Differentiation of Fibroblast-like Cells into Macrophages

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ABSTRACT

Differentiated cells with the morphological, enzymatic, antigenic, and functional characteristics of macrophages formed when a variety of nontransformed and transformed fibroblast-like mouse embryo cell lines were grown in a medium supplemented only with human plasma. Differentiated cells contained numerous lysosomes and phagosomes, nonspecific esterase and acid phosphatase activities, and cell surface la antigens and were capable of phagocytosis of iron particles. Differentiated cells were also growth arrested in the G1 phase of the cell cycle, but both growth arrest and differentiation were reversible processes. These observations suggest that cells with the morphology of fibroblasts have the capacity to undergo nonterminal differentiation into macrophages.

INTRODUCTION

Cellular differentiation is a process of stable biological diversification. During embryological development (13) and during the terminal differentiation of adult stem cells, differentiation is under precise regulation. The process of hematopoiesis clearly demonstrates the phenomena (9). In carcinogenesis, however, many tumors show evidence of aberrant cell differentiation (12). In fact, many tumors are composed of heterogeneous mixtures of cell types the histogenesis of which has been difficult to explain.

Fibrous histiocytoma (1) is one such class of tumors. Both benign (also designated subepidermal nodular fibrosis, giant cell tumor of tendon sheath, pigmented villonodular synovitis, xanthogranuloma, and postirradiation pseudosarcoma) and malignant (also designated dermatofibrosarcoma protubersans and fibroxanthoma sarcoma) tumors of this type are composed of admixtures of fibroblasts and histiocytes or macrophages. Attempts to explain the histogenesis of these tumors have resulted in 3 hypotheses (1, 18): (a) the tumors originate from primitive mesenchymal cells which have the capacity for dual differentiation toward histiocytes and fibroblasts; (b) the tumors are of fibroblastic origin but have the capacity to transform into macrophages; (c) the tumors are purely histiocytic but maintain the capacity to transform into "facultative fibroblasts" under certain conditions. Despite the fact that the latter hypothesis is most widely accepted, no experimental evidence has been presented to support any of these theories.

We now show that nontransformed and transformed fibroblast-like mouse embryo cells can be induced to differentiate into macrophages or histiocytes in vitro under pathophysiological conditions. Based on previous observations that these cell lines represent primitive mesenchymal cells, we suggest that tumors of the fibrous histiocytoma type are derived from mesenchymal stem cells which can differentiate into fibroblasts and into macrophages or histiocytes. We also suggest that other human disease states may be associated with the aberrant differentiation of mesenchymal stem cells into macrophages.

MATERIALS AND METHODS

Cell Culture. Nontransformed BALB/3T3 cells (clone A31) and methylcholanthrene- and SV40-transformed BALB/3T3 cells (MCA3T3, SV3T3) were obtained from Dr. George Todaro (Meloy Laboratories, Springfield, Va.). 3T3-T cells, a nontransformed proadipocyte line derived from BALB/3T3 cells (clone A31), were obtained from Dr. Leila Diamond (Wistar Institute, Philadelphia, Pa.). Nontransformed C3H/10T½ and a methylcholanthrene-transformed derivative (MCA/10T½) were obtained from Dr. Harold Moses (Mayo Clinic, Rochester, Minn.).

Stock cultures of cells were grown in 10% calf serum-Dulbecco's modified Eagle's medium (3T3, SV3T3, MCA3T3) or 10% fetal calf serum-Dulbecco's modified Eagle's medium (3T3-T, C3H/10T½) and maintained in a humidified 5% CO² atmosphere. All cell lines were shown to be free of Mycoplasma contamination by the Hoechst staining method (3). Differentiation of these cells was induced by culture in various blood products described below. Both control and experimental cell lines were maintained on a routine 4-day feeding schedule during the differentiation period. For selected studies, differentiated foci were removed from tissue culture plates by careful aspiration.

Preparation of Blood Products. Blood was obtained from healthy volunteers by venipuncture. The blood was immediately placed in 3.8% sodium citrate to prevent clotting. Human serum, platelet-poor plasma, and platelet-poor plasma-derived serum were also prepared from venipuncture blood specimens.

