MONONUCLEAR PHAGOCYTES FROM CARRAGEENAN-INDUCED GRANULOMAS
Isolation, Cultivation, and Characterization

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It is now accepted that macrophages play an integral role in chronic inflammatory processes (1, 2). They are always present at sites of chronic inflammation, albeit in widely varying proportions, accompanied by a variety of other cell types. The cellular composition of chronic inflammatory lesions is dependent on the nature of the inciting stimulus. The lesions of many clinically important chronic inflammatory diseases such as rheumatoid arthritis are dominated by lymphocytes (3). Some stimuli, e.g. mycobacteria (4) and the intracellular parasites Listeria monocytogenes (5) and Toxoplasma gondii (5) provoke lesions rich in macrophages but also with prominent lymphoid cell components. Lesions caused by indigestible materials such as asbestos (6) and the cell walls of group A streptococci (7, 8) are dominated by macrophages with minimal contribution by cells of the lymphoid system.

Carrageenan is a long chain polysaccharide containing various salts of sulfate esters and is derived from Irish sea moss, Chondrus crispus (9). After being used by Robertson and Schwartz (10) as a stimulus for chronic inflammation, carrageenan has subsequently been used extensively as a model stimulus of both acute (11, 12) and chronic inflammation (13, 14). Chronic inflammatory lesions caused by carrageenan are dominated by macrophages, which have been shown to persist at these sites for prolonged periods of time (15, 16). Although it is likely that these cells are primarily responsible for the formation and evolution of carrageenan granulomas, very little is known about those biochemical properties which allow them to mediate these changes. Some studies with explants of carrageenan granulomas have demonstrated the secretion of collagenase (17) while short-term suspension cultures of such disrupted tissue have been shown to incorporate amino acids and metabolize glucose (18). Clearly, such cells can be characterized best by examination of their properties utilizing stable populations of the dissociated cells in tissue culture. This paper describes such studies which lead us to conclude that cells from carrageenan granulomas can be dispersed enzymatically and maintained in culture and that they display several of the biochemical and functional characteristics used to identify mononuclear phagocytes (19–22).

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Materials and Methods

**Animals.** Male SW-ICR outbred mice were from Hilltop Lab Animals, Inc., Scottdale, Pa. and CBA/J mice were from The Jackson Laboratory, Bar Harbor, Maine. 15-25-g mice were fed a standard pellet diet and water ad libitum.

**Chemicals.** Sodium carrageenan, Viscarin brand, was a gift from Marine Colloids, Inc., Springfield, N.J. Collagenase, CLS II, 175 U/mg was from Worthington Biochemical Corp., Freehold, N.J. Dulbecco’s modified Eagle medium, RPMI medium, lactalbumin hydrolysate (LAH),1 M199, porcine serum, fetal calf serum, and penicillin/streptomycin were from Grand Island Biological Co., Grand Island, N.Y. The porcine and fetal calf sera were inactivated by heating at 56°C for 30 min. (HIPS and HIFCS). 0.80 μm Latex beads, were from Dow Chemical Co., Indianapolis, Ind. Thymidine, [methyl-3H], 6.7 Ci/mmol was obtained from New England Nuclear, Boston, Mass., p-nitrophenyl-N-acetyl-β-D-glucosaminide was from Sigma Chemical Co., St. Louis, Mo. and phenolphthalein-glucuronide from Calbiochem, San Diego, Calif. Microtest II plates were from Falcon, Oxnard, Calif. and Nunclon tissue culture dishes were from Vanguard International, Inc., Neptune, N.J. Lipopolysaccharide (Escherichia coli 0111:B4, Westphal extracted) and phytohemagglutinin P were from Difco Laboratories, Detroit, Mich.

**Induction of Carrageenan Granulomas.** Carrageenan granulomas were induced by subcutaneous injection of 0.5 ml of carrageenan into the abdominal wall of SW-ICR mice. Carrageenan was used as a viscous solution containing 5 mg/ml in sterile distilled H2O.

