FORMATION OF MULTINUCLEATED GIANT CELLS FROM HUMAN MONOCYTE PRECURSORS

Mediation by a Soluble Protein from Antigen- and Mitogen-stimulated Lymphocytes*

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Multinucleated giant cells were first reported in tuberculous granulomas by Rokitansky (1) and Langhans (2, 3) over a century ago. These giant cells are now recognized as a common feature of granulomas induced both by immunological and nonimmunological stimuli (4, 5). Thus, they are found in granulomas associated with the immune response to tuberculosis, leprosy, syphilis, and various fungal and parasitic infections as well as in granulomas associated with nonimmuine responses to toxic agents such as silica, beryllium, and asbestos, and to nontoxic agents such as carbon particles, plastic beads, and iron particles (4).

Nevertheless, the origin and the mechanism of the formation of multinucleated giant cells remain unclear. It is possible, because of the variety of agents that produce granulomas, that these cells are formed by several different mechanisms. Thus, they may arise from the fusion of nonreplicating monocytes or from the mitotic and amitotic division of monocyte nuclei in the absence of cellular division. Several observations suggest that cellular immune mechanisms play a direct role. The giant cells have been found in granulomas of the skin produced by delayed hypersensitivity reactions in humans and in experimental animals (6–8). Moreover, crude supernatants from cultures of antigen-stimulated lymph node cells of rabbits have been shown to cause alveolar macrophages to form giant cells in vitro (9, 10).

In this paper we describe evidence that suggests that a heat-labile protein, ~60,000 mol wt, released from antigen- or mitogen-stimulated lymphocytes promotes the formation of multinucleated giant cells from human monocyte precursors. Autoradiographic studies indicate that the lymphocyte-derived giant cell protein (GCP)[1] induces giant cells by fusion of nonreplicating blood monocytes.

* Supported in part by grants AM16506, AM26034, and AI10085 from the National Institutes of Health; funds from student research grant AM07405-01 from the U. S. Public Health Service; and research funds from the Veterans Administration.

‡ Medical Investigator for the Veterans Administration.

[1] *Abbreviations used in this paper:* GCF, giant cell factor; GCP, giant cell protein; [^{3}H]TdR, tritiated thymidine; LPF, low power fields; MNL, mononuclear leukocytes; PBS, 0.15 M NaCl/0.02 M phosphate, pH 7.4; PHA, phytohemagglutinin; $NaIO_4$, sodium meta periodate; PPD, purified protein derivative of tuberculin; RPMI 1640 + PSH, RPMI 1640 medium containing penicillin (100 U/ml), streptomycin (100 μg/ml), and Hepes buffer (0.001 M), pH 7.4; SBTI, soybean trypsin inhibitor.

Materials and Methods

Giant Cell Assay. Peripheral blood mononuclear leukocytes (MNL) were isolated from venous blood of normal human donors by the Ficoll-Hypaque technique (11). Monocytes were purified from MNL by counter-flow centrifugation performed with a Beckman J-21C centrifuge equipped with the Elutriator Rotor and Sanderson separation chamber (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.). Elutriation was performed by the procedure previously described by Sanderson et al. (12). After elutriation, monocytes were >95% pure as determined by morphology and positive staining for nonspecific esterases (13). Viability was >98% as measured by trypan blue exclusion.

Isolated monocytes were suspended at a density of 10^5 cells/ml in RPMI 1640 medium containing penicillin (100 U/ml), streptomycin (100 μg/ml), and Hepes buffer (RPMI 1640 + PSH), and 30% AB+ human serum (heat inactivated, 56°C for 30 min). Aliquots (200 μl) were dispensed into flat-bottomed wells of microtiter tissue culture plates (Linbro Chemical Co., Hamden, Conn.). Culture supernatants or column fractions being tested for giant cell-forming activity were added in volumes of 50 μl to each well.

After incubation, medium was aspirated from each well and plates were allowed to air dry. The cell layer in each well was stained with Wright's stain (Anderson Laboratories, Inc., Ft. Worth, Tex.), and the number of giant cells (cells containing two or more nuclei) in each well in a total of 10 low power fields (LPF × 200) was determined. Samples were tested in duplicate (column fractions) or quadruplicate. Results were expressed as the mean (duplicate determinations) or the mean ± SEM (quadruplicate determinations).

