INFECTION AND TRANSFORMATION OF MOUSE PERITONEAL MACROPHAGES BY SIMIAN VIRUS 40*

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The study of the effects of small DNA tumor viruses on mammalian cells has been very useful for understanding and clarifying cellular functions during the conversion of normal to tumor cells. One of the most intriguing problems in this regard is the ability of these viruses to stimulate induction of cellular DNA synthesis in cells which have been blocked in a particular period of their cycle by drugs, X-rays, elevated temperature, or contact inhibition (for review, see reference 1). Furthermore, it has been shown that cells in which the state of differentiation is such that DNA replication no longer occurs normally can also be induced to undergo DNA synthesis (2, 3).

Mouse peritoneal macrophages constitute an interesting cell system, because these cells can be maintained for several months when explanted in vitro, but their rate of proliferation is extremely low (4, 5). However, macrophages can be induced to synthesize DNA and replicate when they are incubated with other cells or in medium conditioned by fibroblasts of the same species (6, 7). Thus macrophage cultures constitute a model system in which cell growth is dependent upon interaction with a different cell population.

This paper reports on a study of the infection of mouse peritoneal macrophages by simian virus 40 (SV40),[1] on a comparison between the induction of cellular DNA synthesis by a physiological factor (conditioned medium) and by a viral agent (SV40), and on transformation of macrophages into permanent cell lines which maintain several of the morphological and functional

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[1] Abbreviations used in this paper: AMS, anti-macrophage serum; BME, Eagle's basal medium; 2X BME, double-strength BME; CM, conditioned medium; FBS, fetal bovine serum; PBS, phosphate-buffered saline; PFU, plaque-forming units; SRBC, sheep red blood cells; SV40, simian virus 40; T antigen, tumor antigen; V antigen, viral capsid.

properties of the parental population, but no longer depend on other cells for growth. Preliminary reports of these findings have been made recently (8, 9).

Materials and Methods

Macrophage Cultures.—Macrophages were harvested sterilely from the peritoneal cavity of male C57BL mice 3 days after injection of 1.5 ml of a 2% starch suspension in phosphate-buffered saline (PBS). The cells were kept in ice-cold double-strength Eagle's basal medium (2X BME) supplemented with 10% fetal bovine serum (FBS) and 0.5 IU/ml of heparin; they were centrifuged in the cold and resuspended in medium containing 10% FBS. The suspension was counted in a hemocytometer, and 5×10^6 nucleated cells in 5 ml of medium were plated in 60 mm Petri dishes (Falcon Plastics, Los Angeles, Calif.) containing 12 glass cover slips (11 mm in diameter). The cells were used 2–6 days after seeding.

Conditioned Medium.—Conditioned medium (CM) was obtained in the following manner: 7.5×10^6 L-cells were plated in each of several Blake bottles (bottom area, 160 cm²) with 70 ml BME supplemented with 5% FBS. The medium, harvested 3–5 days after seeding, was tested for DNA synthesis stimulation in macrophages as a function of the concentration, by a method already described (6).

Phagocytic and Enzymatic Activity.—Cover slips of cells to be tested for phagocytosis were incubated in 35 mm Petri dishes (Falcon Plastics) with 3 ml BME supplemented with 10% FBS. Each dish received either 0.4 ml of a 1:20 dilution of Indian ink (Gurr's Indian Ink, Bio-Medical Specialties, Los Angeles, Calif.) in PBS, or 0.4 ml of a 1.25% suspension of twice-washed sheep red blood cells (SRBC) in PBS. To test for the capacity of macrophages to phagocytize opsonized cells, 0.5 ml of 2.5% SRBC in PBS was incubated with 0.5 ml of rabbit anti-sheep hemolysin (Baltimore Biological Laboratory, Baltimore, Md.) diluted 1:3000 for 30 min at 37°C; 0.4 ml of the suspension was then added to each of 35-mm Petri dishes containing four cover slips in 3 ml BME supplemented with 10% FBS. The dishes were left undisturbed at 37°C for 1 hr, then rinsed in PBS under vigorous agitation, dried, and stained with May-Grünwald-Giemsa.

The acid phosphatase test was made according to the Barka procedure (10) after fixation with formalin. The lysozyme test was made by the Osserman method (11) in culture fluids and in cells after three cycles of freezing and thawing; this assay was kindly performed by Dr. Osserman (Dept. of Pathology, Columbia University, New York).

Reactivity with Anti-Macrophage Serum.—Anti-macrophage serum (AMS) was obtained from Dr. M. Virolainen (Dept. of Pathology, University of Helsinki, Helsinki, Finland) or from Miss Joan Press of this Institute. Both antisera were prepared in a similar manner; rabbits were inoculated intravenously three times, 1 wk apart, with approximately 15–20×10^6 C57BL macrophages which had been grown in vitro for approximately 7 days, subjected to trypsinization for the removal of contaminating fibroblasts, and collected by scraping with a rubber policeman. After absorption with red blood cells and lymphocytes, the AMS was cyto-toxic to mouse peritoneal macrophages (cytotoxic index 50%) at a dilution of 1:160, but no toxicity was observed with peripheral lymphocytes or normal fibroblasts at a 1:10 dilution. For indirect immunofluorescence, macrophages on cover slips were fixed in acetone, incubated with rabbit AMS for 30 min, washed three times in PBS, and then incubated with a fluorescein isothiocyanate-labeled anti-rabbit gamma globulin. Only macrophages showed positive staining, either at the cytoplasmic membrane or throughout the cytoplasm.

