Proc. Natl. Acad. Sci. USA
Vol. 77, No. 11, pp. 6773–6777, November 1980
Immunology

Resistance of simian virus 40-transformed hamster cells to the cytolytic effect of activated macrophages: A possible factor in species-specific viral oncogenicity

(viral transformation/tumor antigens/tumor immunity)

JAMES L. COOK*†, JOHN B. HIBBS, JR.*, AND ANDREW M. LEWIS, JR.‡

*Veterans Administration Medical Center and Department of Medicine, Division of Infectious Diseases, University of Utah Medical Center, Salt Lake City, Utah 84148; and ‡National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20205

Communicated by M. M. Wintrobe, July 14, 1980

ABSTRACT **Simian virus 40 (SV40)-transformed hamster cells were relatively resistant to the lytic effect of activated macrophages from animals with chronic intracellular infections. Conversely, SV40-transformed mouse and rat cells and adenovirus 2-transformed hamster cells were highly susceptible to destruction by tumoricidal activated macrophages. The pattern of resistance or susceptibility of SV40-transformed rodent cells was the same whether activated macrophage effectors were obtained from mice, random-bred hamsters, or the inbred LSH hamsters from which some of the SV40-transformed hamster lines were derived. The results suggest that resistance of transformed cells to macrophage-mediated cytolysis may explain in part the species-specific oncogenicity of this DNA virus.**

Simian virus 40 (SV40) is a papovavirus that induces tumors in hamsters and transforms§ cells from many species in tissue culture (1). The ability of SV40-transformed hamster cells, but not of cells from other species transformed *in vitro* by SV40, to induce tumors in the host of origin (2) implies that SV40 may induce a species-specific change during transformation of hamster cells that renders the neoplastic cells resistant to host rejection. The recent observation that SV40-transformed inbred LSH hamster cells induced tumors in histoincompatible CB hamsters almost as efficiently as in syngeneic animals (3) suggests that SV40 may alter the efficiency with which target cells are recognized or destroyed by the host.

The macrophage is one of the key elements in the cell-mediated host immune response to tumor isografts and allografts (4). Furthermore, macrophages from animals with chronic intracellular infections can destroy syngeneic, allogeneic, and xenogeneic neoplastic cells *in vitro* (5). The tumoricidal effect of these "activated" macrophages depends on a nonphagocytic mechanism that requires intimate contact with the target cells (5). To begin to evaluate the reasons for the apparent resistance of SV40-transformed hamster cells to host cell-mediated defenses, we have studied the effects of activated macrophages from animals with chronic intracellular infections on SV40- and adenovirus 2 (Ad2)-transformed rodent cells. The battery of target cells evaluated displayed three different transformed cell phenotypes when inoculated into immunocompetent animals—nononcogenic, oncogenic in the host of origin but not in a histoincompatible host, and oncogenic in both the host of origin and a histoincompatible host—and provided the opportunity to study the relative susceptibilities of these different target cell types to killing by activated macrophages. In this report, we present evidence that suggests that resistance to the cytolytic effect of tumoricidal activated macrophages is one of the mechanisms by which SV40-transformed hamster cells may evade destruction by the host.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Macrophage Monolayers. Peritoneal cells were harvested from female ICR or C3H/HeN mice or from random-bred (RGH) or inbred LSH Syrian hamsters 3 days after stimulation with 10% (wt/vol) proteose peptone (Difco) and 17–22 days after intraperitoneal infection with 10^7 Pasteur strain bacilli Calmette–Guérin (BCG) (TMC 1011; Trudeau Institute) or 8–20 wk after intraperitoneal infection with the C56 strain of *Toxoplasma gondii* (6). Contaminating erythrocytes were lysed with ammonium chloride buffer [NH_4Cl (8.290 g/liter), $KHCO_3$ (1.0 g/liter), and EDTA (0.0372 g/liter) (pH 7.4)] containing 10% (vol/vol) fetal bovine serum and then washed twice with complete medium (see below). Peritoneal cells (4×10^6) in 1 ml of Dulbecco's modified Eagle's medium containing Hepes buffer (20 mM), penicillin G (100 units/ml), and streptomycin (100 μg/ml), supplemented with glucose (final concentration, 15 mM) and 10% (vol/vol) endotoxin-free [<1 ng/ml (7)] fetal bovine serum (Sterile Systems, Logan, UT) (complete medium) were added to 16-mm-well cluster plates (3524; Costar, Cambridge, MA) and allowed to adhere for 1 hr at 37°C in humidified 95% air/5% CO_2. Nonadherent cells were removed by repeated washing with warm medium. Mouse and hamster adherent cell monolayers prepared in this way contained 90–99% macrophages, 1–10% polymorphonuclear leukocytes, and 0–3% lymphocytes, as indicated by Giemsa staining and phagocytosis of heat-killed *Candida albicans*.

