IN VITRO DIFFERENTIATION OF HUMAN MONOCYTES*

Differences in Monocyte Phenotypes Induced by Cultivation on Glass or on Collagen

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Blood monocytes undergo a process of differentiation to macrophages when they migrate into the tissues, and they undergo an additional maturation or activation under inflammatory conditions. Many investigators have studied the differentiation of macrophages in rodents (1–4); lately, similar exeriments have been carried out with human tissue macrophages (5–7). However, the difficulties involved in obtaining human macrophages have limited the number of experiments done and the amount of information available. Therefore, most studies (8–16) in man use peripheral blood monocytes. These cells have been introduced into culture and then allowed to differentiate in vitro.

The identification of distinct cell surface antigens, which may provide markers for the study of monocyte-macrophage differentiation, has been facilitated by the development of monoclonal antibodies (17). Several mononuclear phagocyte lineage antigens have been produced in the mouse (18–20) and in man (21–26).

The aim of the present study is twofold. First, to compare the differentiation of monocytes in vitro during cultivation on collagen matrices (a component of the physiological environment in which monocytes differentiate in vivo), glass, and microexudate-coated glass, to establish whether the tissue culture surface selected for monocyte cultivation has any effect on the pattern of differentiation of the cells. Second, to characterize and correlate the changes in morpholgy and function taking place when human peripheral blood monocytes differentiate to macrophages, epithelioid cells, and giant cells in an established in vitro tissue culture system. In addition, using a new monoclonal antibody directed against human monocytes and macrophages recently produced in this lab,[1] we identified an antigenic change during monocyte differentiation in vitro.

Materials and Methods

Preparation of Mononuclear Leukocytes. Peripheral blood mononuclear leukocytes (MNL)[2] were isolated from venous blood of healthy adult human donors by the Bøyum method (27). Briefly, blood collected into EDTA-containing vacutainers was diluted with an equal volume of

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[2] *Abbreviations used in this paper:* DME, Dulbecco's modified Eagle medium; E, sheep erythrocytes; EIgG, E opsonized with rabbit anti-sheep IgG; EIgMC, E opsonized with rabbit anti-sheep IgM and human complement; FCS, fetal calf serum; MNL, mononuclear leukocytes from peripheral blood; PBS, phosphate-buffered saline; SEM, scanning electron microscopy.

Dulbecco's modified eagles medium (DME) (Gibco Laboratories, Grand Island Biological Co., Glasgow, Scotland), and 30 ml of the mixture was layered onto 15 ml of lymphoprep (density, 1.077 g/ml) (Nyegaard & Co., Oslo, Norway) in 50-ml polypropylene conical tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) and then centrifuged at 1,500 rpm for 30 min at room temperature. The interphase cells were washed twice by centrifugation at 4°C and suspended in DME containing 20% autologous serum, 100 μg/ml streptomycin, and 100 i.u./ml penicillin, to give 2.5×10^6 MNL/ml. The cells were seeded into 16-mm culture wells (24-well tissue culture clusters; Costar Data Packaging, Cambridge, MA) with collagen or untreated, collagen-coated, or microexudate-coated glass cover slips.

Preparation of Hydrated Collagen Matrices. Collagen extracted from rat tail tendons was a kind gift of Dr. Bjørn Obrunk, Department of Medical Chemistry, Uppsala, Sweden. Collagen solutions in 0.1 M acetic acid (1.5 mg/ml) were added to 16-mm culture dishes or 14-mm glass cover slips (250 μl and 60 μl, respectively). To these, 25 μl or 7 μl of 10 × DME medium and 15 μl of 0.142 M NaOH were added simultaneously to bring the pH of the mixture to 7.6 (28). The gels were incubated for 2–24 h at 37°C, washed well with DME medium, and kept hydrated at 37°C until use (29). Collagen matrices used for scanning electron microscopy were prepared on glass cover slips pretreated with 1 mg/ml poly-L-lysine for 1 h at room temperature, to avoid loosening of the collagen from the glass during processing for electron microscopy.

Preparation of Microexudate-coated Surfaces. Rat fibroblasts (R22 CIF) were grown to confluence on 14-mm glass cover slips or 16-mm plastic culture wells in DME with 20% fetal calf serum (FCS) (Gibco Laboratories, Grand Island Biological Co.). The stationary confluent cells were lysed by treatment with 0.25 M NH_4OH for 30 min at room temperature, as described (29). Washed and 70% ethanol sterilized microexudate-coated cover slips were stored dry until use.

Culture of Monocytes. 2½ million MNL in 1 ml DME with 20% autologous serum were seeded in wells with collagen matrices or glass cover slips and washed well with DME after 2 h to remove nonadherent cells. 1 ml of DME with 20% autologous serum was added to each culture. After 7 d, 1 ml of the same medium or 1 ml of DME was added to the cultures. New medium was added every 4 d thereafter.

Opsonization of Sheep Erythrocytes. Sheep erythrocytes (E) stored in Alsevier's solution were obtained from the National Institute of Health, Oslo, and used within 1 wk. Rabbit anti-sheep erythrocyte immunoglobulin G (IgG) and rabbit anti-sheep erythrocyte immunoglobulin M (IgM) were obtained from Cordis Laboratories, Miami, FL. A 5% suspension of E was incubated with subagglutinating concentrations of IgG and IgM as described (30), giving EIgG and EIgM, respectively. The EIgM were incubated with a 1:10 dilution (in veronal-buffered glucose, Ca^{2+} and Mg^{2+}, and 1% gelatin) of zymosan-treated human A^+ serum preadsorbed with E, for 1 h at 4°C to obtain EIgMC (31).

Phagocytosis of Erythrocytes. For attachment, monolayers in 1 ml DME were incubated with 25 μl of a 5% suspension of erythrocytes for 20 min at room temperature. Controls containing E and EIgM did not attach to the monocytes. The cultures were washed well to remove nonattached erythrocytes and incubated in DME for 2 h at 37°C. A 0.14 M solution of NH_4Cl was added to lyse attached, noninternalized erythrocytes. The percentage of phagocytes that attached or internalized 4 or more erythrocytes was counted by scoring at least 300 cells per culture.

