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Multinucleated Giant Cells Generated in Vitro

Terminally Differentiated Macrophages With Down-Regulated c-fms Expression

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Although multinucleated giant cells (MGCs) are a known feature of granulomatous reactions, little is known about their destination and function. In this study human blood monocyte (BM)-derived giant cells were generated by lymphokine stimulation *in vitro*. Their immunophenotype and ultrastructural morphology resembled that of MGCs occurring *in vivo*. Mitotic activity within MGCs could not be established either *in vitro* or *in vivo*. Enzyme equipment of MGCs was elevated in comparison with monocyte-macrophages. In comparison with unfused monocyte-macrophages, MGCs did not reveal a higher level of interleukin-1 production or cytostatic activity. They showed, however, a 20–30-fold increase in the production of oxygen-free radicals in response to zymosan. Transcription of the proto-oncogene c-fms was enhanced in short-term cultivated BM and was rapidly down-regulated in MGCs after fusion had occurred. It is concluded that MGCs represent highly stimulated cells of monocyte-macrophage lineage at a terminal stage of maturation. (Am J Pathol 1988, 130:232–243)

SINCE Langhans[1] described multinucleated giant cells (MGCs) as a common feature of tuberculosis granulomas, MGCs have been observed in many other diseases that led to granulomatous reactions, such as leprosy and listeriosis, foreign body reactions, and in other pathologic states of unknown etiology, such as cancer,[2,3] rheumatoid arthritis and sarcoidosis.[4] Recently, Budka recognized MGCs of possible histiocytic origin in brain tissue of patients with acquired immune deficiency syndrome.[5]

The origin of MGCs has been reported in several investigations.[6–11] Lymphokines produced by antigen- or mitogen-stimulated lymphocytes and interferon have been shown to lead to polykaryon formation of macrophages.[9,12,13] The exact functional significance of macrophage-forming syncytia with up to 100 nuclei, however, is still unclear.[10,11] It has been suggested that they function as antigen-presenting cells,[11] that they represent "activated" macrophages,[10,13] or that they are merely a disposal device for metabolically exhausted macrophages.[14]

In the present study, MGCs were produced *in vitro* by lymphokine stimulation of cultured human blood monocytes (BMs) and compared with MGCs occurring *in vivo* with special reference to the immunophenotype. The functional properties of MGCs generated *in vitro* were investigated, including interleukin-1 production, cytostatic activity, generation of oxygen free radicals, and expression of macrophage-specific growth factor receptor. MGCs are considered to be monocyte-macrophage derivatives at a final stage of maturation.

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Materials and Methods

Cell Separation and Culture

Separation of Normal Human BM, Peritoneal Macrophages (PMs), and Alveolar Macrophages (AMs)

Fresh, heparinized (40 IU/ml) venous blood of healthy volunteers was subjected to density gradient (d = 1.077 g/ml) centrifugation with Ficoll-Urografin (Pharmacia, Uppsala, Sweden; Schering, Berlin, FRG) as described elsewhere.[15] The mononuclear cell fraction of the interphase was separated into lymphocytes and BMs by glass adherence.[16] Resident PM (n = 5) were obtained from young sterile women undergoing diagnostic pelviscopy. Normal effusions of peritoneal cavity (10–50 ml) were aspirated from the Douglas pouch.[17] AMs (n = 5) were obtained from fresh human lungs ectomized because of bullous emphysema or small peripheral carcinoma of bronchus. The bronchial system was rinsed twice with physiologic saline, and AMs obtained from the interphase after density gradient centrifugation.[18] Viability of separated cells was checked by trypan blue exclusion. Purity control was performed on cytospin preparations by Pappenheim (May–Grünwald–Giemsa) staining and enzymecytochemical staining for α-naphthyl acetate esterase.[17,18] Only cell samples with viability and purity of greater than 90% were used for further investigations.

In Vitro *Stimulation of BMs*

Generally, cells were cultured at 37 C in a humidified atmosphere containing 5% CO_2, in RPMI 1640 medium (Serva, Heidelberg, FRG) supplemented with 10% fetal calf serum (Boehringer, Mannheim, FRG) in 75-sq cm culture vessels (Falcon, Oxnard, Calif). Lymphocytes were seeded in a density of 5×10^6 cell/ml and stimulated over 48 hours by addition of 30 μg/ml concanavalin A (Serva, Heidelberg, FRG). Cell-free lymphokine-rich supernatant was stored at −70 C or immediately given to monocyte cultures (5×10^5 to 1×10^6 cells/ml). Unless stated otherwise, monocytes were cultured for 48 hours before addition of the lymphokine-rich supernatants. In some experiments BMs were cultured with 1×10^{-9} M 12-0-tetradodecanoyl-phorbol-13-acetate (TPA, Sigma Chemical Co., St. Louis, Mo) before the addition of lymphokine-rich supernatant. Formation of MGCs was checked with an inverted microscope (Zeiss, Oberkochen, FRG) during the next 24–48 hours. After this period MGCs attaching to the bottom of the culture vessel were harvested by gentle scraping with a rubber policeman. Supernatant conditioned by MGCs was obtained as follows: after removal of nonadherent cells, MGCs were counted invertmicroscopically, and medium was added to a final cell density of $2.5–3 \times 10^4$ cells/ml; subsequent culture lasted 24 hours. Conditioned supernatants of BM transformed into cultured macrophages (referred to in the following as unfused macrophages or as cultured BMs) were prepared in the same manner, except that cells were harvested prior to production of supernatant so that we could count them and were seeded in a density approximately 20-fold of that applied for MGC culture. This ratio of MGCs to unfused macrophages was chosen arbitrarily, because, on the average, 20 nuclei could be observed per MGC.

