SYNTHESIS OF THE SECOND COMPONENT OF COMPLEMENT BY LONG-TERM PRIMARY CULTURES OF HUMAN MONOCYTES*

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Macrophages secrete a wide variety of biologically active products. Some of these, such as lysozyme (1) and certain degradative lysosomal enzymes (2) are well defined and have been purified and characterized physicochemically. Others, including additional lysosomal enzymes (2), collagenase (3), plasminogen activator (4), and elastase (5) have been largely defined in terms of their substrate specificity. A third group of macrophage secretory products has been defined on the basis of biological activity and includes colony stimulating factor (6), endogenous pyrogen (7), a cytolytic factor (8), a lymphocyte mitogen (9), and an inhibitor of cell division (10). Cultures of macrophages have permitted in vitro studies of the control of rates of synthesis (11) and release (12) of these secretory products and have contributed to an understanding of membrane structure (13) and function (14). In vitro studies have indicated that macrophages may help to initiate the antigenic stimulation of lymphocytes (15) and that they modulate lymphocyte secretion of such products as interferon (16) and osteoclast-activating factor (17). Macrophage cultures have also been useful in studies of lymphokine function (18) and host defenses against bacteria (19), mycoplasma (20), viruses (16), and tumors (21).

A stem cell in bone marrow presumably gives rise to blood monocytes which circulate for 24–48 h and then become fixed in tissues as elements of the reticuloendothelial system (22). Blood monocytes may therefore represent a convenient source of cells for the assessment of macrophage function. In previous studies of monocytes, either large volumes of blood were required (16, 23, 24) or the cells could be maintained in vitro no longer than 17 days (1, 25). The present study was made possible by development of techniques for preparation of confluent monolayers of pure monocytes which could be maintained in culture for up to 16 wk.

Initial studies of the biosynthesis of the second component of complement (C2) indicated that hemolytically active C2 was detectable in media harvested from short-term cultures of guinea pig spleen, lung, lymph node, bone marrow, peritoneal exudate (PE),[1] and in some cases liver (26, 27). C2 production was temperature-dependent and was reversibly inhibited by puromycin or cycloheximide; guinea pig spleen was capable of incorporating radiolabeled amino acids into immunochemically and functionally identifiable C2 (27). In subsequent studies with the use of a modification of the Jerne plaque

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[1] *Abbreviations used in this paper:* HAS, heat-inactivated autologous serum; HFCS, heat-inactivated fetal calf serum; PBS, phosphate-buffered saline; PE, peritoneal exudate.

technique, designed to detect guinea pig C2 (28), and by albumin density-gradient separation of human fetal cells (29) it was shown that C2 was produced by mononuclear cells from PE and liver, respectively. C2 production appeared to be restricted to the adherent cell population in guinea pig PE (30).

In the present study, pure cultures of human monocytes synthesized and secreted C2 for up to 16 wk in vitro. In addition, these cells were capable of synthesizing lysozyme, phagocytosing large latex beads, killing *Listeria monocytogenes*, and forming rosettes with IgG or C3-coated erythrocytes.

Materials and Methods

Preparation of Monocyte Monolayers. Circular glass cover slips (15-mm diameter, no. 2, SGA Scientific, Inc., Bloomfield, N. J.) were thoroughly washed by dropping each of approximately 80 slips into a solution of about 9 g of soap (Ivory Snow, Procter and Gamble, Cincinnati, Ohio) in 600 ml of deionized water. To facilitate uniform washing, allundum boiling chips (Fisher Scientific Co., Pittsburgh, Pa.) were placed in the water bath and the mixture was boiled for 4 h. The slips were then rinsed 20 times with deionized water and boiled for an additional 4 h in deionized water. After 10 additional rinses in water they were exposed to 1% Siliclad (Clay Adams, Parsippany, N. J.) in deionized water for 15 min and rinsed again in deionized water five times. Slips were individually dried by placing them on Whatman filter paper and then autoclaved. This washing procedure was essential for preparation of monocyte cultures with a high degree of plating efficiency.

