Kinetics and Ultrastructural Studies of the Induction of Rat Alveolar Macrophage Fusion by Mediators Released From Mitogen-Stimulated Lymphocytes

SABURO SONE, MD, CORAZON BUCANA, PhD, LAWRENCE C. HOYER, ScD, and ISAIAH J. FIDLER, DVM, PhD

From the Cancer Metastasis and Treatment Laboratory and Cancer Biology Program, NCI Frederick Cancer Research Center, Frederick, Maryland

Treatment of F344 rat alveolar macrophages (AMs) *in vitro* with cell-free supernatant fluids obtained from concanavalin-A(Con-A)-stimulated syngeneic lymphocytes induced extensive fusion. The lymphokine responsible for the fusion of AMs (but not other cells) is here referred to as AM fusion factor (Con-A-MFF). Fusion is dependent on the dose of Con-A-MFF and the population density of AM cultures and occurred 10 hours after Con-A-MFF was added to cultures of normal AMs. Con-A-MFF must interact with AMs for more than 8 hours before full expression of fusion is reached at 24 hours. Using a technique allowing for sequential scanning to transmission electron microscopy analysis of cells, the authors determined the relationship of the morphologic characteristics of the surface and the internal structure of cells fusing to form multinucleate giant cells (MGCs). The process of AM fusion begins with the aggregation of AMs, followed by interdigitation of cell processes. Serial sections of MGCs showed lysosomes associated with remnants of plasma membrane in the cytoplasm. The MGCs contained numerous organelles associated with increased secretory activity of cells. (Am J Pathol 1981, 103:234–246)

CELLS of the macrophage–histiocyte series are important in host defense against infections and neoplasms.[1,2] The activation of macrophages to the cytotoxic state is a complex phenomenon and involves many independent features. This multistep process can be initiated by a variety of agents, and the properties displayed by the activated macrophages can depend on the type of activating stimulus.[3,4] Inflammatory conditions are known to lead to recruitment and activation of macrophages. The extent of such activation can vary with the type of inflammation, ie, acute versus chronic. In chronic inflammation, the release of lymphokines by antigen-sensitized lymphocytes is likely to occur.[4] The soluble lymphocyte mediator responsible for macrophage activation is referred to as macrophage-activating factor (MAF), and MAF-treated macrophages can be rendered tumoricidal.[5]

Multinucleated giant cells (MGCs) are commonly observed in inflamed tissues. MGCs are a classical feature of granulomas caused by infectious agents.[6–8] Generally, MGCs are observed in tissues with large numbers of macrophages and a persistent causative agent, ie, chronic inflammation,[7] and evolve from the fusion of mononuclear phagocytes. The formation of MGCs is thought to represent a specialized form of macrophage differentiation by which activated macrophages undergo morphologic changes to form epithelioid cells that subsequently fuse.[4,8–12] Although it has been suggested that many agents can lead to formation of MGCs *in vivo* and *in vitro*,[8] the precise stimulus for and the mechanism(s) of macrophage fusion are still unclear. One stimulus for macrophage fusion is hypothesized to be a soluble mediator(s) released by antigen-sensitized lymphocytes,[10–12] but the relationship of such a macrophage fusion factor (MFF) to other lymphokines and the mode of action of MFF are also unclear.

What is the function of MGCs? Do these cells represent an end point of macrophage differentiation?

Supported by the National Cancer Institute under Contract NO1-CO-75380 with Litton Bionetics, Inc.

Accepted for publication November 21, 1980.

Address reprint requests to Dr. C. Bucana, Cancer Biology Program, NCI Frederick Cancer Research Center, P.O. Box B, Frederick, MD 21701.

0002-9440/81/0508-0234$01.15

What are the conditions that favor formation of MGCs? Do MGCs in the lung, a common site for metastases,[13] have the capacity to destroy tumor cells? To answer these and other questions, we need a simplified and reproducible model for the production of fused alveolar macrophages (AMs) *in vitro*. This report concerns studies on the induction and kinetics of AM fusion *in vitro* following incubation with lymphokines obtained from cell-free supernatant fluids of concanavalin A(Con-A)-stimulated rat lymphocytes. We also describe the ultrastructural characterization of the dynamic process of MGC formation *in vitro*. We have used here a technique developed in our laboratory[14] that allows a sequential scanning to transmission electron microscopic (TEM) analysis of cells. Using this technique, we determined the intimate relationship of the surface morphology and internal structure of MGCs and their satellite cells.

Materials and Methods

Animals

Specific-pathogen-free inbred F344 male rats, 8–10 weeks old, were obtained from the Frederick Cancer Research Center's Animal Production Area.

Mediums

Eagle's minimal essential medium (MEM) was supplemented with sodium pyruvate, nonessential amino acid, penicillin-streptomycin, L-glutamine, and two-fold vitamin solution (CMEM). Hanks' balanced salt solution (HBSS), pH 7.2, was obtained from Grand Island Co., Grand Island, New York. Endotoxin-free fetal calf serum (FCS) was obtained from Reheis Chemical Corporation, Phoenix, Arizona.

Production of Mediators From Con-A–Stimulated F344 Lymphocytes

AM fusion factor (MFF) was obtained from cultures of rat lymphocytes stimulated *in vitro* with Con-A (rat Con-A-MAF) by a modification of a technique described previously.[5,16] Spleen and lymph nodes from normal F344 rats weighing 150 g were collected aseptically, minced in cold HBSS, and pressed through a 60-mesh stainless steel wire sieve (E-C Apparatus Corp., St. Petersburg, Fla). The resulting suspensions were filtered through gauze and centrifuged. The cell pellets were resuspended in CMEM containing 5% FCS. The viability of the non-glass-adherent mononuclear cells was >90%, as determined by the trypan blue exclusion test. Lymph node cells were mixed with spleen cells at a spleen cell/lymph node cell ratio of 10 to 1. In preliminary studies optimal AM fusion activity was consistently obtained when 5×10^6 cells/ml were incubated *in vitro* for 48 hours with 100 μg/ml of insoluble Con-A (Sepharose-bound Con-A, Pharmacia, Uppsala, Sweden). Following incubation, the suspensions were centrifuged for 20 minutes at 5000 rpm. Cell-free supernatants were filtered through a 0.22-μ Millipore membrane and stored until use at −80 C.

Preparation and Purification of AM Cultures

AMs were obtained by a tracheobronchial lavage method as described previously.[15] F344 rats were anesthetized with sodium pentobarbital injected intraperitoneally, immersed in iodine and then alcohol, and placed in a laminar airflow hood. We exsanguinated the rats by severing both renal arteries to minimize postmortem pulmonary edema and reduce trapped blood in the lungs. We opened the chest cavity to produce pneumothorax. The trachea was cannulated with a cut tube from a Butterfly-19 or -21 infusion set (Abbott Laboratories, Chicago, Ill) and anchored by suturing. The lungs were lavaged with 5 ml of Ca^{2+} and Mg^{2+}-free HBSS prewarmed at 37 C. The process was repeated several times to yield a total of 50 ml lavage fluid per rat. The total number of cells collected was determined with a hemocytometer (counting sample diluted in 2% acetic acid solution). The viability of nucleated cells (in HBSS suspension) was determined by trypan blue dye exclusion to be greater than 95%. The yield of AMs obtained from 8–10-week-old F344 rats was approximately 2×10^4 cells/g body weight. The cells recovered by lavage consisted of more than 97% AMs; the remaining cells were either neutrophils or small mononuclear cells. The latter were eliminated, however, during the washing of plated cells.[15] The lavage suspension was centrifuged at 250*g* for 10 minutes. The AM in CMEM containing 5% FCS were plated onto glass coverslips 12 mm in diameter placed into wells 16 mm in diameter (200 sq mm) of tissue culture dishes (Costar, Cambridge, Mass) at a concentration of 1×10^6 AMs/well. After 60 minutes, incubation at 37 C in an atmosphere of 5% CO_2, the monolayers were rinsed and refed with CMEM for the removal of nonadherent cells.

