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The Detection and Characterization of Phagocytic Cells in Established Human Cell Lines Synthesizing Immunoglobulins (33360)

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Long-term suspension cultures derived from the peripheral blood of patients with infectious mononucleosis have been established and characterized in our laboratory (1). The cells in these continuous cultures have light microscopic, histochemical, and ultrastructural features which are characteristically lymphoid (1); and they are capable of immunoglobulin synthesis *in vitro* (2). Preliminary screening studies indicated, however, that some of the cells in these established lines as well as in continuous lines derived from patients with other lymphoproliferative disorders were capable of phagocytosis, a function usually attributed only to monocytes and macrophages. The present investigations were undertaken to quantitate and further characterize the phagocytic cells in these cultures in an effort to discern, if possible, their origin and significance. As part of this characterization the ability of these cells to release leukocytic pyrogen after phagocytosis was also examined, since this is a function previously known to be associated only with granulocytes and monocytes (3).

Materials and Methods. Cell Lines. Seventeen human, long-term cell lines were used. These included AL-1 (derived from a patient with Burkitt's tumor) (4), LK1D (derived from a patient with lymphoblastic leukemia) (5), IM-1 (derived from a patient with lymphosarcoma) (6), and 14 cell lines isolated from patients with heterophile-positive infectious mononucleosis as shown in Table I. These cell lines were maintained in suspension culture containing RPMI 1640 medium (7), with 20% heat-inactivated fetal calf serum (Flow Laboratories, Rockville, Maryland) and were regularly transferred into fresh medium twice weekly.

Phagocytic study. Phagocytic activity was investigated by incubating cell suspensions with both heat-killed *Staphylococcus epidermidis* and polystyrene particles. Cultures of *S. epidermidis* grown in trypticase soy broth at 37° were washed three times in normal saline, heat-killed by immersion in a boiling water bath for 30 min, counted, and then resuspended in normal saline at a concentration of 2×10^9 organisms/ml. The polystyrene particles (1.3-μ diam, Dow Chemical Co., Midland, Michigan) were suspended in normal saline at a concentration of 6×10^8 particles/ml.

TABLE I. Phagocytic Activity of Lymphoid Cell Lines for *S. epidermidis* and Polystyrene Particles.

Cell line[a]	Total cells (%) phagocytic for *S. epidermidis*	Cells (%) with >10 staph./cell	Total cells (%) phagocytic for polystyrene particles	Cells (%) with >10 polystyrene particles/cell
PGLC 33H	29	19	26	8
PGLC 33J	26	9	27	11
PGLC 42D	23	4	19	6
PGLC 42F	30	23	16	5
PGLC 50B	34	22	15	4
PGLC 50D	23	5	28	6
PGLC 53B	35	25	30	13
PGLC 53D	31	18	21	7
PGLC 55B	30	19	25	14
PGLC 55E	34	26	32	12
PGLC 44B	22	6	26	10
PGLC 51D	25	10	23	9
PGLC 54B	29	22	24	7
PGLC 56B	33	25	28	8
AL-1	26	7	37	28
LK1D	24	6	16	4
IM-1	31	21	20	10

[a] Cell lines isolated from the same patient at different times during the course of illness have the same number but different letter coding.

Three days after transfer into fresh medium, cells from actively growing suspension cultures were removed by centrifugation at 250*g* for 30 min and resuspended in 3 ml of fresh medium. Resulting cell concentrations ranged from 0.6×10^7 to 2.2×10^7 cells/ml. One ml of each cell line suspension was incubated with 3×10 polystyrene particles (0.5 ml of suspension) at 37° for 2 hr. An additional 1 ml of each cell line suspension was incubated with 2×10^8 *S. epidermidis* (0.1 ml of suspension) at 37° for 0.5 hr. The ratio of particles to cells ranged from 10:1 to 40:1.

Staining and scoring of slides. Following incubation, all tubes were centrifuged at 250*g* for 10 min and the supernatant was removed. The sedimented cells were resuspended in 2 drops of heat-inactivated fetal calf serum and streaked out on glass slides. The slides were fixed for 5 min in methanol, air dried, and stained for 8 min with fresh Giemsa stain. The percentage of phagocytosis was determined by scoring one hundred consecutive, intact cells.