Venous blood was drawn into a syringe containing 0.2 ml 6% dextran 75 (Travenol Laboratories, Morton Grove, Ill.) in sterile saline with 0.06 ml 0.1 M Na EDTA per ml of blood. The blood was allowed to sediment in the syringe for 3 h after which the leukocyte-rich plasma was removed. The leukocytes were washed twice and then resuspended in Medium 199 (Microbiological Associates, Bethesda, Md.). Between washes the cells were collected by low speed centrifugation (190 g_{max}, 15 min 4°C). The washed cell suspensions were incubated at 4°C for 16 h to permit loss of a substantial portion of polymorphonuclear leukocytes capable of adhering to glass. The leukocytes were then vigorously resuspended by pipetting, to avoid cell clumping, washed again in M199, and a portion resuspended at a concentration of approximately 3×10^6 mononuclear cells/ml in M199 supplemented with 10% heat-inactivated (56°C, 2 h) autologous serum (M199 HAS). 0.2-ml portions were immediately placed on individual cover slips resting on sterile rubber stoppers (plugs from 3-ml. Vacutainer tubes, Becton-Dickinson & Co., Rutherford, N. J.) in plastic petri dishes (Falcon Plastics, Oxnard, Calif.). After 2 h incubation at 37°C in humidified 5% CO_2:95% air atmosphere the slips were rinsed twice by vigorous agitation in each of two beakers containing M199. To obtain confluent monolayers this procedure was repeated using portions of the suspension that were stored at 4°C (in the absence of serum). A third application of 0.2 ml of the cell suspension was performed with the slips in individual wells of a Multi-Dish Disposo-Tray (FB-16-24-TC, Linbro Chemical Co., New Haven, Conn.) to permit settling of monocytes at the periphery of the cover slips. After 30 min at 37°C 0.3 ml of M199 HAS was added to each well and the cells reincubated for 90 min. Monolayers were rinsed again in M199 then incubated for 1 day to 2 wk at 37°C in wells containing fresh M199 HAS (0.5 ml). After rinsing in M199, monolayers were transferred to wells containing 0.5 ml of M199 supplemented with 10% heat-inactivated (56°C, 2 h) fetal calf serum (M199 HFCS) at regular intervals for use in each experiment. Cell viability was assessed with trypan blue (Microbiological Associates).

Fluorometric Assay of DNA. The number of monocytes on a cover slip was determined using a modification of a previously described fluorometric assay for DNA (31). Cover slips were rinsed in isotonic saline, then resubmerged in 0.5 ml of 2% sodium dodecyl sulfate (Matheson, Coleman and Bell, Norwood, Ohio) in deionized water. The slips were incubated at 37°C for 1 h, after which the cell lysate solutions were mixed by repeated pipetting. Aliquots (0.46 ml) of the lysate were stored at −20°C in conical Eppendorf Micro Test Tubes (Brinkman Instruments, Westbury, N. Y.). 20 μl of a 1 mg/ml solution of polyriboguanadylic acid (Collaborative Research, Inc., Waltham, Mass.) in 100 mM Tris, 10 mM EDTA, pH 7.4, was added to each tube. The tubes were gently vortexed and 900 μl of ice-cold absolute ethanol was then added to each sample. The tubes were capped,

vortexed, and stored for at least 18 h at −20°C. Each sample was washed four times in absolute ethanol at 0°C with high speed centrifugation (12,000 g_{max}, 30 min) between washes. The supernatant was removed and the pellet dried at 100°C. 25 μl of deionized water was added to each sample and the tubes vortexed. After 30 min at room temperature, 25 μl of 3,5-diaminobenzoic acid dihydrochloride[2] (DABA) (0.26 g/ml DABA in deionized water, Gold Label, Aldrich Chemical Co., Milwaukee, Wisc.) was added to each tube. The tubes were capped, vortexed, then incubated at 60°C for 30 min. ½ ml of 1.0 N HCl was added to each tube and the relative fluorescence determined in a Perkin-Elmer (MPF-3) fluorescence spectrophotometer (excitation 380–420 nm, emission 500 nm). A standard curve was constructed using the same procedure with known amounts of calf thymus DNA (Sigma Chemical Co., St. Louis, Mo.).

Rosettes. Procedures for rosette formation with IgG-coated human erythrocytes (33), with sheep erythrocytes (34), and with C3-coated sheep erythrocytes (35) were performed as previously described.

Immunofluorescence. Monolayers were rinsed in phosphate-buffered saline (PBS), incubated 1 h at 4°C with fluorescein-conjugated rabbit antihuman immunoglobulin antibody, rinsed in PBS, and examined by fluorescence microscopy. For detection of intracellular lysozyme, cells were fixed for 1 min in ice-cold methanol, dried, and incubated (45 min, room temperature) with rabbit antihuman lysozyme antiserum, rinsed in PBS, reincubated (45 min, room temperature) with a mixture of fluorescein-conjugated goat antirabbit antibody and rhodamine-conjugated bovine serum (Difco Laboratories, Detroit, Mich.), rinsed in PBS, and viewed by fluorescence microscopy. Controls consisted of slips treated in an identical manner except that normal rabbit serum was substituted for the rabbit antilysozyme antiserum.

Phagocytosis. 5.7 μm diameter styrene divinylbenzene beads (Dow Diagnostics, Indianapolis, Ind.) were autoclaved, diluted in M199, and stored at 4°C. A suspension containing approximately 20 beads per monocyte was added to the monolayers, and after incubation at 37°C, the slips were rinsed at timed intervals and examined by phase-contrast microscopy for particle uptake.