Target Cell Lines. The TCMK-1 line of SV40 (strain 777)-transformed C3H/Mai mouse kidney cells (8) was obtained from the American Type Culture Collection. The T2 line of SV40 (strain 776)-transformed BALB/c 3T3 cells (SV3T3) was obtained from G. Todaro (9). SV40HE1, SV40HE2, and SV40HE3 are clonally derived, inbred LSH hamster embryo cell lines transformed by UV-treated SV40 (strain 777) as described (10). The THK-1_t cell line was derived from a subcutaneous tumor induced in a weanling, random bred (NIH strain) golden Syrian hamster by the THK-1 line (11) of SV40

Abbreviations: SV40, simian virus 40; Ad, adenovirus; T antigen, virus-induced nonvirion tumor antigen; BCG, bacillus Calmette–Guérin.

† Present address: Department of Medicine, National Jewish Hospital and Research Center, Denver, CO 80206.

§ Transformation is defined herein as the conversion of normal mammalian cells to cells that have altered appearance and unlimited potential to divide *in vitro*.

(strain 777)-transformed hamster kidney cells. SV40RE1 and SV40RE2 are clonally derived Sprague–Dawley rat embryo cell lines transformed by UV-treated SV40 as described (10). The Ad2HE7 line was derived from a colony of transformed cells arising from LSH hamster embryo cells infected with a UV-treated clinical isolate of Ad2 (10). Ad2HTL3-1 is a tissue culture line derived from the 36th *in vivo* LSH weanling passage of a subcutaneous tumor induced in LSH newborn hamsters by the Ad2HE3 line (10) and adapted to grow in weanling animals by serial transplantation. The cells in each of these transformed lines express virus-specific, nonstructural tumor antigens (T antigens) that are detectable by a microtiter complement fixation test or an indirect immunofluorescent procedure (10).

Cytotoxicity Assay. *In vitro* macrophage-mediated tumor cell killing was evaluated by a modification of a [^{3}H]thymidine radiorelease assay (12) and by observation of stained macrophage/target cell cocultures. Target cells were prelabeled with 0.2 μCi (1 Ci = 3.7×10^{10} becquerels) of [^{3}H]thymidine (specific activity, 1.9 Ci/mmol; Schwarz/Mann) per ml for 18–24 hr during logarithmic phase growth. Labeled target cell monolayers were washed to remove unincorporated label, trypsinized (0.25% trypsin for 3–5 min; Difco), and added (6×10^4 cells) to the prewashed macrophage monolayers or to wells containing no macrophages in complete medium (final volume, 2 ml). To determine the total label incorporated, medium was removed from wells containing adherent targets, the cells were lysed with 2 ml of 0.5% sodium dodecyl sulfate, and 500 μl of this solution (total release) or of medium obtained at the end of 48 hr from the macrophage/target cell cocultures (experimental release) or of target cell cultures alone (spontaneous release) was added to 10 ml of scintillation fluid (Aquasol; New England Nuclear). The samples were assayed for 20 min in a liquid scintillation counter (model LS-330; Beckman Instruments), and the measurements were corrected for quench and recorded as dpm (1 dpm = 16.7 mBq). The arithmetic mean and SEM of the results of triplicate cultures were determined and the percent specific release was calculated by the following formula:

$$\frac{\text{experimental release} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100.$$