AM Fusion Assay

AMs (10^6) were plated into wells, and the cultures were washed 60 minutes later. Con-A-MFF was added to AMs for various incubation periods up to 24 hours. In control experiments, AMs were incubated with

either CMEM containing 10 or 50 μg/ml Con-A or supernatant fluids harvested from cultures of normal rat lymphocytes that were not treated with Con-A. After the incubation period was determined, coverslips were rinsed thoroughly with HBSS and dried immediately. The AMs were fixed with buffered formalin and stained. Coverslips were mounted for microscopy. The percentage of AM fusion was determined by counting the total number of nuclei within MGCs (more than 3 nuclei per cell), and the total number of nuclei within at least 10 microscopic fields was examined at a magnification of × 600.

$$\% \text{ AM fusion} = \frac{\text{Total number of nuclei within MGCs in fields}}{\text{Total number of nuclei in fields}} \times 100$$

Transmission Electron Microscopy (TEM)

Samples on coverslips were fixed for 1 hour with 3% glutaraldehyde and 2% paraformaldehyde buffered with 0.1 M cacodylate buffer, pH 7.3. The samples were washed with buffer and fixed with 1% osmium tetroxide in cacodylate buffer for 30 minutes, washed five times with buffer, and then incubated in a saturated solution of thiocarbohydrazide (Polysciences, Warrington, Pa) for 10 minutes. The samples were then washed five times with distilled water, fixed for 10 minutes in osmium vapor, rinsed with distilled water five times, and stained with 1% aqueous uranyl acetate for 30 minutes. The samples were dehydrated with a graded series of ethanol and were infiltrated and embedded in Spurr's low-viscosity medium. Thin sections were cut with the use of glass knives in an LKB Ultratome III; the sections were stained with Reynold's lead citrate and examined in a Hitachi HU-12A transmission electron microscope at an accelerating voltage of 75 kv. In order to visualize the microfilaments and microtubules, replicate samples were fixed with glutaraldehyde and tannic acid according to Wagner.[17] Ruthenium red staining was done according to Hayat[18] so that we could visualize the cell surface boundaries.

Scanning Electron Microscopy (SEM)

Samples for SEM and TEM were processed simultaneously until the second osmium fixation, after which samples for SEM were dehydrated through a graded series of ethanol, substituted with Freon 113, and critical-point-dried in Freon 13 in a Bomar critical-point dryer SPC-900/EX. The samples were then sputter-coated with gold and examined in a Hitachi HFS-2 field emission scanning electron microscope at an operating voltage of 25 kv.

Sequential SEM–TEM

Specific areas on coverslips that were processed for SEM were scored lightly to provide reference marks for subsequent identification of the location of cells studied. Scanning electron micrographs of several cells were obtained, and the coverslip was removed from the specimen stubs and processed for TEM according to a procedure published elsewhere.[14] The coverslip was removed from the polymerized block with the use of hydrofluoric acid. We observed this process under the dissecting microscope to avoid unnecessary exposure of the sample to acid. Prolonged exposure of the sample to hydrofluoric acid reduces the electron density of the samples. The blocks were washed with water several times and dried in the oven. The cells studied by SEM were identified and sectioned for TEM.

Results

Conditions Required for Optimal Fusion of AMs

Fusion of AMs is dependent both on the dose of Con-A-MFF and the density of the AM monolayer. Moreover, the formation of MGCs is not dependent on AM multiplication but represents a true fusion process. AMs ranging in number from 1×10^4 to 1×10^6 were plated into culture wells with a 200-sq mm area. As shown in Figure 1, Con-A-MFF was added to AM monolayers of differing densities (more than 2500 AMs/sq mm formed a confluent monolayer). The percentage of AM fusion correlated with the population density, suggesting that cell-to-cell contact is an important factor in subsequent fusion. Dilution of Con-A-MFF led to a decrease in AM fusion. In control experiments the addition of 10 or 50 μg/ml soluble Con-A to either CMEM or normal rat supernatant (NRSUP) did not induce AM fusion (data not shown). AM fusion was not associated with DNA synthesis as measured by uptake of DNA precursors [^{3}H]TdR or [^{125}I]IUdR (Table 1). Moreover, visual counts of cells under microscopy revealed no increase in total cell number during the observation period. Collectively, the results indicate that *in vitro* MGC formation induced by Con-A-MFF results from fusion of AMs.

Kinetics of Alveolar Macrophage Fusion

Normal F344 AM were incubated with Con-A-

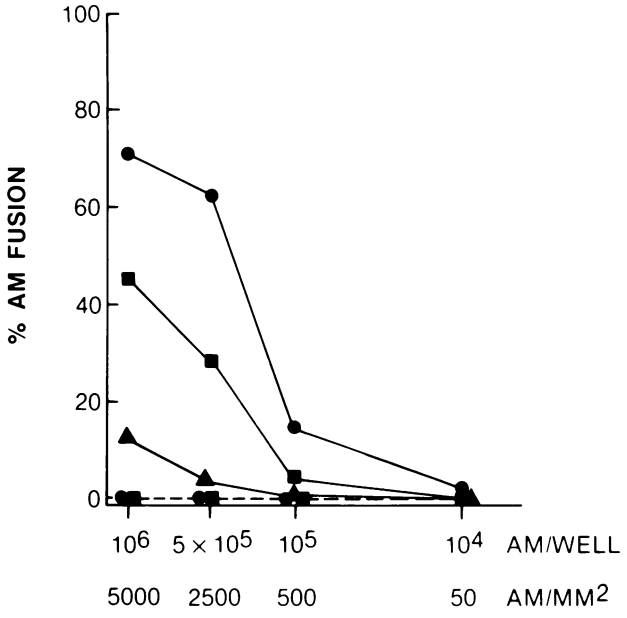

Figure 1—Optimal AM fusion is related to population density and dose of Con-A-MFF. Different numbers of AMs (1×10^4 to 1×10^6) were plated into 200-sq mm wells. Different dilutions of Con-A-MFF were added to the monolayers. The percentage of AM fusion was determined 24 hours later. Con-A-MFF: undiluted ●——●; 1:3 dilution ■——■; 1:9 dilution ▲——▲; normal lymphocyte supernatant fluids ■---■; CMEM ●---●.

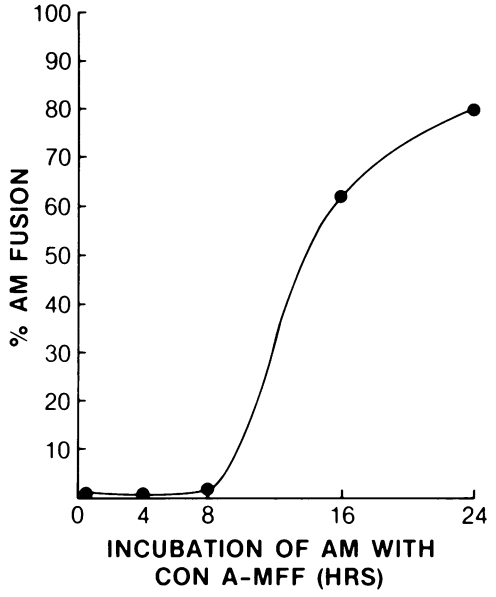

Figure 2—Kinetics of AM fusion by Con-A-MFF. AMs were incubated with Con-A-MFF at 4, 8, 16 and 24 hours, and the percentage of AM fusion was determined.

MFF. At different times, the Con-A-MFF was removed, and the cultures were fixed and stained. Both untreated and Con-A-MFF–treated AMs began to aggregate by 4 hours of *in vitro* incubation. The aggregate size increased with length of incubation. AM clustering peaked by 8 hours and gradually decreased after 24 hours of incubation.

AM aggregation was found to be independent of Con-A-MFF. Such was not the case with AM fusion. The time course of AM fusion is shown in Figure 2. Eight hours' incubation of AMs with Con-A-MFF was not sufficient for fusion to occur. By 16 hours' incubation, significant AM fusion was observed, the peak fusion was reached by 24 hours (Figure 3), whereas untreated AMs remained clustered and did not fuse.