Leukocyte Cultures. MNL were isolated from venous blood of normal or tuberculin-positive donors by the Ficoll-Hypaque technique and suspended in serum-free RPMI 1640 + PSH. Lymphocytes were purified by filtration of MNL through sterile columns packed with Sephadex G-10 (Pharmacia Fine Chemicals, Pharmacia Inc., Piscataway, N. J.) as previously described (14, 15). The eluted population of cells contained <2% esterase-positive cells and >92% formed spontaneous rosettes with sheep erythrocytes (16).

Aliquots (2 ml) of MNL, purified lymphocytes, or purified monocytes at densities of 4×10^6 cells/ml of serum-free RPMI 1640 + PSH were dispensed into different Falcon 3033 tubes (Falcon Labware, Oxnard, Calif.) and cultured at 37°C in a humidified atmosphere containing 5% CO_2 for up to 72 h. Cultures were stimulated with tetanus toxoid 1:1,000 dilution (Eli Lilly and Co., Indianapolis, Ind.), purified protein derivative (PPD), 20 μg/ml (Ministry of Agriculture, Fisheries and Food, Surrey, England), phytohemagglutinin (PHA), 1 μg/ml (Burroughs Wellcome, Greenville, N. C.), or sodium meta periodate ($NaIO_4$), 10^{-3} M for 30 min at 4°C (Sigma Chemical Co., St. Louis, Mo.). Culture supernatants were harvested at appropriate times after cells were pelleted by centrifugation. Supernatants were stored at −70°C until they were tested for giant cell-forming activity.

Lymphocyte transformation was measured by culturing MNL at a density of 2×10^6 cells/ml RPMI 1640 + PSH containing 10% heat-inactivated AB+ serum with and without PPD or PHA. Four aliquots (200 μl) of each were placed in flat-bottomed wells of microtiter tissue culture plates. Cultures were pulsed at 114 h with 0.5 μCi of tritiated thymidine ([^{3}H] TdR, specific activity 1.9 Ci/mM, Schwarz/Mann, Div. Becton, Dickinson & Co., Orangeburg, N. Y.) and harvested 6 h later. Cells from all microcultures were harvested onto Whatmann 3MM paper filters (Arthur H. Thomas Co., Philadelphia, Pa.) with a multiple sample harvester (Brandel, Rockville, Md.). Filters containing harvested cells were placed in vials containing 5 ml Aquasol (New England Nuclear, Boston, Mass.), and radioactivity was measured in a liquid scintillation counter.

Gel Filtration Studies. A column 2.5 cm Diam and 100 cm in length containing Sephadex G-100 was used to fractionate supernatants from cultures of MNL. The column was run with 0.015 M glycylglycine/0.14 M NaCl at pH 7.2. Culture supernatants were concentrated 100-fold by ultrafiltration in an Amicon chamber equipped with a UM 2 membrane (Amicon Corp., Lexington, Mass.) and cleared of insoluble material by centrifugation before they were applied to the column.

Gel filtration was performed at 4°C. The column effluent was continuously monitored for absorbance at 280 nm with an ISCO model UA5 (Instrumentation Specialties Co., Lincoln, Nebr.). Proteins of known molecular weight were used to calibrate the column, and a drop of

tritiated water (New England Nuclear) was mixed with each sample before its application to the column to serve as an internal marker for column volume.

Physicochemical Studies. Heat stability of Sephadex-purified giant cell-forming factor was assessed after exposure to a temperature of 56°C for 45 min. Serially diluted, heated, and unheated factor were tested at the same time in the giant cell assay.

Sephadex-purified giant cell-forming factor was incubated with trypsin (Sigma Chemical Co.) at 37°C for 4 h. The reaction was stopped by addition of a fivefold excess of soybean trypsin inhibitor (SBTI, Sigma Chemical Co.). As a control, the order of addition of trypsin and SBTI to the factor was reversed. Giant cell-forming activity in all samples was determined in the same assay.

Autoradiography. Autoradiographic studies were performed with [^{3}H]TdR in an effort to determine whether giant cells were forming by fusion of nonreplicating monocytes or by repeated amitotic or mitotic nuclear division without cellular division of cytoplasm. Aliquots (500 μl) of elutriator-purified monocytes (10^5/ml) in RPMI 1640 + PSH containing 30% AB+ serum were dispensed into wells of Falcon 3008 multiwell plates in which sterile glass cover slips had been previously placed. [^{3}H]TdR (5 μCi) having a specific activity of 1.9 Ci/mM and supernatant from culture of PHA-stimulated MNL (100 μl) were added to each well and cultured with monocytes for 7 d. Cover slips containing the monocyte monolayers were removed, washed extensively in 0.9% NaCl solution, and then fixed with four 250-ml changes of 2% glutaraldehyde in 0.05 M phosphate buffer, pH 6.8; one 250-ml wash of distilled water; and one 250-ml wash of 95% ethanol. The cover slips were air-dried and mounted on acid-cleaned slides with Pro-Texx (Lerner Laboratories, Stamford, Conn.).