Measurement of C2 Activity. The source and preparation of reagents for hemolytic titration of C2 were given in reference (36) and the assay performed as described in reference 37. To detect synthesis and secretion of C2 by monocyte cultures 0.05-ml aliquots of media were harvested at timed intervals and stored at −90°C. Samples were thawed and then diluted in isotonic Veronal-buffered saline sucrose (μ = 0.065, pH 7.35) for the hemolytic assay. Other complement components were assayed as described in reference 36.

Inhibition of Protein Synthesis. Cycloheximide was purchased from Nutritional Biochemical Corp. (Cleveland, Ohio), dissolved in M199 immediately before use, filtered through a 0.22 μm Millipore filter (Millipore Corp., Bedford, Mass.), and diluted in M199 HFCS.

Incorporation of Radiolabeled Amino Acids into C2 Protein. Incorporation of radiolabeled amino acids into C2 protein was estimated by a procedure outlined elsewhere (29) using ^{14}C-labeled lysine, leucine, isoleucine, and valine (New England Nuclear, Boston, Mass.) at a final concentration of 10 μCi/ml.

Measurement of Lysozyme Activity. Lysozyme activity was measured with the Lyso-Plate assay as previously described (38). *Micrococcus lysodeikticus* was purchased from Sigma Chemical Company. Absolute lysozyme concentration was estimated by comparison with a standard solution of human lysozyme obtained by Dr. David S. Rosenthal from the urine of a patient with monocytic leukemia.

Bacteriolysis of L. Monocytogenes. A previously described technique for the detection of killing of ^{3}H-labeled *Listeria monocytogenes* (19) was used.

Electron Microscopy and Peroxidase Cytochemistry. Cell monolayers were rinsed in normal saline and fixed in 2% purified glutaraldehyde (Polysciences, Inc., Warrington, Pa.) in cacodylate buffer (0.1 M, pH 7.4) containing 0.1 M sucrose for 15 min at room temperature. For endogenous peroxidase, the monolayers were next incubated with 1.4 mM 3,3′-diaminobenzidine tetrahydrochloride (Sigma Chemical Co.) in Tris-HCl buffer (0.05 M, pH 7.1) and 0.01% H_2O_2 for 45 min at room temperature and then rinsed three times in distilled water (39). All monolayers were postfixed in 1.3% OsO_4 in S-collidine (pH 7.4) for 1 h at 4°C (40), stained in situ with 1.5% uranyl acetate in maleate buffer (0.05 M, pH 6.2), dehydrated in graded ethanol, and embedded in Epon (Shell Chemical Co., New York). After polymerization, the disk of polymerized Epon containing

[2] Recrystallized (32) and stored at −20°C until immediately before use.

the cell monolayer was separated from the culture support surface and cut into 4 mm^2 squares and glued to blank Epon blocks. Thin sections were cut parallel to the plane of the cell monolayer using a diamond knife (E.I. duPont de Nemours & Co., Wilmington, Del.) on an LKB Ultrotome III (LKB Instruments, Inc., Rockville, Md.) and picked up on carbon-coated grids. Cells examined for endogenous peroxidase were left unstained, while those viewed by routine electron microscopy were counter-stained with lead citrate (41). Sections were examined in a Phillips 300 electron microscope.

Results

Morphology

After 2 days in culture, monolayers consisted of round, slightly flattened mononuclear cells, less than 10 μm in diameter. All of the cells contained peroxidase positive granules (Fig. 1 a) and fluoresced when treated with rabbit antihuman lysozyme antibody followed by fluorescein-conjugated goat antirabbit immunoglobulin. After 2 wk in culture, the cells had spread to cover the entire cover slip (Fig. 1 b) and measured up to 80 μm in diameter. None of the cells contained peroxidase positive granules at this time. There was heterogeneity in cell shape, and multinucleated giant cells were frequently observed. After 4 wk in culture, the cytoplasm of each monocyte was densely packed with lipid droplets, approximately 0.1–0.8 μm in diameter (Fig. 1 c). Mitotic forms were never observed in these monolayers throughout 16 wk in vitro. On the average, slips contained a mean of 1.1, 0.5, and 0.3 μg of DNA after 2, 4, and 8 wk in culture, respectively. 1.0 μg of DNA corresponds to approximately 1.0–1.5 $\times$ 10^5 monocytes. Based on the DNA content it was calculated that, at maximum density, each slip contained approximately 1–3 $\times$ 10^5 monocytes. Since between 15 and 25 confluent cover slips could be obtained from 30 ml of normal human blood, the efficiency of monocyte collection was approximately 50%.