In another set of experiments, a minimal 8 hours of interaction between Con-A-MFF and AMs was required for subsequent fusion to occur. If AM cultures were treated with Con-A-MFF for less than 8 hours, then washed and refed with CMEM, little or no fusion was observed even 24 hours later (Figure 4).

Table 1—Uptake of [^{3}H]TdR or [^{125}I]IUdR into Untreated and Con-A-MFF–Treated Macrophages Undergoing Fusion

Culture media	% AM fusion*	CPM in AM monolayers†	
		[^{3}H]TdR	[^{125}I]IUdR
CMEM	0	1797 ± 41	1940 ± 110
CMEM and Con-A-MFF	67 ± 4.8	816 ± 42	756 ± 25

* 5000 AMs plated/sq mm. The percentage of fusion was determined after 24 hours of culture.

† 0.2 μCi of [^{3}H]TdR or 2 μCi of [^{125}I]IUdR added to AM cultures. The values represent the mean of 5 wells ± SD.

SEM

We conducted a sequential (SEM) examination of AM fusion. Rat AMs began to aggregate within 2 hours after the addition of Con-A-MFF to the cultures. At 8 hours, tight clusters of cells were seen, and by 10–12 hours the clusters were composed of very large cells with satellite single cells attached to them. At 15 hours, MGCs were predominant in the field, and only occasional clusters of cells and isolated single cells could be seen. Several changes in the cell surface of AMs accompanied the formation of MGCs. Initially, AMs showed extensive ruffling of the cell surface, and this surface architecture was maintained even when the cells began to aggregate. At 8 hours, AMs in the cluster showed a predominantly ruffled cell surface, but in some cells the ruffles were replaced by short, flat plates frequently terminating in short finger-like processes (Figure 5). The latter appeared to be a transition stage from ruffles to filopodia. Occasionally, very long filopodia emanated from the tip of the flat plates. These long processes appeared to be directed toward other cells in the same cluster as well as toward cells from an adjacent cluster. Within a cluster, AMs were usually of uniform size except for a few

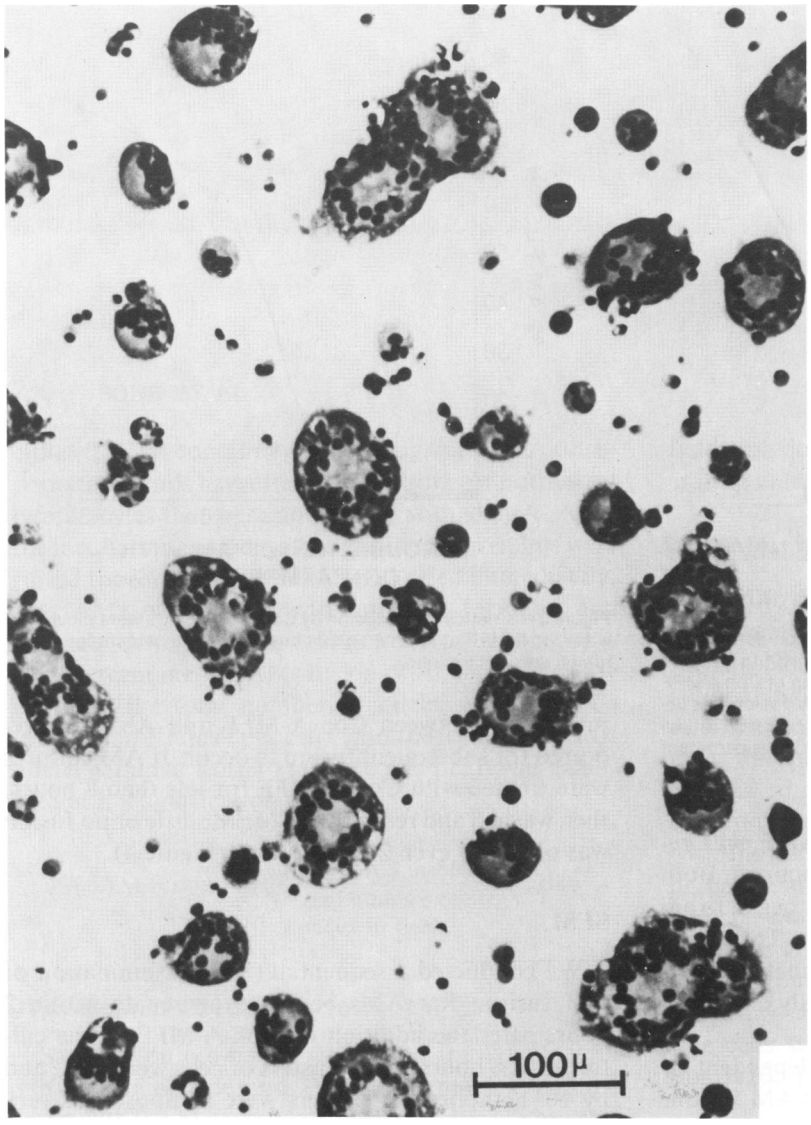

Figure 3—MGCs formed by the fusion of normal AMs that were cultured in Con-A-MFF for 24 hours.

occasional cells that were about two times the size of isolated single cells. As shown by TEM, these large cells were binucleated. At 10 hours, the cell aggregate was composed of very large cells (three to four times larger than single cells) and attached satellite single cells with discrete boundaries (Figure 6). The cell surface of both MGCs and satellite cells was covered with ruffles and thin platelike processes (Figure 6). This was the earliest time point (10 hours) at which we found MGCs by sequential SEM–TEM analyses. After 10 hours, AMs assumed a very pleomorphic appearance. The cell surface was covered by ruffles, plates, or long filopodia or any combination of these projections. New MGCs were formed eventually from clusters of cells that were farther away from the initial MGCs. Cells that were not involved in MGC formation and remained as single, isolated cells assumed a ruffled cell surface or occasionally a mixed ruffled surface with platelike processes.

By 16–24 hours, 50–70% of the cell population was composed of MGCs or tight clusters of cells. In general, the cell surface of MGCs and single cells remained ruffled. Interpretation of the surface topography of cells is frequently difficult and requires replicate samples from different donors. In this study, 1 out of 8 replicate samples from different animals showed AMs that were strikingly very different from other replicates, in that aggregates of AMs exhibited long fingerlike processes (filopodia) interdigitating with similar processes from neighboring cells (Figure 7). The overall surface topography of these MGCs and single cells after 24 hours' incubation showed numerous filopodia that were in marked contrast to the ruffled surface of cells in other replicates. We believe that

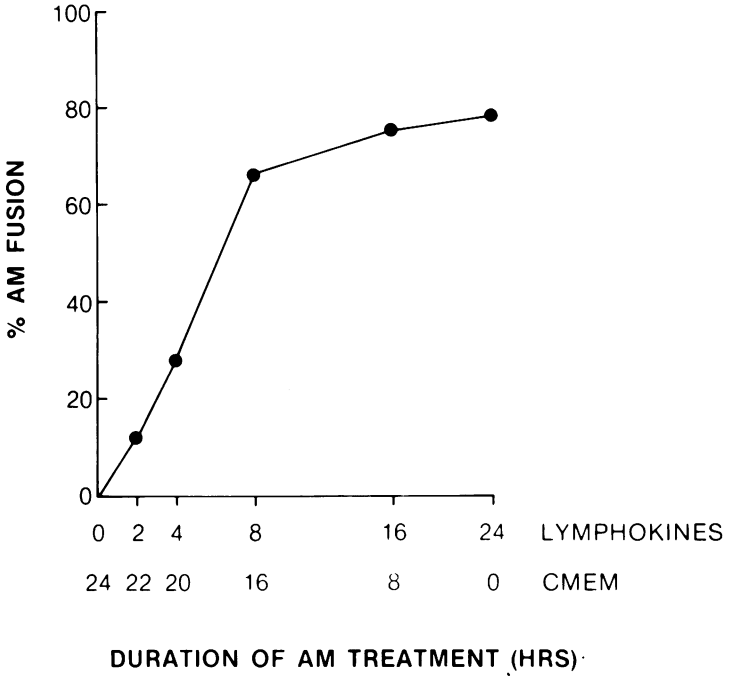

Figure 4—Duration of Con-A-MFF interaction with AMs necessary for fusion. AMs were incubated with Con-A-MFF for indicated times (0, 2, 4, 8, 16, or 24 hours). When the cultures were washed and refed with CMEM, the percentage of fusion was determined after 24 hours of the total (Con-A-MFF and CMEM) incubation period.

this AM sample was obtained from animals with a respiratory infection of unknown etiology that led to a change in the differentiated phenotype of these AMs.[19] Nonetheless, MGC formation was also observed here.