Medium samples removed from such cultures have been shown to be cell-free by microscopic examination. After sampling of the medium from plates used in the release assays, the cells were fixed with methanol and stained with 10% (wt/vol) Giemsa to permit comparison between release results and morphologic observations. The significance of observed differences in the percent specific [^{3}H]thymidine release from various target cell lines was estimated using the Student *t* test.

RESULTS

Relative Susceptibility of SV40-Transformed Mouse, Hamster, and Rat Cells to Lysis by Activated Mouse Macrophages. To compare the susceptibilities of nononcogenic and oncogenic SV40-transformed cells from different species to killing by activated macrophages, a number of well-characterized mouse, rat, and hamster lines were obtained. The tumor-inducing capacities of these lines in syngeneic and histoincompatible adult hosts are summarized in Table 1.

In initial experiments, the effect of activated macrophages from BCG-infected mice on the nononcogenic TCMK-1 SV40-transformed mouse cell line (8) was compared to the effect on SV40HE1, an oncogenic SV40-transformed LSH hamster cell line that induces tumors in syngeneic, LSH, and histoincompatible CB hamsters with approximately equal efficiency (3) (Table 1). The results of the macrophage/target cell cocultures were evaluated visually after 48 hr by fixing and staining the remaining cells. In repeated experiments in which TCMK-1 cells were almost completely destroyed at 48 hr (<1 target cell per 200× field), numerous SV40HE1 cells (30–50 cells per 200× field) exposed to the same macrophage populations remained. Greater than 90% of the SV40HE1 target cells were viable by trypan blue exclusion at the end of the cocultivation period, and when macrophage-exposed SV40HE1 cells were reseeded in fresh medium, macroscopic target cell colonies were visible within 3–7 days. Wells containing only target cells, fixed and stained 2–4 hr after seeding, usually contained 40–70 cells per 200× field, which suggested that cultivation of SV40HE1 cells on activated macrophage monolayers had resulted in transient cytostasis but not significant cytolysis of these target cells. Attempts to enhance the destruction of SV40HE1 cells in this system by adding endotoxin (1 ng/ml to 10 μg/ml, *Escherichia coli* O128:B12, phenol extract; Sigma) (7) or sodium periodate-induced mouse macrophage activating factor (10% vol/vol) (13) were unsuccessful (data not shown).

Table 1. Tumor-inducing capacity of virus-transformed rodent cells

Cell line	Species, strain of origin	Cells (log)/TPD_{50}*: Host of origin	Cells (log)/TPD_{50}*: Histoincompatible host (strain)†
TCMK-1	Mouse, C3H/Mai	>8.5	
SV40RE1	Rat, Sprague–Dawley	>8.5	
SV40RE2	Rat, Sprague–Dawley	>8.5	
SV40HE1	Hamster, LSH	4.2	5.0 (CB)
SV40HE2	Hamster, LSH	3.6	5.5 (CB)
SV40HE3	Hamster, LSH	3.5	3.5 (CB)
$THK\text{-}1_t$	Hamster, NIH	<2.5	<2.5 (CB)
Ad2HE7	Hamster, LSH	>8.5	
Ad2HTL3-1	Hamster, LSH	4.1	>7.5 (CB)

* Cells (log)/TPD_{50}, logarithm of number of tissue culture cells required to produce subcutaneous tumors in 50% of the surviving adult animals. TPD, tumor-producing dose. $TPD_{50} > 8.5$ = no tumors developed during a 3-mo observation period after subcutaneous challenge with 10^8 tissue culture cells. For tumor challenge procedure, see ref. 10.