Infection with SV40.—SV40 was derived from strain RH-911 obtained from Dr. A. J. Girardi of The Wistar Institute. Virus pools were prepared by infecting confluent CV-1 cells (12) at a low multiplicity of infection (0.5–1.0 plaque-forming units [PFU] per cell). The material used was the supernatant from cultures harvested after cell lysis and frozen and thawed three times. The titers of the various pools in CV-1 cells varied between 3×10^7 and 5×10^8 PFU/ml.

For infection of the macrophages, the original medium was replaced with 0.2 ml of a suspension of SV40 in BME with 5% FBS, diluted to a concentration of 50–100 PFU/cell (based on an estimated 0.6–1.2 $\times$ 10^6 macrophages/60 mm Petri dish). After virus adsorption for 90 min at 37°C, cover slips were transferred to individual 35 mm dishes containing 3 ml of BME with 10% FBS. For mock infection, the supernatant of noninfected CV-1 cells was used.

Tumor Antigen (*T*) *and Viral Capsid* (*V*) *Antigen Determination.*—To determine the presence of T or V antigens, cover slip cultures were rinsed in two changes of PBS, air-dried, fixed without delay in acetone: methanol (70:30, v/v) at −70°C for 10 min, and then stored at −20°C until use (not more than 48 hr). The cells were then stained with fluorescein-conjugated antibodies as already described (13). The percentage of antigen-positive cells was determined on counts of 500 cells.

To test for SV40 infectivity, supernatant medium and 2–5 $\times$ 10^6 cells, frozen and thawed three times, were assayed by the plaque method on CV-1 cells (14). The inducibility of SV40 was tested by fusing transformed macrophages with permissive CV-1 cells in the presence of inactivated Sendai virus, by the method already described (15).

Extraction of SV40 DNA from Infected Macrophages.—Two 100 mm Petri dishes were seeded with 25 $\times$ 10^6 macrophages, infected with 100 PFU of SV40/cell. After a 1 hr adsorption period, 10 ml of medium containing a total of either 0.5 μCi of thymidine (TdR)-^{14}C or 20 μCi of TdR-^{3}H were added. The cultures were harvested at 72 hr after infection when either TdR-^{3}H or ^{32}P-labeled SV40 DNA (component I) was added as a marker. The DNA was extracted by the procedure of Hirt (16); 0.2 ml of the extracted DNA was layered on 3 ml of CsCl solution ($\rho = 1.50$ g/cm^3), and centrifuged at 35,000 rpm for 3.5 hr at 25°C (Spinco rotor SW 39). Five-drop fractions were collected from the bottom of the tube directly onto filter paper discs. After they were dried, the radioactivity was measured in a Beckman liquid scintillation counter.

Radioactivity Counts and Radioautography.—Cover slips with cells were grown in the presence of tritiated thymidine (TdR-^{3}H, specific activity 6 mCi/mmole; Schwarz Bio Research Inc., Orangeburg, N.Y.); concentrations of 0.1–0.2 μCi/ml of TdR-^{3}H were used for continuous labeling experiments, and 1–2 μCi/ml for pulse labeling experiments. The cover slips were rinsed in two changes of PBS, and air-dried. If the presence of T antigen was to be determined, the cover slips were first fixed as described above and, after immunofluorescence, further fixed in Carnoy's solution for 20 min at room temperature, rinsed in 70% ethanol, thoroughly dried at 80°C for 15 min, dropped in vials containing 5 ml of scintillation fluid (Liquifluor, New England Nuclear Corp., Boston, Mass.), and counted in a well-type scintillation spectrometer (Beckman). The cover slips were then removed from the scintillation vials, rinsed in two changes of 95% ethanol, dried, and mounted (cells facing upward) on gelatin-coated microscope slides. The slides were covered with Kodak AR10 stripping film and exposed for 4 days at 4°C. After being developed, the slides were stained with Giemsa.

RESULTS

The infection of macrophages with SV40 induced DNA synthesis, which became evident from 24 to 40 hr after infection (Fig. 1), whereas in the mock-infected cultures only very few cells showed incorporation of TdR-^{3}H, even after several days of exposure to the precursor. The DNA labeling indexes and the percentage of T antigen-positive cells at a given time varied widely from experiment to experiment, depending on the particular pool of virus (Table I). DNA synthesis was generally found to lag slightly behind the appearance of T antigen. The percentage of T antigen-positive cells and of tritium-labeled

nuclei in a typical experiment are shown in Fig. 1. Under these conditions, 85–100% of the cells were positive for both T antigen and DNA synthesis within 4 days after infection. However, with certain pools of virus, both T antigen and DNA synthesis were induced at a much slower rate (Table I, Experiments V13 and V14).