Morphological Studies. Monocytes on cover slips or collagen matrices were washed with phosphate-buffered saline (PBS) and then fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer with 0.1 M sucrose, pH 7.3, at room temperature. For phase contrast microscopy, the cultures were washed, mounted, and photographed with Kodak Panatomic-X black and white film (Eastman Kodak Co., Rochester, NY). For scanning electron microscopy (SEM), the fixed cultures were dehydrated in alcohol, transferred to amyl-acetate, and critical point dried (Hitachi CPI, Tokyo) in carbon dioxide. The specimens were coated with gold (Polaron SEM coating unit E 5000) and examined with a high resolution Hitachi scanning electron microscope (HHS/2R) at 20 kv and a tilt angle of 30°. Pictures were taken on Kodak Plus-X film (Eastman Kodak Co.)

Monoclonal Antibodies. Three murine monoclonal antibodies reactive with human monocytes were used. 1D5 is a monoclonal anti-human monocyte antibody.[1] It was obtained after immunization of BALB/c mice with purified viable human monocytes. This antibody reacts

with human monocytes and macrophages but not with the histiocytic lymphoma cell line U937, granulocytes, normal peripheral blood B and T cells, or established cell lines with T and B cell characteristics.[1] BRL clone 63D3 is a monoclonal anti-human monocyte antibody (22, 33) that recognizes human monocytes and granulocytes but does not react with T or B cells or human macrophages. 63D3 was obtained from BRL Molecular Diagnostics, Gaithersburg, MD. A monoclonal antibody against human DR (Ia-like) antigen was kindly provided by Dr. Russel Curry of the University of Minnesota, Minneapolis, MN. This antibody is directed against a "backbone" DR antigen of all individuals and precipitates the 29-33K polypeptide chains from radiolabeled B cells. The antibody reacts with B cells, cultured B cell lines, and monocytes (33).

Detection of Antigen Expression on Cultured Cells. The expression of 1D5, 63D3, and DR antigen on cultured monocytes-macrophages was detected by binding of monoclonal antibodies, using an indirect immunofluorescence technique. Monocytes on glass or collagen were incubated with 250 μl of monoclonal antibodies for 45 min at 37°C. The concentrations of antibody used (10-fold higher than the concentrations determined by titrations to give optimum immunofluorescent intensities) were: for 1D5, a 1/150 dilution of ascites fluid; for 63D3, a 1/50 dilution of ascites fluid; and, for anti-DR, a 1/50 dilution of tissue culture supernatant. Controls, including tissue culture supernatants and ascites from nonreactive hybridoma clones, were used at the same dilutions. The cells were washed three times with 2.0 ml of warm washing fluid and overlaid with 250 μl of a 1/50 dilution of fluorescein-conjugated IgG fraction of rabbit anti-mouse IgG (Miles, Yeda Ltd., Rehovot, Israel). The cells were incubated for 30 min at 4°C and washed four times with cold washing fluid. The percent of fluorescing cells was determined on wet mounted culture slides with a Leitz Orthoplan fluorescence microscope equipped with a phloemopak illuminating system (E. Leitz, Inc., Rockleigh, NJ). Cells were photographed with Kodak Tri-X pan film (Eastman Kodak Co.). All washes and dilutions of antisera were done in DME containing 10% FCS. In some experiments, the simultaneous expression of Fc receptor-mediated phagocytosis and 1D5 or DR antigen was assessed. In this case, cultured cells were first allowed to phagocytose opsonized erythrocytes, as described above, and then processed for immunofluorescence and photographed.

