Brief Definitive Report

FUNCTIONAL AND BIOCHEMICAL STUDIES OF MULTINUCLEATED GIANT CELLS DERIVED FROM THE CULTURE OF HUMAN MONOCYTES*

BY LIANA SCHLESINGER, ROBERT A. MUSSON, AND RICHARD B. JOHNSTON, JR.

From the Department of Pediatrics, National Jewish Hospital and Research Center/National Asthma Center, and the Departments of Pediatrics and Pathology, University of Colorado School of Medicine, Denver, Colorado 80206

Multinucleated giant cells (MGC)[1] are found in a variety of inflammatory disease processes (1, 2). Both monocytes and macrophages appear in the lesions of these diseases before the MGC; and monocytes are thought to be the precursor to both the macrophages and multinucleated cells (1–3). The phagocytic capacity of MGC derived in vivo has been studied using cells adherent to coverslips planted under the skin of rodents (1, 3–6), and MGC derived from cultured mouse macrophages have also been shown to be phagocytic (7, 8). It has been reported that human monocytes can form variable numbers of multinucleated cells in vitro (9–12). However, functional capacities of human MGC that might relate to removal of foreign material or microorganisms have not been defined, and such functions have not been thoroughly studied with MGC of any species. We report here some functional and biochemical characteristics of MGC derived from human blood monocytes cultured in vitro. The characteristics examined are potentially important in mediating the resolution of, or in propagating, the variety of pathologic lesions in which these cells are found.

Materials and Methods

Isolation and Culture of Monocytes. Mononuclear cells were isolated from the blood of healthy adult volunteers (13), and 4×10^6 cells were added to 16-mm-diameter culture wells (10, 13). Monocytes (>98% esterase positive [14]) were allowed to adhere for 1–2 h, washed, and either studied immediately or cultured for 9–14 d in either M199 plus 20% heat-inactivated (56°C, 30 min) autologous serum or in RPMI 1640/McCoy's 5a (1:1) containing 10% heat-inactivated serum (10, 13). These concentrations of cells and of serum in the designated medium, and heat inactivation of the serum, gave optimal MGC formation. The medium with serum was changed after 24 h and then every third

This work was supported by grants AI 14148 and CA 26846 from the National Institutes of Health. This work was completed during the tenure of an Established Investigatorship awarded to Dr. Musson from the American Heart Association, with funds contributed in part by the Colorado Heart Association. Dr. Schlesinger's current address is: Universidad de Chile, Instituto de Nutricion y Technologia de los Alimentos, Santiago, Chile. Reprint requests should be addressed to Dr. Musson.

[1] *Abbreviations used in this paper:* E, sheep erythrocytes; EIgG, sheep erythrocytes sensitized with rabbit IgG anti-E; EIgMC, sheep erythrocytes sensitized with rabbit IgM anti-E and complement; FACS, fluorescence-activated cell sorter; HBSS, Hank's balanced salt solution; MGC, multinucleated giant cells; NBT, nitroblue tetrazolium; O_2^-, superoxide anion; PBS, phosphate-buffered saline; PMA, phorbol myristate acetate.

day. By days 9 to 14, 25–50% of the cultured cells were MGC, defined as large cells having two or more nuclei. Both foreign body–type and Langhans-type MGC were observed (1, 2, 12). In four preparations the average number of nuclei per MGC was 4.5 (range, 2–30); 72% of MGC had 2–4 nuclei, 94% had 2–10 nuclei. The remainder of the cells, which we term "macrophages," were large uninucleated cells with the appearance of macrophages. In two experiments, 100% of both cell types stained intensely for esterase (14).