The reason for the variations between different experiments at the same multiplicity of infection is unclear. Either the condition of the virus suspension,
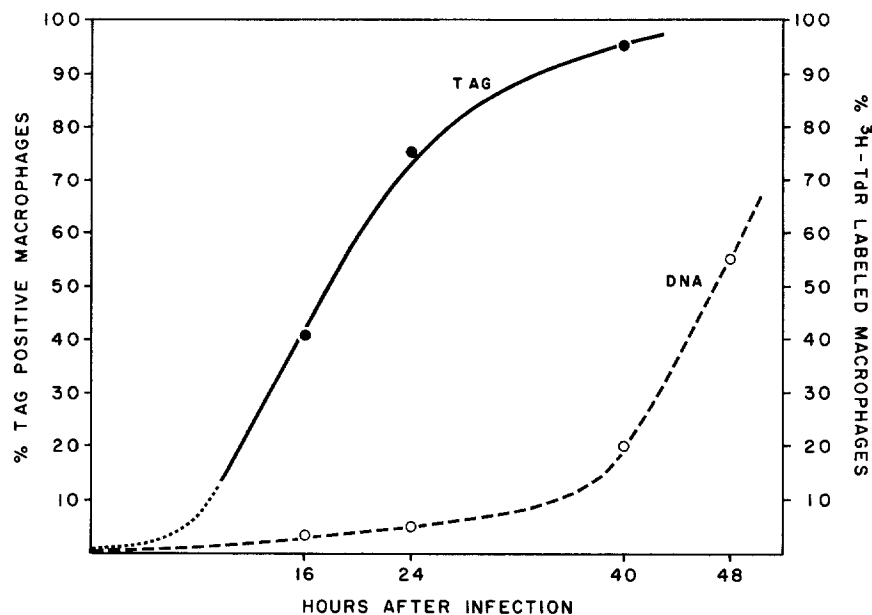

Fig. 1. T antigen (TAG) and DNA synthesis in macrophages infected with SV40 at 50–100 PFU/cell and incubated in the continuous presence of TdR-^{3}H (0.1 μCi/ml). T antigen determinations and radioautography were done on the same cell populations.

i.e. whether it was rich or poor in cellular debris, or the different phagocytic activity of the macrophages from experiment to experiment may account for the discrepancy.

At various intervals after infection, cover slips were also fixed and tested by immunofluorescence for SV40 V antigen. In no case were any positive cells observed. To determine whether replication of SV40 DNA occurred, DNA was extracted from the macrophage cultures by the Hirt method, as described under Materials and Methods. No newly synthesized SV40 DNA could be detected under conditions in which about 95–97% of the marker SV40 DNA could be recovered in the supernatant fraction. Thus, it appears that mouse

peritoneal macrophages, like other mouse cells (17), are essentially nonper-missive for SV40.

Comparison of DNA Synthesis Induced by SV40 and Conditioned Medium.—The induction of DNA synthesis in C57BL macrophages exposed to either SV40 virus or CM was followed by pulse labeling (1 μCi/ml for 100 min) at 24-hr intervals for 11 days. Both treatments led to the synthesis of DNA at comparable rates after a lag phase of at least 24 hr (Fig. 2). Synthesis reached

TABLE I

Kinetics of Induction of T Antigen and DNA Synthesis in Macrophages after Infection with SV40

Exp. No.	Virus pool	Time after infection	Per cent cells positive for		
			T antigen	DNA synthesis	
				SV40-infected	Mock-infected
		(days)			
V6	15 son.	2	83	10 (P)	<1 (P)
		4	100	15 (P)	<2 (P)
		8	100	50 (P)	NT
V8	15 son.	4	85	90	<1
		6	100	100	<2
		10	100	100	12
V13	G	1	0	3	<1
		2	13	8	1
		3	22	20	2
		4	22	25	4
		5	60	49	NT
		9	NT	89	10
V14	P 155 SN	2	8.5	<2	<1
		3	12	7.5	2
		4	39	12	2
		5	27	23	NT

NT, not tested; (P), pulse labeling.

the peak of 40% labeled nuclei at the same time in both preparations. However, while macrophages in CM incorporated a negligible amount of thymidine after 9 days in culture, SV40-infected cells still showed a considerable level of DNA synthesis 11 days after infection. The percentage of labeled cells in control cultures (macrophages incubated with regular BME plus 10% FBS) remained below 0.3% throughout the experiment (Fig. 3 a).

Morphological Aspects of the Infected Cells.—In the first days after infection, the cells undergoing DNA synthesis remained morphologically indistinguishable from control cells incubated in normal medium; as the incubation period was extended from 4 to 9 days after infection, however, morphological changes