Sequential SEM–TEM

In order to determine the earliest time point for AM fusion, we examined the same cell or groups of cells first by SEM and then sequentially processed them for TEM. At the SEM level, very large AMs (three to four times larger than the isolated single cells) were observed after 10 hours of incubation with Con-A-MFF. These large cells were identified by TEM as multinucleated, with processes interdigitating with satellite single cells (Figure 8). Serial sections of AM clusters including MGCs revealed that interdigitation of processes occurred across a fairly wide area of the cell surface, but, in most instances, no fusion could be seen among them. In sequential analysis of other MGCs, we observed single cells that were completely surrounded by the MGCs but were not fused with them (Figure 9). The enclosed unfused cell, which was elec-

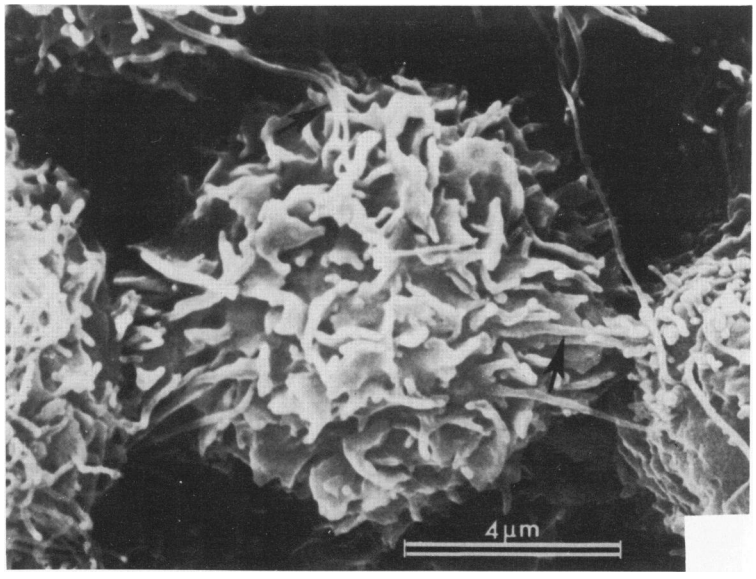

Figure 5—SEM of AMs showing transition form of cell surface from ruffles to plates to filopodia. *Arrow* indicates a long filopodium emanating from a plate.

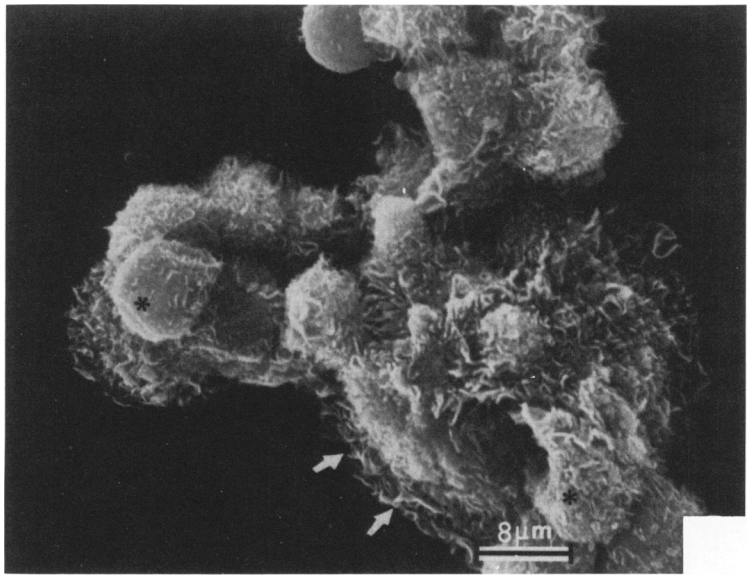

Figure 6—SEM of an AM cluster 10 hours after incubation with Con A-MFF. Note the large cells (*arrows*) that are two to three times larger than the satellite cells (*asterisk*).

tron-dense as compared with the giant cell *per se*, appeared to be trapped in the membranous labyrinth of the giant cell. The satellite cells exhibited prominent cytoplasmic processes directed toward the MGCs as shown by TEM. Serial sections indicated that these directional processes were of the ruffled-membrane type, as deduced from previous sequential SEM–TEM studies and the presence of seemingly branched cytoplasmic processes shown in Figures 10 and 11. Further details of the internal structure of MGCs are provided in the TEM study.

TEM

Normal rat AMs exhibit a marked heterogeneity in their electron density. This heterogeneity was exaggerated when cells were treated with tannic acid (TA) during the fixation procedure (Figure 12). This fixation procedure allowed for the demonstration of a direct correlation between functional and morphologic heterogeneity of AMs. After TA treatment, 45–50% of normal AMs were electron-dense, and the remainder were electron-lucent cells or cells of intermediate electron density. Electron-dense cells generally showed numerous vacuoles, endocytosed material, inclusion or residual bodies, secondary lysosomes, electron-dense eccentric nuclei, and very electron-dense cytoplasm. Electron-lucent cells exhibited the normal complement of cell organelles, sometimes with prominent rough endoplasmic reticulum and electron-lucent

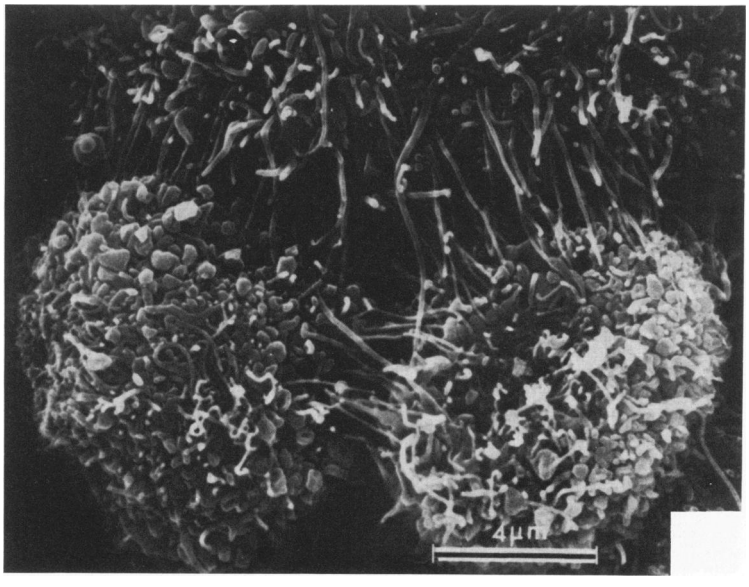

Figure 7—SEM of a cluster where AMs exhibit prominent filopodia that interdigitate with processes from neighboring cells.