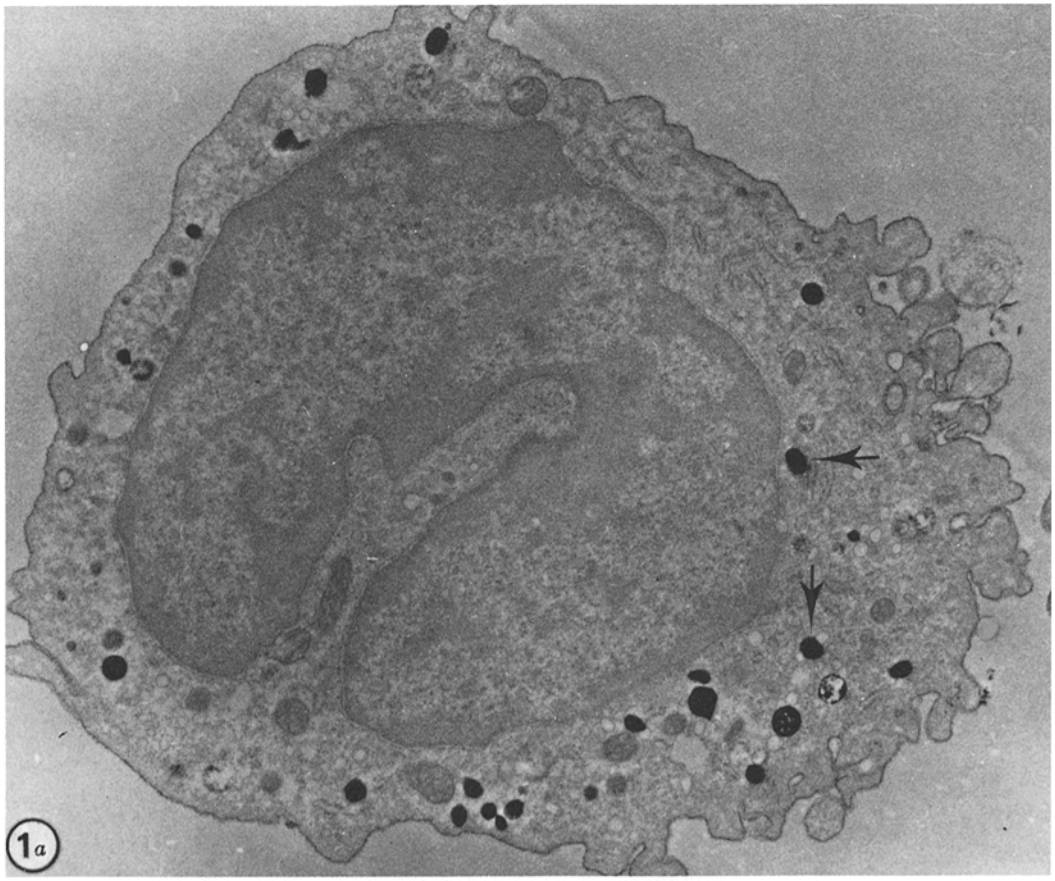
Cell Surface Properties

ROSETTE FORMATION. After 8 wk in culture, separate monolayers (approximately 10^4 cells/slip) were incubated for 30 min at room temperature in PBS (pH 7.4) containing approximately 10^7 sheep erythrocytes, erythrocytes sensitized with IgM antiForssman antibody and the third component of human complement (EC3), or with human 0^+ erythrocytes sensitized with IgG anti-D antibody. The monolayers were then rinsed and each slip scanned for rosette formation. All the cells in the monolayer preparations rosetted with EC3 and with IgG-coated erythrocytes, but none formed rosettes with sheep erythrocytes.

SURFACE STAINING. Monolayers (2 wk in culture, approximately 10^5 cells/slip) were incubated with fluorescein-conjugated rabbit antihuman immunoglobulin and examined by fluorescence microscopy. None of the cells stained for surface immunoglobulins.

Phagocytosis

Monolayers in culture 2 days–10 wk were rinsed in M199 then incubated with approximately 20 5.7 μm diameter latex beads per cell in M199 HFCS for 24 hr at 37°C, the cover slips rinsed, and each cell examined for uptake of the latex particles under phase-contrast microscopy. Under these conditions nearly 100% of the cells had ingested from 1–40 latex beads.