Phagocytosis of EIgG and EIgMC. Sheep erythrocytes (E) were opsonized with the IgG and IgM fractions of rabbit anti-E, and with complement (15). Phagocytosis was measured by incubating the adherent cells with 0.5–1 ml of HBSS containing 1×10^7 EIgG or EIgMC for 1 h at 37°C (E/phagocyte ≅ 20:1, based on microscopic estimates of cell number). Uningested E were lysed by brief exposure to distilled water, the cultures were processed (16), and at least 200 MGC and macrophages were counted, using 1,000× magnification. Neither MGC nor macrophages ingested unopsonized E or EIgM. For analysis of rate of uptake, the reaction was stopped at 5, 10, 20, 30, 40, and 60 min. In some experiments, nuclei were stained with ithidium iodide, 20 μg/ml in HBSS containing 1 mg/ml ethylene diamine, and counted with fluorescence microscopy.

Phagocytosis and Killing of Candida. C. albicans (ATCC 18804) was used (17). For phagocytosis, a ratio of ~5 candida to one cell was used, and incubation was for 1 h. The cells were stained (Wright's-Giemsa), and at least 200 of both MGC and macrophages (or 200 monocytes) were counted using 1,000× magnification. ~95% of cell-associated candida could be seen within a phagocytic vacuole (17).

The candidacidal assay employed a ratio of ~2 candida per cell. After 1 h, the cells were washed and stained with Giemsa stain (18). At least 200 of both MGC and macrophages were counted at 1,000× magnification, and the percentage of dead candida inside the cells was determined (17, 18).

Reduction of Nitroblue Tetrazolium (NBT). The assay was that of Bryant et al. (19), using 100 ng/ml of phorbol myristate acetate (PMA) (Consolidated Midland Corp., Brewster, NY) as stimulus, incubation at 37°C for 30 min, fixation with 10% formalin, and nuclear staining with 0.5% safranin (19). Superoxide dismutase (Diagnostic Data Inc., Montview, CA), 200 μg/ml was present in some wells. At least 200 of both cell types were examined for the presence of prominent blue color at a magnification of 1,000×.

Determination of HLA-Dr(Ia)Phenotype. Cells were incubated with anti-Ia serum, then stained with fluorescein-conjugated goat antibody to mouse Ig (20). The percentage of fluorescing cells was determined (20). No cells fluoresced when treated with the second antibody alone.

Separation of MGC and Macrophages. Cells cultured for 9–14 d were washed twice in PBS without Ca^{++} and Mg^{++}, then incubated with 10 μM Hoechst dye 33342 (Calbiochem-Behring, San Diego, CA) in M199 for 20 min at 37°C in 5% CO_2-95% air. This stain binds to DNA, causing the nucleus to fluoresce without affecting cell viability. The cells were then washed twice with PBS, incubated in a solution of trypsin (2.5 mg/ml), EDTA (1 mg/ml), and DNAse (10 μg/ml) for 15 min at 37°C in 5% CO_2, and then gently scraped from the dish using a rubber policeman. The trypsin reaction was stopped by adding 5% fetal calf serum, and cells were centrifuged at 275 *g* for 10 min. The supernatant was discarded and the cells were resuspended in PBS containing 10 μM Hoechst stain, incubated at 37°C for 15 min, and washed twice in PBS at 4°C. In three preparations using the fluorescent microscope, the same percentage of the cells were MGC before and after removal from the culture dish. The cells were then separated into two populations in a fluorescence-activated cell sorter (FACS IV, Becton Dickinson, Sunnyvale, CA). Viability of both populations was 90–95% as measured by trypan blue exclusion. When used for enzyme assays, 5×10^4 cells/ml were lysed in 0.05% Triton X 100.

Enzyme Assays. Cell protein content and activities of *N*-acetyl-β-D-glucosaminidase, acid phosphatase, and β-glucuronidase were measured as previously described (10).