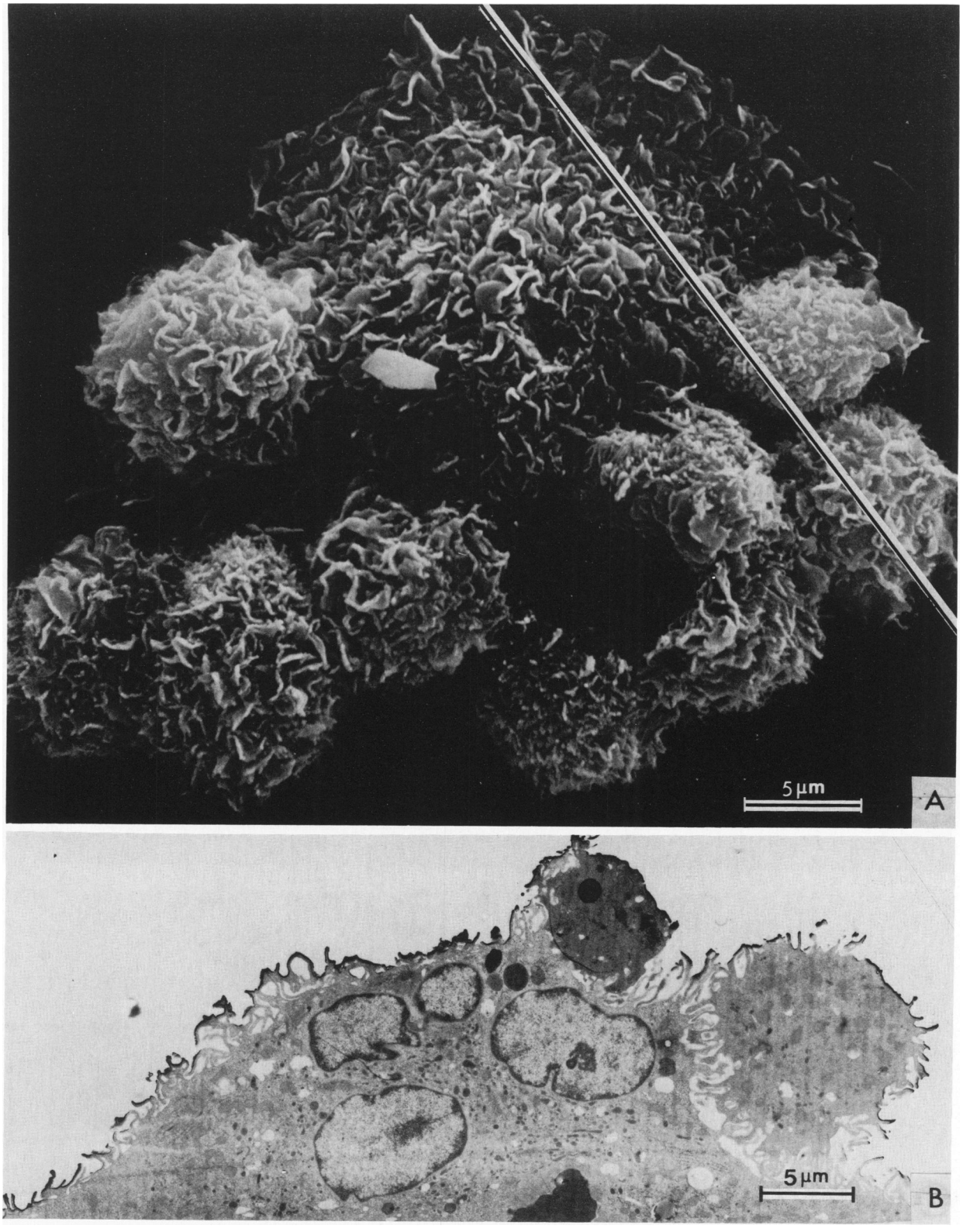

Figure 8—Sequential SEM–TEM analysis of an MGC observed after 10 hours' incubation. **A**—SEM of an MGCs as well as the single cells attached to it show numerous ruffles on the surface. **B**—TEM of the same cell shows the large cell as multinucleated and the two smaller cells transsected at the area indicated by the *white line* in the SEM picture show processes interdigitating with processes from the MGC. Serial sections show no fusion of the single cells with the MGC at this point. (With a photographic reduction of 13%)

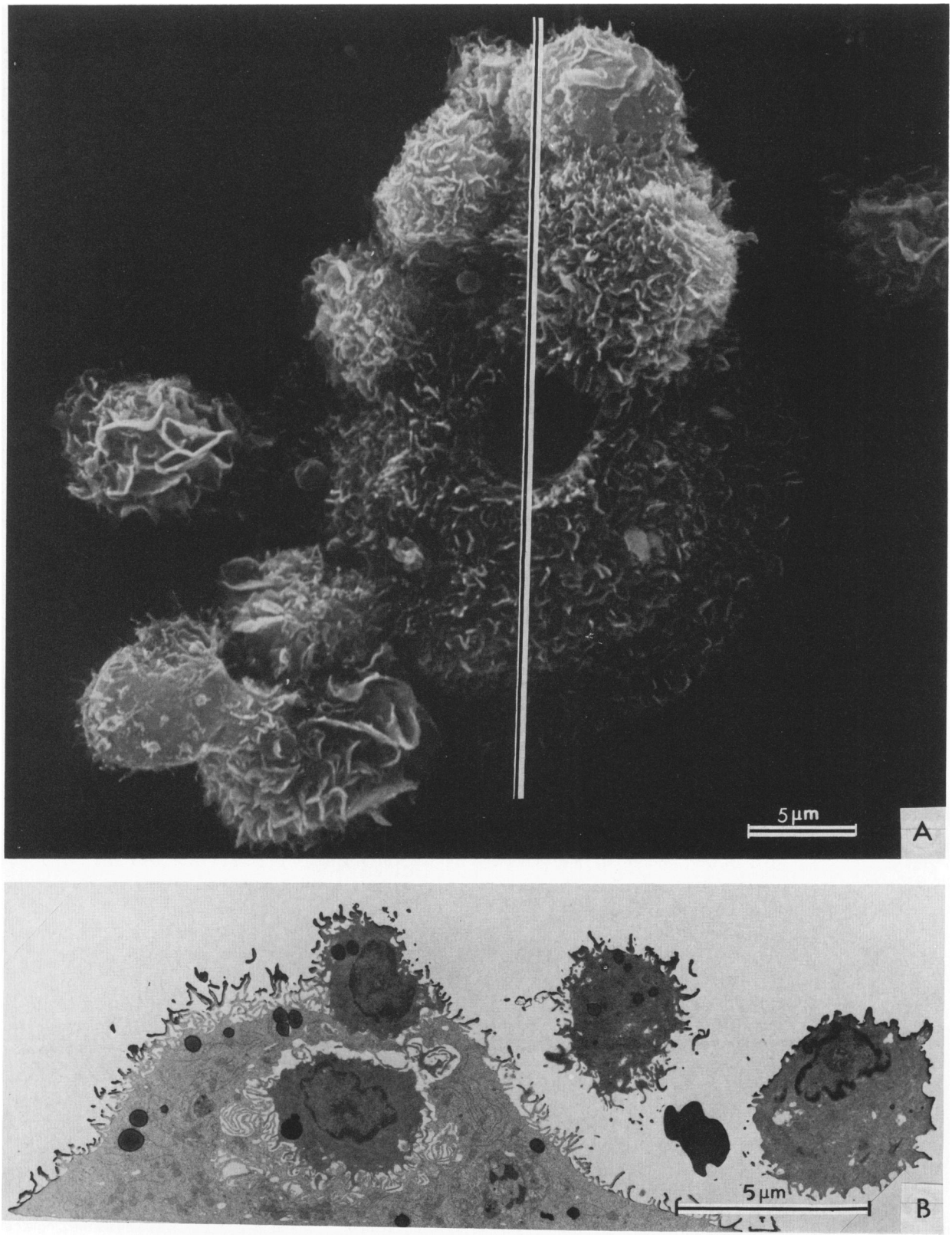

Figure 9—SEM-TEM of an MGC showing an unfused AM trapped in the membranous labyrinth of the MGC. (With a photographic reduction of 15%)