became detectable. In some cultures, the original macrophage population was gradually replaced by large epithelial-like cells with greatly enlarged nuclei, which always showed strong DNA-synthesizing activity in labeling experiments (Fig. 3 *b*). In addition, cells of fibroblastic morphology could be observed on some cover slips. When cover slips of infected macrophages were kept in culture for extended periods, a confluent layer eventually formed in which both cell types could be recognized. No definite succession of events could be established
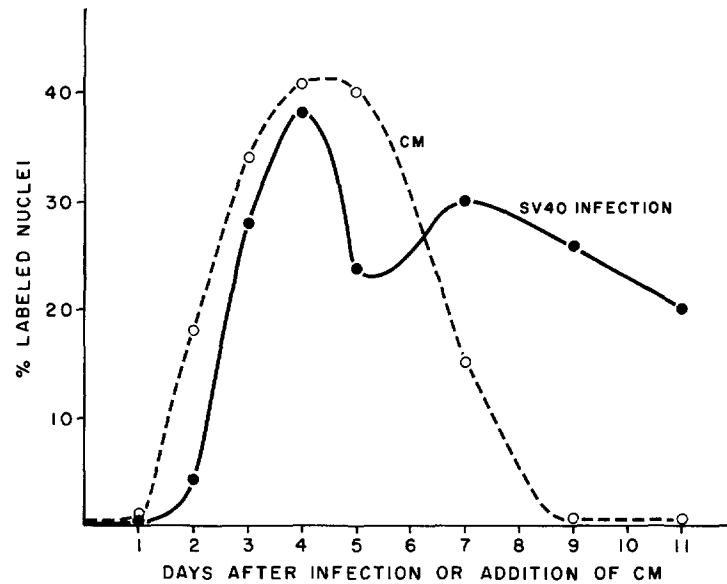

FIG. 2. Induction of DNA synthesis in mouse peritoneal macrophages as measured at various times after SV40 infection or addition of CM. Pulse labeling (TdR-^{3}H, 1.0 μCi/ml for 100 min).

on a morphological basis, since different cover slips from the same experiment had different ratios of cell types at a given time; moreover, when a particular cover slip was observed for a long period, the ratio between cell types seemed to vary from day to day. This modulation in morphology was observed even in established lines kept in culture for several months.

Establishment of a Line of Transformed Macrophages.— 7 days after infection a confluent monolayer of cells was selected and the cells were detached with 0.25% trypsin in 0.1% ethylenediaminetetraacetate (EDTA) (in calcium- and magnesium-free PBS). Cells from each 11 mm round cover slip were redistributed into two 30 mm Petri dishes. 3–6 days later, at confluence, the cells were detached with EDTA-trypsin, as above, and one 25 cm^2 plastic bottle
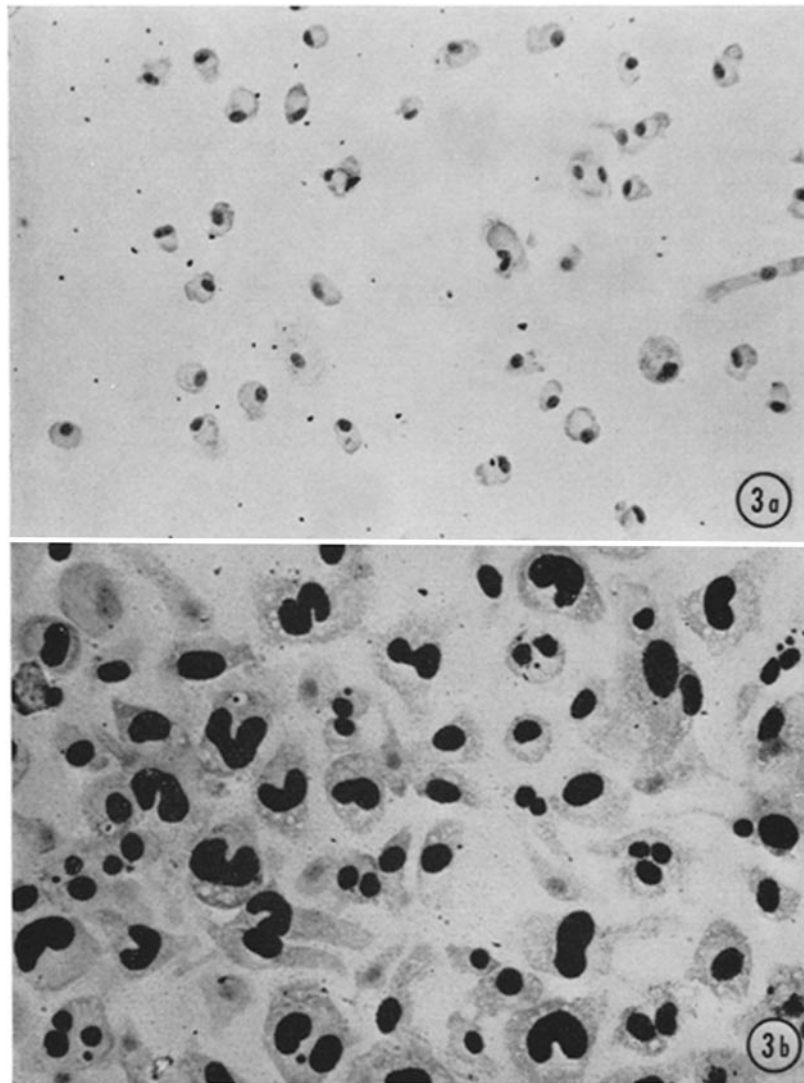
FIG. 3 a. Radioautography of mouse peritoneal cells maintained for 9 days in BME containing 10% FBS and 0.1 μCi/ml of TdR-^{3}H. No nuclear labeling. $\times$ 250.