Results

The maturation of human monocytes into macrophages and MGC within the same culture dishes permitted us to compare certain functional activities under

TABLE I

*Comparison of Functional and Antigenic Characteristics of Macrophages and Multinucleated Giant Cells**

Characteristic	Macrophages	MGC
	% of cells that were phagocytic‡	
Phagocytosis of:		
EIgG	79 ± 3 (8)	67 ± 6
EIgMC	41 ± 8 (5)	45 ± 8
Candida albicans	89 ± 6 (7)	88 ± 8
	% of ingested fungi killed	
Killing of *Candida albicans*	21 ± 5 (6)	24 ± 4
	% of cells positive	
Reduction of NBT		
No stimulus	11 ± 3 (12)	13 ± 3
PMA	67 ± 5 (12)	70 ± 5
PMA + superoxide dismutase	12 ± 5 (6)	12 ± 4
	% of cells positive	
Presence of HLA-Dr (Ia) antigen	75 ± 5 (4)	74 ± 2

* Studies were performed on the two cell types present within the same culture dishes. The values represent mean ± SEM; the number in parentheses in the macrophage column indicates the number of experiments performed.

‡ The percentage of cells that ingested at least one particle is shown.

identical conditions. The percentage of macrophages and MGC that ingested EIgG or EIgMC was comparable (Table I). Ingestion of EIgG by freshly isolated monocytes was slightly less than that by the two cultured cell types (64 ± 12%, n = 4); phagocytosis of EIgMC by monocytes was weak (8 ± 2%, n = 4), as reported (16). In studies of the rate of phagocytosis, there was no ingestion of EIgG by macrophages or MGC at 20 min, comparable slight ingestion by 30 min, and equivalent ingestion at 40 min and 60 min (n = 2, data not shown).

At 40 and 60 min the mean number of EIgG ingested per nucleus was approximately four times higher for macrophages than for MGC, and ingestion varied inversely with the number of nuclei. The approximate number of EIgG ingested per nucleus after incubation for 60 min was 19 with macrophages, 11 with MGC containing 2 nuclei, 8 with 3 nuclei, 7 with 4 nuclei, 6 with 5 nuclei, and 5 with 6 nuclei (15 d of culture, at least 16 cells counted at each point).

The percentage of MGC and macrophages ingesting *C. albicans* and the percentage of the ingested candida that was killed were the same for the two cell types (Table I). 83% of fresh monocytes ingested one or more candida (±2%, SEM; n = 3); and monocytes killed 33 ± 2% (n = 3) of the candida they had ingested. The extent of ingestion of candida by the three cell types is compared in Fig. 1. Unlike the uptake of EIgG, uptake of candida was present at the earliest time point studied (5 min). The number of candida ingested per cell was greatest for MGC at each time point and by 60 min was almost twice as high in the MGC as in the macrophages or monocytes. The number of candida ingested per nucleus was ~45% as high in MGC as in macrophages (mean, n = 2).

An equivalent percentage of MGC and macrophages reduced NBT dye when stimulated with PMA (Table I). 98% of fresh monocytes reduced NBT in response to PMA. The stimulated reduction of NBT by macrophages and MGC was due to superoxide anion (O_2^-) since the inclusion of superoxide dismutase

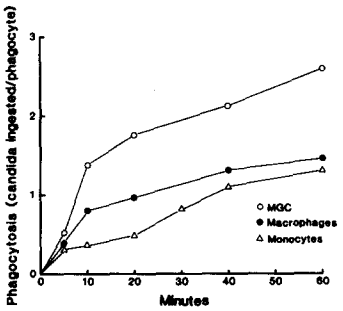

FIGURE 1. Phagocytosis of *Candida albicans* by MGC, macrophages, and adherent freshly isolated monocytes. The average number of candida ingested per cell is plotted as a function of incubation time. Results are shown with cells of a single donor studied at the same time; MGC and macrophages were in the same culture dishes, monocytes were in separate dishes. The results are representative of those obtained with cells from three donors.

reduced the percentage of positive cells to that seen in unstimulated cultures.

In order to examine concentrations of lysosomal enzymes in these cells, we enriched for each cell type by separating on the basis of nuclear fluorescence. The larger MGC had significantly higher levels of three lysosomal enzymes when compared on the basis of cell number (Table II). However, since the amount of protein per cell was approximately fourfold higher in the MGC preparations (Table II), specific activities of the enzymes were higher in macrophages.