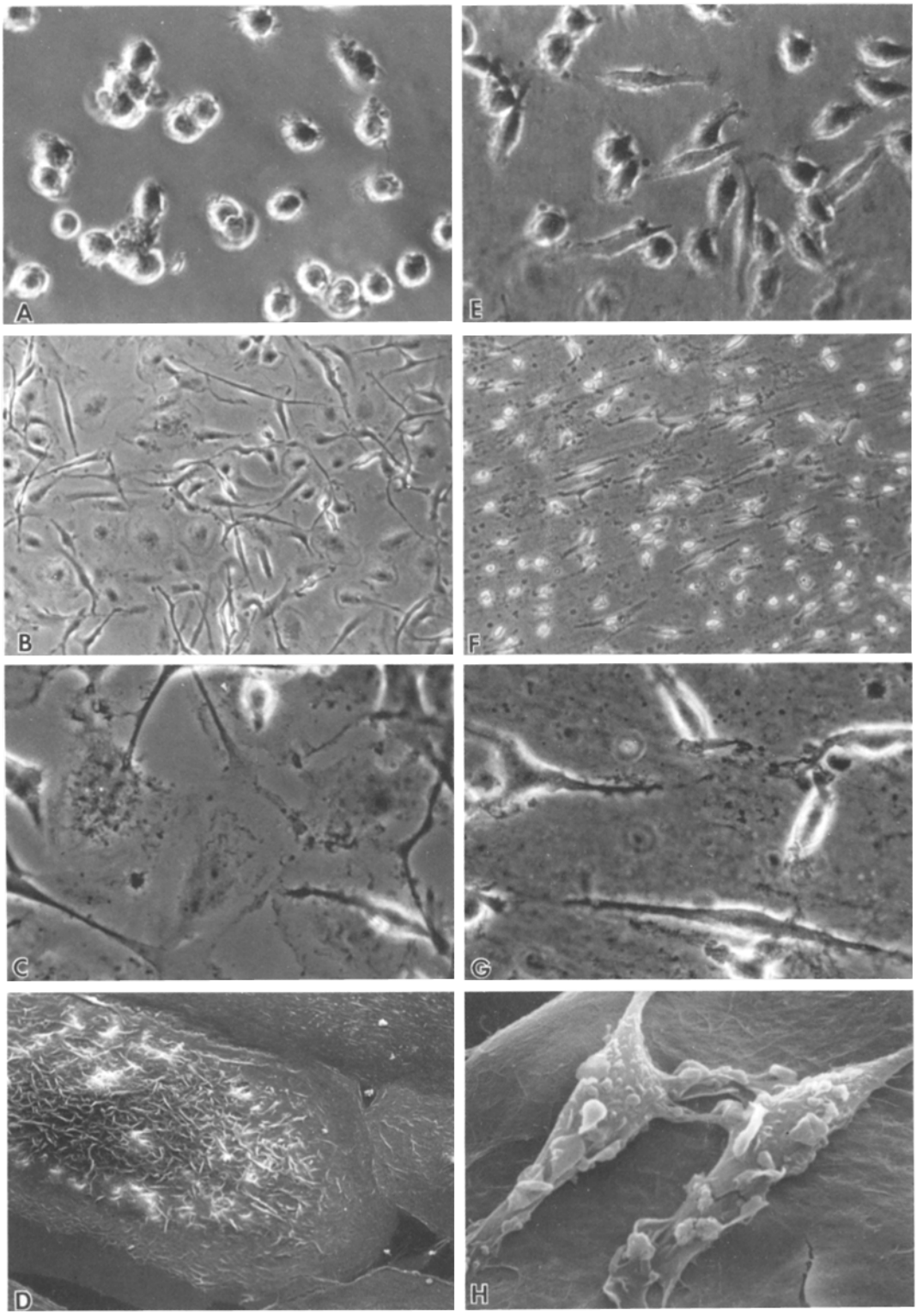
Results

Differences in Morphology of Monocytes Cultured on Glass and on Collagen. Adherence of the monocytes to collagen gels was as efficient as adherence to glass or microexudate-coated glass in the presence of serum. When serum was absent, the adherence to the culture surfaces was reduced. Under the culture conditions used, the monocytes in all cultures remained viable and metabolically active for over 4 wk. At the start of culture (2 h), all the adherent monocytes were still rounded with prominent membrane ridges and folds (observed by SEM) (not shown).

By 24 h, some of the glass-adherent monocytes had started to spread (Fig. 1 A). By days 4–5, most of the cells were well spread and appeared much larger. No organization or alignment of the cells was observed (Fig. 1 B, C). From day 4 to day 7, the cultures changed to more or less confluent monolayers of epithelioid-like cells with highly folded and ruffled surfaces (see Fig. 4 C). During this time, some binuclear and multinuclear cells could be distinguished. The latter became the most prominent cell type by ~17–20 d (Fig. 1 D). The formation of multinucleated giant cells involved a stretching of the membranes. The surface of the cells became less irregular, and fewer large folds and ruffles were observed (Fig. 1 D). Different donors' monocytes on glass matured into macrophages and epithelioid cells, but did so over varied lengths of time in culture.

Monocytes on microexudate-coated glass differentiated in a similar way, but faster than cells on glass. Typical epithelioid cells were found by days 5–6, and giant cells, on average larger and more numerous than on glass, appeared as early as 7–8 d.

The monocytes on collagen gels differentiated differently from those on glass and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microexudate-coated glass. Spreading of the cells was observed already by 24 h in culture (Fig. 1 E). From 2–3 d in culture, an alignment of the cells along the collagen fibers was observed. With time, the cells became longer and more ruffled (Fig. 1 F–H). When aggregates of cells were formed, the monocytes did not loose their elongated polar shape, and no epithelioid-like cells or multinucleated giant cells were ever observed.

Phagocytic Receptor Modulation Induced by Cultivation on Glass or Collagen. The development of Fc and C_3 receptor function during monocyte differentiation on glass cover slips was found to be very similar to that reported by others (34). Monocytes from different donors gave different rates of receptor function modulation. In some cases, a reduction in the percent of phagocytosis-positive cells was observed between 2 to 4 d. This reduction seemed to be caused by a period of weak adherence of the phagocytosing cells to the glass surfaces (observed by morphological examination). In general, the binding of EIgG to human monocytes was followed by ingestion of the bound particles (Fig. 2 A). EIgG could be shown to bind to ~40% of the 2-h glass adherent monocytes. The capacity for binding and ingestion of particles by the Fc receptor went up to 60%, remained at this level, and then gradually dropped to ~20% of the cells (Fig. 2 A).

Newly separated human monocytes, cultured on glass for 2–24 h, expressed a receptor for C_3 that mediated binding but not ingestion of EgMC (Fig. 2 B). By the 3rd d in culture, monocytes seeded on glass were also capable of ingesting EIgMC by their C_3 receptor. This capacity was maintained for ~14–16 d (Fig. 3 B). From ~20 d, the differentiated monocytes lost their capacity to phagocytose via their C_3 receptor (Fig. 2 B, Fig. 3 D).

When human monocytes were cultured on microexudate-coated cover slips, the phagocytic function of the cells was modulated in a manner very similar to that of the monocytes differentiated on glass (Fig. 2 C and D). Binding and ingestion were ~10% higher, and the variation fom donor to donor was lower both for EIgG (Fig. 2 C) and EIgMC (Fig. 2 D) binding and ingestion.

In comparison, the differentiation of human monocytes on collagen matrices gave rise to a different pattern of Fc and C_3 receptor function (Fig. 2 E and F). Already, by 2 d in culture, all the monocytes bound and ingested particles by their Fc (Fig. 2 E) and their C_3 (Fig. 2 F) receptors. This activity was very stable and reproducible throughout the entire culture period. In all cultures, binding seemed to lead to very effective ingestion (Fig. 3 E–H).

Expression of 1D5 Antigen on Monocytes Cultured on Glass. The kinetics of 1D5 antigen

FIG. 1. Morphology of human monocytes cultured on glass (A–D) or collagen matrices (E–H). Micrographs A–C and E–G are of phase contrast microscopy; D and H are of scanning electron microscopy. (A) 24-h-old monocytes on glass. The cells are still mostly rounded. Magnification, × 440. (B) 11-d-old monocytes on glass. The cells are larger and heterogeneous in shape. While some are large and flat (epitheloid), others are elongated and more polar. Magnification, × 100. (C) 11-d-old monocytes on glass. Typical epitheloid cells together with some elongated cells are observed. Magnification, × 440. (D) 21-d-old monocytes on glass. A large flat cell (multinucleated giant cell) is observed. Surface ridges are prominent in the perinuclear region of the cell. Magnification, × 450. (E) 24-h-old monocytes on collagen. The cells have started spreading and have acquired an elongated polar appearance. Magnification, × 440. (F) 11-d-old monocytes on collagen. Elongated aligned cells are observed. Magnification, × 100. (G) 11-d-old monocytes on collagen. The cells are elongated and polar. Magnification, × 440. (H) 21-d-old monocytes on collagen. The cells are elongated and aligned parallel to each other. Surface ruffles and villi are observed. Magnification, × 1,000.