Human serum was prepared by dialysis of citrated plasma against phosphate-buffered saline (pH 7.4) containing 0.75 mM calcium and 0.5 mM magnesium ions. Platelet-poor plasma was made by centrifugation of citrated plasma at 25,000 x g for 30 min according to the procedures described by Ross et al. (16). Platelet-poor plasma-derived serum was prepared by dialysis of platelet-poor plasma against phosphate-buffered saline containing 0.75 mM calcium and 0.5 mM magnesium with removal of the clot by filtration (Whatman no. 1 filter paper; W. & R. Balston, Ltd., England). Heat-inactivated plasma was prepared by incubation at 60° for 1 hr.

Plasma collected in acid-citrate dextrose anticoagulant was also obtained from a therapeutic plasmapheresis of a patient with Goodpasture's disease. This plasma contained platelets and platelet products, including platelet-derived growth factors which were released as a result of platelet lysis during plasmapheresis. Results of experiments using normal human plasma and plasma from the patient with Goodpasture's dis-

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were used at 25 to 30% concentration (v/v) in Dulbecco's medium (Grand Island Biological Co., Grand Island, N. Y.) unless otherwise designated.

In selected experiments, the above-described medium was supplemented with one or more of the following agents: heparin, 15 units/ml (Panheparin, 1000 USP units/ml; Abbott Laboratories, North Chicago, Ill.); insulin, 50 μg/ml (Sigma Chemical Co., St. Louis, Mo.); epidermal growth factor, 10 ng/ml (Collaborative Research, Inc., Waltham, Mass.); fibrinogen, 0.5 or 1.0 mg/ml (Sigma); thrombin, 25 units/ml (Sigma); plasmin, 0.1 unit/ml (Sigma); or plasminogen, 0.1 unit/ml (Sigma).

Enzyme Cytochemistry. Acid phosphatase activity was performed according to a modification of the method described by Lillie (11). Briefly, a suspension of foci of differentiated cells or foci in situ were fixed in 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.4), rinsed, and incubated for 40 min in 40 mM β-glycerophosphate buffer (pH 7.0). They were then incubated at room temperature in 0.2% lead nitrate in 0.07 M Tris-maleate buffer, pH 5.0. Specimens were then rinsed in distilled water, followed by a 2% acetic acid and a 2% ammonium sulfide rinse. A brown lead sulfite precipitate indicated acid phosphatase activity. Acid phosphatase activity could be inhibited with NaF as previously described.

Nonspecific (naphthyl butyrate) esterase activity was assayed by the method of Li et al. (10). Studies were performed on both differentiated and undifferentiated cells. These specimens were trypsinized (0.25%) for 10 min at 37° and then allowed to adhere to glass slides prior to staining. Red cytoplasmic staining primarily localized to intracellular granules characterized nonspecific esterase-positive cells.

Antiserum. Antibodies reactive with either Ia or H-2 alloantigens were generously provided by Drs. Chella David and G. Garrison Fathman (Mayo Clinic). The reagents used in this study included anti-Ia (B10 × HT), anti-B10.A(5R), anti-H-2D<sup>+</sup> (CBA/J × B10), anti-B10.A(18R) (4). Normal BALB/c serum was used as a negative serum control. Fluoresceinated rabbit anti-mouse IgG antibody was purchased from Cappel Laboratories, Cochranville, Pa.

Indirect Immunofluorescence. Standard methodology was used for detection of indirect immunofluorescence indicating the presence of either Ia or H-2D<sup>+</sup> surface alloantigens on these BALB/c-derived mouse embryo cells using the alloantisera outlined above. Anti-H-2D<sup>+</sup> antibodies were used as positive controls, and mouse serum from normal BALB/c mice was used as the negative serum control. Cells to be tested were grown on glass coverslips in medium containing human plasma. After differentiated foci formed, the coverslips were rinsed in phosphate-buffered saline (0.136 M NaCl-0.003 M KCl-10 mM phosphate, pH 7.4), and each coverslip was incubated in one of the alloantisera listed above. Several different concentrations of each alloserum were utilized in each test. The cells and antibody were incubated at 37° for 30 min and then rinsed in phosphate-buffered saline to remove unbound antibody. The coverslips were then covered with a 1:10 dilution of the fluoresceinated rabbit anti-mouse immunoglobulin reagent and incubated for 30 min at 37°. Excess antibody was again removed by rinsing the coverslip with phosphate-buffered saline, and the coverslips were then inverted onto glass slides and examined under a fluorescence microscope. Cells which showed a pattern of ring fluorescence outlining the cell membrane were considered positive for the antigen in question.