Discussion

Although MGC are present in many chronic granulomatous processes, their role in inflammation is still in question. In this study, we report that MGC derived from human blood monocytes, when compared on the basis of cell number, were able to ingest EIgG, EIgMC, and candida as efficiently as macrophages and more efficiently than monocytes, suggesting that receptors for Fc, cleaved C3, and, perhaps, mannose-terminated glycoconjugates are present on their surface. This observation agrees with previously published demonstrations that rodent MGC formed in vitro or on implanted coverslips can phagocytose EIgG (5, 7, 8) and EIgMC (5), and that MGC present in cryostat sections of lesions from patients with leprosy can form rosettes with complement-coated E (21). Although the number of particles ingested per nucleus has been less in MGC than in macrophages in some studies (4, 6, 8), including ours with EIgG and candida, the data indicate that rodent and human MGC can phagocytose at least as effectively as macrophages when considered as individual cells, and that they can do so through a variety of receptors.

The MGC and macrophages that arose in our cultures of human monocytes were also similar in their capacity to kill *Candida albicans* and in O_2^--dependent reduction of NBT. The extent of killing of *C. albicans* by MGC and macrophages achieved in our studies (24% and 21% of ingested organisms killed, respectively) was almost identical to that reported previously for activated mouse macrophages (23–27%) (17). These results suggest that MGC and macrophages are equally capable of participating in host defense against infection through phagocytic killing. The presence of Ia antigen on 74% of MGC suggests that MGC could

TABLE II

*Activity of Cellular Lysosomal Enzymes in Macrophages and Multinucleated Giant Cells**

Enzyme	Macrophage-rich fraction	MGC-rich fraction
	μmol substrate cleaved/10^6 cells	
β-Glucosaminidase	7,753 ± 1,080	18,286 ± 2,027
Acid phosphatase	4,311 ± 531	13,523 ± 1,465
β-Glucuronidase	90 ± 22	247 ± 22
	μmol substrate cleaved/μg cell protein	
β-Glucosaminidase	26.6 ± 9.05	9.9 ± 0.08
Acid phosphatase	12.2 ± 2.05	7.8 ± 0.03
β-Glucuronidase	0.23 ± 0.05	0.14 ± 0.07

* Values represent mean ± SEM. A total of three experiments was performed in which cells were separated according to nuclear fluorescence, using a FACS, into a fraction rich in macrophages (95%, 97%, and 97% macrophages; mean 96%) and a fraction rich in MGC (60%, 85%, and 86% MGC; mean 77%). Enzyme activities were calculated on the basis of cell number for all three experiments (upper set of values). For two of these experiments, enzyme activity was expressed on the basis of cell protein (lower set). In these two experiments one fraction was 97% macrophages and the other was 86% MGC (means). Protein content for 10^6 cells was 436 μg for macrophages and 1,615 μg for MGC (means, $n = 2$).

interact effectively with lymphocytes during the immune response. Specific activities of three lysosomal enzymes were higher in macrophages than in MGC. However, specific activities in MGC were 3–39 times higher than those reported by us previously for these enzymes in monocytes (10, 14), which is compatible with a strong role for MGC in digestion of internalized debris.

Summary

We compared phagocytic and metabolic activities of multinucleated giant cells (MGC) and macrophages derived from human monocytes after 9–14 d in culture. Phagocytosis of sheep erythrocytes (E) coated with IgG, of E coated with IgM and complement, and of *Candida albicans* was comparable in MGC and macrophages. The same percentage of ingested fungi was killed by MGC (24 ± 4%) and macrophages (21 ± 5%). ~70% of MGC and macrophages exhibited superoxide-dependent reduction of nitroblue tetrazolium during stimulation. Ia antigen was present on ~75% of both cell types. Analysis of cell populations separated by nuclear fluorescence indicated that β-glucosaminidase, acid phosphatase, and β-glucuronidase activity per cell was higher in MGC, but specific activity of these enzymes was greater in macrophages. These results suggest that MGC have the capacity to function like macrophages in host defense against infection.