**Isolation of Granuloma Cells.** The 7-day old granulomatous nodules were dissected under sterile conditions from the subcutaneous tissue underlying the skin and scraped into sterile Dulbecco’s phosphate-buffered saline (PBS) supplemented with 100 U/ml of penicillin and streptomycin. As outlined in Fig. 1 the tissue was sliced further into small pieces and added to Petri dishes containing 0.2% collagenase in Dulbecco’s modified Eagle medium (DMEM). The dishes were placed on a stirrer inside a 37°C incubator equilibrated with 5% CO2 in air and stirred for 1 h. At this time the supernatant fluid containing released cells was removed and chilled. The digestion step was repeated and the two supernates combined. The cells were pelleted by centrifugation at 2,000 rpm for 10 min at 4°C and washed three times with PBS. In three successive experiments the yield was 4 × 106-5 × 106 cells/granuloma with 75-95% of cells excluding trypan blue. The cells were then resuspended in DMEM + 10% HIPS and added to 35 or 60 mm Nunclon tissue culture dishes or to 15-mm glass cover slips and incubated for 2 h. The nonadherent cells were removed by washing the cell sheet four times with PBS and the adherent cells incubated for 24 h in fresh DMEM containing 10% HIPS. After 24 h in the presence of serum the cells were washed and subsequently maintained in DMEM + 0.2% LAH.

**Cells.** The mouse fibroblast cell line, CCL-1, NCTC clone 929 was obtained from the American Type Culture Collection and maintained in Eagle’s medium containing 10% fetal calf serum. The cultures were split 1 to 10 every week. Mouse peritoneal macrophages were obtained by intraperitoneal lavage from mice injected 4 days previously with thioglycollate broth or from untreated mice and adherent cells maintained in DMEM or M199 containing HIPS. Lymphocytes were prepared from the thymus of normal 8-wk-old male CBA/J mice by teasing into RPMI-1640 media and culturing the cells in microtest plates.

**Enzyme Assays.** Lactate dehydrogenase (LDH) was assayed by determining the rate of oxidation of reduced nicotinamide adenine dinucleotide at 340 nm. N-acetyl-β-D-glucosaminidase (NAG) was assayed by the method of Woolen et al. (23), β-glucosaminidase by the method of Talalay et al. (24), and β-galactosidase by the method of Conchie et al. (25). Enzyme activity for hydrolytic enzymes is expressed as nanomoles of substrate cleaved/hour at 37°C. Lysozyme was assayed by the method of Gordon et al. (20). 5′-Nucleotidase was assayed by the method of Edelson and Cohn (21) and leucine aminopeptidase by the method of Wachsmuth (22). Cathepsin D was assayed by the method of Williams and Lin (25) using methyl[14C]glycinated hemoglobin as the substrate. Acid phosphatase was assayed by the method of Walter and Schutt (27). 1 U of acid phosphatase is defined as the amount of enzyme required to liberate 1 nmol of p-nitrophenol at 25°C. Protein was determined by the method of Lowry et al. (28) by using human serum albumin as standard. DNA was assayed by the method of Setaro and Morley (29).

1 Abbreviations used in this paper: LAH, lactalbumin hydrolysate; HIPS and HIFCS, heat-inactivated porcine serum and heat-inactivated fetal calf serum; NAG, N-acetyl β-D-glucosaminidase; DMEM, Dulbecco’s modified Eagle medium; LPS, lipopolysaccharide; LAF, lymphocyte activating factor; PBS, phosphate-buffered saline; PHA, phytohemagglutinin.
Inject 2.5 mg of Carrageenan 7 days, (lissect granuloma)
Tissue rinsed in PBS containing antibiotics
Tissue digested in DMEM + collagenase

37°C/5% CO₂
in air

1 h incubation

Supernate

Combine and Chill

Add fresh digesting medium to tissue
1 h incubation

Supernate

Centrifuge, Wash cells 3x with PBS

Resuspend in DMEM + 10% serum
and culture for 2 h at 37°C in
5% CO₂ in air

Remove nonadherent cells by washing with PBS
Maintain culture without serum

FIG. 1. Schematic drawing illustrating the procedure for enzymatic dispersion of 7-day subcutaneous carrageenan granulomas.

Phagocytosis. Granuloma cells were maintained on 60-mm dishes for 4 days under standard culture conditions. At this time the cells were washed and fresh DMEM medium containing 1% HIPS was added. Washed latex beads, 320 μg/ml, were added to a duplicate set of plates and cells incubated for 4 h. Control cultures and cells exposed to latex were washed three times in PBS and examined by phase contrast microscopy.