Immunophenotyping of MGCs

For immunophenotyping we used the monoclonal antibodies (MAbs) Ki-M1, Ki-M2, Ki-M3, Ki-M4, Ki-M6, and Ki-M8, which were established by immunizing female BALB/c mice with purified lysosomes of the human histiocytic cell line U-937 cells or cell suspensions of human lymph nodes, then by fusing murine spleen cells with the nonsecreting myeloma line 63-Ag8.653 as described elsewhere.[19] These antibodies show a restricted reactivity to the monocyte-macrophage lineage.[20] Thus, Ki-M1 reacts with BMs, macrophages, and interdigitating reticulum cells (IRCs),[21] whereas Ki-M4 selectively stains dendritic reticulum cells (DRCs; also termed follicular dendritic cells) and a small subset of BMs.[22] Ki-M6 and Ki-M8 bind antigens intracytoplasmically localized in monocytes and macrophages of various tissues.[23,24] In addition, the MAb OKM1[25] (Ortho Pharmaceuticals, Raritan, NJ) was applied recognizing a monocyte-macrophage related antigen. T-lymphocyte-specific MAbs used were OKT4, OKT8, and OKT11 (Ortho[26]), whereas To15[27] served as B-lymphocyte-specific reagent. For evaluation of mitotic activity of MGCs, we applied the MAb Ki67, which defines a nuclear localized antigen selectively expressed by proliferating cells during S, G_2, and M phases of the cell cycle.[28]

Tissues of sarcoidosis (n = 3), foreign body reactions (n = 3), and giant cell epulis (n = 1) were deep frozen in nitrogen, and cryostat sections were prepared for immunohistochemical analysis. Immunostaining of cytospin preparations of separated cell populations and of tissue sections was done according to the method of Stein et al,[29] with peroxidase-conjugated rabbit anti-mouse Ig diluted 1 : 10 in PBS, supplemented with 2% heat-inactivated normal human serum (Dako, Copenhagen, Denmark) and peroxidase-conjugated goat anti-rabbit IgG diluted 1 : 10 in PBS (Medac, Hamburg, FRG) as secondary and ter-

tiary antisera, respectively. After visualization of peroxidase activity with 0.06% diaminobenzidine (Walter, Kiel, FRG) and 0.01% H_2O_2 in PBS, preparations were counterstained with hematoxylin and mounted with glycerine gelatin.

Electron Microscopy

Cell suspensions were fixed in 5% glutaraldehyde for 30 minutes, postfixed in osmium tetroxide for 1 hour, dehydrated, and embedded in araldite. Ultrathin sections were contrasted with uranyl acetate and lead citrate and investigated in a Siemens Elmiscop 101 (Siemens, Berlin, FRG).

Functional Analysis

Enzymecytochemistry

Cytospin preparations were used for visualization of α-naphthyl acetate esterase, acid phosphatase, and tartrate-resistant acid phosphatase as described elsewhere.[30]

Assay for Interleukin-1

Single-cell thymocyte suspensions from female C3H/HeJ mice (6–10 weeks old) in a density of 2.5×10^6 cells/ml were cultured for 72 hours in RPMI 1640 medium supplemented with 5% fetal calf serum, 2.5×10^{-5} M 2-mercaptoethanol (Sigma) and 2 μl/ml phytohemagglutinin A (PHA; Wellcome, Dartford, England) in 96-microwell trays (200 μl/well; Nunc, Roskilde, Denmark) with conditioned supernatant of cultured BMs and MGCs in final dilutions of 10% and 1%. Eight hours before the end of the incubation period thymocyte proliferation was assessed by labeling with 0.2 μCi/well ^{3}H-thymidine (20 Ci/mmol; Amersham, Braunschweig, FRG). Cells were collected with a cell harvester (Cambridge Technology, Cambridge, Mass), and incorporated radioactivity was measured in a Beckman scintillation counter (Beckman, Berkely, Calif). Supernatants of LPS stimulated (50 μg/ml; Difco, Detroit, Mich) freshly isolated mononuclear cells (5×10^6/ml for 24 hours) were used as interleukin-1 standard.

Assay for Cytostatic Activity

Three human cell lines were used as target cells: K-562 cells were the generous gift of Dr. Anderson (Kiel); BJAB cells derived from a Burkitt lymphoma were the generous gift of Prof. Wolf, Munich; and HeLa cells were received from the American Tissue Type Collection. Cells in logarithmic phase of growth were seeded in varying concentrations (1×10^5/ml to 5×10^5/ml) in 96-microwell trays and cultured for 30 hours in conditioned medium of cultured BMs and MGCs (50% final dilution) or cocultured for the same period with MGCs (5×10^4/ml) or cultured BM (1×10^6/ml). During the final 6 hours of incubation ^{3}H-thymidine labeling was performed followed by cell harvesting and scintillation counting as described above for the interleukin-1 assay.

Assay for Chemiluminescence Response

Five hundred microliters of cell suspensions in RPMI 1640 were pipetted into dark adapted vials of a Biolumate LB-9505 (Berthold, Wildbad, FRG). To enhance chemiluminescence, 50 μl of Luminol (2×10^{-17} M, Sigma) were added. To initiate phagocytosis, 2 mg zymosan from Saccharomyces cerevisiae (in 50 μl, Sigma) was added. Chemiluminescence was registered immediately at 37 C. Counts per minute were related to the cell number and plotted by an interface-connected Apple calculator (Apple, Calif).

Northern Blot Analysis of Proto-oncogene c-fms

Total cellular RNA was purified under ribonuclease-inactivating conditions by the guanidine thiocyanate-cesium chloride method[31] and further analyzed by electrophoresis of 10 μg RNA through 1.2% agarose formaldehyde gels followed by Northern Blot transfer to coated nylon membranes (Gene Screen, New England Nuclear, Boston, Mass).[32] The 1.5 kb Pst I fragment of the cloned v-fms gene pSM3[33] was isolated by preparative gel electrophoresis and nick-translated with ^{32}P-labeled desoxycytidine triphosphate (3000 Ci/mmol; Amersham, Braunschweig, FRG) to specific radioactivities of $2–4 \times 10^8$ cpm/μg DNA. Membranes were prehybridized at 42 C for 24 hours in buffer consisting of 50% formamide, 5 × SSC, 1% sodium dodecyl sulfate (SDS), 2× Denhardt's solution, 25 mM phosphate buffer (pH 7.0), and 200 μg/ml salmon sperm DNA. The RNA blots were hybridized for 24–48 hours at 42 C with 1×10^7 cpm nick-translated probes per milliliter hybridization buffer (the same as the prehybridization buffer). After hybridization, the blots were washed twice in 2 × SSC and 0.5% SDS for 5 minutes at room temperature and twice in 0.1 × SSC and 0.5% SDS for 30 minutes at 55 C. After drying blots were exposed to X-ray films (Kodak XAR 5) with an intensifying screen at −70 C. The integrity of RNA samples analyzed was checked by methylene blue staining of the nylon membranes and evaluation of the 28S/18S ratio.[34]