† The inbred CB strain of hamsters differs from the LSH strain at a major histoincompatibility locus.

To quantitate the differences in the susceptibilities of these target cells to the tumoricidal effect of activated macrophages, the 48-hr [^{3}H]thymidine radiorelease assay was used. TCMK-1 cells cocultured with macrophages from C3H/HeN mice with chronic *T. gondii* or BCG infection were lysed, whereas SV40HE1 cells were relatively resistant to lysis (Table 2, experimental series 1). Visual evaluations of the wells from which the samples were taken were compatible with these results. SV3T3—an SV40-transformed BALB/c mouse cell line that was also included in these experiments—was destroyed as efficiently (89.3% ± 2.2% specific release) as TCMK-1. Thus by this radiorelease assay, activated macrophages from C3H/HeN ($H\text{-}2^k$) mice lysed H-2 identical (TCMK) or H-2 different (SV3T3, $H\text{-}2^d$) SV40-transformed mouse cells but were ineffective in destroying xenogeneic SV40HE1 cells.

Activated mouse macrophages can destroy xenogeneic SV40-transformed rat cells (14). To verify this observation under the conditions of these experiments, a nononcogenic SV40-transformed Sprague–Dawley rat embryo cell line (SV40RE1, Table 1) derived under the same conditions used for the SV40HE1 line [i.e., UV-treated transforming virus and cells transformed under agar (10)] was exposed, along with TCMK-1 and SV40HE1 cells, to activated macrophages from

Table 2. Specific release of [^{3}H]thymidine from SV40-transformed mouse, rat, and hamster cells cocultured with activated mouse macrophages

Series†	Percent specific [^{3}H]thymidine release*					
	TCMK-1	SV40RE1	SV40HE1	SV40HE2	SV40HE3	THK-1$_t$
1 (6)	85.2 ± 3.4		11.9 ± 1.5			
2 (4)	95.4 ± 2.3	89.2 ± 5.7	11.6 ± 1.7			
3 (5)	93.7 ± 3.2‡		16.8 ± 2.1	47.7 ± 2.1	28.6 ± 3.4	27.8 ± 3.4

* Percent spontaneous release (mean ± SEM) of the target cells in these experiments was as follows: TCMK-1, 13.8 ± 1.2; SV40RE1, 23.7 ± 2.2; SV40HE1, 13.8 ± 1.0; SV40HE2, 15.2 ± 1.3; SV40HE3, 11.7 ± 2.5; and THK-1$_t$, 14.1 ± 1.4.

† Number in parentheses = number of experiments in each series.

‡ Significance of the differences in the percent specific release for these cell lines was as follows: TCMK-1 vs. each hamster line, $P < 0.001$; SV40HE1 vs. SV40HE2, $P < 0.001$; SV40HE1 vs. SV40H3 or THK-1$_t$, $P < 0.05$; and SV40HE2 vs. SV40HE3 or THK-1$_t$, $P < 0.01$.

either C3H/HeN or random-bred ICR mice (Table 2, experimental series 2). Whereas activated mouse macrophages killed SV40-transformed, xenogeneic rat cells and TCMK-1 cells, the effectors were relatively ineffective in destroying SV40HE1 cells. SV40RE2, another SV40-transformed Sprague–Dawley rat embryo cell line that was included in these experiments, was destroyed as efficiently (93.6% ± 3.5% specific release) as were TCMK-1 and SV40RE1. Thus, it appeared that the inability of activated mouse macrophages to lyse SV40HE1 cells was not solely because of the derivation of the target cells from a different species, and the data suggested that this relative target-cell resistance to macrophage-mediated destruction was not independently related to UV treatment of the transforming virus.