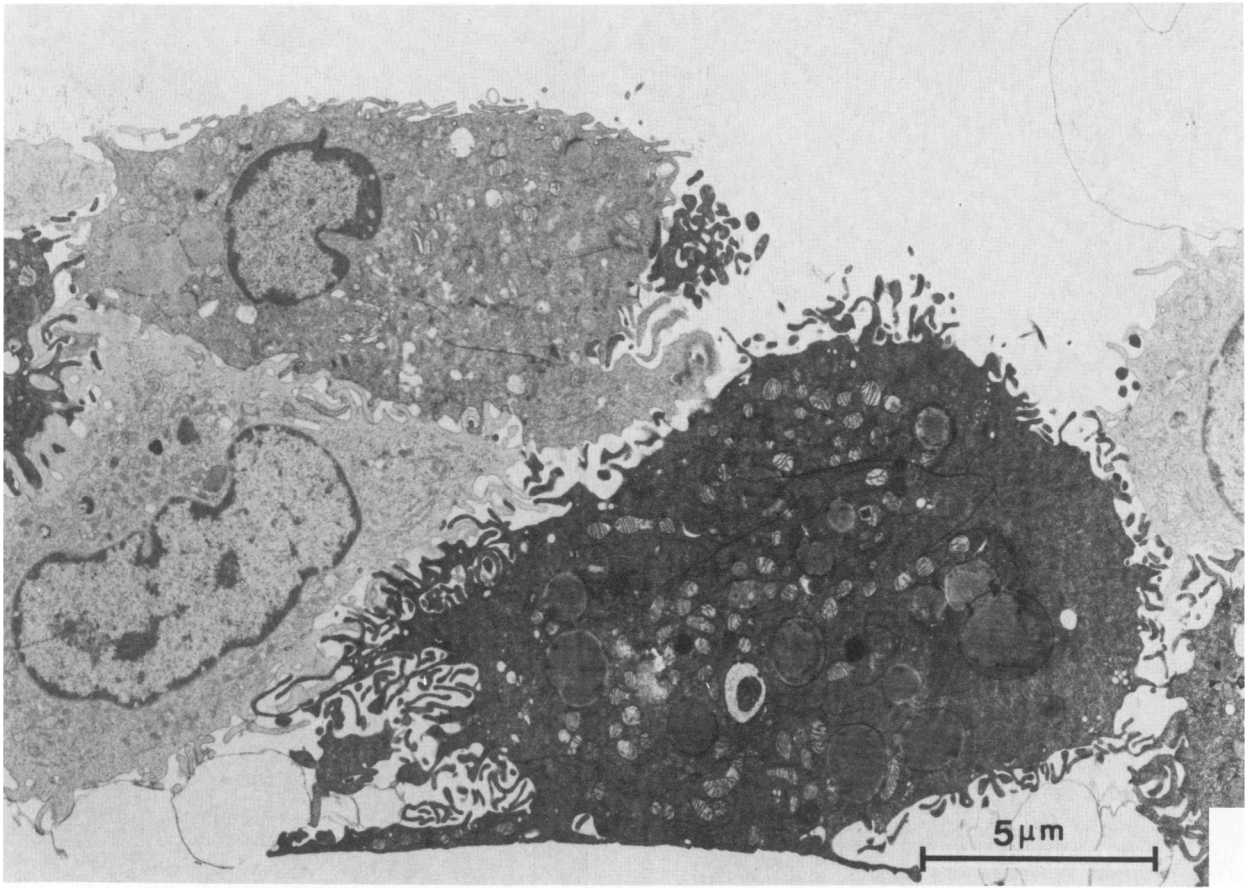

Figure 10—TEM of AM clusters after TA fixation showing cells of various electron densities.

cytoplasm. Certain sections showed both types of cells with microtubules and microfilaments. At the end of 18 hours' incubation with Con-A-MFF, 20–30% of the MGCs were electron-lucent, and almost all of the very large MGCs (>20 nuclei) were electron-dense. MGCs often have both electron-dense and electron-lucent cells as satellite cells. By using TA in the fixative, we were also able to dramatically demonstrate the interdigitation of processes between cells of different electron densities (Figure 11).

Serial sections from the 10-hour incubation sample showed that the very large cells were, in fact, multinucleated. Sometimes lysosomes associated with remnants of plasma membrane in the cytoplasm were seen in the newly formed MGCs (Figure 13). The cytoplasm also contained numerous Golgi bodies, an extensive endoplasmic reticulum, and numerous vesicles. In a favorable cut, several centrioles could be seen in the cytoplasm. It appears that AM fusion occurred only at certain sites of the contiguous membranes, such that vacuoles and cytoplasmic processes were often trapped in the cytoplasm of the MGCs (Figues 13 and 14). Lysosomes were seen along areas of fusion and were closely associated with vacuoles, remnants of plasma membrane, or trapped cytoplasmic processes. Ruthenium red staining was used to delineate the boundaries of single cells in the cluster. We observed that cytoplasmic processes and large vacuoles in the MGCs stained for ruthenium red, which suggested that these structures were trapped during fusion. None of the giant cells examined showed cells in mitosis.

Discussion

The present experiments demonstrate that rat lymphocytes stimulated *in vitro* with Sepharose-bound Con-A release into the culture supernatant fluids factors that can induce the fusion of rat AMs. Previously, it was shown that other lymphokines such as MAF or migration inhibition factor (MIF) could be released by both antigen and mitogen-stimulated lymphocytes.[4,5,16,20] The present data suggest that such is also the case for MFF. Lymphocytes from animals sensitized to BCG and then exposed to BCG but not purified protein derivative (PPD)[10,21] or lymphocytes

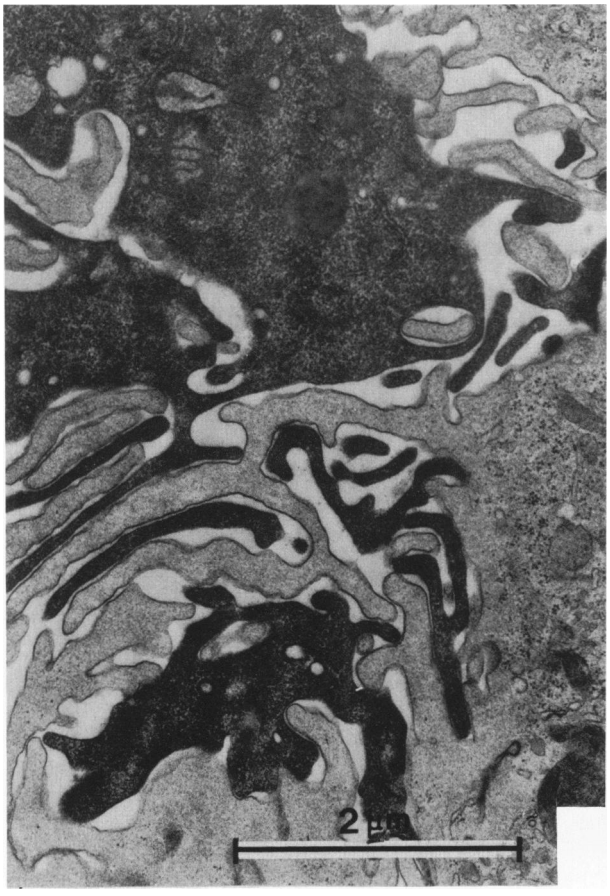

Figure 11—TEM of interdigitating processes of light and dark staining AMs.

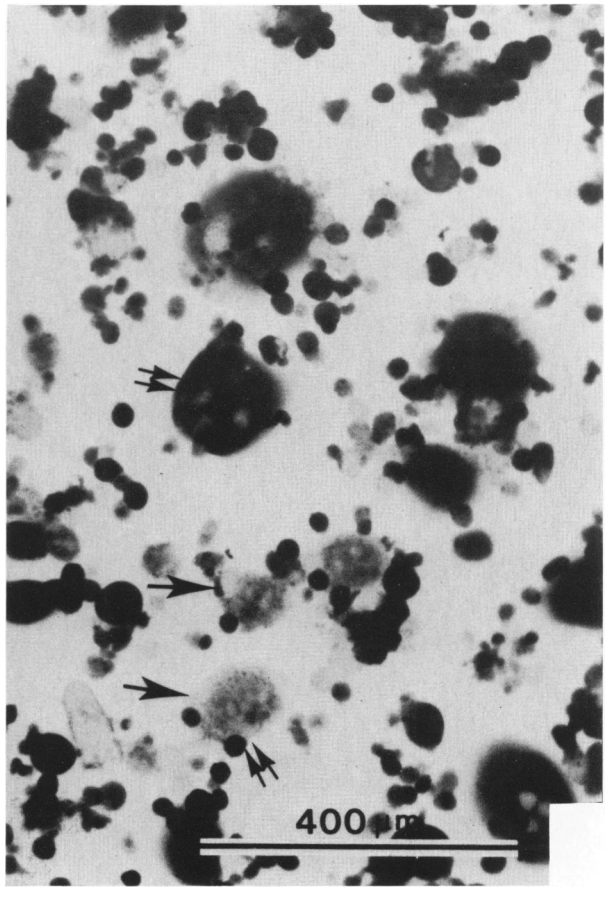

Figure 12—Light micrograph of resin-embedded sample showing light (*arrows*) and dark-staining MGCs. Note dark MGCs with light AM cells and light MGCs with dark AM satellite cells (*double arrows*).

from guinea pigs sensitized to bovine gamma globulin[12] were shown to release MFF in response to a challenge by the specific antigen. We now demonstrate that mitogen stimulation under appropriate lymphocyte concentration and source conditions, ie, spleen cells and lymph node cells at a ratio of 10:1, 5×10^6 cells/ml, can release MFF.