Autoradiography was performed as previously described by Boren et al. (17). Slides were dipped in melted NTB2 nuclear tract emulsion (Eastman Kodak Co.) at 46°C, drained, cooled, and stored for 6 wk at 4°C in a taped box containing Drierite (W. A. Hammond Drierite Co., Zenia, Ohio). Exposed slides were developed in D-19 (Eastman Kodak Co.) at 15°C for 6 min and fixed with acid-hardened fixer for 10 min at 15°C. Fixer was removed with 20 changes of distilled water, and slides were stained with hematoxylin.

Results

Formation of Multinucleated Giant Cells. MNL from a donor with immunity to tetanus toxoid and PPD elaborated a soluble giant cell factor (GCF) when stimulated with these antigens or with the T cell mitogen, $NaIO_4$ (Fig. 1). The giant cells contained discrete nuclei as illustrated in Fig. 2. In some experiments we have observed as many as 16 nuclei in a single giant cell. The nuclei were unusually arranged in a random

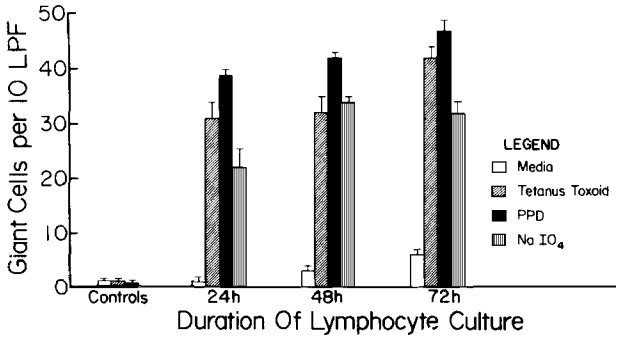

FIG. 1. MNL were obtained from a donor with cell-mediated immunity to tetanus toxoid and PPD (confirmed by positive lymphocyte transformation to these antigens). MNL were cultured 24, 48, and 72 h with and without tetanus toxoid, PPD, and after treatment with $NaIO_4$ (see Materials and Methods). Aliquots (25 μl) of harvested MNL supernatant and mitogen control samples (RPMI 1640 + PSH to which tetanus toxoid or PPD were added) were tested in the same experiment for their ability to induce giant cell formation. LPF, low power fields.

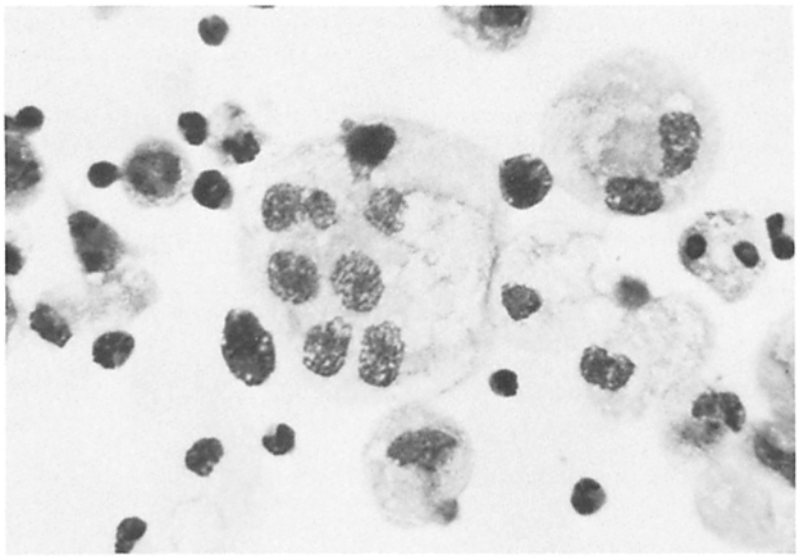

FIG. 2. Photomicrograph (× 1,000) of human blood monocytes grown for 7 d in the presence of a 1:6 dilution of supernatant from culture of MNL stimulated 72 h with PHA. One large multinucleated giant cell is surrounded by single and binucleated monocytes.