Results

Formation of MGCs

MGCs appeared within 24–48 hours after addition of lymphokine-rich supernatants to cultures of adherent BMs. Obviously, the readiness of BMs to fuse into polykaryons increased during the first days of culture, because more MGCs could be obtained from BMs cultured for 2 days than from freshly isolated BMs (Figure 1). On the average, 80–90% of stimulated BMs were multinucleated after 48 hours, when preculture lasted between 2 and 4 days. In some experiments up to 150 nuclei could be observed in the syncytia, most of the MGCs showing between 10 and 50 nuclei (Figure 2). On the average, MGCs harbored about 20 nuclei. Figure 3 gives typical examples of MGCs obtained after *in vitro* stimulation of BMs. Interestingly, formation of MGCs could be almost completely inhibited when BMs were exposed to 1×10^{-9} M TPA prior to lymphokine stimulation (Figure 1). The ability of cultured BMs to generate MGCs in response to lymphokine stimulation was also reduced after 3 weeks of culture (Figure 1).

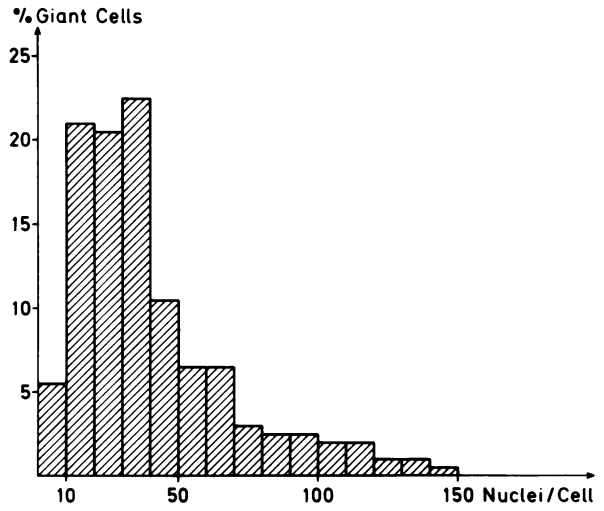

Figure 2—Nuclei of MGCs were counted 48 hours after addition of lymphokine rich supernatant. Some MGCs contained more than 100 nuclei, and more than 50% harbored between 10 and 40 nuclei.

Immunophenotype of MGCs *in Vitro* and *in Vivo*

Antigen Profile

The reaction patterns of the applied MAbs are summarized in Table 1. In comparison with freshly isolated BMs and BMs cultured for 2 days, MGCs elicited *in vitro* exhibited a reduced reactivity with MAbs Ki-M1 and Ki-M2, whereas they were strongly stained by Ki-M6 (Figure 4). In this they resembled MGCs found *in vivo* (Figure 4), which, however, completely lacked positivity for Ki-M2 in most cases. A comparably strong reactivity for Ki-M6 was only observable in long-term-cultured BMs and AMs (Table 1). Neither the MGCs elicited *in vitro* nor the MGCs appearing *in vivo* in granulomas and foreign body reactions revealed a positive staining pattern for Ki-M4, which selectively recognizes DRCs presumed to be the accessory cells of B-cell immune response. Lymphocytic markers (OKT4, OKT8, OKT11, and To15) could not be detected on MGCs. T cells surrounding the MGCs were constantly observed *in vitro* (Figure 5).

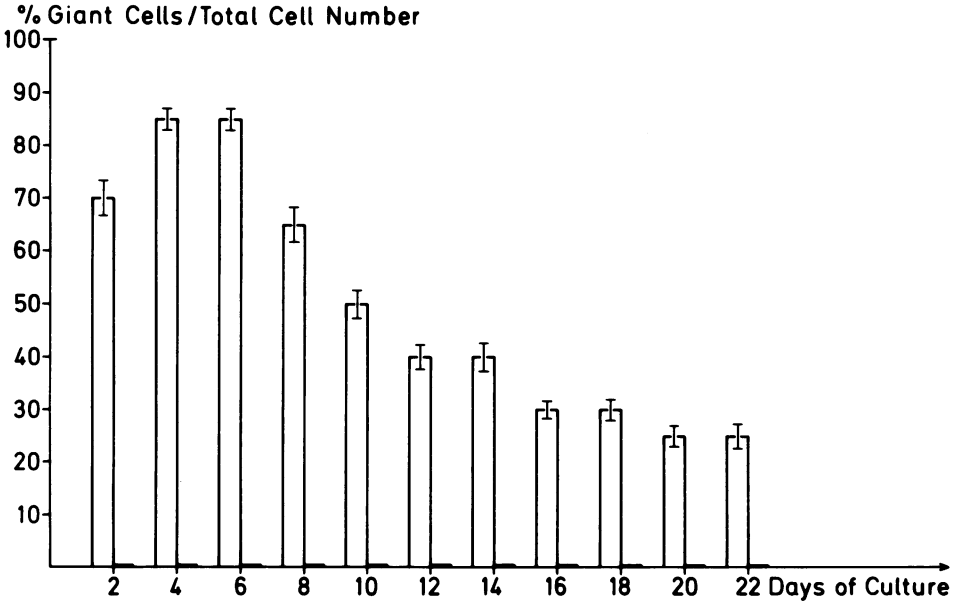

Figure 1—Multinucleated giant cells with more than three nuclei per cell were counted 48 hours after addition of lymphokine-conditioned supernatant. The highest yield of MGCs could be observed when BMs were cultured for 2–4 days prior to addition of supernatant. Total culture times consisting of preculture and 48-hour stimulation are given. Preculture with medium containing TPA (1×10^{-9} M) almost completely blocks giant cell formation (*black columns*).