FIG. 3 b. Radioautography of mouse peritoneal cells 9 days after infection with 50–100 PFU of SV40/cell. Culture in BME containing 10% FBS and 0.1 μCi/ml of TdR-^{3}H. $\times$ 250. Note the greatly enlarged size and that all the cells including multinucleated ones are heavily labeled.

was seeded with the cells originating from one Petri dish. No further growth of the transformed cells could be observed until 5–8 wk later, when in some bottles there appeared two types of colonies that could be easily distinguished on the basis of their morphology: (a) colonies of large cells of fibroblastic character, and (b) colonies of smaller cells, often presenting multiple cyto-plasmic filaments. When these cells were tested for T antigen, acid phospha-tase staining, and carbon uptake, the same pattern was always observed;
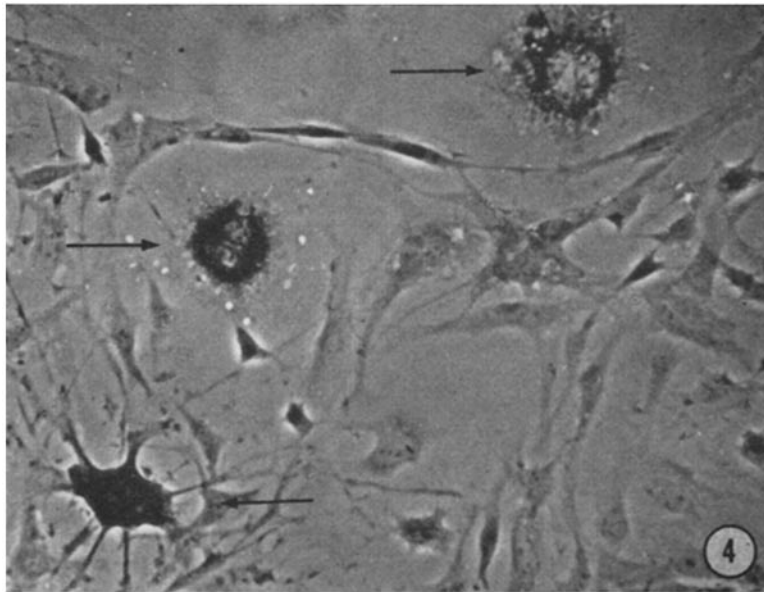

FIG. 4. Acid phosphatase staining of a culture of mouse peritoneal cells 7 wk after infection with SV40, before cloning. Arrows point to positive cells; note absence of staining in cells of the fibroblast type. Phase contrast; × 400.

although both cell types were T antigen-positive, cells of the epithelioid type gave a strong positive reaction in the acid phosphatase test and showed pro-nounced phagocytic activity, whereas cells of the fibroblastic type were nega-tive in both tests (Fig. 4).

Cloning was attempted by seeding the transformed cells in 60 mm plastic Petri dishes and changing half the medium weekly. No colony of significant size could be found until 3–4 wk after seeding. Colonies appearing after this lag phase were allowed to grow to approximately 300 cells. They were then removed by the ring technique and plated in 25-ml bottles. Out of 23 attempts at transplanting colonies, only 2 proved successful; 1 of these clones (1C-21),

obtained 11 wk after seeding, has been maintained successfully in continuous
culture for more than 2 yr. The medium used for this cell line is the same as
that used for the initial macrophage culture and the cultures are split at a
ratio of 1:5 twice weekly.

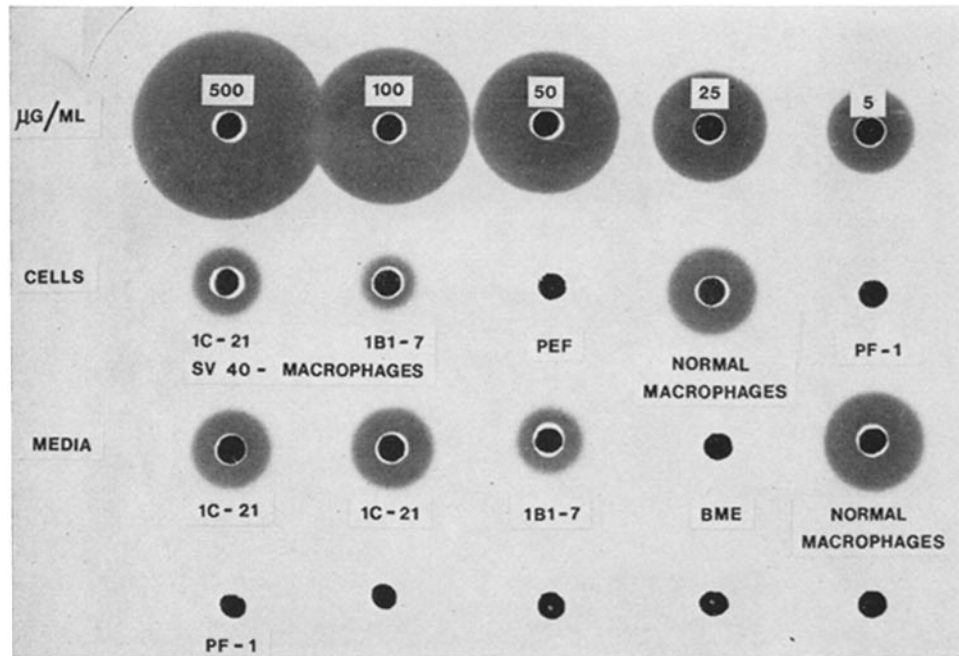The properties of this cell population were determined by several experi-