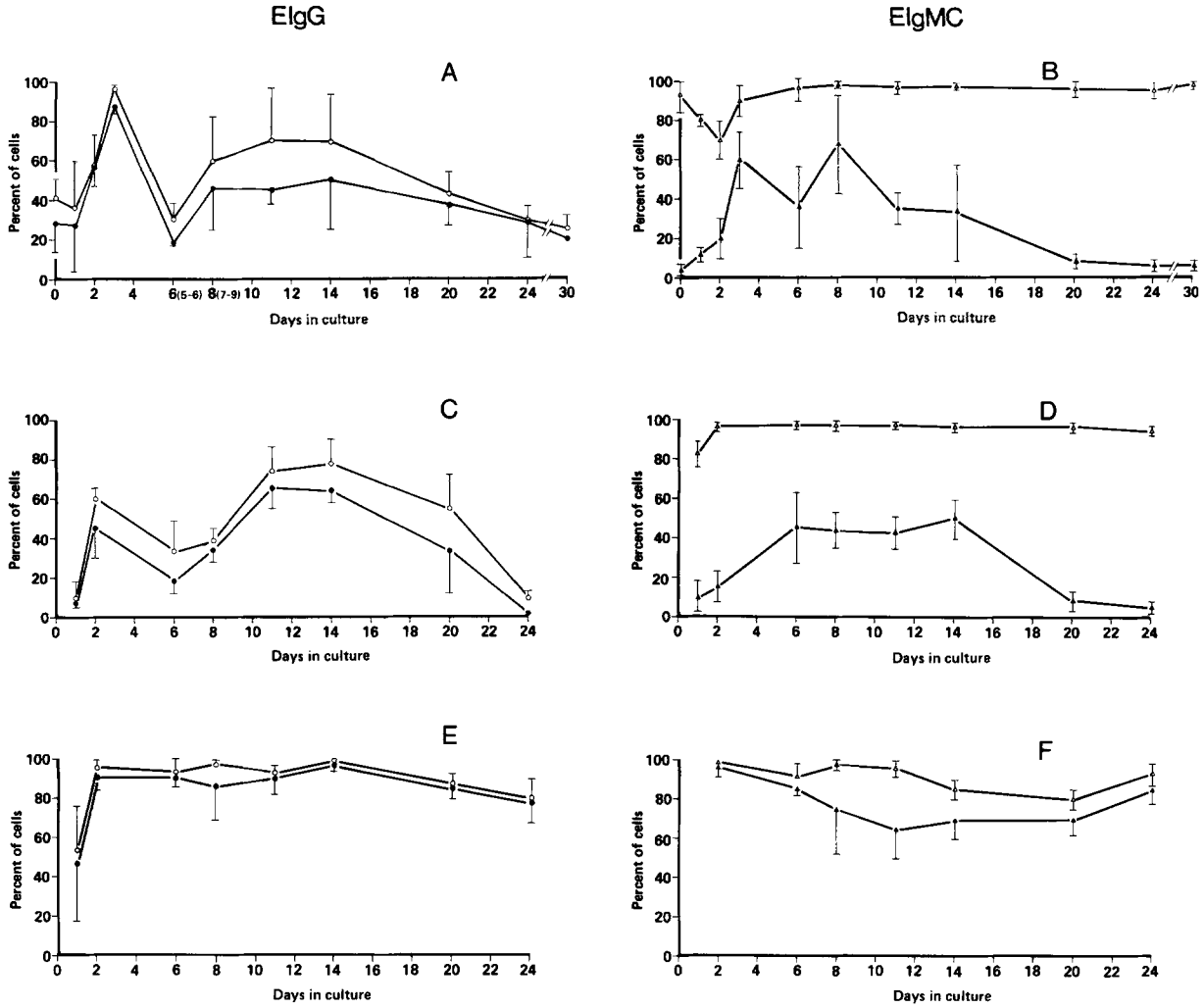


FIG. 2. Phagocytosis of EIgG (A, C, and E) and EIgMC (B, D, and F) by human monocytes cultured on glass cover slips (A and B), microexudate-coated glass (C and D), and collagen matrices (E and F). ○, Binding of EIgG; ●, ingestion of EIgG; △, binding of EIgMC; ▲, ingestion of EIgMC. The results are means of six different donors monocytes ± SD.