Electron Microscopy. Differentiated foci were prepared for transmission and scanning microscopic examination by standard procedures. For transmission electron microscopy, differentiated foci were fixed in situ in 2.5% glutaraldehyde-0.2 M cacodylate buffer for 2 hr at 22°. The specimen was then rinsed and postfixed in 1% osmium tetroxide for 30 min. Specimens were dehydrated in a graded ethanol series of solutions. The specimens were over laid with Spurr, a low viscosity epoxy resin embedding medium for electron microscopy, and thick and thin sections were cut. Sections were stained with toluidine blue or uranyl acetate and lead citrate, respectively. A Philips 201 electron microscope was used to examine specimens by transmission electron microscopy. For scanning electron microscopy, specimens which were grown on glass coverslips were fixed in situ in glutaraldehyde as above, dehydrated in ethanol, and then critical-point dried in CO<sub>2</sub>. Specimens were shadowed with palladium and carbon and then examined in an ETEC scanning electron microscope.

Cell Cycle Analysis. Flow microfluorimetric analysis of the DNA content of individual cells was determined in cells of differentiated foci which were removed from tissue culture flasks by aspiration. Foci were pooled, and the cell aggregates were dispersed by trypsinization (0.25%) in Dulbecco's phosphate-buffered saline containing 0.1% EDTA. The dispersed cells were diluted with an equal volume of a solution containing DNase (0.01 mg/ml), soybean trypsin inhibitor (0.2 mg/ml), and bovine serum albumin (1.0 mg/ml) in phosphate-buffered saline (pH 7.4). Cells were incubated in this solution for 2 min and then sedimented by centrifugation at 100 × g for 10 min. The cell pellet was resuspended in 500 μl phosphate-buffered saline, and then 14.5 ml cold 70% ethanol were added. Cells were stained with mithramycin (100 μg/ml) and analyzed by flow microfluorimetry. Cell cycle distributions were determined by previously described methods (5).

Phagocytosis Studies. Undifferentiated 3T3 cells and differentiated 3T3 cells were assayed for their ability to phagocytose carbonyl iron particles as described by Falk et al. (6). Cells attached to the plastic substrate were washed twice in phosphate-buffered saline containing 0.75 mM Ca<sup>2+</sup> and 0.5 mM Mg<sup>2+</sup>. They were then cultured in Dulbecco's modified Eagle's medium containing 10% calf serum in which was suspended carbonyl iron, 10 mg/ml (Sigma). Cells were incubated with occasional gentle rocking at 37° for 1 hr. Thereafter, the undifferentiated cells were either scraped from the flask with a rubber policeman or trypsinized (0.25% for 30 min at 37°) and sedimented by centrifugation at 100 × g for 5 min. The cell pellet was then fixed with Trump's fixative. These studies on undifferentiated cells were performed both on low-density rapidly growing cells and on quiescent G<sub>i</sub>-arrested high-density cells with comparable results. Differentiated foci were processed similarly; however, individual foci were individually aspirated and pooled to concentrate them prior to centrifugation and fixation. The fixed specimens were processed as described above, and both thick sections stained with toluidine blue and thin sections stained with uranyl acetate and lead citrate were examined. Due to the presence of iron particles in the specimens, the quality of the sections was not ideal but was adequate to draw an unequivocal conclusion from the data. To validate the presence of iron within differentiated cells, the cells were analyzed by energy-dispersive X-ray microanalysis.
for heavy metals.

Measurements were made in a Philips 400 electron microscope equipped with a Kevex 30-sq mm silicone lithium-drifted detector (resolution, 145 eV) and interfaced with a Kevex 7000 multichannel analyzer. Accelerating voltage was 60 kV, beam current was 50 to 60 μA, and a beam spot size was 0.5 μm. Live counting time was 100 sec.