FIG. 5. Detection of muramidase (lysozyme) in normal and SV40-transformed macro-
phages. Dark areas correspond to zones of lysis of *Micrococcus lysodeikticus* in 1% agar. Top
row: lysis obtained with different quantities of purified enzyme. Second row: lysis by extracts
of cells (three cycles of freezing and thawing). *PEF*: primary C57BL mouse embryo fibroblasts.
PF-1: SV40-transformed C57BL mouse embryo fibroblasts. Third and fourth rows: lysis by
culture supernatants of same cells.

mental tests. All of the cells showed a strong fluorescence for SV40 T antigen,
but none for viral coat antigen. In three separate experiments no infectious
virus could be demonstrated by cocultivation or fusion of 1C-21 cells with
permissive cells.

To test for the transplantability of the SV40-transformed macrophages,
7×10^5 1C-21 cells in 0.1 ml BME were injected subcutaneously into the backs
of six 8 day old C57BL mice previously irradiated with 650 R. Similar conditions
have been reported to allow tumor formation by 3T3 cells in Swiss mice (18).
The animals were examined twice weekly until their deaths 3–5 wk later.

No nodule formation could be detected in any animal during this observation period.

Macrophage Properties of 1C-21 Cells.—The 1C-21 cells showed as positive a reaction for acid phosphatase as peritoneal macrophages maintained for 3–4 days in vitro; fibroblasts tested in parallel gave a completely negative response.

Lysozyme (or muramidase) is an enzyme which in man has been found to be associated essentially with normal or tumorous monocytes, macrophages,
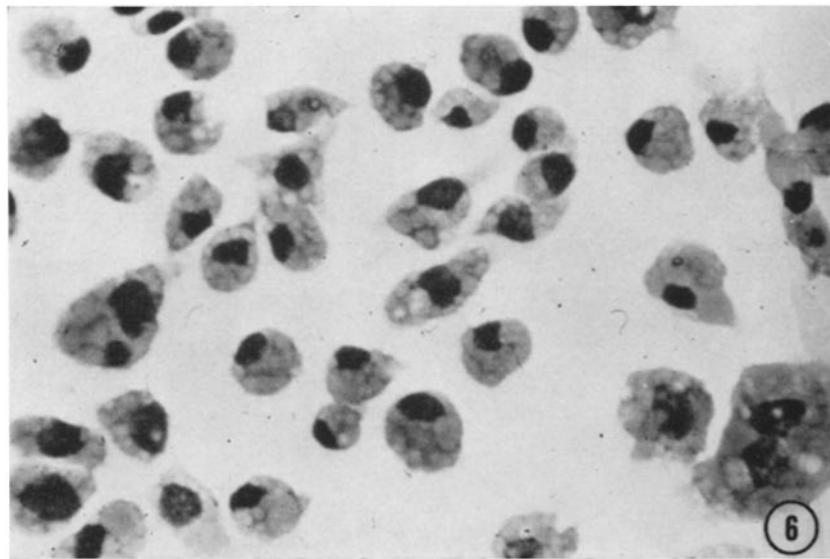

Fig. 6. Phagocytosis by 1C-21 cells. SV40-transformed macrophages were incubated for 1 hr in the presence of opsonized SRBC. 100% of the cells have phagocytized SRBC.

and polymorphs (11). In mice it has been reported that a myelomonocytic leukemia has a high lysozyme content, while little activity has been detected in other tumors, such as reticulum cell sarcomas, fibrosarcomas, and lymphoid cell leukemias (19). Since normal macrophages release detectable amounts of the enzyme in their culture fluid, SV40-transformed macrophages were expected to produce it in similar quantities. As shown in Fig. 5, SV40-transformed macrophages (at their 21st passage in vitro) contained and released measurable quantities of muramidase, as did normal macrophages. No activity could be detected in extracts or culture fluids of normal or SV40-transformed C57BL mouse fibroblasts.

When SV40-transformed macrophages, in the 17th passage, were tested for phagocytic activity, the cells were found to engulf both carbon particles and