We thank Dr. John Lehman for guidance with the FACS, Dr. Anthony Hayward for assistance in measuring Ia antigen, and Dr. Zanvil Cohn for helpful discussions.

Received for publication 20 December 1983 and in revised form 23 January 1984.

References

1. Papadimitriou, J. M., and M. N. I. Walters. 1979. Macrophage polykarya. *CRC Crit. Rev. Toxicol.* 6:211.

2. Adams, D. O. 1982. The biology of the granuloma. *In* Differential Diagnosis of Granulomas. F. O. Schmitt, S. J. Bird, and F. E. Bloom, editors. Raven Press, New York. p. 1.
3. Mariano, M., and W. G. Spector. 1974. The formation and properties of macrophage polykaryons (inflammatory giant cells). *J. Pathol.* 113:1.
4. Papadimitriou, J. M., T. A. Robertson, and M. N. I. Walters. 1975. An analysis of the phagocytic potential of multinucleate foreign body giant cells. *Am. J. Pathol.* 78:343.
5. Mariano, M., T. Nikitin, and B. E. Malucelli. 1976. Immunological and nonimmunological phagocytosis by inflammatory macrophages, epithelioid cells and macrophage polykaryons from foreign body granulomata. *J. Pathol.* 120:151.
6. Chambers, T. J. 1977. Studies on the phagocytic capacity of macrophage polykaryons. *J. Pathol.* 123:65.
7. Gordon, S., and Z. Cohn. 1970. Macrophage-melanocyte heterokaryons. I. Preparation and properties. *J. Exp. Med.* 131:981.
8. Weinberg, J. B. 1983. Macrophage polykaryon formation *in vitro* by peritoneal cells from mice given injections of sodium periodate. *Am. J. Pathol.* 110:182.
9. Lewis, M. R. 1925. The formation of macrophages, epithelioid cells and giant cells from leukocytes in incubated blood. *Am. J. Pathol.* 1:91.
10. Musson, R. A., H. Shafran, and P. M. Henson. 1980. Intracellular levels and stimulated release of lysosomal enzymes from human peripheral blood monocytes and monocyte-derived macrophages. *J. Reticuloendothel. Soc.* 28:249.
11. 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.
12. Postlethwaite, A. E., B. K. Jackson, E. H. Beachey, and A. H. Kang. 1982. Formation of multinucleated giant cells from human monocyte precursors. *J. Exp. Med.* 155:168.
13. Musson, R. A., and P. M. Henson. 1979. Humoral and formed elements of blood modulate the response of peripheral blood monocytes. *J. Immunol.* 122:2026.
14. Musson, R. A. 1983. Human serum induces maturation of human monocytes in vitro: changes in cytolytic activity, intracellular lysosomal enzymes, and nonspecific esterase activity. *Am. J. Pathol.* 111:331.
15. Yamamoto, K., and R. B. Johnston, Jr. 1984. Dissociation of phagocytosis from stimulation of the oxidative metabolic burst in macrophages. *J. Exp. Med.* 159:405.
16. Newman, S. L., R. A. Musson, and P. M. Hensen. 1980. Development of functional complement receptors during in vitro maturation of human monocytes into macrophages. *J. Immunol.* 125:2236.
17. Sasada, M., and R. B. Johnston, Jr. 1980. Macrophage microbicidal activity: correlation between phagocytosis-associated oxidative metabolism and the killing of candida by macrophages. *J. Exp. Med.* 152:85.
18. Lehrer, R. I. 1970. Measurement of candidacidal activity of specific leukocyte types in mixed cell populations. *Infect. Immun.* 2:42.
19. Bryant, S., R. E. Lynch, and H. R. Hill. 1982. Kinetic analysis of superoxide anion production by activated and resident murine peritoneal macrophages. *Cell. Immunol.* 69:46.
20. Mori, M., and A. R. Hayward. 1982. Phenotype and function of human milk monocytes as antigen presenting cells. *Clin. Immunol. Immunopathol.* 23:94.
21. 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.