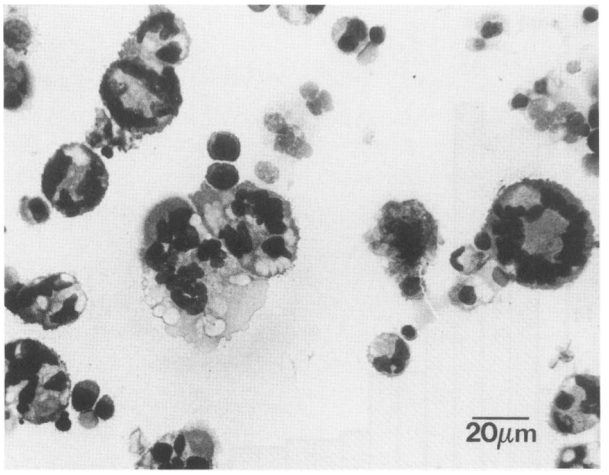

Figure 3—Typical MGCs obtained *in vitro* 48 hours after stimulation of cultured BMs with lymphokine-conditioned supernatant. May–Grünwald–Giemsa. (Cytospin preparation, ×500)

Mitotic Activity of MGCs in Vitro *and* in Vivo

In order to clarify whether giant cells originate by nuclear division without cytoplasmic division (endomitosis), we looked for the nuclear expressed Ki67 antigen, known to be associated with proliferation. We chose this immunocytochemical procedure because it allows evaluation *in vitro* and *in vivo*. As shown in Figure 6, Ki67 positivity was missing in nuclei of MGCs *in vitro* as well as *in vivo*, which indicated that MGCs were not undergoing mitosis or endomitosis. Some nuclei of surrounding lymphocytes showed a regular positive staining with Ki67 (Figure 6).

Ultrastructural Morphology of MGCs

Besides revealing the confirmation of true polykaryons, electron microscopy of MGCs generated *in vitro* revealed a close ultrastructural simularity with Langhans' giant cells of tuberculosis and other granulomatous inflammations.[35–37] Many round nuclei with small nucleoli and thin rims of heterochromatin were found lying next to the outer cell membrane, which appeared to be corrugated by long processes. The cytoplasm contained many lysosomelike dense bodies and electron-lucent vacuoles (Figure 7). Occasional phagocytosed cells could be observed (Figure 7).

Functional Activities

Enzyme Equipment of Multinucleated Giant Cells

In comparison with BMs and short-term-cultured unfused macrophages, MGCs exhibited an enhanced activity for α-naphthyl acetate esterase, acid phosphatase, and acid phosphatase tartrate-resistant. The latter could also be observed in long-term-cultured BMs and alveolar macrophages, which suggests that this enzyme can be looked upon as a criterion of maturity (Table 2).

Interleukin-1 Production

The capacity of conditioned supernatant to stimulate ^{3}H-thymidine incorporation of mouse thymocytes treated with a suboptimal dose of PHA can serve as a criterion for its content of interleukin-1.[38] Supernatants conditioned by MGCs over a period of 24 hours enhanced ^{3}H-thymidine incorporation of mouse thymocytes when compared with unconditioned medium. Interleukin-1 activity of supernatants obtained from cultures of unfused macrophages, however, reached a comparable level. Neither MGCs nor unfused macrophages exhibited the interleukin-1 activity of LPS-treated interphase cells (IL-1

Table 1—Immunophenotyping of Multinucleated Giant Cells *in Vivo* and *in Vitro* in Comparison With Resident Macrophages and Cultivated Monocytes

	Multinucleated giant cells (granuloma)	Multinucleated giant cells (*in vitro*)	Blood monocytes	Blood monocytes (cultured 2 days)	Blood monocytes (cultured 3 weeks)	Peritoneal macrophages	Alveolar macrophages
Ki-M1	−/(+)	−/(+)	+	++	−/(+)	++	+
Ki-M2	−	−/(+)	+	+	−/(+)	++	−
Ki-M3	+	+	+	+	−	−	−
Ki-M4	−	−	0,1%*	−	−	−	−
Ki-M6	++	++	+	+	++	+	++
Ki-M8	+	+	(+)	+	++	+	++
OKM1	+	+	+	+	+	+	+

*Percentage of nonadherent blood monocytes; (+), only faintly and inconstantly stained; + − + +, degrees of immunoreactivity as revealed by microscopic evaluation.

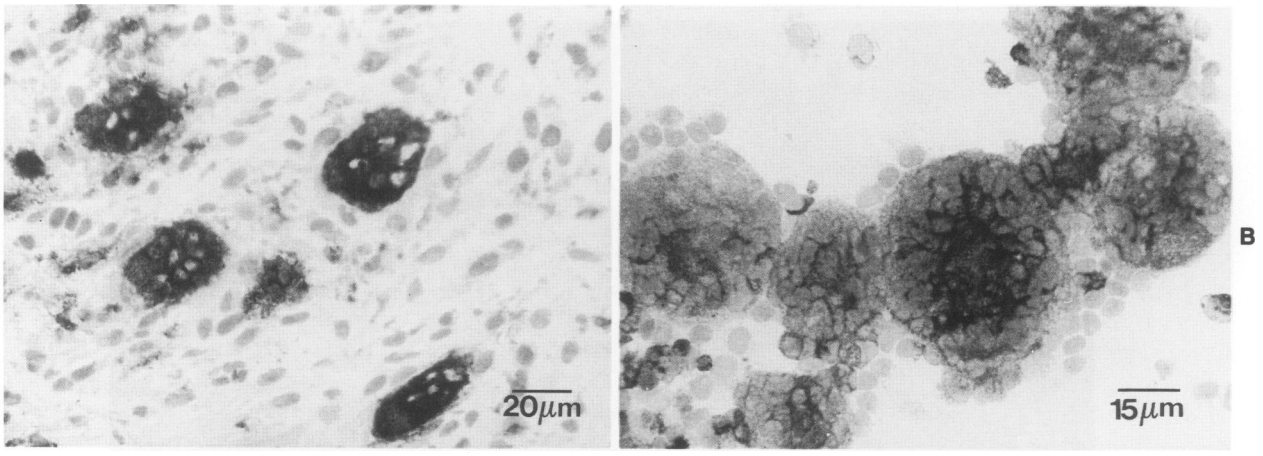

Figure 4—MGCs occurring in a giant cell epulis (**A**) and MGCs obtained by stimulating cultured BMs with lymphokine-rich supernatants (**B**) strongly express the monocyte-macrophage-specific antigen recognized by the MAb Ki-M6. Immunoperoxidase reaction. (**A**, cryostat section, ×750; **B**, cytospin preparation, ×500)

