, and could be inhibited at any time by puromycin. Mononuclear tissue cells, of unknown type, were presumably responsible for production of pyrogen by these tissues. Recent evidence suggests that blood monocytes, as opposed to PMN leucocytes, require continuing protein synthesis for pyrogen production (Bodel, unpublished observations). The low fevers induced by supernatants from many of the lymph node preparations may perhaps be explained by the small amount of tissue available for study. On the other hand, since lymphocytes and derived lymphoid cell lines do not make pyrogen, even after stimulation (7), the numbers of cells capable of pyrogen production in most lymph nodes may be small.

The reasons for the differences in pyrogen production by tissues from patients with Hodgkin's disease compared to those from patients with nonmalignant diseases are not clear. Involvement of spleen tissue by the lymphomatous process, as determined by standard pathologic criteria, was clearly not related to its capacity for significant, early pyrogen production, since tissue from patients with stage III or IV disease was often less active than that from patients with apparently localized disease. Involvement of lymph nodes by Hodgkin's disease, however, appeared to be related to spontaneous early pyrogen production *in vitro.*

Eight of 16 patients undergoing splenectomy in the nonmalignant group had received corticosteroids preoperatively, agents which have antipyretic effects both *in vivo* and *in vitro* (6). However, there is no difference between the results of incubations of tissues from patients receiving or not receiving steroids when initial

pyrogen release, total pyrogen release, or release after a phagocytic stimulus is compared. Lymphangiography, a procedure often followed by fever (18), could have contributed to the "spontaneous" activity of three nodes in the Hodgkin's disease group, and the one positive node in the lymphoma group. However, nodes from two other patients subjected to lymphangiography were not active.

Recent theories (19, 20) have postulated that in Hodgkin's disease altered lymphocytes act as an autoimmune stimulus to normal lymphoid tissue cells, producing a kind of graft vs host reaction, with ensuing lymphoid hyperplasia, immunological deficiency, and eventual neoplastic transformation. After stimulation of sensitized rabbit lymphocytes by antigen, pyrogen release is induced from blood leucocytes, perhaps by the action of a lymphokine (21); this mechanism may explain certain experimental fevers of hypersensitivity in rabbits. By a similar pathway, tissue lymphocytes from patients with Hodgkin's disease, altered perhaps by virus or tumor-associated antigen (22), may induce release of pyrogen from tissue PMN or mononuclear cells. This interaction *in vitro* could lead to the observed early "spontaneous" release of pyrogen from tissue cell suspensions. The decreased reactivity of tissue from patients with late stage disease may reflect suppression or deficiency of this immune mechanism.

The failure of the *in vitro* results to correlate with fever in the patient may perhaps be due to differences between *in vivo* and *in vitro* conditions. Pyrogen release from tissue cells could be enhanced or suppressed *in vivo,* depending on several variables, including opportunities for cell activation and presence of factors modifying pyrogen production, release, or transport. Such variables could play an important role in determining the occurrence of fever in patients with potentially febrile conditions such as Hodgkin's disease.

ACKNOWLEDGMENTS

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