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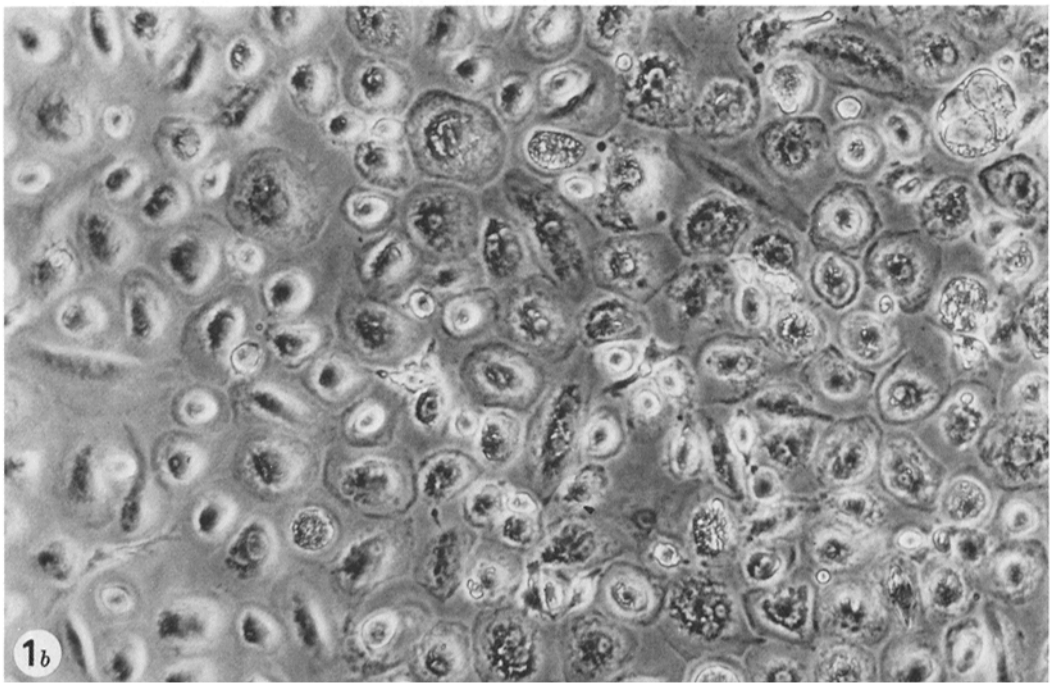

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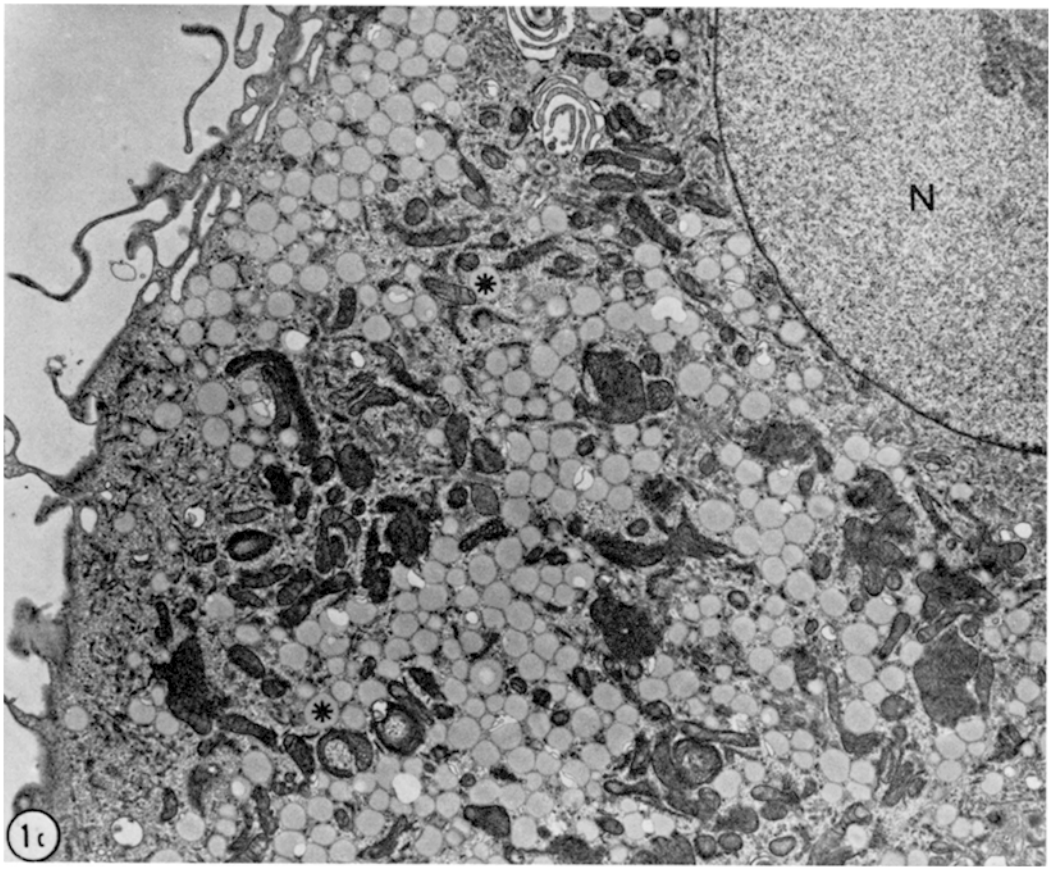

FIG. 1. (a) Human monocyte in culture for 2 days, fixed and incubated for endogenous peroxidase. Numerous small, round vesicles containing electron-dense reaction product (arrows) are present in the cytoplasm. × 12,200. (b) Human monocytes in culture for 2 wk. Phase-contrast × 157. (c) Human monocyte in culture for 4 wk, fixed and stained for routine electron microscopy. The cell is greatly enlarged as compared to the one shown in Fig. 1 a. The nucleus (N) contains loose chromatin and a prominent nucleolus. The cytoplasm is replete with numerous lipid droplets (asterisks). × 6,600.