RESULTS

Dulbecco’s modified Eagle’s medium containing citrated human plasma was added to nontransformed 3T3, 3T3-T, or 10T½ cells or to transformed SV3T3, MCA3T3, or MCA/10T½ cells. As a result of activation of the clotting process by calcium ions in the medium, a plasma clot formed shortly after incubation at 37°. Thereafter, clot lysis was observed within 48 to 72 hr depending on the cell type. Approximately 5 days later, foci of tightly aggregated cells were observed. These aggregates, which were designated MF for reasons described below, measured approximately 0.1 mm in diameter (Fig. 1). Analysis of MF by scanning electron microscopy (Figs. 2 and 3) showed aggregates of cells containing numerous cell surface projections. Many MF appeared to be attached to the plastic dish only by a small stalk; others detached from the plate and were found free floating in the tissue culture medium.

Table 1 shows that the formation of MF occurred only when cells were incubated in human plasma. If cells were incubated in medium containing serum, in platelet-poor plasma-derived serum, or in heat-inactivated plasma, no MF were observed. Heparin (15 units/ml) anticoagulation of the plasma also prevented MF formation. In addition, MF were not observed in medium containing calf serum or fetal calf serum. Addition of insulin, epidermal growth factor, thrombin, plasminogen, fibrinogen, or combinations of these factors at various concentrations to human plasma did not increase or decrease the quantity of MF formed.

Light microscopy demonstrated that cells in MF contained numerous cytoplasmic granules and large dense bodies (Fig. 4). Transmission electron microscopy demonstrated that these granules were enclosed by a unit membrane and contained either homogeneous material or membrane fragments. Granules were morphologically similar to lysosomes (Figs. 5 and 6). Large dense bodies consisting of what appear to be fused lysosomes resembling phagolysosomes or autophagosomes were also observed. Acid phosphatase cytochemical staining established that many of these granules and phagolysosomes in cells of MF contained acid phosphatase activity (Fig. 7; Table 2). By contrast, significant acid phosphatase activity was not detected in undifferentiated cells. Acid phosphatase activity in MF was completely inhibited by NaF (data not shown).

Cells in MF derived from nontransformed 3T3-T cells also contained nonspecific esterase activity, an enzyme marker characteristic of macrophages (Fig. 8) (8). MF formed from transformed SV3T3 and MCA3T3 cells, however, stained only poorly for this enzyme. Undifferentiated nontransformed 3T3 or 3T3-T cells did not stain (Table 2).

Levels of cell surface la antigens. Both cells in MF and undifferentiated cells, however, did stain with antibodies to H-2Dd histocompatibility antigens which they share in common. Fig. 9 demonstrates ringed fluorescence produced by anti-la7 on MF cells.

Flow microfluorimetric analysis showed that a high proportion of cells in MF were growth arrested in the G1 phase of the cell cycle (Chart 1; Table 3), and the percentage of cells in G1 by this assay represents a low estimate of the actual quantity of G1 cells because approximately 10% of the cells assayed by the flow microfluorimetric analysis method were morphologically undifferentiated cells. Differentiation of mouse embryonic cells into MF and the associated process of G1 arrest were found to be reversible if MF were trypsinized and then replated in medium containing either 10% fetal calf serum or 10% calf serum. Loss of differentiation characteristics and the reinitiation of cell growth were observed over a 3-day interval following this treatment.

The data presented in Figs. 10 to 12 show that the cells in the differentiated foci also function as macrophages. They show the ability to phagocytose carbonyl iron particles. By contrast, undifferentiated 3T3 cells whether growing or non-growing at high or low cell density did not show significant phagocytic activity. Fig. 10 illustrates the appearance of undifferentiated 3T3 cells which had been incubated with iron, trypsinized, and then fixed. No evidence of significant phagocytosis is apparent. Comparable results were obtained when

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The abbreviation used is: MF, macrophage foci.
The majority of cells are in the G1 phase of the cell cycle. This chart is representative of the data derived on other cell types presented in Table 3.

