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Origin of Multinucleated Giant Cells in Long-Term Diffusion Chamber Cultures (41442)

LAWRENCE N. SHULMAN[1] AND STEPHEN H. ROBINSON

Charles A. Dana Research Institute and the Harvard–Thorndike Laboratory of the Beth Israel Hospital, Department of Medicine, Beth Israel Hospital and Harvard Medical School, Boston, Massachusetts 02215

Abstract. Multinucleated giant cells (MNGCs) form spontaneously in long-term *in vivo* diffusion chamber cultures of murine bone marrow. Their appearance coincides with the growth of macrophages which become the predominant cells in culture after an initial phase favoring granulopoiesis. Host mice bearing diffusion chamber cultures were given regular injections of [^{3}H]thymidine beginning prior to the development of MNGCs. The nuclei in MNGCs in these cultures were nonuniformly labeled. In any given MNGC all, none, or some of the nuclei might be labeled and the percentage of labeled nuclei was similar to the percentage labeling of the mononuclear macrophages in these cultures. These findings demonstrate that under these experimental conditions MNGCs arise primarily from cell fusion rather than nuclear division and that they are the random fusion products of both quiescent and actively dividing macrophages.

Cultures of leukocytes *in vitro* have long been known to produce multinucleated giant cells (MNGCs). Lambert in 1912 showed that cultures of chick embryo spleen cells produced MNGCs in significant numbers when foreign bodies (lycopodium spores) were added (1). In 1925 Lewis studied conversion of leukocyte cultures to macrophages and MNGCs, using cells from a variety of species including man (2). MNGCs are seen in sites of chronic inflammation, with viral infections, and as part of the host defense against foreign bodies such as talc or plastics (3–8). The microstructure of these MNGCs has been thoroughly studied with the electron microscope (8, 9). These giant cells are felt to arise from fusion of macrophages, although the mechanism of their formation has usually been studied under complex *in vivo* or *in vitro* situations involving the above-mentioned stimuli.

We have observed the spontaneous formation of MNGCs in long-term diffusion chamber (DC) cultures of murine bone marrow implanted into the peritoneal cavities of previously irradiated mice. Granulocytes and macrophages grow preferentially under these conditions. There is an increase in macrophages during the first 1 1/2 weeks of culture, after which these cells persist in roughly constant numbers for 7 additional weeks of culture. By transplanting cells from these DC cultures into new DCs in new host mice (secondary cultures), cultures composed of virtually 100% macrophages are found. These conditions also lead to the development of MNGCs, permitting us to study the mechanism of MNGC formation.

Materials and Methods. The DC culture system has been described in detail previously (10). CD-1 virgin female mice (Charles River Laboratories, Wilmington, Mass.) were used as bone marrow donors and DC recipients. DC recipients were irradiated with 475 rads whole body irradiation 24 hr prior to implantation of the cultures. DCs were harvested at various times after the initiation of culture, incubated in 0.5% Pronase (Calbiochem) at room temperature for 55 min and the cells removed with a Pasteur pipet. Slide preparations of cells were made using a Shandon cytocentrifuge and were stained with Wright–Giemsa stain.

Cells to be implanted into secondary DC cultures were grown for 8 days in primary culture and harvested as described above using sterile technique. Cells from several chambers were pooled, inoculated into new

[1] To whom reprint requests should be addressed.

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DCs, and implanted into newly irradiated host mice.

In order to study the genesis of MNGCs, animals bearing secondary cultures were injected with 2 μCi of [^{3}H]thymidine (New England Nuclear, 6.7 Ci/mmol) in PBS intraperitoneally every 12 hr from the 11th to the 19th day of culture. This schedule approximates a continuous infusion of [^{3}H]thymidine (11). DCs were harvested every 2 days from Days 11–25. Cytocentrifuge preparations were made of the cell suspensions and slides were fixed in methanol. Kodak emulsion was applied and the slides were exposed for 7 days at 4°. They were developed with Kodak D-19 developer and stained with modified Giemsa stain. Nuclei with five or more grains over them were considered labeled.

Results. In primary cultures (data not shown) macrophages reach maximal number by Day 10 and then remain more or less constant. Small numbers of MNGCs begin to appear at 3 weeks. When cells from primary cultures are transplanted into secondary culture macrophages quickly increase in number and eventually account for 100% of the DC population. Figure 1 demonstrates the increase in the number of macrophages, binucleate cells, and cells with three or more nuclei in secondary culture as a function of time. On the 11th day of secondary culture, prior to the initiation of [^{3}H]thymidine labeling, no MNGCs are present. Over the next 17 days these cells become increasingly frequent and also demonstrate an increase in the number of nuclei per cell. By the 28th day 23.3% of the cells have at least two nuclei and 2.5% have three or more nuclei.