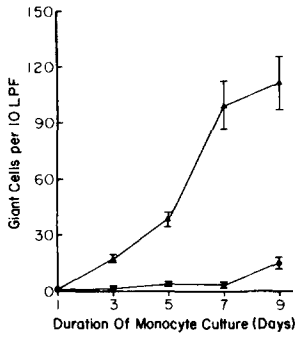

FIG. 3. MNL from a normal donor were cultured for 72 h with and without PHA. PHA was added to supernatant of the unstimulated MNL culture after harvest. Aliquots (25 μl) of supernatants from PHA-stimulated (▲) or PHA-control (●) cultures were added to a series of wells of a microtiter plate containing elutriator-purified monocytes (see Materials and Methods). Culture medium was removed from each of three wells containing PHA-stimulated and PHA-control supernatant 1, 3, 5, 7, and 9 d after initiation of monocyte cultures. The number of giant cells in each well was quantitated by microscopy.

fashion as illustrated in Fig. 2, but on rare occasions they assumed a circular distribution as in Langhans' type giant cells. The effect of time on the generation of giant cells by the factor was studied by exposing monocytes to supernatants from PHA-stimulated or unstimulated cultures of MNL. Supernatant from PHA-stimulated MNL cultures induced formation of significant numbers of giant cells by the third day of monocyte culture (Fig. 3). The number of giant cells formed progressively increased throughout 9 d of monocyte culture (Fig. 3). Only a small number of giant cells was seen in day 9 monocyte cultures treated with supernatants to which PHA was added after harvesting from unstimulated MNL cultures (Fig. 3). This probably

represents spontaneous formation of giant cells; however, generation of the giant cell factor by PHA stimulation of lymphocytes contaminating the elutriator-purified monocytes cannot be excluded.

The effect of duration of monocyte exposure to the GCF on the number of nuclei present per giant cell was determined by microscopic examination of the giant cells

TABLE I

Effect of Duration of Monocyte Exposure to GCF on the Number of Nuclei per Giant Cell

Duration of monocyte culture*	Percent giant cells containing indicated numbers of nuclei								
	Nuclei per giant cell								
	2	3	4	5	6	7	8	9	10
d									
1	100	—	—	—	—	—	—	—	—
3	83	9	6	1	—	1	—	—	—
5	62	17	12	4	5	—	—	—	—
7	46	22	11	11	5	1	1	3	—
9	44	17	12	9	7	6	2	2	1

* Microtiter plates used in the experiment described in the legend to Fig. 3 were used in this study. Giant cells formed in wells containing elutriator-purified monocytes cultured for 1–9 d with aliquots of supernatant from cultures of PHA-stimulated MNL were examined microscopically, and the number of nuclei per giant cell was quantitated. A total of 100 giant cells were examined for each time period except for day-1 cultures. Only 25 giant cells were present in all three wells from day-1 cultures.

TABLE II

GCF Production and [^{3}H]TdR Incorporation by Tuberculin-positive and -negative Subjects

Subjects*	Giant cells/10 LPF (mean + SEM)			[^{3}H]TdR incorporation (mean + SEM)		
	Media	PHA	PPD	Media	PHA	PPD
Tuberculin-positive						
A	5 ± 1	66 ± 4	46 ± 4	52 ± 2	7,467 ± 262	3,459 ± 106
B	5 ± 1	44 ± 5	45 ± 6	217 ± 48	6,724 ± 288	6,232 ± 129
C	4 ± 1	55 ± 9	46 ± 3	59 ± 5	6,190 ± 199	3,539 ± 102
D	4 ± 1	52 ± 6	49 ± 4	73 ± 4	9,100 ± 231	2,726 ± 190
E	7 ± 1	53 ± 6	43 ± 5	125 ± 14	9,160 ± 843	5,523 ± 388
Mean ± SEM	5 ± 1	54 ± 4	45 ± 1	105 ± 30	7,728 ± 607	4,295 ± 670
Tuberculin-negative						
F	7 ± 1	77 ± 3	4 ± 1	74 ± 24	3,648 ± 99	501 ± 90
G	5 ± 1	41 ± 4	7 ± 1	92 ± 6	6,621 ± 126	81 ± 44
H	3 ± 1	72 ± 4	6 ± 1	32 ± 1	7,879 ± 220	380 ± 60
I	5 ± 2	30 ± 4	5 ± 3	442 ± 21	18,873 ± 660	1,390 ± 158
Mean ± SEM	5 ± 1	55 ± 11	6 ± 1	160 ± 94	9,255 ± 3326	588 ± 281