Leukocytic pyrogen production. Several of the cell lines (LKID(PGLC 33H, PGLC 33J, PGLC 42D, PGLC 42F) were studied for their ability to release leukocytic pyrogen following phagocytosis of heat-killed *Staphylococcus albus* or polystyrene particles. Cells from actively growing suspension cultures were centrifuged at 250*g* for 15 min (4°). They were washed once in either pyrogen-free modified Hanks' solution (MHS) or Krebs-Ringer phosphate buffer (KRP) (8). Aloquots containing $200–400 \times 10^6$ viable cells, were resuspended in 10 ml of medium consisting of 15% human plasma plus KRP or MHS, 200 mg/100 ml of glucose, 1000 units of heparin, and 10,000 units of penicillin G. Heat-killed *S. albus* or polystyrene particles were added in a ratio of 15–55:1 particles to phagocytic cells, and the mixtures were incubated at 37° for 18 hr in a shaker incubator. At the conclusion of the incubation period the cells were removed by centrifugation at 2500*g* for 15 min (4°). The remaining unphagocytized particles were removed by an additional centrifugation at

2500*g* for 45–60 min. The supernatants were then stored at 4° until tested for pyrogen content. Appropriate controls consisting of cells in medium and medium with particles were processed in a similar manner and the supernatants were tested for pyrogen. As a further control, suspensions of mixed peripheral blood leukocytes obtained from normal volunteers and patients with infectious mononucleosis were also incubated with particles, and the cell-free supernatants were assayed for pyrogen content.

Leukocytic pyrogen assay. New Zealand albino rabbits from the NIH colony were used for all pyrogen assays. Details of their housing, feeding, and recording of temperatures have been previously reported (9). Aliquots of the cell-free supernatants were injected into a marginal ear vein. The presence of leukocytic pyrogen was detected by a prompt rise in temperature greater than 0.3° reaching a peak within the first hour after injection and returning to the baseline by 2–3 hr after injection. Dosages were determined by the number of phagocytic cells present in the suspensions prior to centrifugation since previous studies of human peripheral leukocytes had shown that phagocytes are the only cells capable of pyrogen production (3, 10).

Results. The cell lines studied were composed of a heterogeneous population of actively dividing lymphoblast-like cells. In addition, there were cells present in the population with the light microscopic features of small lymphocytes, lymphocytoid plasma cells, and atypical lymphocyte, as well as occasional multinucleated giant cells (1, 11). The phagocytic activity of these human lymphoid cell lines for *S. epidermidis* and polystyrene particles is shown in Table I. In general, from 15 to 35% of cells in the cell lines demonstrated phagocytosis. The phagocytic cells were morphologically indistinguishable from other nonphagocytic cells in the lines and could be distinguished only following the ingestion of particles. In general, the cells demonstrating phagocytosis were intermediate in size, ranging from 8 to 15 μ in diameter, and morphologically similar to the atypical lymphocytes seen in the peripheral blood

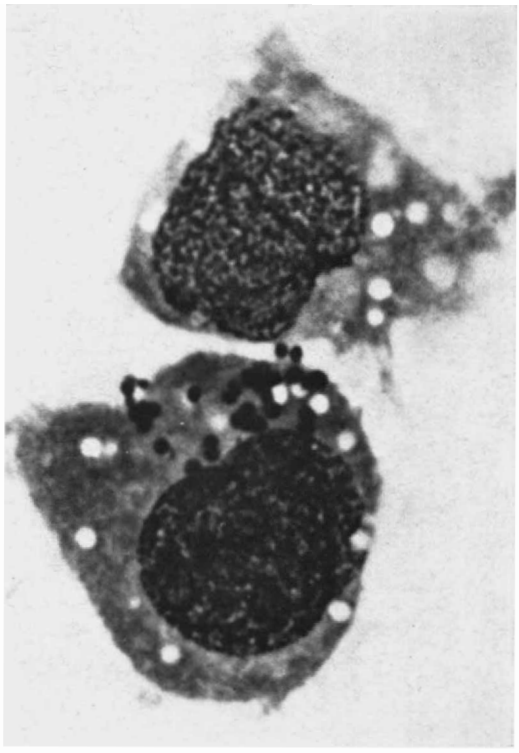

FIG. 1. Phagocytic cell with ingested *S. epidermidis*.

of patients with infectious mononucleosis. Photomicrographs of typical phagocytic cells from the lymphoid cell lines are shown in Figs. 1 and 2, respectively. Percentage phagocytosis appeared to be independent of the type of particle used and the length of time the cell lines had been in culture. In the lines derived from patients with infectious mononucleosis the percentage phagocytosis was not significantly different whether the cell lines had been isolated early or late in the course of clinical illness. The three cell lines derived from patients with other lymphoproliferative disorders had phagocytic percentages similar to the infectious mononucleosis cell lines.