sheep erythrocytes at a very high rate (Fig. 6). Usually 95–100% of the 1C-21 cells engulfed carbon when incubated for 1 hr in the presence of Indian ink. To compare the phagocytic capability of normal and transformed macro-
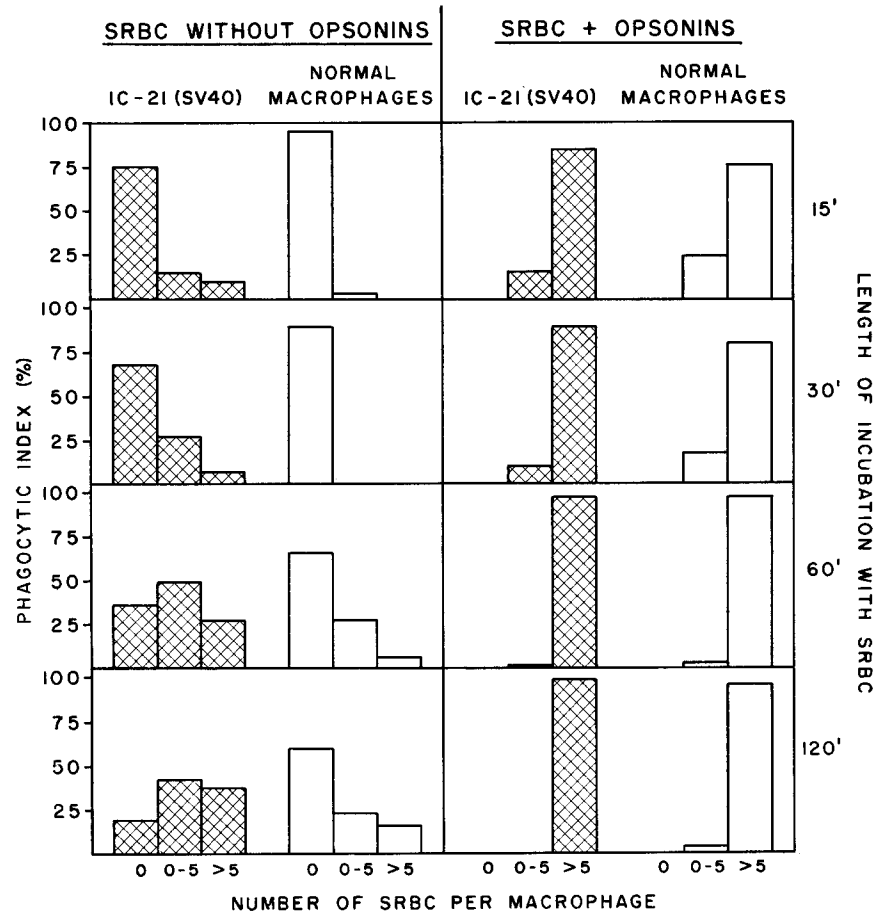

Fig. 7. Comparison between the phagocytic properties of normal and SV40-transformed mouse macrophages. Cultures of freshly explanted macrophages and of 1C-21 cells were incubated for various periods with opsonized (right of diagram) or nonopsonized (left of diagram) SRBC. Columns indicate the percentages of phagocytosis by SV40-transformed (cross-hatched areas) or normal (plain areas) macrophages.

phages, cells were incubated for various periods with opsonized or nonopsonized sheep erythrocytes. After the cells were fixed and stained with May-Grünwald-Giemsa, the percentages of cells containing various numbers of erythrocytes were determined. As shown in Fig. 7, the transformed macrophages

consistently showed a slightly higher phagocytic activity than the normal cells, as measured with both types of erythrocytes. As with normal macrophages, 1C-21 cells presented a much higher phagocytic capacity for opsonized than for nonopsonized erythrocytes, with nearly 100% of the cells having incorporated red cells after 1 hr of incubation. These results indicate that the 1C-21 cells have the capacity characteristic of other monocytes to bind and phagocytize immune complexes, suggesting the presence on these cells of IgG receptors (20).
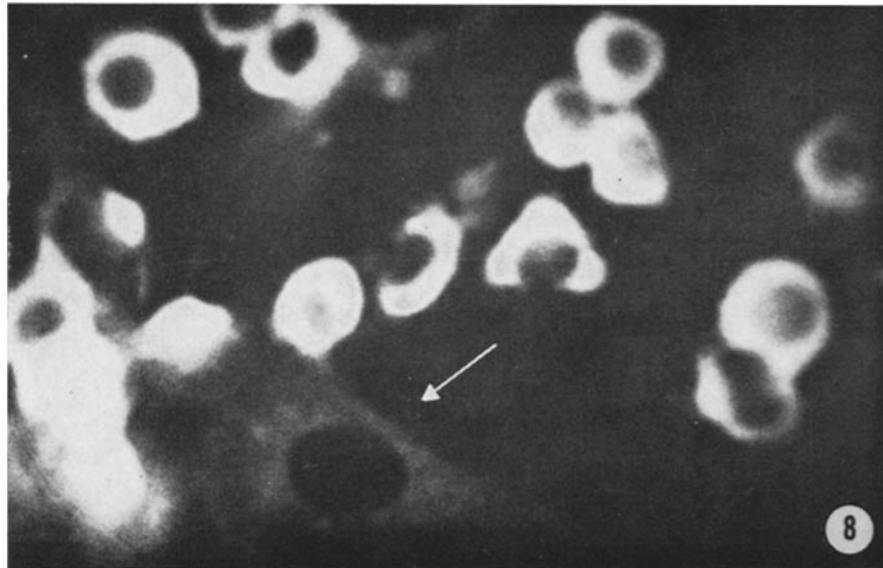

Fig. 8. Specific fluorescent staining with anti-mouse macrophage serum of SV40-transformed macrophages. Mixtures of 1C-21 cells and normal mouse embryo fibroblasts were incubated with rabbit anti-mouse macrophage serum, then stained with fluorescein-conjugated anti-rabbit globulin. Note complete absence of staining in fibroblasts (arrow).