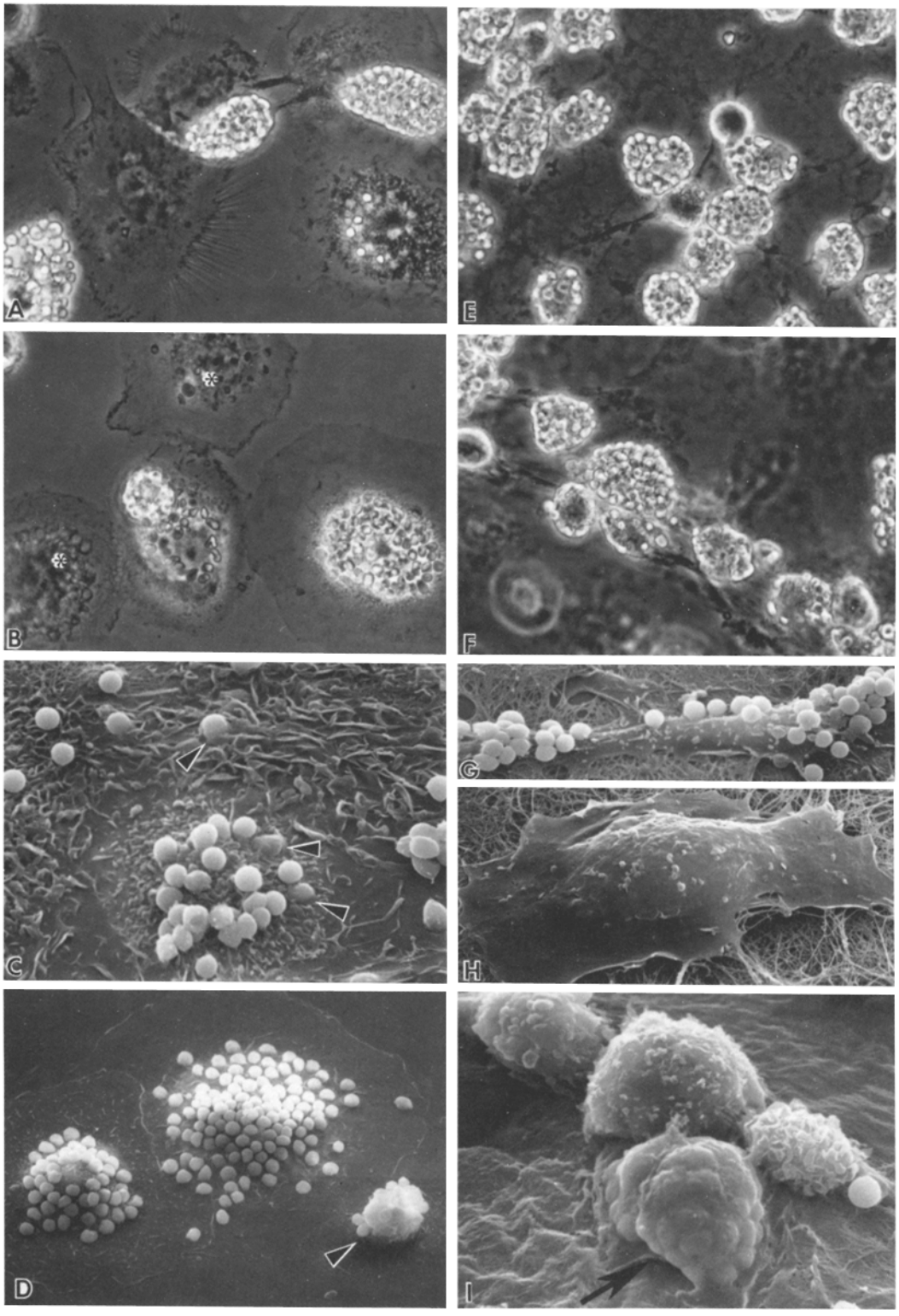
expression on monocytes cultured on glass cover slips are shown in Fig. 4. All freshly isolated monocytes were 1D5 positive, although individual cells expressed varying amounts of antigen (Fig. 5). During the first 4–5 d of culture, all the cells continued to express the 1D5 antigen, and generally an increase in staining intensity was observed. After this period, the antigen was gradually lost, and by the 2nd and 3rd wk in culture, the majority of the cells did not stain for the antigen. Monocytes from different donors varied greatly in the rate at which they lost the antigen, but the pattern of antigen expression was similar for all donors. Cells with intermediate and very weak staining could be observed in the cultures before the appearance of negative cells. Several typical features were apparent in cultures from all donors. Giant cells were always negative, whereas cells with epithelioid morphology were negative or

weakly positive. In old cultures, strongly positive cells were always found among the smallest cells (Fig. 5 D and F), often in clusters or "nests," located on top of the epithelioid monolayer.

Comparison of Macrophage Antigen Expression on Cells Cultured on Glass or Collagen. The antigen expression of monocytes undergoing differentiation to macrophages on glass or on collagen is presented in Table I. All freshly isolated monocytes were $1D5^+$, $63D3^+$, and DR^+. The intensity of staining of these antigens was, as observed for 1D5, highly variable from cell to cell. In particular, the monocytes showed great heterogeneity with respect to DR antigen expression. The heterogeneity of DR intensity was observed with all donors tested so far (>50) and persisted for the entire culture time. A prominent feature of the glass cultures was the presence of some cells showing very intense DR staining from day 2 on. High amounts of DR antigen were not restricted to a subset of small cells, but were observed on all cell types, including the cells of epithelioid or giant cell morphology. A difference was observed for the expression of the two macrophage-specific antigens 1D5 and 63D3, when macrophages allowed to differentiate on glass or collagen were compared. Both antigens were progressively lost from the cells differentiating on glass, and the 1D5- and the 63D3-positive cells seemed to belong to a population of small cells in these cultures. In the collagen cultures, cells negative for 63D3 appeared early, and by 3 wk all the cells on collagen had lost this antigen. Differentiation of monocytes to macrophages on collagen thus seems to be linked to disappearance of the surface antigen recognized by the 63D3 antibody. On the other hand, the 1D5 antigen was present on the majority, if not all, of the cells on collagen during the entire culture period. The loss of Fc receptor function in glass cultures was paralleled by the loss of the surface antigens recognized by the 1D5 and 63D3 antibodies (Table I). To establish whether the same subpopulation of cells in long-term cultures of monocytes simultaneously expressed FcR and 1D5 antigen, double labeling experiments were performed. FcR-positive cells found in late glass cultures were either $1D5^+$, $63D3^+$ or $1D5^-$, $63D3^+$, whereas FcR-positive macrophages in late collagen cultures were $1D5^+$, $63D3^-$. When double-label experiments were performed to study the coexpression of FcR and DR antigen, no correlation could be observed.

The Effect of Contact with Glass on Monocyte Differentiation. To establish whether the presence of collagen or the absence of contact with glass was the cause for the different phenotypes expressed by the monocyte in culture, three controls were included. In some cases, very loose (dilute) collagen gels were prepared, and many of the cells fell through the collagen matrix and adhered to the glass below the collagen. The cells adherent to the glass had very close contact with collagen fibers on their side or upper surface (observed by SEM) (not shown). In other experiments, the collagen gels were loosened from the glass, so that a large number of cells adhered to the glass below the collagen meshwork. Finally, some collagen gels were allowed to form in the central ¾ of the cover slip, leaving a rim of uncoated glass at the periphery of the glass cover slip.

In all these cases, the monocytes that had any contact with the glass cover slip differentiated like cells seeded on glass. The presence of collagen in the cultures and even close contact between the cells and collagen did not inhibit their glass-induced differentiation. It was therefore concluded that the contact or lack of contact with the glass gave rise to the differences observed in monocyte differentiation.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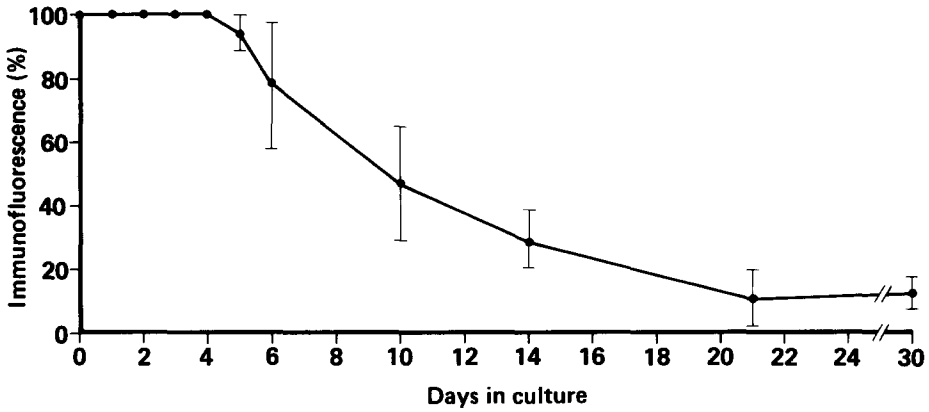

FIG. 4. Expression of 1D5 antigen on human monocytes cultured on glass cover slips. The results are expressed as the percent of adherent cells that stain by immunofluorescence. Mean of four experiments ± SD.