Synthesis and Secretion of C2

KINETICS. Separate monolayer preparations (cell density 1–30 × 10^4/slip) from 10 normal individuals were rinsed and then incubated in 0.5 ml of fresh M199 HFCS after 1 day, 2 wk, 6 wk, and 14 wk in culture. At timed intervals, portions of the media were removed and assayed for functional C2 with the hemolytic assay. After each 0.05-ml sample was removed, an equal volume of fresh M199 HFCS was added to the wells. The resulting dilution was taken into account in calculating total C2 production. After a 6 day lag, C2 production was linear in monolayers examined after 1 day in culture (Fig. 2 a). No lag in C2 production was detected in monolayers studied at 2–4 wk (Fig. 2 b) or 6–8 wk (Fig. 2 c) in vitro. Moreover, the average rates of synthesis and secretion of C2 per cell were twice and three times the initial rate in the 2–4 and 6–8 wk

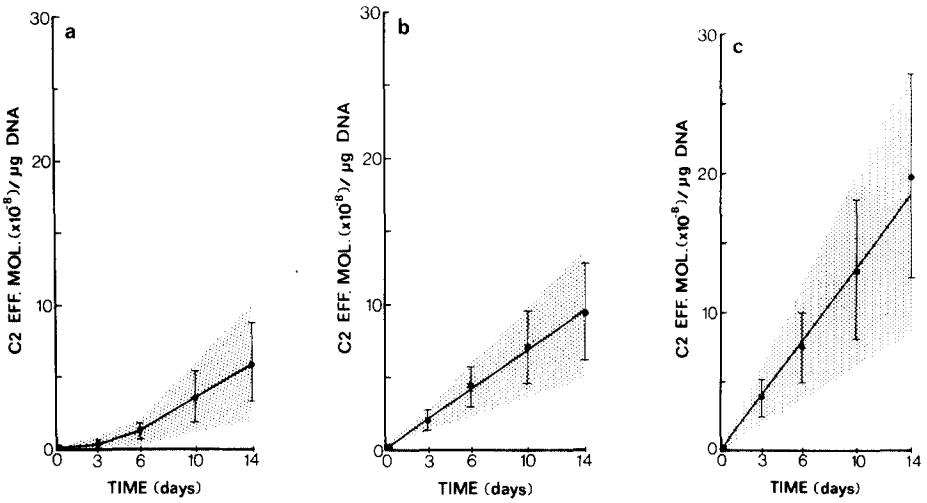

FIG. 2. Kinetics of C2 production by peripheral blood monocytes from 10 normal individuals during 2-wk intervals in culture (a) 0–2 wk in vitro; (b) 2–4 wk in vitro; (c) 6–8 wk in vitro. Points, mean C2 production; vertical bars, two standard deviations; shaded areas, range; Eff. Mol., hemolytically effective molecules.

cultures, respectively. In the latter preparations, C2 was secreted at an average rate of approximately 30 mol/cell per min. To test whether the progressive loss of cells with time and resulting change in cell density affected rates of C2 synthesis, monolayers were established at densities varying up to 12-fold (6–72 × 10^3 cells/slip). The rate of C2 production was unaffected by cell density when examined during the 6th and 7th wk in culture. Significant production of hemolytic C2 was detected during the 15 and 16 wk in culture, however, the fluorometric assay was insufficiently sensitive to measure the small amount of DNA on each slip after 16 wk in vitro, precluding calculation of a per cell synthetic rate during that period. Lysates of normal monocyte monolayers (7 wk in culture) contained no detectable intracellular hemolytically active C2. No functionally active C1, C4, C3, C8, or C9 were detected in media from monocyte monolayers.

EFFECT OF CYCLOHEXIMIDE. Replicate monolayers from a single individual (3 wk in culture) were incubated in M199 HFCS or M199 HFCS containing cycloheximide (0.5 µg/ml) for 48 h at 37°C, after which each monolayer was rinsed. One of the preparations incubated in the presence of cycloheximide was fed with fresh M199 HFCS lacking the inhibitor and the other incubated again in medium with cycloheximide. C2 secretion was monitored with the hemolytic assay. As shown in Fig. 3, cycloheximide inhibited C2 production by approximately 80%; C2 production was restored by removing the inhibitor.

INCORPORATION OF ^{14}C-LABELED AMINO ACIDS. Extensively dialyzed and concentrated medium harvested from monolayers (6 wk in vitro) incubated for 5 days in the presence of ^{14}C-labeled amino acids was mixed with normal human serum (to provide carrier protein) and placed in a well opposite rabbit antihuman C2, C3, and C4 antisera. Radioautography of the washed and dried Ouchterlony plates revealed labeling of the C2, C3, and C4 precipitin bands (Fig. 4).