Table 3
Flow microfluorimetric analysis of cell cycle distribution of undifferentiated and differentiated cells

<table>
<thead>
<tr>
<th>Cell cycle distribution (%)</th>
<th>G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontransformed cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3T3-T, undifferentated*</td>
<td>54</td>
<td>38</td>
<td>8</td>
</tr>
<tr>
<td>3T3-T, differentiated</td>
<td>80</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Transformed cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCA3T3, undifferentated</td>
<td>50</td>
<td>35</td>
<td>15</td>
</tr>
<tr>
<td>MCA3T3, differentiated</td>
<td>80</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>SV3T3, undifferentated</td>
<td>52</td>
<td>36</td>
<td>12</td>
</tr>
<tr>
<td>SV3T3, differentiated</td>
<td>75</td>
<td>16</td>
<td>9</td>
</tr>
</tbody>
</table>

* Undifferentiated cells were examined during the state of exponential growth.

differentiated foci. Cells in these foci differed from adjacent undifferentiated cells in that they contained numerous cytoplasmic granules, nonspecific esterase and acid phosphatase activity, and a cell surface antigens. The data also show that the differentiated cells have phagocytic activity and store phagocytosed carbonyl iron particles in cytoplasmic phagolysosomes. The majority of these macrophage-like cells also had a DNA content characteristic of cells arrested in the G1 phase of the cell cycle. Both growth arrest in G1 and expression of the differentiated phenotype were shown to be reversible processes.

These results represent the first experimental evidence that macrophages or histiocytes can be derived from cells with a fibroblast morphology. The possibility that macrophages can be derived from fibroblast-like "primitive mesenchymal reticular cells" in the bone marrow (14) and even from adipocyte-like cells similar to 3T3-T proadipocytes has, however, been proposed (9). Whether benign and malignant fibrous histiocytomas, which consist of a mixture of fibroblasts and histiocytes, are derived from primitive mesenchymal cells which can differentiate into both fibroblasts and histiocytes or whether fibroblast or histioyte precursor cells in these tumors have the capacity to differentiate into the complementary cell type remains to be determined.

Our results suggest that macrophages can indeed be derived from mesenchymal precursor cells with the morphology of fibroblasts in an experimental model system. The conclusion that nontransformed 3T3 and related mouse embryo cells represent such multipotential mesenchymal precursor cells is the undifferentiated cells were removed from the flask by scraping. In contrast, Fig. 11 shows that cells from differentiated foci contain large black inclusions within the cytoplasm in apparent phagosomes. Fig. 12 establishes that these particles are within the cell, and energy-dispersive X-ray microanalysis establishes that this material is iron (Chart 2).

**DISCUSSION**

Nontransformed 3T3 and related mouse cell lines which have the morphology of fibroblasts have been shown in this paper to be capable of differentiation into cells with morphological, enzymatic, antigenic, and functional characteristics of macrophages. Culture of these cells in tissue culture medium supplemented only with human plasma resulted in the formation of...
supported by several recent studies. Clones of nontransformed mouse embryo cells have been isolated which possess the potential to differentiate into mature adipocytes (7), and transformation of such cells has been shown to result in their differentiation into cells with the characteristics of cartilage, hemangiopericytes, and possibly endothelial cells (2, 19). The observation that both nontransformed and transformed cells can differentiate into various cell types, including macrophages, is compatible with numerous in vitro and in vivo studies which show that malignant transformation is not necessarily associated with a loss of the ability to differentiate rather with aberrant differentiation.

We suggest that differentiation of mesenchymal precursor cells into macrophages may also occur in various disease processes. For example, in atherosclerosis it has long been known that foam cells are present in so-called ‘fatty streaks.’ These are structures which may precede coronary artery atherosclerosis (15). It has been debated whether these cells are derived from circulating macrophages that migrate into the arterial wall or whether they are derived by conversion of intimal fibroblasts or myoblasts into macrophage-like cells (15). The results of our studies raise the possibility that foam cells and macrophages in atheromatous plaques may be derived from a common mesenchymal precursor cell type which is localized to the arterial wall.

Two important questions are relevant to the observation that mesenchymal precursor cells may be capable of differentiating into macrophages. What extrinsic factors influence the differentiation of macrophages from mesenchymal precursor cells, and what are the cellular mechanisms which control macrophage differentiation?

Macrophage differentiation in our studies occurred only when 3T3 and related cells were cultured in medium containing human plasma. Human serum, calf serum, and fetal calf serum did not promote differentiation. Furthermore, heparinized or heat-inactivated human plasma also failed to promote differentiation. This suggests that specific plasma factors may promote macrophage differentiation. Interestingly, we were unable to mimic the induction of macrophage differentiation with a variety of purified or partially purified plasma components.