Table II shows the results of leukocytic pyrogen assays performed on the supernatants obtained following incubation of certain of the cell lines with either *Staphylococcus albus* or polystyrene particles. For comparison, pyrogen release following phagocytosis of *S. albus* by suspensions of peripheral leukocytes from normal volunteers and from pa-

tients with infectious mononucleosis is also shown. No pyrogen could be detected in the cell line supernatants, despite the assay of supernatant volumes from numbers of phagocytic cells far in excess of that required to demonstrate pyrogen production by peripheral leukocytes.

Discussion. Mononuclear cells in the peripheral blood have traditionally been classified as lymphocyte or monocyte based on morphological and functional criteria (12, 13). These criteria include appearance under the light microscope (size, nuclear shape, chromatin configuration, and staining properties); ultrastructural features; presence or absence of phagocytic activity; histochemical analysis; and the capability to synthesize immunoglobulins *in vitro* (14). The cell lines used in this study display features characteristic of lymphoid cells. By light and electron microscopy these cells are morphologically heterogeneous ranging in appearance from cells which resemble atypical lympho-

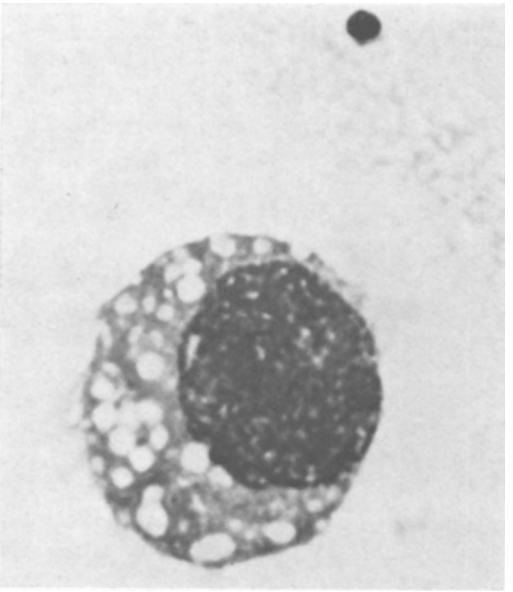

FIG. 2. Phagocytic cell with ingested polystyrene particles.

TABLE II. Pyrogen Release by Cell Lines as Compared to Peripheral Leukocytes.

Cell	Particle[a]	Total no. of cells assayed /rabbit ($\times 10^6$)[b]	Phagocytosis (%)[c]	No. of phagocytes assayed /rabbit ($\times 10^6$)[b]	No. of test animals	Mean ΔT (°)
Normal peripheral leukocytes	*S. albus*	8.3–10.6	60–47	5.0	8	1.05 ± .08
Infectious mononucleosis peripheral leukocytes	*S. albus*	7.1	70	5	4	0.53 ± .11
PGLC 33H	*S. albus*	50	17	8.5	4	0.18
LK1D	*S. albus*	50	1	0.5	4	<0.1
PGLC 33H	*S. albus*	100	30	30	3	<0.1
PGLC 42F	*S. albus*	70	50	35	2	<0.1
PGLC 33J	*S. albus*	150	23	35	1	0
PGLC 42D	*S. albus*	80	25	20	2	0.1
PGLC 42F	Polystyrene particles	180	19	34	2	<0.1

[a] In studies on peripheral leukocytes *S. albus* were added in a ratio of 20 bacteria to 1 phagocyte. Ratio of particles/phagocytes in the cell line studied ranged from 15–55:1.

[b] Doses of supernatant tested are expressed in terms of the total number of cells from which they were derived.

[c] For peripheral leukocytes the percentage of phagocytes is equivalent to the cumulative percentage of monocytes and granulocytes. When examined after 2 hr of incubation 95–96% of these cells had ingested 1 or more *S. albus* organisms.

cytes to cells which resemble lymphoblasts and phytomitogen transformed lymphocytes, all of which are fundamentally lymphoid in character (1, 11). On histochemical analysis the cells stain positively for acid phosphatase, but there is a complete absence of staining for peroxidase and leukocyte alkaline phosphatase (1). By radioimmunoelectrophoresis active synthesis of immunoglobulins by these cells has been demonstrated (2). Nevertheless, a certain percentage of cells in each line are capable of a well-defined cellular function, phagocytosis, which is usually attributed only to monocytes and macrophages.