Effects of Tumoricidal Activated Mouse Macrophages on Several Different SV40-Transformed Hamster Cell Lines. The relative resistance of the SV40HE1 cell line to activated macrophage-induced cytolysis implied that this phenotype may be a general property of SV40-transformed hamster cells. However, the possibilities that this phenomenon was a peculiarity of this cell line, that UV treatment of the transforming virus may have played a role, and that macrophage resistance was only manifested in SV40-transformed cells from the hamster strain tested (i.e., LSH) had to be considered. To evaluate these alternative explanations, three other SV40-transformed hamster cell lines were tested for susceptibility to activated macrophage-induced destruction (Table 2, experimental series 3). Two of these lines, SV40HE2 and SV40HE3, arose under agar from different clones of transformed cells in the same transformation experiment from which the SV40HE1 line was derived. The third line, THK-1$_t$ was derived from a subcutaneous tumor induced by the THK-1 cell line of random-bred Syrian hamster kidney cells transformed by infectious (non-UV-treated) SV40. All of the SV40-transformed hamster lines in these experiments were significantly less susceptible to the tumoricidal effect of activated mouse macrophages than was the TCMK-1 line ($P < 0.001$), which suggested that the resistant phenotype was not a unique property of SV40HE1, of transformed LSH cells, or of hamster cells transformed by inactivated virus. Although all of the SV40-transformed hamster lines tolerated exposure to activated mouse macrophages much better than did TCMK-1 cells, there was significant variability in the degree of lysis observed with the different hamster lines (Table 2, experimental series 3). This finding supports the conclusion that the SV40HE1, SV40HE2, and SV40HE3 lines are the products of different transformation events and suggests that there may be phenotypic variability in different cells transformed by the same viral inoculum.

Effects of BCG-Activated Hamster Macrophages on Virus-Transformed Mouse, Rat, and Hamster Cell Lines. Because it was still possible that the inefficient destruction of SV40-transformed hamster cell lines was due to the species difference between the effector and target cells and not an inherent resistance of the target cells, the experiments were repeated using macrophages from BCG-infected random-bred (RGH) and inbred (LSH) Syrian hamsters (Table 3). Several observations are derived from these data. First, macrophages from uninfected hamsters injected with peptone had little cytolytic effect when cocultivated with TCMK-1 or SV40HE1 cells, whereas peptone-elicited macrophages from BCG-infected hamsters effectively lysed TCMK-1 but not SV40HE1 cells (Table 3, experimental series 1). Second, the general pat-

Table 3. Specific release of [^{3}H]thymidine from SV40-transformed mouse, hamster, and rat cells and Ad-2-transformed hamster cells cocultured with activated hamster macrophages

Series†	PEC‡ source	Percent specific [^{3}H]thymidine release*							
		TCMK-1	SV3T3	SV40HE1	SV40HE2	SV40RE1	SV40RE2	Ad2HE7	Ad2HTL3-1
1 (3)	Peptone-RGH	12.8 ± 2.5		3.5 ± 1.8					
	BCG-RGH	91.1 ± 3.5		13.9 ± 3.5§					
2 (5)	BCG-RGH	98.5 ± 0.9	98.0 ± 0.5	25.1 ± 2.1§					
3 (4)	BCG-RGH	97.9 ± 2.0		25.2 ± 2.1§		97.6 ± 0.9	90.9 ± 1.0		
4 (6)	BCG-RGH	94.5 ± 2.9		14.2 ± 3.1§	14.3 ± 2.6§				
5 (6)	BCG-RGH			16.2 ± 3.2§				95.8 ± 4.2	96.2 ± 2.1
6 (2)	BCG-RGH	91.3 ± 8.7		16.5 ± 9.4§	12.5 ± 5.6§				
	BCG-LSH	98.0 ± 1.4		11.6 ± 4.6§	13.7 ± 2.9§				

* Mean ± SEM percent specific release. Percent spontaneous release (mean ± SEM) from the target cells in these experiments was as follows: TCMK-1, 20.8 ± 1.1; SV3T3, 19.5 ± 2.4; SV40HE1, 11.0 ± 0.8; SV40HE2, 13.9 ± 1.9; SV40RE1, 27.6 ± 3.4; SV40RE2, 29.0 ± 4.0; Ad2HE7, 12.8 ± 1.7; Ad2HTL3-1, 18.5 ± 2.1.