Macrophage fusion can occur as a response to a large number of independent agents.[4,8] Lymphokines are probably only one stimulus for the phenomenon. The formation of MGCs has been observed following implantation of glass coverslips in normal and athymic nude mice.[22] Clearly, the formation of MGCs under these conditions did not require the interaction of functional T lymphocytes.[22,23] Mitogens such as Con-A or PHA have been reported to directly induce *in vitro* fusion of human and hamster macrophages.[24,25] This was not the case in our studies with rat AMs. Incubation of AMs with free Con-A did not induce any AM fusion by 24 hours' incubation. AM fusion was dependent on the density of the monolayer. This finding agrees with clinical observations that the formation of MGCs *in vivo* occurs in tissues where large numbers of monocytes concentrate.[8]

The extent of AM fusion was related to the density of the population. Nonetheless, it is difficult to envision that fusion resulted from the simultaneous endocytosis of particles by adjacent AMs. First, not all AMs in the culture aggregated during the initial incubation period that preceded fusion. Second, treatment with Con-A-MFF was mandatory for fusion (but not aggregation) to take place. In any case, not all AMs were involved in the fusion process. Throughout the observation period, there were many uninvolved, solitary AMs. Because MGCs consisted of more electron-dense cells than electron-lucent cells, it is possible that macrophages at a certain stage of their maturation are more susceptible to the effects of Con-A-MFF. Moreover, almost all of the very large MGCs (>20 nuclei) observed at 18–24 hours were always electron-dense. This suggests a rapid mixing of cytoplasm that probably occurs as fast as the intermixing of cell surface antigens reported in other systems.[27]

Optimal AM fusion induced by Con-A-MFF was also dose-dependent. Thus, serial dilutions of Con-A-MFF would lead to a decreased efficiency of AM fusion incubated at identical densities. In our present studies, AM fusion did not involve nuclear division,[26] since we were unable to demonstrate DNA synthesis in

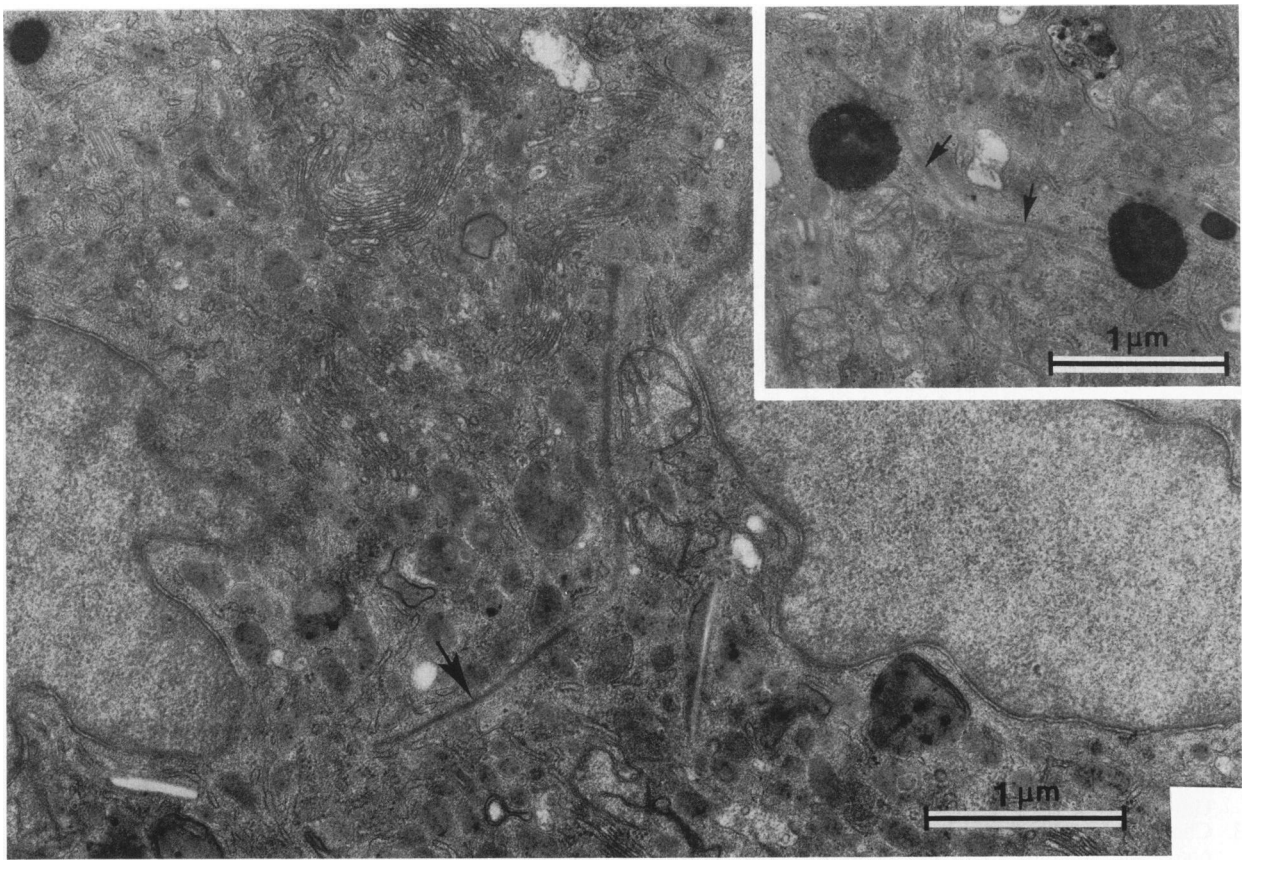

Figure 13—TEM of areas of fusion showing remnants of membranes between cells (*arrow*). Serial sections of this area show lysosomes associated with the membrane remnant (**inset**). Numerous Golgi bodies and lysosomes are also seen.