It has been generally accepted that monocytes are phagocytic, whereas circulating lymphocytes are nonphagocytic in nature. Recent work, however, has shown that a fraction of normal lymphocytes under certain conditions are capable of erythrophagocytosis (15) as well as adherence to glass (14,16), suggesting a functional overlap between lymphocytes and monocytes. The structural features and functional capabilities of lymphocytes and monocytes overlap more noticeably in certain disease states. The atypical lymphocytes in the peripheral blood of patients with infectious mononucleosis, first described by Downey in 1923 (17), are basically lymphoid in character but also possess some of the cytoarchitectural features of the monocyte (18). In 1939, Hertzog showed that circulating atypical lymphocytes from patients with infectious mononucleosis, as well as some lymphocytes from patients with lymphocytic leukemia or unexplained lymphocytosis, were capable of phagocytizing bacteria (19). More recently, phagocytosis has been demonstrated in circulating lymphocytes in certain disease states following intravenous administration of carbon particles (20). In the present study phagocytic activity in long-term suspension cultures of lymphoid cells derived from peripheral blood or lymphoid tissue was demonstrated.

Recent studies on human peripheral leukocytes have demonstrated that they will release endogenous pyrogen following phagocytosis or stimulation with endotoxin (10, 21). When phagocytes are removed from peripheral leukocyte suspensions by passage through a glass wool column, the nonphagocytic lymphocytes obtained do not release pyrogen when stimulated with endotoxin (10). Thus, the ability to release leukocytic pyrogen following appropriate stimulation appears to be limited to granulocytes and monocytes (21). The fact that the cell lines did not release leukocytic pyrogen despite active phagocytosis lends further support to this concept, and also suggests that the phagocytic cells seen in these cell lines are of lymphoid origin.

The cytoarchitectural features of the phagocytic cells in the established cell lines appear similar to those of the atypical lymphocytes seen in the peripheral blood of patients with infectious mononucleosis. Some of these peripheral atypical lymphocytes have been shown to have phagocytic activity (19). However, whether or not the phagocytic cells seen in our cell lines represent direct descendants from circulating atypical lymphocytes is a question for speculation at this time. A more likely explanation for these findings may lie in the possibility that both monocytes and lymphocytes may originate from a common stem cell (22), and that in the process of cytodifferentiation lymphocytes may assume the properties usually attributed to monocytes and macrophages. Finally, it is intriguing that these cell lines are synthesizing immunoglobulins at the same time that they are demonstrating phagocytic activity. A considerable body of evidence has been accumulated which suggests that an important function of macrophages in cellular immune reactions is to process antigen for antibody synthesis (23). Whether or not the phagocytic cells observed in the cell lines may be serving such a function awaits further investigation.

Summary. Long-term suspension cultures of lymphoid cell lines were studied for phagocytic activity and pyrogen production. These cell lines were shown to actively phagocytize both polystyrene particles and *Staphylococcus epidermidis*, a function generally not attributed to lymphoid cells. We have observed that release of leukocytic pyrogen, normally associated with phagocytic activity of granulocytes and monocytes, did not accompany phagocytosis by these lymphoid cell lines.

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Reversible Nature of Atropine Blockade of Acetylcholine in Vascular Beds* (33361)

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During the course of some studies of autonomic nervous system function, we used atropine to prevent the cardiovascular effects of injected acetylcholine. Atropine never failed to produce the familiar persistent blockade of cholinergic effects on the heart, but the blockade of cholinergic effects on blood vessels appeared to be temporary. That is, the first postatropine injection of acetylcholine produced no vasodilation, while a second or third usually did. This was unexpected because while the very substantial literature on the pharmacology of atropine contains many references to variation in sensitivity of cholinergic receptors to atropine, it does not include any reports of an atropine blockade which is reduced or terminated after several injections of acetylcholine. In this report we present data from a large number of experiments which document this effect.

Materials and Methods. Cats anesthetized with pentobarbital sodium (30 mg/kg) were used. Bood pressure was recorded from the femoral artery by means of a Statham pressure transducer connected to a Sanborn end-recorder. Vascular changes in local beds were monitored by the electrical impedance technique, the basic principles of which have

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