† Number in parentheses = number of experiments in the series.

‡ PEC, peritoneal exudate cell.

§ Mean percent specific release from the SV40-transformed hamster lines is significantly lower than that from the other lines tested ($P < 0.001$).

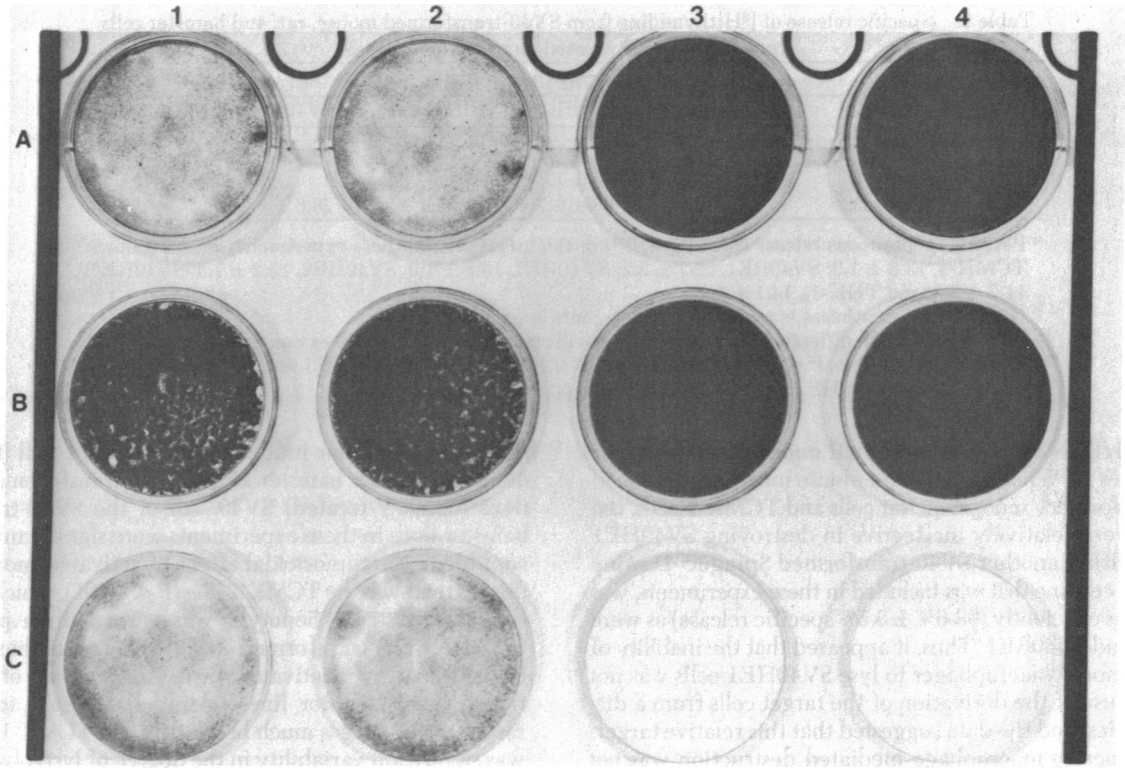

FIG. 1. Low-power photograph of a Giemsa-stained cluster plate in which each circle represents an individual culture. In row A, TCMK-1 cells (6×10^4 per well) were either cultured on a monolayer of BCG-activated RGH hamster macrophages (columns 1 and 2) or alone (columns 3 and 4). In row B, SV40HE1 cells (6×10^4 per well) were either cultured on a monolayer of the same macrophages (columns 1 and 2) or alone (columns 3 and 4). In row C, columns 1 and 2, the same macrophage population was cultured without added target cells. The cultures were incubated for 4 days in complete medium. The cells which grew in the wells in rows A and C, columns 1 and 2, were contaminating fibroblasts from the hamster peritoneal cell population that did not contain SV40 T antigen when tested by indirect immunofluorescence.