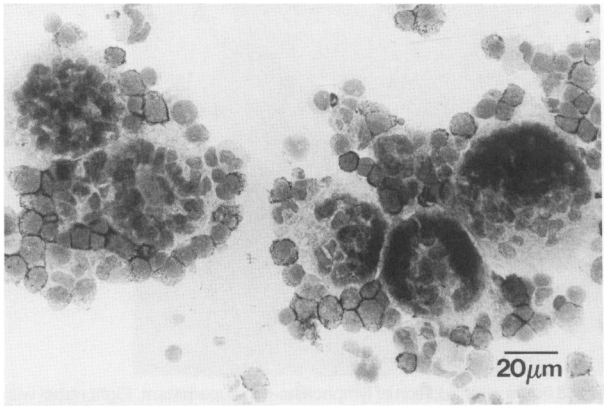

Figure 5—MGCs obtained by *in vitro* stimulation of cultured BMs with lymphokine-rich supernatants do not express the OKT-11 antigen, which is positive in the surrounding lymphocytes. Immunoperoxidase reaction. (Cytocentrifuge preparation, ×500)

standard) that showed an almost undiminished activity at a dilution of 1 : 100 (Table 3).

Cytostatic Activity

Two tests using three different transformed cell lines of human origin were performed to measure the possible cytostatic activity of cultured MGC. Tables 4 and 5 summarize the data obtained by culture of target cells in MGC-conditioned medium and by coculture of target cells and MGCs, indicating factor-bound and cell-mediated cytostatic activity, respectively. No significant differences in cytostatic activity between unfused macrophages and MGCs could be established in either assay. On the contrary, occasionally an enhancement of ^{3}H-thymidine incorporation of target cells occurred.

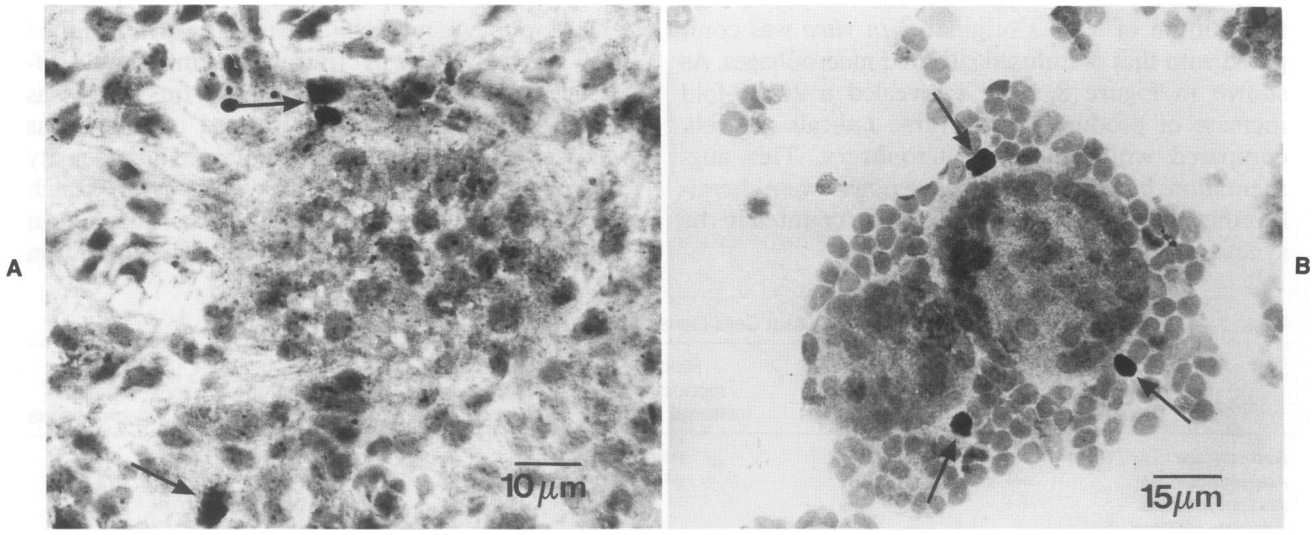

Figure 6—The nuclear expressed antigen recognized by the MAb Ki-67 is associated with cell proliferation. This antigen is not expressed by MGCs *in vivo* (**A**) or *in vitro* (**B**). *Arrows* indicate positively stained lymphocytes. (Immunoperoxidase reaction, **A**, cryostat section, ×750; **B**, cytocentrifuge preparation, ×1000)