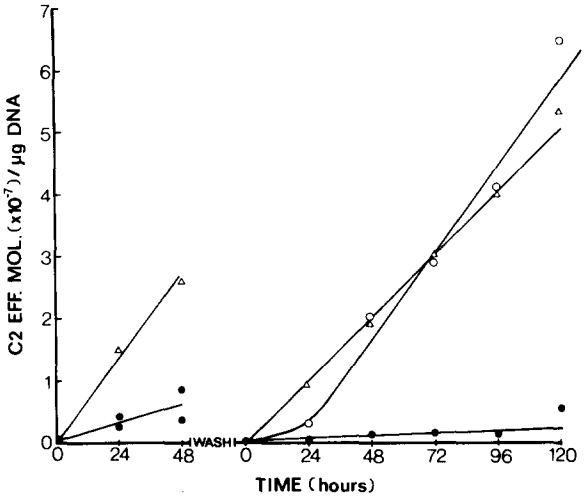

FIG. 3. Reversible effect of cycloheximide on C2 production by human monocytes: Three monolayers (3 wk in culture), one incubated in medium alone (△) and two in medium plus 0.5 µg/ml cycloheximide (●). After 48 hr all slips washed and one of the two inhibited monolayers placed in medium lacking cycloheximide (○).

Using the same procedure no labeled C2 was detected in medium harvested from monolayers incubated in the presence of cycloheximide (2 µg/ml) or in medium to which ^{14}C-labeled amino acids were added after harvesting from the culture slips.

Production of Lysozyme

Secretion of lysozyme by monocyte monolayers was detected after 2 days in culture. The maximum rate of lysozyme secretion was approximately 50,000 enzymatically active molecules per cell per min, and there was no significant change in rate of secretion by cells maintained from 2 to 10 wk in vitro (Table I).

Bacteriolysis of L. monocytogenes

Monolayers (5 wk in culture) were incubated at 37°C in M199 containing approximately 10^7 [^3H]thymidine-labeled *L. monocytogenes* per slip. After 10 min the slips were rinsed vigorously in M199 and reincubated in M199 at 37°C. At timed intervals the monolayers were rinsed, lysed, and assayed for viable bacteria. The results (Table II) showed a significant decrease in viable organisms up to 5 h, after which an increase in bacterial count was detected.

Discussion

Several lines of evidence indicate that the method described in this report yields pure human monocyte cultures. None of the cells in these preparations rosetted with sheep erythrocytes, making T lymphocyte contamination unlikely. None of the cells in the monolayers bound fluoresceinated anti-IgG antiserum, but all formed rosettes with IgG- or C3-coated erythrocytes. The absence of surface IgG on the monocyte monolayers makes B lymphocyte contamination unlikely. All of the cells, at 2 days, contained peroxidase-positive granules and lysozyme, and throughout the time in vitro were capable of

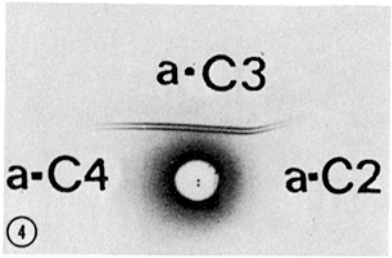

FIG. 4. Radioimmunodiffusion analysis of incorporation of ^{14}C-labeled amino acids into complement proteins by human monocytes (6 wk in culture). Center well: dialyzed, concentrated monocyte culture medium plus normal human serum. Outer wells: rabbit antisera to human complement components.

ingesting latex particles. These features are characteristic of monocytes and not of lymphocytes (1, 25, 42). Finally, none of the cells had morphologic features of polymorphonuclear leukocytes, eosinophils, or basophils. Several technical details were essential to the preparation (with a high degree of plating efficiency) of pure monocyte monolayers which survived in culture for as long as 16 wk; (*a*) incubation of washed leukocytes at 4°C in medium without serum for 12–18 h before plating to decrease the number of glass adherent polymorphonuclear leukocytes in the cell suspension, (*b*) resuspension of cell pellets before centrifugation to prevent clumping, (*c*) careful preparation of cover slips to permit efficient and uniform plating of the monocytes, and (*d*) vigorous washing of cover slips after adherence of the monocytes to remove cell debris and contaminating, but less firmly adherent, cell types.