In the case of monocyte differentiation on microexudates, a similar point was noted. The exudates coating the glass were not continuous or thick enough to stop the cells from coming into some direct contact with the glass. SEM revealed a patchy distribution of microexudate substances, as already reported by Werb et al. (35).

Discussion

The evidence presented in this paper suggests that two different processes can take place during in vitro cultivations of human monocytes, depending upon the substrate used for culture. The first appears to be a maturation of monocytes to tissue macrophage-like cells, which occurs during cultivation on collagen matrices. The monocytes develop in vitro into a highly phagocytic population of cells. Both Fc and C_3 receptor-mediated binding and ingestion are enhanced.

A second pathway of monocyte differentiation occurs when the cells are cultured on glass, plastic, or micro-exudates. These surfaces seem to induce the formation of epithelioid-like, multinucleated giant cells identical to those found in foreign body

FIG. 3. Morphology of phagocytosis by 11-d-old human monocytes cultured on glass (A–D) or collagen matrices (E–I). A, B, E and F are phase-contrast microscopy; C, D, G, H, and I are scanning electron microscopy. (A) Ingestion of EIgG by monocytes on glass. Three of the cells have ingested >20 particles, one monocyte has Ingested 5 particles, and the other three have not ingested particles. Magnification, × 440. (B) Ingestion of EIgMC by monocytes on glass. Two well-spread and one rounded cell have ingested the particles; two of the cells that bound the particles did not ingest them. This is evident by the presence of the ghosts of the lysed particles visibly attached to the surface of these cells (*). Magnification, × 440. (C) Monocytes phagocytosing EIgG. Typical epithelioid cells are observed with extensive surface ridges and folds. Some of the attached particles are in the process of being ingested (arrows). Magnification, × 1,250. (D) EIgMC attached to the surface of monocytes on glass. The particles were not ingested by the large and spread or the rounded monocytes (arrow). Magnification, × 600. (E) Ingestion of EIgG by monocytes on collagen. All the cells besides one have ingested the particles. Magnification, × 440. (F) Ingestion of EIgMC by monocytes on collagen. All the cells besides one have ingested the particles. Magnification, × 440. (G) Attachment of EIgG to monocyte on collagen. A large number of particles are attached to the cell. Magnification, × 1,000. (H) Ingestion of EIgG by monocyte on collagen. All the attached particles have been ingested and the monocyte is liftng off the collagen. Magnification, × 1,500. (I) Ingestion of EIgMC by monocytes on collagen. The internalized particles can be seen outlined in the cell (arrow). Magnification, × 1,500.