* Peripheral blood MNL were obtained from tuberculin-positive or tuberculin-negative subjects and cultured as described in Materials and Methods in serum-free RPMI 1640 for 72 h with and without PPD (20 μg/ml) or PHA (1 μg/ml). Culture supernatants were tested for their ability to cause formation of giant cells. Media alone and to which PPD or PHA were added induced formation of 6 ± 1, 4 ± 1, and 6 ± 1 giant cells per 10 LPF, respectively. [^{3}H]TdR incorporation was measured as described in Materials and Methods.

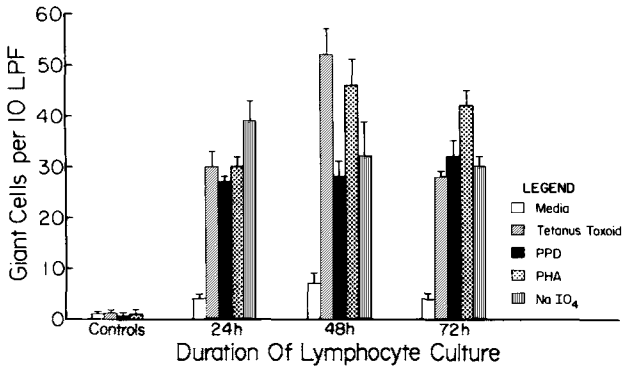

FIG. 4. MNL from the same donor described in Fig. 1 were passed over Sephadex G-10 columns to remove monocytes and other adherent cells (see Materials and Methods). The eluted cells contained <2% esterase-positive mononuclear cells, and 93% formed spontaneous rosettes with sheep erythrocytes. This enriched T lymphocyte population was cultured for 24, 48, and 72 h with and without tetanus toxoid, PPD, and PHA, and a portion was treated with $NaIO_4$ (see Materials and Methods). Aliquots (25 μl) of harvested culture supernatants and control samples (RPMI 1640 + PSH to which tetanus toxoid, PPD, or PHA were added) were tested in the same assay for GCF activity.

TABLE III

*Inability of Monocytes to Produce GCF**

Duration of culture	Giant cells/10 LPF	
h		
	Monocytes + media	Monocytes + PHA
24	3 ± 2	3 ± 1
48	4 ± 2	3 ± 1
72	2 ± 1	4 ± 1
	MNL + media	MNL + PHA
72	6 ± 2	68 ± 7
	Media	Media + PHA
	4 ± 1	3 ± 2

* MNL and monocytes (>95% esterase positive) purified by centrifugal elutriation were cultured in serum-free RPMI 1640 + PSH for up to 72 h with and without PHA (1 μg/ml) as described in Materials and Methods. Harvested supernatants were diluted 50% with serum-free RPMI 1640 + PSH and tested for their ability to induce giant cell formation.

formed in wells containing elutriator-purified monocytes cultured for 1–9 d with aliquots of supernatants from culture of PHA-stimulated MNL (Table I). Giant cells formed by monocytes exposed to the GCF for 24 h contained only two nuclei per cell (Table I). Exposure of monocytes to the GCF for 3–9 d resulted in the formation of giant cells containing from 2 to 10 nuclei (Table I). These data suggest that giant cells containing many nuclei per cell tend to arise in monocyte cultures exposed to the GCF for long periods of time (i.e., 5–9 d).

GCF Production by Tuberculin-positive and Tuberculin-negative Subjects. MNL from tuberculin-positive and tuberculin-negative donors were cultured with PPD to determine whether production of the GCF by antigen-stimulated MNL correlated with

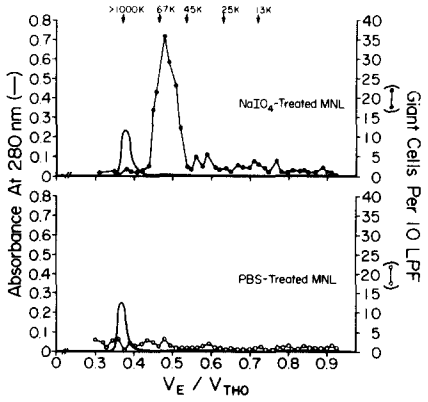

FIG. 5. MNL (8×10^8) from a normal donor were divided into two equal aliquots. One aliquot was treated with $NaIO_4$ and the other with 0.15 M NaCl/0.02 M phosphate (PBS) as described in Materials and Methods. $NaIO_4$-treated and PBS-treated MNL were cultured at a density of 4×10^6 cells/ml of serum-free RPMI 1640 + PSH for 72 h. Harvested supernatant (100 ml) from each culture was concentrated by ultrafiltration to a volume of 3 ml and subjected to gel filtration on the same column of Sephadex G-100 (see Materials and Methods). Aliquots (50 μl) from each column run were tested in the same assay for GCF activity.