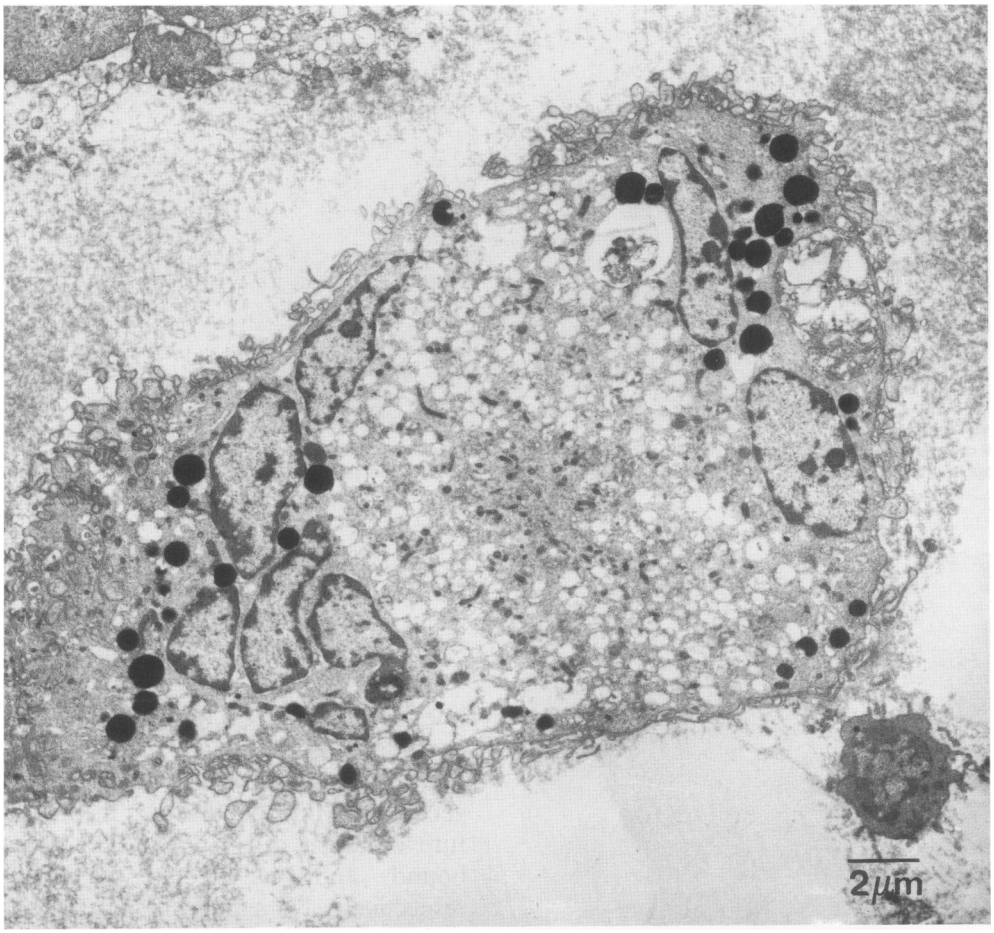

Figure 7—Electron micrograph of an MGC generated by stimulation of cultured BMs 48 hours after addition of lymphokine-rich supernatant. Eight nuclei with small nucleoli and narrow rims of heterochromatin, a ruffled outer cell membrane, and lysosome-like dense bodies in the cytoplasm can be seen. (Glutaraldehyde fixation, uranyl acetate and lead citrate counterstaining, ×5000)

Chemiluminescence Response

The generation of oxygen free radicals in response to zymosan of MGCs originated *in vitro* was compared with that of unfused cultured macrophages. As shown in Figure 8, MGCs revealed a 20–30-fold increase of produced oxygen-free radicals per cell, compared with unfused macrophages. This augmentation, however, could not be noted when chemiluminescence was related to the nuclei counted in the syncytia.

Northern Blot Analysis of Proto-oncogene c-fms

When equal amounts of total cellular RNA of different monocyte-macrophage subsets were examined for c-fms mRNA by hybridization, considerable differences could be noted (Figure 9). c-fms RNA was clearly detectable in freshly isolated monocytes as well as in cells of the interphase obtained after density centrifugation (monocytes and lymphocytes), which indicates that c-fms expression was constitutive in BMs and did not result from artificial stimulation

Table 2—Enzymecytochemistry of Multinucleated Giant Cells Generated *in Vitro*

	Multinucleated giant cells	Blood monocytes	Blood monocytes (cultured 2 days)	Blood monocytes (cultured 3 weeks)	Peritoneal macrophages	Alveolar macrophages
Acid esterase	++	+	++	++	++	++
Acid phosphatase	++	+	++	++	++	++
Acid phosphatase tartrate-resistant	++	−	−	+	−	++

+, −, ++, degrees of enzyme activity as revealed by light microscopic evaluation.

Table 3—Interleukin-1 Production of Multinucleated Giant Cells Generated *in Vitro*

Source of culture supernatant	Incorporated[3] H-thymidine (cpm)		
	1:10	Dilution of supernatant	1:100
IL-1 standard	21139 ± 870		31897 ± 725
Unconditioned medium	641 ± 156		641 ± 156
MGC I	7513 ± 283		2069 ± 37
MGC II	4609 ± 166		1318 ± 210
MGC III	2733 ± 709		884 ± 173
MGC IV	4282 ± 803		798 ± 186
MGC V	6949 ± 105		652 ± 76
MGC VI	2656 ± 486		778 ± 142
MGC VII	2702 ± 952		760 ± 309
MGC VIII	11880 ± 1102		1903 ± 402
Macrophages* III	4743 ± 345		1467 ± 94
Macrophages* IV	7614 ± 970		879 ± 123
Macrophages* V	8965 ± 622		2124 ± 279
TPA-treated monocytes	11523 ± 914		1138 ± 513

*Unfused BM of the same donors cultured for the same time as MGCs without any further stimulation in a cell density exceeding that of MGC culture 20-fold.

Table 4—Cytostatic Activity of Multinucleated Giant Cells Generated *in Vitro* (Factor-Mediated Cytostatic Activity)

Source of culture supernatant	Incorporated[3] H-thymidine		
	K-562	BJAB	HeLa
Unconditioned medium	13873 ± 857	17327 ± 536	8850 ± 2636
MGC I	13522 ± 612	ND†	ND
MGC II	13621 ± 465	ND	ND
MGC III	18737 ± 934	23859 ± 1371	8686 ± 1133
MGC IV	19750 ± 918	26371 ± 1389	5720 ± 1113
MGC V	17108 ± 114	23407 ± 185	6649 ± 1040
MGC VI	15652 ± 205	ND	ND
MGC VII	15622 ± 370	19929 ± 2579	4490 ± 766
MGC VIII	11345 ± 452	16049 ± 791	7666 ± 1534
Macrophages* III	16439 ± 988	16637 ± 665	10865 ± 1198
Macrophages* IV	18086 ± 1204	14719 ± 1595	10540 ± 198
Macrophages* V	13211 ± 696	ND	ND

*Unfused BMs of the same donors cultured for the same time as MGCs without any further stimulation in a cell density exceeding that of MGC culture 20-fold.
ND, not done.