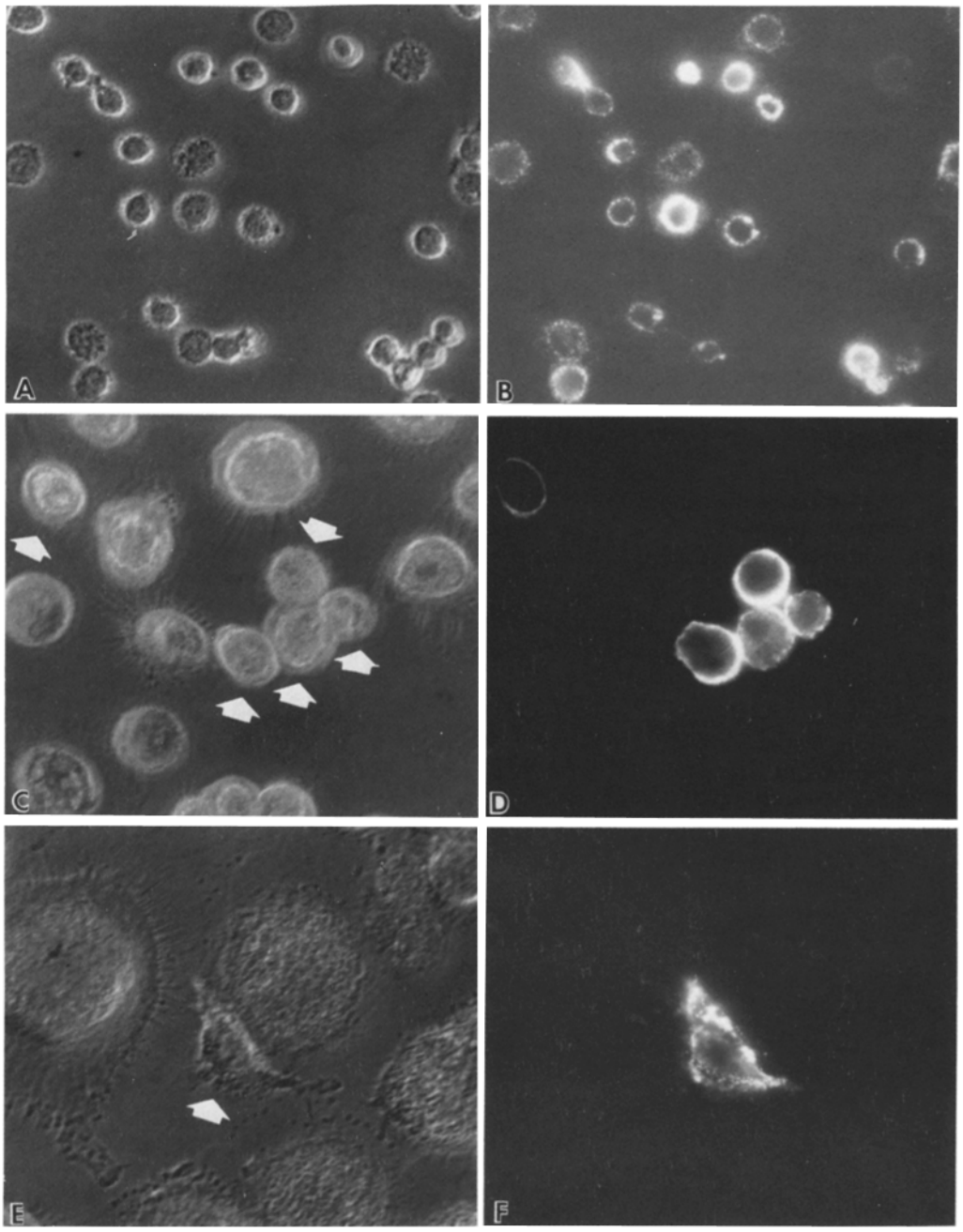

FIG. 5. Expression of the 1D5 antigen on monocytes cultured on glass cover slips. A, C, and E show phase contrast microscopy. B, D and F, immunofluorescence. Magnification, × 440. A and B show 24-h-old monocytes. Most of the cells express the 1D5 antigen. Heterogeneity in the intensity of fluorescence is observed. C and D show 10-d-old monocytes. The smaller cells (arrows) are those that express the 1D5 antigen.

TABLE I

Comparison of Macrophage Antigen Expression on Monocytes Cultured on Glass or Collagen

Substrate	Time in culture	Phagocytosis (FcR)	Immunofluorescence		
			1D5	63D3	anti-DR
		%	%	%	%
Glass	2 h	NT*	100	100	100‡
Glass	2 d	67	100	100	83
Glass	8 d	35	18	31	67
Glass	21 d	16	5	21	75
Collagen	2 d	>90§	>90§	90	>90
Collagen	8 d	>90	>90	11	>90
Collagen	21 d	>84	>90	0	>70

* Not tested.

‡ Variable staining.

§ Accurate quantitative measurements of phagocytosis or immunofluorescence could not be obtained on unfixed collagen gels.

granulomas. These cells lose their phagocytic capacity as well as the 1D5 antigen recognized by a monoclonal antibody against human monocytes. Foreign body granuloma cells (derived from in vivo inflammatory sites) have been shown (37–39) to be poorly phagocytic and to loose their Fc receptor function, while maintaining their ability to bind particles via their C_3 receptors.

The monoclonal antibody 63D3 has been reported to recognize human monocytes but not tissue macrophages (22–32). This antibody binds to a cell surface antigen present on all freshly seeded monocytes that is progressively lost as the monocytes mature or differentiate into macrophages in vitro both on glass and on collagen gels. The 1D5 monoclonal antibody, which also binds to all freshly seeded monocytes, has been shown to bind to some human myelomonocytic leukemias and to the U937 histiocytic cell line differentiated in vitro by treatment with the tumor promoter TPA as well as synovia and synovial fluid phagocytes, human milk macrophages, and umbilical cord macrophages.[1] All 3-wk-old collagen macrophages also express the antigen recognized by the 1D5 antibody. In contrast, only a subpopulation of 3-wk-old glass macrophages still binds the 1D5 antibody. The 1D5 antigen thus seems to be a marker for the monocyte and macrophage stages of mononuclear phagocytes, but not foreign body granuloma cells.

It is not clear from our results whether the differentiation of monocytes on collagen is dependent on some specific signal, or is a preprogrammed time-dependent process. Because contact with glass seems to have a dominant effect on monocyte differentiation in vitro when monocytes are exposed to glass and collagen together, it is important that any studies on monocyte differentiation or activation should consider the influence the tissue culture substrate has on the in vitro findings.

Summary

We demonstrated that the in vitro differentiation of human peripheral blood monocytes to macrophages is dependent on the environment and conditions of monocyte culture. Cultivation of monocytes on glass or microexudate-coated glass gave rise to cells resembling foreign body granuloma macrophages. After an initial rise in Fc receptor- and C_3 receptor-mediated phagocytosis, a progressive loss of Fc

receptor expression and C_3-mediated ingestion were observed. The monocyte surface antigens recognized by the anti-human monocyte monoclonal antibodies 1D5 and 63D3 were lost from the surface of the majority of cells cultured on glass and microexudates. A subpopulation of Fc receptor-positive cells that were 1D5 and 63D3 positive was retained in fully differentiated cell populations.

In comparison, monocytes cultivated on collagen matrices gave rise to highly phagocytic cells resembling human resident tissue macrophages. Both Fc- and C_3-mediated phagocytosis were enhanced and remained so during the entire length of culture. The surface antigens recognized by the 1D5 antibody, expressed on all freshly seeded monocytes, was maintained on the macrophages. The antigen recognized by the 63D3 antibody was not expressed on mature cells. The present evidence would indicate that variations in expression of phagocytic receptors and the surface antigens 1D5 and 63D3 can be ascribed to the stage of development of the macrophage or its stage of activation, rather than to independent subsets of mononuclear phagocytes.

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References

1. Cohn, Z. A., and B. Benson. 1965. The differentiation of mononuclear phagocytes: morphology, cytochemistry, and biochemistry. *J. Exp. Med.* **121:**153.
2. van Furth, R. 1976. Origin and kinetics of mononuclear phagocytes. *Ann. N. Y. Acad. Sci.* **278:**161.
3. Munthe-Kaas, A. C., and G. Kaplan. 1980. Endocytosis by macrophages. From the reticuloendothelial system. I. Carr and W. T. Daems, editors. Plenum Publishing Corp., New York. **1:**19.
4. Kaplan, G. 1981. The role of receptors in endocytosis. *In* Cell Biological Aspects of Disease. W. T. Daems et al., editors. Martinus Nijhoff, The Hague. 185.
5. Chang, Y., and C. Yao. 1979. Investigation of the human macrophages I. Collection and in vitro cultivation. *Eur. J. Immunol.* **9:**517.
6. Hammerstrøm, J. 1979. Human macrophage differentiation in vivo and in vitro. A comparison of human peritoneal macrophages and monocytes. *Acta. Pathol. Microbiol. Scand. Sect.* **87:**113.
7. Balkwill, F. R., and N. Hogg. 1979. Characterization of human breast milk macrophages cytostatic for human cell lines. *J. Immunol.* **123:**1451.
8. Lewis, M. R. 1925. The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated blood. *Am. J. Pathol.* **1:**91.
9. Carrel, A., and A. H. Ebeling. 1926. The fundamental properties of the fibroblast and the macrophages. II. The macrophage. *J. Exp. Med.* **144:**285.
10. Lewis, W. H. 1927. The formation of giant cells in tissue cultures and their similarity to those in tuberculosis lesions. *Am. Rev. Tuberculosis.* **15:**616.
11. Bennett, W. E., and Z. A. Cohn. 1966. The isolation and selected properties of blood monocytes. *J. Exp. Med.* **123:**145.
12. Johnson, W. D. Jr., B. Mei, and Z. A. Cohn. 1977. The separation, long-term cultivation, and maturation of the human monocytes. *J. Exp. Med.* **146:**1613.
13. Wuest, D., R. Crane, and J. J. Rinehart. 1981. Enhancement of Fc receptor function during human monocyte differentiation in vitro. *J. Reticuloendothel. Soc.* **30:**147.
14. Nakagawara, A., C. F. Nathan, and Z. A. Cohn. 1981. Hydrogen peroxide metabolism in