tern of target cell susceptibility to activated macrophage-induced cytolysis with hamster macrophages was similar to that observed in the experiments in which activated mouse macrophages were used; SV40-transformed mouse and rat cells were susceptible, whereas SV40-transformed hamster cells were relatively resistant (Table 3, experimental series 2–4). Third, two different lines of LSH hamster embryo cells transformed by Ad2 (Ad2HE7 and Ad2HTL3-1) were as susceptible to activated hamster macrophage-induced lysis as SV40-transformed mouse or rat cells (Table 3, experimental series 3 and 5). Ad2HE7 does not induce tumors in syngeneic newborn hamsters (10). Ad2HTL3-1 induces tumors in adult syngeneic hamsters; however, unlike SV40HE1 and SV40HE2, this line will not induce tumors in histoincompatible CB hamsters (Table 1). Fourth, activated macrophages that are syngeneic to the SV40-transformed hamster cell lines (SV40HE1 and SV40HE2 are of LSH origin), when compared to those from random-bred hamsters, did not cause increased lysis of these targets (Table 3, experimental series 6).

These data support the conclusion that SV40-transformed hamster, but not mouse or rat, cells are resistant to the tumoricidal effects of activated macrophages irrespective of the histocompatibility differences between effector and target cells. The susceptibility of the two Ad2-transformed LSH cell lines to lysis further suggests that the resistant phenotype is SV40-specific and that resistance to macrophage-mediated destruction does not necessarily correlate with tumor-inducing capacity in the syngeneic adult host.

Fate of SV40-Transformed Hamster Cells Exposed to Activated Macrophages. The relative resistance of the SV40-transformed hamster cell lines studied has been defined by their failure to release incorporated [^{3}H]thymidine during the 48-hr period of cocultivation with activated mouse and hamster macrophages. However, the long-term fate of the resistant hamster target cells could not be determined from these data. That SV40-transformed hamster target cells had been "injured" during their exposure to activated macrophages was suggested by the target cell cytostasis observed visually (not shown). The following question arose: Would these injured targets recover and proliferate or would they eventually die? When parallel cocultures of BCG-activated hamster macrophages and SV40HE1 cells were followed for 2–3 days beyond the termination of the 48-hr release assay, the target cells, whose growth was initially slowed compared to control cultures, eventually formed a confluent multilayer, whereas cocultures containing TCMK-1 cells had little or no regrowth of target cells (Fig. 1). Thus, many of the SV40HE1 target cells resumed division after a temporary period of cytostasis, whereas the TCMK-1 target cells were efficiently lysed. Studies have shown that tumoricidal, activated macrophages lose some of their cytotoxic potential after 24–72 hr in culture (15). Therefore, the prolonged culture of activated macrophage/target cell cocultures may have had the functional effect of removing the targets from the cytostatic influence of the macrophages.

DISCUSSION

Although SV40 can infect and transform hamster, mouse, rat, rabbit, guinea pig, bovine, monkey, and human cells *in vitro* (1), this virus is inefficient in inducing tumors in species other than hamsters and mastomys. SV40-transformed hamster cells are highly tumorigenic when transplanted into adult animals. Attempts to transplant SV40-transformed mouse cells into

syngeneic animals are usually unsuccessful unless the transformed cells have been maintained in tissue culture for long periods of time (16) or the immune response of the host has been depressed (17). The reasons for the unusual susceptibility of Syrian hamsters to the oncogenic effect of SV40 virus and for the increased tumor-inducing capacity of SV40-transformed hamster cells compared with SV40-transformed cells from other species are unknown.