Scanning Electron Microscopy. Cells grown on cover slips were fixed with 3% phosphate-buffered glutaraldehyde. After postfixation in 1% buffered osmium-tetroxide, the cover slips were dehydrated in ethanol, and dried by the critical point method of Anderson (30), by using solvent-substituted liquid CO₂ in a Denton DCP-1 device (Denton Vacuum, Inc., Cherry Hill, N. J.). Approximately 200 Å of gold was deposited by sputtering with a Denton DSM-1 device and the specimens examined with an AMR-900 scanning electron microscope operating at 10 kV.

Transmission Electron Microscopy. Granuloma cells maintained on 60-mm tissue culture dishes were fixed with 3% phosphate-buffered glutaraldehyde and postfixed in 1% OsO₄. After dehydration in ethanol the cell layer was removed by the addition of propylene oxide which dissolves the polystyrene and allows the cell sheet to float off the surface of the dish. The material was rinsed with propylene oxide and embedded in Epon. Approximately 800-Å sections were cut with an LKB ultratome, stained with uranyl-magnesium acetate and lead citrate, and examined on a Zeiss 9S-2 electron microscope.

Histology of Carrageenan Granulomas. Explanted granulomas were fixed in Bouins solution. Two samples from each nodule were embedded in paraffin and 6-μm sections prepared. The sections were stained either with hematoxylin/eosin or Giemsa before examination.

125I-IgG Preparation. Soluble aggregates of human immunoglobulin (IgG) were prepared as described by Fletcher and Lin (31). The aggregates were radiolabeled with 125I by the lactoperoxidase reaction (31) and repurified for binding studies.

Assay for Lymphocyte Activation Factor (LAF). The LAF activity of granuloma cells, macrophage, and fibroblast culture supernates were evaluated by their effects on thymocyte cultures. Both mitogenic and potentiating effects with mitogen were assayed by a modification of the method described by Gery et al. (32). 0.1-ml culture supernates were added with or without PHA, to thymocyte cultures set up in triplicate in Microtest II plates. The cultures contained 1.5 x 10⁶ thymocytes in RPMI-1640 medium, with 5% HIFCS in a final vol of 0.2 ml per well. The cultures
were incubated for 72 h at 37°C in 5% CO₂ atmosphere in air and their response determined by the incorporation of [³H]thymidine (1 μCi/well) during the last 6 h of incubation. The cultures were harvested by a MASH II automatic harvester.

Results

Induction of Carrageenan Granuloma. Preliminary experiments demonstrated that subcutaneous injections of 0.5 ml of an aqueous suspension of carrageenan (5 mg/ml) produced granulomatous lesions within 6–8 days. The great majority of the cells composing the lesion were identified by morphological and histological means as being mature mononuclear phagocytes as described previously by Spector (33). Also found were small numbers of immature macrophages, fibroblasts, and rarely, polymorphonuclear leukocytes.

Characterization of Cultured Granuloma Cells

MORPHOLOGY. The enzymatically-dispersed granuloma cells can be maintained in a variety of cultured media including DMEM, M199, and a modified Waymouth's medium. The presence of serum was found to improve attachment of the cells but after 48 h the presence of serum was no longer critical and cultures could be maintained for up to 3 wk in its absence. There was no increase in cell number over this culture period as shown by microscopic evaluation. The morphology of cultured granuloma cells was very similar to that of mouse peritoneal macrophages as demonstrated by light microscopy. Observation of the cells with a scanning electron microscope revealed that they also had similar surface characteristics to those of peritoneal macrophages. Cells from both sources were well spread on the surface of the glass cover slips, had many cytoplasmic projections and showed ruffled membranes (Fig. 2 A) similar to that seen for stimulated peritoneal macrophages (Fig. 2 B).

Transmission electron micrographs of cultured granuloma cells show cells to have microvilli, compact mitochondria, rough endoplasmic reticulum, considerable amounts of Golgi elements, and many lysosomes (Fig. 2 C). In addition, large vacuoles in the cytoplasm appear to contain the inciting stimulus, carrageenan (Fig. 2 D).