Table 5—Cytostatic Activity of Multinucleated Giant Cells Generated *in Vitro* (Cell-Mediated Cytostatic Activity)

Coculture conditions	Target cell concentration (cells/ml)	Incorporated[3] H-thymidine/% growth reduction		
		K-562	BJAB	HeLa
Without MGC or macrophages	1×10^5	58662 ± 3777	24968 ± 2190	28550 ± 897
Without MGC or macrophages	5×10^5	103790 ± 4444	104626 ± 2442	41594 ± 3143
With MGCs*	1×10^5	17715 ± 3841	17320 ± 2023	10737 ± 823
		−87%	−72%	−98%
With MGCs	5×10^5	45352 ± 4291	48190 ± 4291	12863 ± 1564
		−67%	−67%	−94%
With macrophages†	1×10^5	20685 ± 3362	16209 ± 1977	13476 ± 851
		−82%	−75%	−88%
With macrophages	5×10^5	40369 ± 1780	47275 ± 1974	11386 ± 5215
		−71%	−64%	−97%

*MGCs generated from 1×10^6 monocytes/ml incorporated m = 10243 ± 1028 cpm, 1×10^6 unfused monocytes/ml incorporated 9968 ± 1828 cpm.
†Unfused BMs of the same donor cultured for the same time as MGCs without any further stimulation.

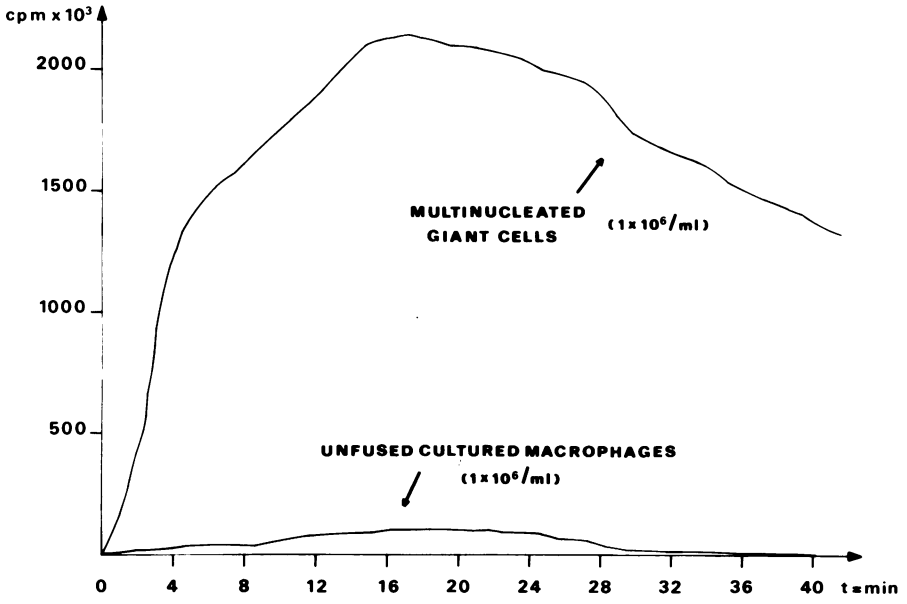

Figure 8—Chemiluminescence of MGCs derived from cultured BMs after stimulation with lymphokine-rich supernatant is elevated in comparison with unfused cultured macrophages. Oxygen free radical release per cell in response to zymosan is about 20 times higher in MGCs than in unfused macrophages.

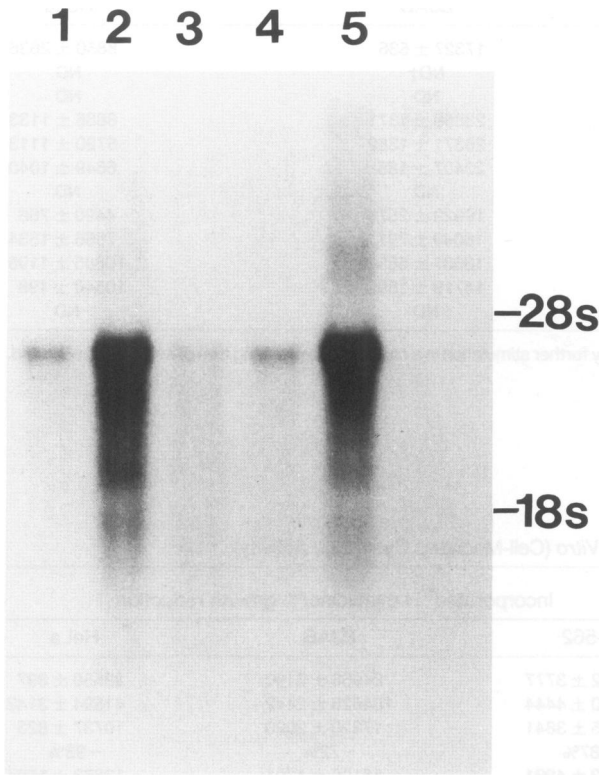

Figure 9—Autoradiography of total cellular RNA (10 μg), separated electrophoretically according to size, blotted to a coated nylon membrane, and hybridized with a radioactively labeled v-fms DNA probe. Lane 1, mononuclear cells; Lane 2, 48-hour cultured BMs; Lane 3, MGCs generated *in vitro*; Lane 4, BMs separated from mononuclear cells by glass adherence; Lane 5, PMs. A high level of c-fms expression is observable in PMs and BMs cultured for 48 hours. Freshly isolated BMs exhibit a lower level of c-fms transcription. A down-regulation of c-fms expression occurs in cultured BMs that underwent fusion into MGCs by the addition of lymphokine-rich supernatant.

during the separation procedure. Purified lymphocytes were negative (data not shown). During the first days of culture, BMs exhibited a drastic increase of c-fms transcription when transformed into adherent macrophages. Interestingly, some but not all PM samples revealed a comparably high level of c-fms expression. When culture of BMs transformed into macrophages exceeded more than 3 weeks, c-fms expression declined until it was no longer detectable; c-fms transcripts were also barely detectable in AMs (data not shown). MGCs generated from 2-day-cultured highly c-fms-expressing BMs exhibited also a very faint band after hybridization, which indicated that down-regulation had occurred during generation of polykaryons.