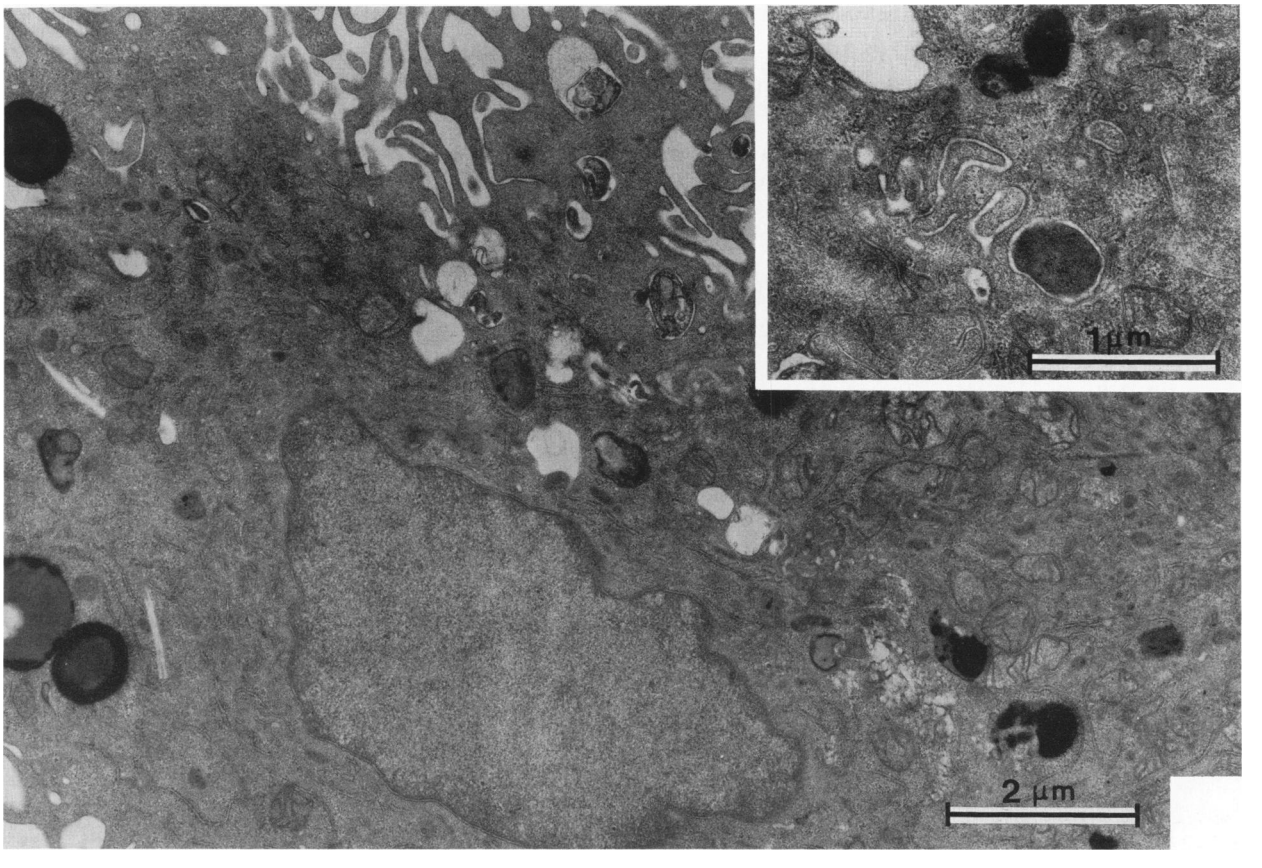

Figure 14—TEM showing presence of lysosomes along areas of fusion. Trapped vesicles and cytoplasmic projections are seen as a consequence of fusion at certain sites on the cell surface. **Inset** shows lysosomes closely associated with trapped cytoplasmic processes.

cultures undergoing active fusion process. The TEM data presented here confirm previous reports that MGCs result from macrophage fusion.[2,3,8] Numerous Golgi bodies, vesicles, polysomes, and rough endoplasmic reticulum can be found within the MGCs.

In vitro fusion of macrophages following treatment with lymphokines released by antigen-stimulated lymphocytes have been reported previously.[10,12] Nonetheless, the release of MFF by mitogen-stimulated lymphocytes provides a useful and reproducible means of achieving *in vitro* fusion of rat AMs. The use of this lymphokine allowed us to investigate the pathogenesis of MGCs. These studies, as well as the functional properties of MGCs and their possible role in host defense against neoplasms, are described in the companion paper.[28]

References

1. Hibbs JB Jr: Role of activated macrophages in nonspecific resistance to neoplasia. J Reticuloendothel Soc 1976, 20:223-231
2. Eccles SA: Macrophages and cancer, Immunological Aspects of Cancer. Edited by JE Castro. Baltimore, University Park Press, 1978, pp 123-154
3. Chapman HA Jr, Hibbs JB Jr: Modulation of macrophage tumoricidal capability by components of normal serum: A central role for lipid. Science 1977, 197: 282-285
4. Poste G: The tumoricidal properties of inflammatory tissue macrophages and multinucleated giant cells. Am J Pathol 1979, 96:595-610
5. Fidler IJ, Darnell JH, Budmen MB: Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res 1976, 36:3608-3615
6. Hanna MG Jr, Snodgrass MJ, Zbar B, Rapp HJ: Histological and ultrastructural studies of tumor regression in inbred guinea pigs after intralesional injection of *Mycobacterium bovis* (BCG). Natl Cancer Inst Monogr 1973, 39:71-84
7. Adams DO: The granulomatous inflammatory response. A review. Am J Pathol 1976, 84:164-191
8. Papadimitriou JM: Macrophage fusion in vivo and in vitro: A review, Membrane Fusion. Edited by G Poste, GL Nicolson. New York, Elsevier-North Holland, 1978, pp 181-218
9. Chambers TJ: Multinucleate giant cells. J Pathol 1978, 126:125-148
10. Galindo B, Lazdins J, Castillo R: Fusion of normal rabbit alveolar macrophages induced by supernatant fluids from BCG-sensitized lymph node cells after elicitation by antigen. Infect Immun 1974, 9:212-216
11. Parks DE, Weiser RS: The role of phagocytosis and natural lymphokines in the fusion of alveolar macrophages to form Langhans giant cells. J Reticuloendothel Soc 1975, 17:219-228
12. Warfel AH, Hadden JW: Macrophage fusion factor elicited from BCG-sensitized lymphocytes. Am J Pathol 1978, 93:758-766
13. Fidler IJ: Tumor heterogeneity and the biology of cancer invasion and metastasis. Cancer Res 1978, 38:2651-2660
14. Bucana C, Hoyer LC, Hobbs B, Breesman S, McDaniel M, Hanna MG Jr: Morphological evidence for the translocation of lysosomal organelles from cytotoxic macrophages into the cytoplasm of tumor target cells. Cancer Res 1976, 36:4444-4458
15. Sone S, Poste G, Fidler IJ: Rat alveolar macrophages are sensitive to free and liposome-encapsulated lymphokines. J Immunol 1980, 124:2197-2202
16. Fidler IJ, Darnell JH, Budmen MB: In vitro activation of mouse macrophages by rat lymphocyte mediators. J Immunol 1976, 117:666-673
17. Wagner RC: Tannic acid as a mordant for heavy metal stains. Proceedings of the Electron Microscopy Society of America, 34th Annual Meeting, Miami Beach, Florida, 1976, pp 316-317
18. Hayat MA: Positive Staining for Electron Microscopy. New York, Van Nostrand Reinholdt, 1975, pp 162-176
19. Leake EA, Wright MJ: Variation on the form of attachment of rabbit alveolar macrophage to various substrata as observed by scanning electron microscopy. J Reticuloendothel Soc 1979, 25:417-441
20. Ruco LP, Meltzer MS: Macrophage activation for tumor cytotoxicity: Induction of tumoricidal macrophages by supernatants of PPD-stimulated Bacillus Calmette Guérin immune spleen cell cultures. J Immunol 1977, 119:889-896
21. Galindo B, Rodriguez A: Affinity of macrophage fusion factor for macrophages. J Reticuloendothel Soc 1979, 26:221-227
22. Papadimitriou JM: The influence of the thymus on multinucleate giant cell formation. J Pathol 1976, 118:153-156
23. Machdo EA, Lair SV: Giant multinucleate macrophages in methyl cellulose-stimulated athymic nude mice. J Reticuloendothel Soc 1978, 23:383-387
24. Smith CW, Goldman AS: Macrophages from human colostrum: Multinucleated giant cell formation by phytohemagglutinin and concanavalin A. Exp Cell Res 1971, 66:317-320
25. Chambers TJ: Fusion of hamster macrophages induced by lectin. J Pathol 1977, 123:53-61
26. Hadden JW, Sadlik JR, Hadden EM: The induction of macrophage proliferation in vitro by a lymphocyte-produced factor. J Immunol 1978, 121:231-238
27. Frye LD, Edidin M: The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. J Cell Sci 1970, 7:319-335
28. Sone S: Functions of alveolar macrophages fused after interaction with lymphokines. (Manuscript submitted)

Acknowledgments

We thank W. E. Fogler and Diane Plentovich for excellent technical assistance and Elynor Sass for preparation of the manuscript.