It is, however, possible that proteolysis of plasma proteins might yield polypeptides which are differentiation-promoting agents. This is so because the appearance of differentiated foci is chronologically related to fibrinolysis of the plasma clot which overlies the cell monolayer. Immediately following addition to cell monolayers of medium containing human plasma, a plasma clot formed. Two to 4 days later, clot lysis occurred as a result of fibrinolysis. This liquid medium was typically decanted, and fresh medium containing plasma was again added. Coincidental with the lysis of the second plasma clot, differentiation occurred. This suggests that labile plasma components released during fibrinolysis might indeed have a specific metabolic effect which induces the differentiation process, but it has not been possible to identify these molecules as yet. It is also possible that activation of the fibrinolytic system could affect the adherence properties of the cells and their shape. This could directly induce the differentiation response. We have preliminary data which suggest that the culture of 3T3 cells on nonadherent surfaces can in fact induce morphological changes in the cells which are similar to the macrophage foci described in this paper. A major goal of subsequent studies will be to determine the actual mechanism(s) responsible for the induction of macrophage differentiation.

Recent studies in our laboratory have also established that cellular differentiation is coupled to growth arrest at a distinct state in the G0 phase of the cell cycle which we have designated G0 (17). We showed that differentiation of proadipocyte cell lines occurs only following growth arrest at G0 and not following growth arrest at other states in the G1 phase of the cell cycle. The data presented in this paper that macrophage foci contain a 2N content of DNA characteristic of G0-arrested cells raise the interesting question of whether macrophage differentiation is also regulated by specific metabolic events that occur at a G0-like state.

Note Added in Proof

We have established recently that cells which undergo shape changes induced by culture on nonadherent surfaces do not have the characteristics of macrophages.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Phase-contrast micrograph of C3H/10T1/2 macrophage focus and the surrounding monolayer of undifferentiated cells. × 250.

Fig. 2. Scanning electron micrograph of SV3T3 macrophage focus showing a compact mass of round cells distinct from the monolayer. × 380.

Fig. 3. High-magnification scanning electron micrograph of MCA3T3 macrophage focus demonstrating polymorphic cell surface projections. × 2,520.

Fig. 4. Light microscopic micrograph of thick section of MCA3T3 macrophage focus cut perpendicular to the plate showing that differentiated cells contain numerous dark pleomorphic cytoplasmic granules (arrows) which are primarily located in the center of the focus and are rarely seen in the outermost cell layers. × 200.

Fig. 5. Transmission electron micrograph of 3T3-T cells in a macrophage focus showing numerous cytoplasmic granules varying in size, shape, and electron density and large phagolysosomes-autophagic vacuoles (×). × 4,500.

Fig. 6. High-magnification electron micrograph of cytoplasmic granules showing that they are membrane bound and contain various membrane fragments. × 37,500.

Fig. 7. Cytochemical demonstration of acid phosphatase activity in 3T3-T cell in macrophage foci. Small arrows, lysosomes; large arrows, phagolysosomes-autophagic vacuoles. × 900.

Fig. 8. Dispersed cells of 3T3-T macrophage focus showing a positive staining reaction for maphthyl acetate esterase (solid arrows) in contrast to undifferentiated cells (dashed arrows). The positive reaction in fact was indicated by bright red cytoplasmic staining. × 400.

Fig. 9. Fluorescent micrograph of MCA3T3 macrophage focus stained with anti-la7 alloantisera. Cells with la antigen are outlined by a ring of fluorescence (arrows). × 500.

Fig. 10. Specimen of undifferentiated 3T3 cells processed as described in Fig. 11 showing no evidence of iron particles within the cytoplasm. × 1,680.

Fig. 11. Light micrograph of a toluidine blue-stained thick section of Spurr-embedded 3T3 cells from a macrophage focus which had been incubated with carbonyl iron. Large quantities of dense material, i.e., iron, are present in the cytoplasm. × 1,680.

Fig. 12. Transmission electron micrograph of a differentiated macrophage that contains a large cuboidal dense particle shown in Chart 2 to be iron. A and B, sites in this cell which were analyzed for the presence of iron by energy-dispersive X-ray microanalysis. × 10,800.
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