Discussion

As yet, the functional significance of macrophage-derived MGCs occurring in various granulomatous reactions has not been established.[9,10,11,37] In order to answer this question, we produced MGCs with up to 150 nuclei *in vitro* by treating cultivated human BMs with lymphokine-conditioned supernatants of concanavalin A-stimulated peripheral lymphocytes. On morphologic and immunocytochemical testing, no significant differences could be observed between MGCs *in vitro* and *in vivo*. Immunostaining with a panel of monocyte-macrophage-specific MAbs confirmed the monocyte-macrophage origin of MGCs (Figure 4; Table 1); lymphocyte-related antigens were

missing (Figure 5). The antigenic properties of MGCs differed clearly from the BM and macrophage populations analyzed in this study (Table 1). MGCs especially showed a reduced or missing reactivity for the MAb Ki-M1, which defines an antigen on macrophages and IRCs,[21] and for the Ki-M2 antigen, whereas the intracytoplasmically localized monocyte-macrophage-specific antigen recognized by the MAbs Ki-M6[23] and Ki-M8[24] were enhanced. The Ki-M4 antigen exclusively found on DRC and a small subset of BM[22] was not expressed by MGCs. It has been suggested that MGCs are engaged in presentation of antigens.[10,11] The reduced or missing reactivity for the MAbs Ki-M1 and Ki-M4, respectively, which recognize IRC and DRC as the antigen presenting cells of T- and B-cell immune response, respectively, suggest that MGCs do not belong to these compartiments of immune accessory cells. The nuclear antigen recognized by the MAb Ki67 and selectively expressed by proliferating cells[28] could not be found in MGCs either *in vitro* or *in vivo,* which further underlines the view that MGCs result from the fusion of nondividing cells of the monocyte-macrophage lineage.[9,13,39]

The similarity between MGCs occurring *in vitro* and *in vivo* and the high percentage of MGCs generated *in vitro* prompted us to further analyze the functional activities of these cells. MGCs clearly exhibited a higher level of enzyme equipment than did monocyte-macrophages. Like Weinberg et al,[13] we found a strong tartrate-resistant acid phosphatase activity in MGCs (Table 2).This enzyme has been observed in highly stimulated macrophages.[30] Kobayashi et al[40] found a positive correlation between the interleukin-1 content of experimentally induced granulomas and the extent of granulomatous inflammation, suggesting that interleukin-1 might be involved in its initiation and maintenance. Because ultrastructural findings have pointed to a secretory activity of MGCs,[37] we assayed the interleukin-1 secretion of MGCs. Our results show that MGCs produce an amount of interleukin-1 similar to that produced by unfused macrophages, indicating that the release of this inflammatory mediator is not a specific function of MGCs. Because MGCs of histiocytic origin can be found in a variety of neoplasias,[2,3] and because the capacity of monocyte-macrophages to produce cytostatic or cytotoxic factors has been clearly shown,[41,42] MGCs were screened in a further step for their factor-bound or cell-mediated cytostatic activity. MGC-conditioned supernatants failed to induce a growth reduction of the permanent cell lines used as targets (Table 4). Similar results were obtained by Poste,[43] who investigated MGCs of mice. Hence, it seems unlikely that MGCs occurring in various tumors play a significant role in factor-mediated tumor defense. Under coculture conditions, a significant reduction of ^{3}H-thymidine uptake of target cells could be noticed when macrophages or MGCs were used as effector cells (Table 5). No differences between unfused macrophages and polykaryons could be established in cytostatic capacity under these circumstances. It seems, therefore, unlikely that MGCs present in some tumors represent a specialized form of immunologic defense.

Measuring the production of oxygen free radicals in response to zymosan, we found a 20–30-fold increase in MGCs when compared with unfused macrophages (Figure 8). This elevation of oxygen generation seems to result mainly from summing up the activity of single cells that underwent cell fusion. The functional significance of giant cell formation, however, could lie in the synchronization and concentration of oxygen free radical release. Together with the enhanced equipment with lysosomal enzymes (Table 2), this finding points to a role of such MGCs in host defense in infectious diseases. Thus, it has been shown that despite reduced phagocytic activity,[10,44] MGCs exhibited an efficient killing of ingested fungi.[10] Nevertheless, MGCs are generated under various pathologic conditions and can be formed by differently differentiated macrophages. Macrophages confronted with indigestable material build up foreign body giant cells. Exposure to media rich in lipids results in foamy cells seen in xanthomatous reactions with generation of Touton giant cells. Acid-fast bacteria such as mycobacteria induce the formation of epithelioid cells which give rise to Langhans' giant cells. Whether this morphologic heterogeneity reflects a functional diversity remains to be shown.

In a further step we studied the transcription of c-fms in MGCs. The gene product of the c-fms protooncogene is a transmembrane protein with tyrosine kinase activity that is obviously related to the receptor for CSF-1 and its human relative urinary CSF exclusively expressed by monocytes/macrophages.[45,46] Consequently, analysis of c-fms transcription on the RNA level could provide information about the expression of monocyte/macrophage specific growth factor receptor. The c-fms expression was found to be high in 48-hour cultivated BMs and decreased after cell fusion into MGCs had occurred, indicating that growth factor receptor expression was rapidly down regulated in MGC (Figure 9). It cannot be ruled out that down-regulation of the c-fms expression is the initial event during lymphokine stimulation and possibly provides a prerequisite for macrophage fusion. Differences in the readiness to generate MGCs be-

tween AMs and BMs that exhibit considerable differences in c-fms expression have been described.[47] On the other hand, a very low level of c-fms transcription, as revealed by the AMs and long-term cultured BMs and MGCs, could be typical for a terminal stage of maturation within the monocyte-macrophage differentiation pathway. Maturation was evidenced by enzymecytochemical and immunocytochemical criteria such as tartrate-resistant acid phosphatase activity and reduced Ki-M1 and Ki-M2 antigen expression (Tables 1 and 2). Down-regulation of c-fms expression in macrophages after culture or stimulation was recently reported by two other groups.[48,49] Because it has been shown that besides proliferation, functional activities are regulated via the CSF-1 receptor,[50,51] we conclude from these *in vitro* findings that the fully developed mature macrophage (long-term cultivated BM, AM, MGC) shows a down-regulation of c-fms transcription, while the developing and maturing macrophage (short-term cultivated BM) shows a high level of expression of this gene. From this point of view, MGCs must be considered as activated macrophages at a final stage of maturation.

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