Cellular content of lysosomal acid hydrolases. The enzymatic activity of three lysosomal acid hydrolases present in cultured granuloma cells were compared to that found in unstimulated peritoneal macrophages and in confluent L929 fibroblasts. The retention of the cytoplasmic marker enzyme LDH was used to demonstrate the viability of the cells during 24 h of culture in serum-free media. Table I shows that in cultured granuloma cells three representative lysosomal acid hydrolases are present at high specific activity. The specific activity of these enzymes found in granuloma cells is comparable with that found in peritoneal macrophages and four to eight times higher than that found in fibroblasts. In addition to lysosomal enzymes capable of hydrolyzing carbohydrate substrates two additional lysosomal hydrolases were found in the granuloma cells at high specific activity. After 48 h of culture the specific activity of cathepsin D and acid phosphatase was similar to that found in thioglycollate-stimulated macrophage and higher than that found in unstimulated macrophages (Table II).

Lysozyme secretion. Cultured granuloma cells were found to secrete lysozyme continuously and at a constant rate for at least 72 h (Fig. 3) while the
Fig. 2. Scanning electron micrographs of thioglycollate-stimulated macrophages and granuloma cells cultured for 24 h (A and B). Transmission electron micrographs of cultured granuloma cells after 3 days of culture (C and D).
Table I

Cellular Content of Lysosomal Acid Hydrolases in Granuloma Cells, Unstimulated Macrophages and L929 Fibroblasts

| Cultured cells | LDH mU/mg cell protein | β-Glucuronidase U/mg cell protein | β-Galactosidase U/mg cell protein | NAG μg/mg cell protein |
|----------------|------------------------|---------------------------------|---------------------------------|-----------------------|
|                | Cell | Media | Cell | Media | Cell | Media | Cell | Media |
| Granuloma      | 595 ± 35 | <10 | 123 ± 12 | 81 ± 4.4 | 73.8 ± 1.0 | 41.0 ± 3.4 | 33.6 ± 1.4 | 6.8 ± 0.9 |
| Macrophage     | 530 ± 87 | <10 | 112 ± 2.5 | 32.3 ± 5.8 | 93.9 ± 10.3 | 30.5 ± 3.9 | 21.7 ± 0.4 | 1.9 ± 0.1 |
| Fibroblast     | 390 ± 30 | <10 | 24 ± 1 | 0.6 ± 0.4 | 9.9 ± 0.7 | 0.6 ± 0.0 | 9.9 ± 0.7 | 0.6 ± 0.0 |

The cells were maintained for 24 h in M199 without serum. Media were collected and the cells lysed with 0.1% Triton X-100 in saline.

Table II

Content of Acid Phosphatase and Acid Proteinase in Cultured Granuloma and Peritoneal Macrophages

| Cells                  | Time in culture | Acid protease | Acid phosphatase |
|------------------------|-----------------|---------------|------------------|
|                        | h   | µg | Hb hydrolyzed/min/mg protein | mU/mg protein |
| Unstimulated peritoneal macrophage | 48  | 14.9 ± 4.5 | 4.2 ± 1.3 |
| Stimulated peritoneal macrophage | 48  | 36.6 ± 2.7 | 12.7 ± 3.9 |
| Granuloma cells      | 24  | 11.6 ± 5.4 | 12.6 ± 3.7 |
|                        | 48  | 20.0 ± 5.5 | 6.97 ± 1.7 |
|                        | 72  | 24.3 ± 6.7 | -               |

Peritoneal macrophages were isolated from unstimulated or thioglycollate-stimulated mice and cultured in an identical way as the granuloma cells. The data are the average ± standard deviation, n = 3. The values for the granuloma cells after 48 h of culture are the average of two separate experiments.

Lysozyme activity found in the cells remained at a relatively constant low level. The cells remained viable throughout the 72 h incubation as judged by retention of the cytoplasmic marker enzyme LDH. The amount of lysozyme secreted by granuloma cells did not differ greatly from that released by macrophages. Granuloma cells secreted 0.8 µg lysozyme per µg of DNA in 48 h compared to 1.3 µg lysozyme per µg DNA in 48 h for peritoneal macrophages.

Phagocytosis. Macrophages phagocytose latex particles both in vivo and in vitro. We therefore compared the phagocytic ability of cultured granuloma cells with that of peritoneal macrophages and of fibroblasts. As expected, at least 90% of the peritoneal macrophages phagocytosed large numbers of latex beads during a 4-h incubation period in serum-free medium as determined by phase contrast microscopy. On the other hand L929 fibroblasts showed no detectable phagocytic activity. We found that nearly 90% of the granuloma cells also ingested many latex particles per cell. The granuloma cells remained viable in culture for an additional 72 h after phagocytosis of the latex.