TABLE IV
Heat Stability Studies

Condition*	Dilutions	Giant cells/ 10 LPF
GCF untreated	Undiluted	47 ± 5
	1:2	51 ± 2
	1:4	42 ± 4
	1:8	23 ± 2
GCF + 56°C (45 min)	Undiluted	7 ± 1
	1:2	8 ± 1
	1:4	5 ± 1
	1:8	9 ± 1
Buffer		3 ± 1

* GCF purified by gel filtration of supernatant from PHA-stimulated MNL was serially diluted and aliquots of each dilution were heated to 56°C for 45 min. Heated and unheated samples of GCF were tested in the same assay for their ability to induce giant cell formation.

the presence of delayed hypersensitivity to antigen. The degree of cell-mediated immunity in the donor to PPD was assayed by lymphocyte transformation (Table II). PPD-stimulated MNL from tuberculin-positive donors all produced GCF, whereas those from tuberculin-negative donors did not (Table II). Similar amounts of GCF were produced by PHA-stimulated MNL both from tuberculin-positive and from tuberculin-negative donors (Table II). Thus, the production of GCF in response to antigen may be an in vitro correlate of cell-mediated immunity.

Production of GCF by Monocyte-depleted Lymphocytes. It was of interest to determine whether peripheral blood lymphocytes enriched in T cells and depleted of monocytes by passage through Sephadex G-10 columns could produce the GCF when stimulated by antigens or mitogens. Monocyte-depleted lymphocytes from a tuberculin- and tetanus toxoid-immune donor elaborated the GCF when stimulated by these antigens

TABLE V
Trypsin Sensitivity Studies

Condition*	Added after incubation	Giant cells/10 LPF
Buffer + HBSS‡	HBSS	3 ± 1
GCF + HBSS	HBSS	51 ± 2
GCF + trypsin	SBTI	2 ± 1
GCF + SBTI	Trypsin	40 ± 3

* GCF (200 μl) purified by gel filtration of supernatant from PHA-stimulated MNL was incubated at 37°C with trypsin (12.5 μg in 25 μl HBSS) for 4 h, and the reaction was stopped by addition of a 5-times excess of SBTI. As a control, the order of addition of trypsin and SBTI to GCF was reversed.
‡ Hanks' balanced salt solution.

or by PHA or $NaIO_4$ (Fig. 4). In contrast, PHA-stimulated monocytes were not capable of generating the GCF (Table III). These data support the conclusion that GCF is of T cell origin and that it is not a monokine.

Gel Filtration Studies. In an effort to determine the approximate molecular weight of the giant cell factor, supernatants from cultures of $NaIO_4$-treated MNL were pooled and fractionated on a column packed with Sephadex G-100. Column fractions were tested for their ability to induce the formation of giant cells. A single peak of giant cell-forming activity eluted from the column after the excluded proteins (Fig. 5). As determined by gel filtration, the GCF was found to be ~60,000 mol wt. As a control, supernatant from 0.15 M NaCl/0.02 M phosphate, pH 7.4 (PBS)-treated MNL from the same donor was fractionated on the same column of Sephadex G-100, and column fractions were tested for giant cell-forming activity. Gel filtration of the control supernatant did not yield active fractions (Fig. 4). In additional studies, we found that GCF produced by streptokinase-streptodornase- or PHA-stimulated MNL also has a molecular weight of ~60,000 (data not shown).

Physicochemical Characterization of Partially Purified GCF. Physicochemical studies were performed on partially purified giant cell factor isolated by Sephadex G-100 chromatography. It was found to be heat labile, because its biological activity was destroyed after incubation at 56°C for 45 min (Table IV). Its biological activity also was destroyed by proteolytic digestion with trypsin (Table V). Taken together, our data indicate that the GCF is a heat-labile protein of ~60,000 mol wt.