Evidence that these pure monocyte monolayers synthesized the second component of complement was based on observations that (*a*) biologically active C2 was secreted into the medium in cultures maintained for several months; (*b*) C2 secretion was reversibly inhibited by low concentrations (0.5 μg/ml) of cycloheximide, a well known inhibitor of protein synthesis; (*c*) production of C2 was accompanied by incorporation of ^{14}C-labeled amino acids into C2 protein. The rates of C2 synthesis varied as a function of time in vitro. The rate of synthesis of C2 by monocytes maintained for 8 wk in culture was approximately three times the rate during the 2nd wk in culture. Moreover, a significant lag in onset of C2 secretion was noted in the early cultures but was not detected in preparations maintained for 2 wk or more before measuring C2 synthesis. This lag may be a result of maturation of the monocytes early in culture. Morphologic evidence of maturation in vitro included loss of peroxidase positive granules and formation of extensive cytoplasmic processes.

Local synthesis of C2 by monocytes at a site of an inflammatory exudate may be of importance in supporting complement dependent host defenses. C2 is a limiting component in the activation of the classical complement pathway, inasmuch as the concentration of C2 in serum (43) is relatively low (on a molar basis 10-fold less than C4 [44] and 40-fold less than C3 [45]) and the $C\overline{42}$ complex is unstable (46). $C\overline{42}$, the enzyme that cleaves C3 in the classical pathway, decays, liberating an inactive C2 molecule and a C4 site capable of reaccepting active C2 to regenerate the enzyme. Local production of C2 may circumvent

TABLE I
Lysozyme Production by Human Monocytes In Vitro

Time In vitro*	Lysozyme‡
wk	*μg/monolayer*
0–2	1.5
2–4	2.5
4–6	2.5
6–8	2.0
8–10	2.4

* Culture media pooled at 2-wk intervals from multiple monolayers obtained from each of eight normal individuals.
‡ Mean lysozyme production per monolayer (10^4 – 3×10^5 monocytes/monolayer) during a 2-wk interval.

TABLE II
Bacteriolysis by Human Monocytes In Vitro

Hours In vitro*	Viable *L. monocytogenes*/μg monocyte protein‡	
	Exp. 1	Exp. 2
0	1,100	1,000
5	29	12
24	5.6×10^5	4.4×10^5

* 10^7 *L. monocytogenes* added to each of six monolayers (5-wk-old monolayers, approximately 10^5 monocytes/monolayer). After 10 min all monolayers were rinsed and two assayed for viable bacteria and protein (0 hr). Remaining monolayers were rinsed and assayed in duplicate after 5 h and 24 h, respectively.
‡ Mean of duplicate determinations.

these limiting effects by increasing the C2 concentration at a site of inflammation.

Earlier studies suggested that the human peritoneal macrophage was a site of synthesis of the fourth (C4) and third (C3) components of complement (47). This conclusion was based solely on detection of radiolabeled precipitin arcs with immunoelectrophoresis of culture media mixed with normal serum as carrier protein. In the present study, radiolabeled C3 and C4 bands were detected by immunoprecipitation of media harvested from monocytes incubated with ^{14}C-labeled amino acids. Nonetheless, production of functional C3 and C4 was not detected, although the functional assays are capable of measuring less than 1 ng/ml of the respective proteins. In addition, synthesis of biologically active C3 and C4 was easily demonstrated in short-term cultures of human liver (29). Several possible explanations for the discrepency between the immunochemical and functional data have been considered. Among these is the possibility that, since serum was used as a source of carrier protein, some other labeled macrophage product coprecipitated with carrier (unlabeled) C3 and C4. On the other hand, postsynthetic degradation or inhibition of C3 and C4 activities may have

occurred. Thus it is not possible at the present time to determine whether human monocytes are in fact capable of synthesizing C3 and C4.

Recently Gordon et al. (1) reported that mouse peritoneal macrophages and human monocytes synthesize lysozyme, and that this protein accounts for approximately 2% of the secreted mouse macrophage products. We have confirmed their observations in human monocytes and find that lysozyme secretion persists up to at least 12 wk in vitro. In the present study, the rate of lysozyme secretion was approximately 1,500 times greater, on a molar basis, than the maximal rate of C2 synthesis. All of the cells in the culture were apparently producing lysozyme, as judged by immunofluorescent antibody studies, but no estimate has been obtained of the proportion of cells per culture that synthesize C2. Therefore, a precise ratio of the relative rates of synthesis of C2 and lysozyme must await further studies.

Summary

A method has been developed for preparation of confluent monolayers of human monocytes from small volumes of blood and for maintenance of these pure monocyte cultures for up to 16 wk in vitro. These cells phagocytosed 5.7 μm diameter latex beads, rosetted with erythrocytes coated with IgG or with C3, killed *Listeria monocytogenes*, and synthesized both lysozyme and the second component of complement. Lysozyme was secreted at a rate of approximately 50,000 mol/min per cell for at least 12 wk in cultures. The maximal rate of C2 synthesis and secretion was considerably less; i.e., approximately 30 mol/min per cell between the 2nd and 12th wk in culture. Monocytes produced little C2 during the first 6 days in culture after which a marked increase in the rate of C2 production was noted. This increase was coincident with morphologic evidence of monocyte maturation.

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