Resistance to trypsin detachment. L929 fibroblasts can be removed completely from the culture dish by brief treatment with trypsin-EDTA in M199 medium at 37°C. On the other hand, granuloma cells were not detached by the trypsin-EDTA treatment prolonged for 24 h. When the trypsin-EDTA-contain-
ing medium was removed from the granuloma cells after 24 h the granuloma cells were shown subsequently to be capable of phagocytosing latex particles.

**Binding of soluble aggregates of IgG.** Peritoneal macrophages and granuloma cells maintained in culture in glass cover slips under identical conditions bound \(^{125}\text{I}-\text{labeled soluble aggregates of IgG (Fig. 4). This uptake appeared to be specific with respect to cell type since the L929 mouse fibroblasts did not bind aggregated IgG (Fig. 5). In addition, the binding of }^{125}\text{I-IgG aggregates to granuloma cells was inhibited 70% by the addition of a twofold excess of unlabeled aggregated IgG indicating specificity of binding.}

This cell-associated radioactivity could be due to binding to the Fc receptor, or to subsequent phagocytosis of the aggregates, or both. One way to distinguish these two processes is to measure the uptake of the labeled aggregates at low temperature, conditions which have been shown to inhibit phagocytosis. Glass
FIG. 4. Binding of soluble \textsuperscript{125}I-IgG aggregates by granuloma cells and macrophages. Granuloma cells and unstimulated peritoneal macrophages were cultured on 15-mm glass cover slips in M199 + 1% HIPS for 2 days. The cells were washed twice with PBS and fresh medium devoid of serum but containing various levels of \textsuperscript{125}I-IgG aggregates was added. The cells were incubated at 37°C in a 5% CO\textsubscript{2} incubator for 20 min. The cover slips were removed and washed four times, blotted, and counted in a Beckman Gamma Counter. The data represents the average ± SD of three cover slips.

cover slips bearing granuloma cells were transferred to fresh media containing 50 mM Hepes buffer pH 7.4 and the dishes cooled at 0-2°C on ice. Under these conditions the granuloma cells were still capable of binding \textsuperscript{125}I-IgG aggregates and the binding could be completely inhibited by a 20-fold excess of unlabeled IgG (Table III).

Release of a Factor from Granuloma Cells which Stimulates Thymocytes. Macrophages have been shown to secrete factors which modulate the response of both T lymphocytes (32) and B lymphocytes (34) to mitogenic stimuli. The question as to whether cells isolated from a chronic inflammatory lesion secrete such factors is relevant to an understanding of cellular interactions at sites of inflammation. Therefore, adherent cells from peritoneal exudates and from carrageenan-granulomas were cultured for 24 h in RPMI-medium containing 5% fetal calf serum with or without LPS. The medium was collected and assayed for its ability to stimulate thymidine incorporation by cultured thymocytes. The results of a representative experiment are presented in Table IV. The fibroblasts were employed here for controls.
FIG. 5. Comparison of the binding of $^{125}$I-IgG aggregates by fibroblasts, macrophages, and granuloma cells. The three cell types were cultured on glass cover slips as described in the legend to Fig. 4. 60~µg of the $^{125}$I-IgG aggregates were added to cultures containing cover slips without cells and to cover slips containing $0.3 \times 10^6$ cells and incubated for 20 min. The data represents the average ± SD of three coverslips.

|           | $^{125}$I-IgG | cpm-Blank |
|-----------|--------------|-----------|
| A. 37°C   | 75 µg        | 390 (380–400) |
|           | 75 µg + 300 µg cold IgG | 51 (40–62) |
| B. 0°C    | 150 µg       | 810 (740–880) |
|           | 150 µg + 3 mg cold IgG | 0 |

Cells were cultured on 60-mm Nunclon dishes for 3 days. At this time 50 mM Hepes buffer was added to the culture media and the cultures split. One half was incubated at 37°C and the other cooled to 0–2°C by setting the dishes on ice. $^{125}$I-IgG aggregates were added to the medium and the cells incubated for 20 min at 37°C and 60 min for cells at 0°C. Blanks are dishes without cells incubated with the same labeled media and are subtracted from the experimental points. The results are expressed as cpm associated with the cells and are the average of duplicate determinations. The numbers in brackets are the individual values.