Autoradiographic Studies. Monocytes were cultured for 7 d with supernatants from PHA-stimulated MNL to induce the formation of giant cells. [^{3}H]TdR was added at the beginning to the monocyte cultures to label newly synthesized DNA. Autoradiography was then performed on the monocyte monolayers. After 6 wk exposure, stained monolayers overlayed with emulsion were examined under the light microscope. Microscopic fields (× 1,000) were selected at random until a total of 500 cells were counted. Uptake of [^{3}H]TdR into nuclear DNA was observed in 4 of 377 mononuclear cells. 123 multinucleated giant cells were examined. In 120 giant cells, no detectable [^{3}H]TdR was incorporated into nuclear DNA. In the remaining three giant cells, [^{3}H]TdR was incorporated into only one nucleus of each cell.

These autoradiographic data suggest that lymphocyte-derived GCP promotes the formation of multinucleated giant cells by facilitating the fusion of nonreplicating

monocytes rather than by inducing repeated amitotic or mitotic nuclear division without cellular division of monocyte cytoplasm.

Discussion

A quantitative in vitro assay that measures the formation of multinucleated giant cells from precursor human peripheral blood monocytes is described. By using this assay, we have found that human peripheral blood lymphocytes, when stimulated by T cell mitogens or antigens, produce a soluble factor that causes monocytes to form multinucleated giant cells.

The production by lymphocytes of giant cell factor in the presence of PPD in vitro correlated with delayed hypersensitivity to this antigen in vivo. Monocyte-depleted lymphocytes were shown to elaborate GCF when they were cultured with specific antigen or mitogen, suggesting that there is little or no requirement for monocytes for the production of the GCF. Physicochemical studies indicated that this GCF is a heat-labile protein of ~60,000 mol wt. Our autoradiographic studies suggest that the GCP promotes the formation of giant cells by fusing nonreplicating monocytes.

Earlier studies in the rabbit have demonstrated that alveolar macrophages from normal animals form multinucleated giant cells in vitro when they are exposed to supernatants of lymphocyte cultures rich in lymphokines (9, 10). Our finding that human peripheral blood monocytes under the influence of a soluble lymphocyte-derived factor fuse and form multinucleated giant cells suggests that multinucleated giant cells present in cell-mediated immune granulomas in different body tissues and organs could develop from circulating monocytes. We have previously demonstrated in the guinea pig that at least three lymphokines (lymphocyte mitogenic factor, lymphocyte-derived chemotactic factor for monocytes, and macrophage migration inhibitory factor) are released in vivo at sites of cell-mediated immune reactions (18–20). These studies could provide a basis for postulating that in man, circulating monocytes might accumulate in granulomas at sites of cell-mediated immune reactions by chemotactic migration in response to lymphocyte-derived chemotactic factor for monocytes. Macrophage migration inhibitory factor could act to retain monocytes at the granuloma site. Lymphocyte-derived GCF then could cause accumulated monocytes to fuse and form multinucleated giant cells.

The morphology of the multinucleated giant cells formed in vitro after exposure of blood monocytes to the lymphocyte-derived factor is generally different from that of the classic giant cells of the Langhans' type found in vivo at sites of delayed hypersensitivity reactions. The reason for this discrepancy is not apparent from our study, although Langhans' type giant cells are usually associated with removal of necrotic tissue (4). It is possible that factors released from necrotic tissue or from other sources cause the nuclei of the Langhans' giant cells to assume a peripheral location.

The functions of multinucleated giant cells in granulomas remain to be clarified. Our study demonstrates the feasibility of inducing the formation of multinucleated giant cells in vitro from precursor human blood monocytes. These observations may facilitate future studies of the biological features and functions of human multinucleated giant cells.

Summary

Multinucleated giant cells are associated with granulomas arising from immunological and nonimmunological inflammatory reactions. They are an integral part of

the host immune response to chronic infectious diseases. In the present study we have demonstrated that human lymphocytes when stimulated by specific antigens or T cell mitogens produce a soluble factor that causes peripheral blood monocytes to fuse and form multinucleated giant cells in vitro. Production of the giant cell factor by antigen-stimulated peripheral blood lymphocytes correlates with the existence of cell-mediated immunity to specific antigen. Monocyte-depleted blood lymphocytes, but not purified blood monocytes, produce the giant cell factor when cultured with antigens or T cell mitogens. Gel filtration and physicochemical studies indicate that the lymphocyte-derived giant cell factor is a heat-labile protein of ~60,000 mol wt. These findings suggest that multinucleated giant cells in granulomas may be formed by fusion of circulating monocytes in response to the release of a 60,000-mol wt protein from antigen-stimulated T lymphocytes.