The presence of a macrophage-specific antigen on SV40-transformed macrophages was detected by indirect immunofluorescence with rabbit anti-macrophage serum. In experiments in which a mixture of 1C-21 cells was mixed with normal C57BL mouse embryo fibroblasts, transformed macrophages presented a strong fluorescence comparable to that of normal macrophages, but the fibroblasts showed only a marginal reaction (Fig. 8). No fluorescence could be detected when the cells were incubated with normal rabbit serum.

DISCUSSION

Macrophages can be stimulated to undergo DNA synthesis by infection with SV40. Since virus replication is not detectable, the majority of newly synthesized DNA must be cellular. The kinetics of DNA synthesis induction

in macrophage cultures stimulated by SV40 or by CM are essentially the same, except that with the latter, after the initial wave of DNA synthesis, the cells do not continue to multiply unless new CM is added (6, 7). In the SV40-treated cultures, DNA synthesis persists, a phenomenon that is probably reflected in the fact that many infected macrophages become tetraploid within the first cell generation (21). Since the number of mitoses observed is much lower than would be expected if all the induced cells were able to replicate, it is quite clear that a large proportion of the stimulated population is not viable.

In CM-stimulated cultures and, at time, in SV40-stimulated cultures, DNA synthesis is preceded by a characteristic stretching of the cells over the surface with numerous pseudopods (6). This phenomenon also occurs after treatment of macrophages with phorbol esters, a class of cocarcinogens that directly interacts with the cell membranes and also has the capacity to stimulate macrophages to undergo DNA synthesis, although to a lesser extent than SV40 or CM (Mauel, J. and V. Defendi, unpublished observation).

In experiments in which the efficiency of infection of macrophage was high, synthesis of T antigen always preceded induction of DNA synthesis. In other cell systems, it has always been difficult to determine the exact chronological sequence between these two expressions of SV40 infection, probably because no other cell population can be as uniformly in a G_0 phase of the cell cycle as macrophages. It would be interesting to determine at which level in the chain of events leading to the initiation of DNA synthesis stimuli as diverse as the CM factor and SV40 act.

Induction of DNA synthesis in mouse macrophages has also been reported after infection with polyoma virus (22); however, it was not clear in these experiments if the cells were eventually destroyed by the virus. In several experiments in our laboratory, polyoma virus was cytocidal for C57BL peritoneal macrophages (Mauel, J. and V. Defendi, unpublished observation).

The pleomorphism exhibited by the transformed macrophages is intriguing, particularly with regard to the question of the relationship between fibroblast-like cells and macrophages. We cannot completely exclude the possibility that some fibroblasts were present in the original population obtained from the peritoneal cavity, although in some experiments care was taken to remove fibroblasts by repeated trypsinization over several days after the cell population had become attached to the cover slips. However, the persistence of both cell types in colonies that could be interpreted reasonably as having arisen from single cells suggests that the macrophages may be capable of considerable modulation in their morphological, as well as physiological, properties. Morphological variations associated with functional compartmentalization, i.e. immunoglobulin production, have been reported in a line of human lymphoid cells (23).

Macrophages stimulated by the CM factor are capable of several divisions,

but permanent lines have not yet been established despite repeated attempts (Mauel, J. and V. Defendi, unpublished observation). After infection of macrophages with SV40, however, it is possible to select a cloned cell population that replicates indefinitely and has many properties of primary macrophages, i.e., high acid phosphatase and phagocytic activity, lysozyme production, and specific (?) antigenic determinants. Nonetheless, these cells are transformed according to the following criteria: they contain SV40 T antigen, they can be removed from glass surfaces by trypsin, they do not require a CM factor to maintain replication, and they are heteroploid (Lehman, J., and V. Defendi, unpublished observation). Their failure to produce tumors when inoculated into syngeneic, conditioned animals is not surprising since SV40-transformed mouse fibroblasts are likewise very rarely tumorigenic (24). The maintenance of specialized functions after transformation by SV40 has been reported for several other cell types, particularly those from endocrine organs (25, 26). In other cases, however, synthesis of specific products, such as collagen, has been found to be considerably decreased (27). The availability of a stable population of macrophages in vitro is particularly useful for studying their role in viral immunity and in the immune response; experiments to elucidate some of these functional properties are now in progress.

During the preparation of this manuscript, Stone and Takemoto (28) published a report in which the establishment of lines from macrophages infected with SV40 was described. These lines also have several characteristics of primary macrophages, such as high phagocytic and acid phosphatase activity, and the capacity to produce β-1-C globulin.

SUMMARY

The stimulation of DNA synthesis in mouse (C57BL) macrophages explanted in vitro was demonstrated after treatment with conditioned medium or infection with SV40. In the latter case, induction of SV40 T antigen was detected before TdR-^{3}H incorporation. Even though all macrophages were infected (T antigen-positive), they exhibited considerable pleomorphism, accompanied by functional differences. Permanent lines of SV40-transformed macrophages were eventually established, and one clone was isolated which replicates indefinitely and has many properties of primary macrophages: high acid phosphatase and phagocytic activity, lysozyme production, and specific antigenic determinants. These cells differ from normal macrophages in that they contain the SV40 genome, can be trypsinized, and do not require conditioned medium for continued replication.

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