Figure 2 shows an example of an MNGC during labeling with [^{3}H]thymidine. In some MNGCs there was no nuclear labeling, in some all nuclei were labeled, and in most an intermediate number of nuclei was labeled. From the 17th to the 21st day of culture an average of 36.4% ± 2.0 (SEM) of all mononuclear macrophages was labeled. During this same period of culture 26.0% ± 1.5 (SEM) of all nuclei in MNGCs was labeled. The percentage of nuclei labeled in both macrophages and MNGCs was relatively constant during this time, as shown in Table I.

Discussion. Culture of murine marrow in the DC system leads to a progressive predominance of macrophages with time. In secondary culture 70–100% of the cells were macrophages during the time studied with autoradiography. The reason for development of MNGCs in these cultures is not clear. There is no evidence for either chemical or viral stimulation. It is possible that the material comprising the DC (the lucite ring or Millipore filter) may constitute a foreign stimulus, leading to the formation of MNGCs.

The data presented here demonstrate that these multinucleated cells are primarily the products of cell fusion and not cell division. Since no MNGCs were present prior to initiation of [^{3}H]thymidine injections and since the schedule of injections simulated a continuous infusion of this precursor (11, 12), all nuclei in MNGCs should have been labeled if they were the products of the nuclear division of mononuclear cells. In MNGCs containing two or more labeled nuclei it is possible that some of the nuclei arose by division of some of the nuclei within that cell. Those MNGCs with no labeled nuclei or with only one labeled nucleus must have been the products of cell fusion; a clear example is a binucleate cell

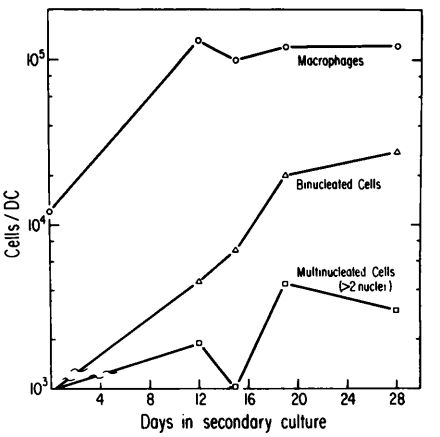

FIG. 1. Growth of mononuclear macrophages and multinucleate cells in secondary diffusion chamber cultures established from 8-day primary cultures of normal murine bone marrow.