The authors are grateful for the excellent technical assistance of Susan Turner and Elizabeth Schaeffer. Helpful suggestions of Dr. James Roszell were appreciated.

Received for publication 20 July 1981 and in revised form 29 September 1981.

References

1. Rokitansky, C. 1855. Lehrbuch der pathologischem Anatomic. 3rd edition. Braumüller, Vienna. **I:**295.
2. Langhans, T. 1868. Ueber Riesenzellen mit wandständigen Kernen in Tuberkeln und die fibröse form des Tuberkels. *Virchows Arch. Pathol. Anat. Physiol. Klin. Med.* **42:**382.
3. Langhans, T. 1870. Beobachtungen über resoystion der extravasate und Pigment-bildung in denselben. *Virchows Arch. Pathol. Anat. Physiol. Klin. Med.* **49:**66.
4. Turk, J. L. 1980. The Role of Delayed Hypersensitivity in Granuloma Formation. Research Monographs in Immunology. Elsevier North-Holland Biomedical Press, Amsterdam. **1:** 275.
5. Spector, W. G., and N. Heesam. 1969. The production of granulomata by antigen antibody complexes. *J. Pathol.* **98:**31.
6. Turk, J. L., and D. Parker. 1977. Sensitization with Cr, Ni, and Zr salts and allergic type granuloma formation in the guinea pig. *J. Invest. Dermatol.* **68:**341.
7. Turk, J. L., P. Badenoch-Jones, and D. Parker. 1978. Ultrastructural investigations on epithelioid cell granulomas induced by zinconium in the guinea pig. *J. Pathol.* **124:**45.
8. Papadimitriou, J. M., and W. G. Spector. 1971. The origin, properties and fate of epithelioid cells. *J. Pathol.* **105:**187.
9. Galindo, B., J. Lazdins, and R. Castillo. 1974. Fusion of normal rabbit alveolar macrophages induced by supernatant fluids from BCF-sensitized lymph node cells after elicitation by antigen. *Infect. Immun.* **9:**212.
10. Parks, D. E., and S. W. Russell. 1975. The role of phagocytosis and natural lymphokines in the fusion of alveolar macrophages to form Langhan's giant cells. *J. Retianloendothel. Soc.* **17:**219.
11. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **21**(Suppl):77.
12. Sanderson, R. J., F. T. Shepperdson, A. E. Vatter, and D. W. Talmage. 1977. Isolation and enumeration of peripheral blood monocytes. *J. Immunol.* **118:**1409.
13. Yam, L. T., C. Y. Li, and W. H. Crosby. 1971. Cytochemical identification of monocytes and granulocytes. *Am. J. Clin. Pathol.* **55:**283.

14. Ly, I., and R. I. Mishell. 1974. Separation of mouse spleen cells by passage through columns of Sephadex G-10. *J. Immunol. Methods.* **5:**239.
15. Postlethwaite, A. E., and A. H. Kang. 1979. Latent lymphokines: isolation of guinea pig latent lymphocyte-derived chemotactic factor for monocytes. Its activation by trypsin and a soluble factor from macrophages. *J. Immunol.* **123:**561.
16. Jondall, M., G. Holm, and H. Wigzell. 1972. Surface markers on human T and B lymphocytes. I. A large population of lymphocytes forming nonimmune rosettes with sheep red blood cells. *J. Exp. Med.* **136:**207.
17. Boren, H. G., E. C. Wright, and C. C. Harris. 1974. Quantitative light microscopic autoradiography. *Methods Cell Biol.* **8:**277.
18. Postlethwaite, A. E., and R. Snyderman. 1975. Characterization of chemotactic activity produced *in vivo* by a cell-mediated immune reaction in the guinea pig. *J. Immunol.* **114:**274.
19. Postlethwaite, A. E., A. S. Townes, and A. H. Kang. 1976. Characterization of macrophage migration inhibitory factor activity produced *in vivo* during a cell-mediated immune reaction in the guinea pig. *J. Immunol.* **117:**1716.
20. Postlethwaite, A. E. 1980. Isolation and characterization of lymphocyte mitogenic factor released *in vivo* during a cell-mediated immune reaction in the guinea pig. *J. Immunol.* **125:** 1955.