Supernates from unstimulated cultures of the three cell types tested had similar effects on the thymocyte cultures, i.e., increasing the incorporation of thymidine, as compared to the values obtained with the control medium. Peritoneal macrophages and granuloma cells differed, however, from the fibroblasts, when stimulated with LPS. Supernates from the LPS-stimulated fibroblasts resembled the control medium (+ LPS), while the LPS-stimulated macrophages and granuloma cells released mitogenic activity into the medium.
TABLE IV
Secretion of LAF by Cultured Granuloma Cells

| Cells     | LPS  | -PHA cpm ± SD | +PHA cpm ± SD |
|-----------|------|--------------|---------------|
| Fibroblast L929 | -    | 473 ± 136    | 2,182 ± 184   |
|           | +    | 613 ± 155    | 11,808 ± 705  |
| Macrophage | -    | 454 ± 90     | 2,603 ± 236   |
|           | +    | 1,327 ± 633  | 45,158 ± 504  |
| Granuloma | -    | 295 ± 25     | 2,688 ± 270   |
|           | +    | 2,776 ± 1,226| 51,581 ± 3,752|
| None      | -    | 183 ± 38     | 1,137 ± 72    |
|           | +    | 902 ± 96     | 8,002 ± 1,061 |

Cells were cultured in RPMI-1640 media supplemented with 5% HIFCS with or without 15 μg/ml of LPS. Macrophage cultures contained 4 x 10⁶ cells and the granuloma culture, 2 x 10⁶ cells. The fibroblasts cultures were confluent. Control media contained no cells. The media were collected after 24 h and assayed for LAF as described in Materials and Methods. The results are expressed as cpm/well ± SD, n = 3.

TABLE V
Enzyme Activities Characteristic of Stimulated and Unstimulated Macrophages

| Macrophages        | Enzyme specific activity | 5'-Nucleotidase | Aminopeptidase |
|--------------------|--------------------------|-----------------|---------------|
| Unstimulated       | 13.28 ± 1.33             | 9.41 ± 4.50     |
| Thioglycollate     | 0.21 ± 0.15              | 41.67 ± 1.61    |
| Granuloma          | 0.31 ± 0.12              | 36.67 ± 2.81    |

Cells were cultured for 24 h in serum containing medium, washed twice with PBS, and fresh M199 devoid of serum was added. After 24 h of incubation the media were removed, the cells washed three times with PBS, and harvested in triton-saline. The enzymes were assayed as described in Materials and Methods. The results are the average ± SD, n = 3.

which potentiated the thymocyte response to PHA by up to six times over the controls.

Cultured Granuloma Cells Behave as Stimulated Macrophages. The data presented above strongly indicate that cultures of dispersed granuloma cells are comprised mainly of macrophages. Since these mononuclear cells present in the lesion are in direct contact with and have ingested the inciting stimulus carrageenan (Fig. 2D) it is likely that the cells are highly stimulated. Two recent publications (21, 22) describe changes in plasma membrane enzyme activities which are characteristic of stimulated and unstimulated macrophages. Specifically, 5'-nucleotidase is decreased in stimulated macrophages (21) whereas leucine aminopeptidase is increased (22). We therefore compared the activities of these enzymes in unstimulated and thioglycollate-stimulated peritoneal macrophages with that found in granuloma cells. The results shown in Table V demonstrate that the granuloma cell membrane enzyme activities
closely resemble that found in thioglycollate-stimulated cells. Thus, based on these criteria the mononuclear phagocytes isolated from granulomas are stimulated cells.