human monocytes during differentiation in vitro. *J. Clin. Invest.* **68:**1243.
15. Postlethwaite, A. E., B. K. Jackson, E. H. Beachey, and A. H. King. 1982. Formation of multinucleated giant cells from human monocyte precursors. Mediation by a soluble protein from antigen- and mitogen-stimulated lymphocytes. *J. Exp. Med.* **155:**168.
16. Yem, A. W., and M. J. Parmely. 1981. Modulation of Ia-like antigen expression and antigen-presenting activity of human monocytes by endotoxin and zymosan A. *J. Immunol.* **127:**2245.
17. Köhler, G., and C. Milstein. 1975. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (Lond.).* **256:**494.
18. Springer, T., G. Galfré, D. S. Secher, and C. Milstein. 1979. Mac-I: a macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immunol.* **9:**301.
19. Austyn, J. M., and S. Gordon. 1981. F4/80, a monoclonal antibody directed specifically against the mouse macrophage. *Eur. J. Immunol.* **11:**805.
20. Hirsch, S., J. M. Austyn, and S. Gordon. 1981. Expression of the macrophage-specific antigen F4/80 during differentiation of mouse bone marrow cells in culture. *J. Exp. Med.* **154:**713.
21. Ault, K. A., and T. A. Springer. 1981. Cross-reaction of a rat anti-mouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. *J. Immunol.* **26:**359.
22. Ugdini, V., G. Nunez, R. G. Smith, P. Stastny, and J. D. Capra. 1980. Initial characterization of monoclonal antibodies against human monocytes. *Proc. Natl. Acad. Sci. U. S. A.* **77:**6764.
23. Breard, J., E. L. Reinherz, P. C. Kung, G. Goldstein, and S. F. Schlossman. 1980. A monoclonal antibody reactive with human peripheral blood monocytes. *J. Immunol.* **124:**1943.
24. Perussia, B., D. Lebman, S. H. Ip, G. Rovera, and G. Trinchieri. 1981. Terminal differentiation surface antigens of myelomonocytic cells are expressed in human promyelocytic leukemia cells (HL 60) treated with chemical inducers. *Blood.* **58:**836.
25. Zola, H., P. McNamara, M. Thomas, I. J. Smart, and J. Bradley. 1981. The preparation and properties of monoclonal antibodies against human granulocyte membrane antigens. *Br. J. Haematol.* **48:**481.
26. Todd III, R. F., J. D. Griffin, J. Ritz, L. M. Nadler, J. Abrams, and S. F. Schlossman. 1981. Expression of normal monocyte-macrophage differentiation antigens on HL 60 promyelocytes undergoing differentiation induced by leukocyte-conditioned medium or phorbol diester. *Leuk. Res.* **5:**491.
27. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. *Scand. J. Clin. Lab. Invest.* **97** (suppl. 21):77.
28. Elsdale, T., and J. Bard. 1972. Collagen substrate for studies on cell behavior. *J. Cell. Biol.* **54:**626.
29. Werb, Z., M. J. Banda, and P. A. Jones. 1980. Degradation of connective tissue matrices by macrophages. I. Proteolysis of elastin, glycoproteins, and collagen by proteinases isolated from macrophages. *J. Exp. Med.* **152:**1340.
30. Kaplan, G. 1977. Differences in the mode of phagocytosis with Fc and C_3 receptors in macrophages. *Scand. J. Immunol.* **6:**797.
31. Wong, L., and J. D. Wilson. 1975. The identification of Fc and C_3 receptors on human neutrophils. *J. Immunol. Methods.* **7:**69.
32. Bethesda Research Laboratories, Inc. 1982. Monoclonal antibody to human monocyte. Brochuer Molecular Diagnostics Division Bethesda Research Laboratories, Inc.
33. Gaudernack, G., R. Curry, and R. P. Messner. 1981. Rapid identification of human B lymphocytes and monocytes with rhodamine-labeled *Brucella melitensis*. *J. Immunol. Methods.* **43:**351.

34. Newman, S. L., R. A. Musson, and P. M. Henson. 1980. Development of functional complement receptors during in vitro maturation of human monocytes into macrophages. *J. Immunol.* **125:**2236.
35. Werb, Z., D. F. Bainton, and P. A. Jones. 1980. Degradation of connective tissue matrices by macrophages. III. Morphological and biochemical studies on extracellular, pericellular, and intracellular events in matrix proteolysis by macrophages in culture. *J. Exp. Med.* **152:**1537.
36. Mariano, M., T. Nikitin, and B. E. Malucelli. 1976. Immunological and non-immunological phagocytosis by inflammatory macrophages, epithelioid cells and macrophage polykaryons from foreign body granulomata. *J. Pathol.* **120:**151.
37. Ridley, M. J., D. S. Ridley, and J. L. Turk. 1978. Surface markers on lymphocytes and cells of the mononuclear phagocyte series in skin sections in leprosy. *J. Pathol.* **125:**91.
38. Wellhausen, S. R., and D. L. Boros. 1980. Comparison of Fc, C_3 receptors and Ia antigens on the inflammatory macrophage isolated from vigorous or immunomodulated liver granulomas of Schistosome-infected mice. *J. Reticuloendothel. Soc.* **30:**191.
39. Smith, B. R., and K. A. Ault. 1981. Increase of surface Ia-like antigen expression on human monocytes independent of antigenic stimuli. *J. Immunol.* **127:**2020.