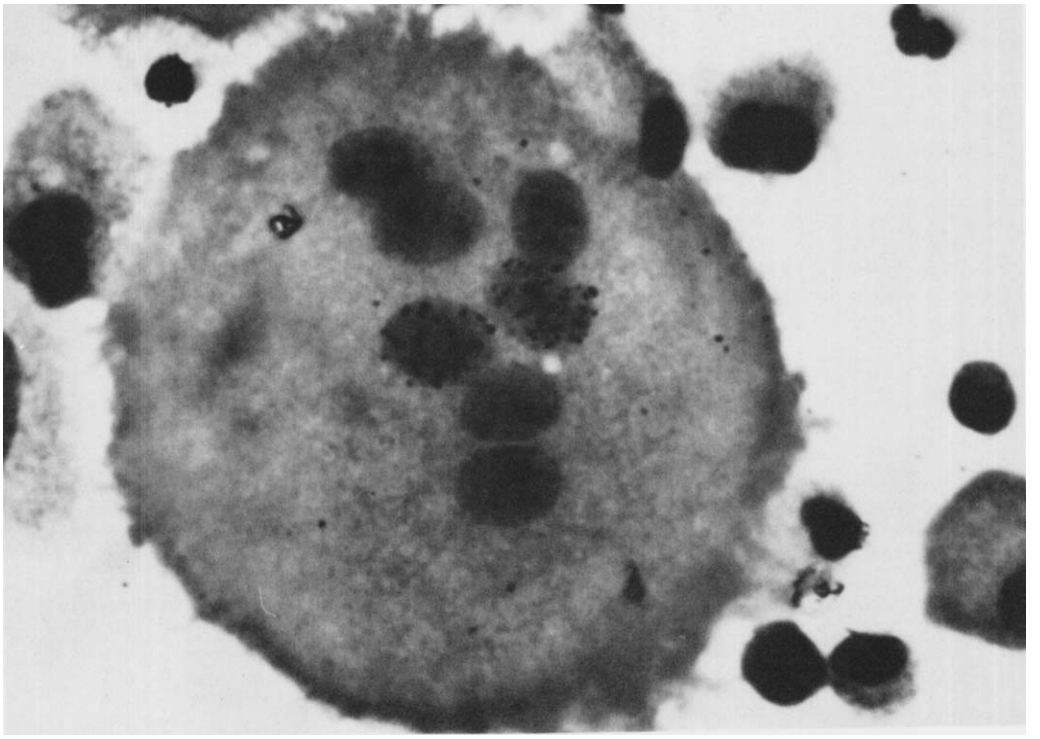

FIG. 2. Multinucleate giant cell. Two of the seven nuclei are labeled with [^{3}H]thymidine.

with one labeled and one unlabeled nucleus, a not uncommon finding in this study.

The fact that the MNGCs are composed of both labeled and unlabeled nuclei demonstrates that they are the fusion products of both nondividing and dividing macrophages, and not only aged and inactive cells. However, there may be a slightly greater propensity for quiescent cells to fuse since the percentage of labeled nuclei in MNGCs is slightly less than that in the mononuclear macrophage population, 26 versus 36%. Since these values remain roughly constant during the period of [^{3}H]thymidine administration (Table I), it is unlikely that much nuclear division was occurring in MNGCs during this time.

This closed *in vivo* culture system provides an opportunity for further study of the development and the functional capabilities of macrophages and MNGCs.

TABLE I. PERCENTAGE NUCLEI LABELED IN MACROPHAGES AND MNGCS AS A FUNCTION OF TIME IN SECONDARY CULTURE

Day[a]	15	17	19	21
Macrophages	32[b]	32	39	37
MNGCs	25	27	28	23

[a] [^{3}H]Thymidine injections were given from Days 11–19 of secondary culture.
[b] Results are percentages.

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1. Lambert RA. The production of foreign body giant cells in vitro. J Exp Med 15:510–515, 1912.
2. Lewis MR. The formation of macrophages, epithelioid cells and giant cells from leucocytes in incubated blood. Amer J Pathol 1:91–99, 1925.
3. Papadimitriou JM, Sforsina D, Papaelias L. Kinetics of multinucleate giant cell formation and their modification by various agents in foreign body reactions. Amer J Pathol 73:349–361, 1973.
4. Dreher R, Keller HU, Hess MW, Roos B, Cottier

H. Early appearance and mitotic activity of multinucleated giant cells in mice after combined injection of talc and prednisone acetate: A model for studying rapid histiocytic polykarion formation in vivo. Lab Invest **38**:149–156, 1978.

5. Elias PM, Epstein WL. Ultrastructural observations on experimentally induced foreign-body and organized epithelioid-cell granulomas in man. Amer J Pathol **52**:1207–1216, 1968.
6. Black MM, Epstein WL. Formation of multinucleate giant cells in organized epithelioid cell granulomas. Amer J Pathol **74**:263–270, 1974.
7. Carter RL, Roberts JDB. Macrophages and multinucleate giant cells in nitrosoquinoline-induced granulomata in rats: An autoradiographic study. J Pathol **105**:285–288, 1971.
8. Sapp JP. An ultrastructural study of nuclear and centriolar configurations in multinucleated giant cells. Lab Invest **34**:109–114, 1976.
9. Sutton JS, Weiss L. Transformation of monocytes in tissue culture into macrophages, epithelioid cells, and multinucleated giant cells: An electron microscope study. J Cell Biol **28**:303–330, 1966.
10. Marmor JB, Russell JL, Miller AM et al. Modulation of murine granulocyte proliferation in diffusion chamber cultures. Blood **46**:39–50, 1975.
11. Cronkite EP, Bond VP, Fliedner TM, Killman KS. Use of tritiated thymidine in the study of haemopoietic cell proliferation, in Ciba Foundation Symposium on Haemopoiesis. Boston, Little Brown, pp70–92, 1960.
12. Robinson SH, Brecher G, Lourie IS, Haley J. Leukocyte labeling in rats during and after continuous infusion of tritiated thymidine: Implications for lymphocyte longevity and DNA reutilization. Blood **26**:281–295, 1965.

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