Discussion

Granulomas induced by carrageenan have been shown to contain macrophages which persist at the sites of inflammation for prolonged periods (15). There has been extensive study on the pathology (15, 16, 35, 36) and biology (36, 37) of granuloma induction, progression and resolution but there have been only a few investigations of the biochemical and functional characteristics of the cells composing such lesions. Cells dispersed from carrageenan granulomas would be expected to display functional and biochemical characteristics of mononuclear phagocytes. In this study we have characterized the inflammatory cells found at the sites of inflammation caused by the injection of carrageenan, from a biochemical, morphological, and functional point of view. This has been made possible by utilizing the techniques of enzymatic dispersion which has found wide use for dispersing such tissues as liver (38, 39), synovial tissue (40, 41), and various neoplastic tissues (42, 43). We have shown in this study that cells obtained by enzymatic dispersion of carrageenan granulomas do indeed exhibit several properties of mononuclear phagocytes. Cells dispersed from such granulomas show comparable specific activities of three representative lysosomal acid hydrolases to that seen in mouse peritoneal macrophages. That the secretion of lysozyme is a constitutive function of macrophages obtained from a variety of sources was clearly established by the study of Gordon et al. (20). The ability of the granuloma cells to secrete lysozyme is therefore a strong indication that the cultures contain mononuclear phagocytes. The phagocytic activity of granuloma cells was demonstrated by their ability to ingest latex beads. This activity permitted a quantitative estimate of the percentage of cells in the culture that were phagocytic. In several experiments we found that more than 90% of the cells were capable of ingesting latex and that mouse fibroblast cells were not phagocytic. These findings are in accordance with light microscope as well as scanning and electron microscopic studies which showed that the fibroblast content of such cultures was minimal. Macrophages are resistant to detachment from the surfaces upon which they are cultured; both proteolytic enzymes and chelating agents which remove cells such as fibroblasts do not detach macrophages from culture surfaces (44). Like peritoneal macrophages, the granuloma cells are resistant to detachment by a combination of trypsin and EDTA. The presence of Fc receptors on mononuclear phagocytes is well established (45, 46). These receptors can be detected by the formation of rosettes with antibody-coated erythrocytes or alternatively by the binding of $^{125I}$-labeled aggregated immunoglobulins (46). The specific binding of soluble aggregates of IgG by granuloma cells suggests the presence of a Fc receptor on these cells. In addition, the demonstration of specific binding under the same conditions to peritoneal macrophages but not to fibroblasts is in support of the granuloma cultures being composed of macrophage-like cells. Detection of carrageenan in the cultured granuloma cell vacuoles (Fig. 2) is consistent with the findings of Catanzaro et al. (47). These workers demonstrated that cultured guinea pig
macrophages endocytose carrageenan and that it was resistant to total degradation due to its unique structure.

Cultured granuloma cells were found to secrete an elastinolytic activity similar to that found from thioglycollate-stimulated macrophages but not from unstimulated macrophages. Thus, granuloma cells maintained in serum-free medium secreted an activity which was capable of hydrolyzing tritium-labeled insoluble elastin (28.4 μg elastin hydrolyzed/18 h/ml culture media). Mouse fibroblasts were found not to secrete this activity. This finding is in agreement with that found by Werb and Gordon (48).

Therefore, the demonstration of a series of characteristic features of granuloma cells described above which are in common with criteria used to define mononuclear phagocytes (19-22) provides strong evidence of their common identity. Furthermore, data on plasma membrane enzyme activities in comparison with unstimulated and thioglycollate-stimulated mouse peritoneal macrophages (Table V) indicate that the granuloma mononuclear phagocytes are stimulated cells.

Although carrageenan granulomas contain small numbers of lymphocytes most other types of granulomas contain large numbers of these cells. It was therefore relevant to examine the effect of mononuclear phagocytes from carrageenan granulomas on lymphoid cell function. It is clear that like other mononuclear phagocytes the granuloma cells stimulated with LPS release soluble factors which enhance the response of thymocytes to a mitogenic stimulus. Such responsiveness gives further indication of the importance of the interaction of mononuclear phagocytes and lymphoid cells at sites of immune-based chronic inflammation.

Studies are in progress on other functions of mononuclear phagocytes isolated from carrageenan granulomas to define further the role that they play in the evolution of granulomatous inflammatory lesions.

Summary

Stable cultures of mononuclear phagocytes from carrageenan-induced granulomas in mice have been established after enzymatic dispersion of these lesions. The cells can be maintained for up to 3 wk without division in serum-free media. The mononuclear phagocytes were identified by several criteria. The cells are adherent, phagocytic, contain lysosomal acid hydrolases at high specific activities, secrete lysozyme, and bind soluble aggregates of IgG. The activities of 5'-nucleotidase and leucine aminopeptidase in the cultured granuloma cells showed that they resembled macrophages from thioglycollate-stimulated mice but not unstimulated macrophages in these respects. Supernates from the cultured granuloma cells contain factor(s) which induce the proliferation of thymocytes; the release of such factors by the cells is stimulated by lipopolysaccharide.

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