The recent observation that SV40-transformed inbred LSH hamster cells were able to induce tumors in histoincompatible CB hamsters almost as efficiently as in syngeneic animals (3) suggested that SV40 either may alter the immunologic recognition of the hamster cells it transforms or may modify the cells in such a way that they can withstand an attack by the cell-mediated host defenses. The data presented here, which show that SV40-transformed hamster cells are relatively resistant to *in vitro* cytolysis by the activated macrophage—an effector cell component of cellular immune reactions, suggest that the latter postulated SV40 mechanism may operate *in vivo*.

Because previous *in vivo* studies suggest that macrophages play an important role in the host response to SV40-transformed mouse cells (18), the resistance of SV40-transformed hamster cells, but not mouse or rat cells, to activated macrophage-induced cytolysis may also offer one explanation for the species-specific oncogenicity of this DNA virus. The SV40-transformed mouse, rat, and hamster cells used in these studies all contain serologically detectable, virus-specific T antigens; however, the function of these proteins is still being defined. If SV40 T antigens are responsible for the resistant phenotype of transformed hamster cells, there may be a functional difference in the T antigens expressed in cells from different species that has not been detected serologically.

Others have postulated different mechanisms by which SV40-transformed cells might evade the host immune response, such as (*i*) insufficient amounts of virus-specific transplantation antigen in the developing focus of tumor cells to sensitize the host, (*ii*) blockage of antibody protection of the antigenic sites of transformed cells from recognition by host effector cells (19), and (*iii*) development of suppressor cells that reduce the intensity of the host cellular immune response to transformed cells (20, 21). None of these mechanisms is incompatible with the existence of an inherent resistance of SV40-transformed hamster cells to the mechanisms by which activated macrophages cause tumor cell lysis. The existence of suppressor cells in the tumor cell microenvironment may, in fact, explain why SV40-transformed hamster cells that are resistant to cytolysis may be able to proliferate after exposure to activated macrophages. Macrophage activation *in vivo* may depend on continuously high levels of the lymphokine, macrophage-activating factor in the tumor environment. Furthermore, it has been shown that activated macrophages may function not only as tumoricidal effector cells but also as suppressors of lymphocyte function and, thus, it has been postulated that activated macrophages may exert negative feedback control over their own activation (22).

SV40-transformed hamster cells that survive exposure to activated hamster macrophages *in vitro* resume replication after a period of temporary cytostasis presumably caused by a loss of cytotoxic potential by the macrophages during *in vitro* cultivation (Fig. 1). If persistence of activated macrophages is required for maintenance of tumor cell cytostasis *in vivo*, then the feedback inhibition of lymphocyte function by these effector cells would favor the proliferation of a target cell, such as the SV40-transformed hamster cell, that could survive the initial cytolytic effect of the macrophage. In this regard, two recent studies suggest that the specific immune response of lymphoid cells from tumor-bearing hamsters to SV40 transplantation antigen disappears between the fourth and seventh days after tumor challenge and that suppression of lymphocyte responses in such animals may be in part macrophage-mediated (21, 23).

In addition to a possible role in the tumor-inducing capacity of SV40-transformed rodent cells, the susceptibility or resistance of a transformed cell to host effector mechanisms, such as tumoricidal activated macrophages, could be of more general importance in the study of tumor biology. If, in addition to antigenic changes that might result during the transformation process, neoplastic cells acquire a heritable resistance to destruction by activated macrophages and other types of effector cells, such as cytotoxic lymphocytes and natural killer cells, then rejection of the incipient malignancy would be more difficult. An understanding of the mechanisms by which tumor cells resist host defenses, such as activated macrophage-induced cytolysis, could prove useful in defining alterations in the tumor cell microenvironment that might enhance the effect of the host response and result in tumor cell destruction.

We thank Ruth Bartlett for secretarial assistance. This work was supported by the Veterans Administration, by American Cancer Society Grant CH-139, and by National Institutes of Health Training Grant 5-T-32-AI07011.

1. Butel, J. S., Tevethia, S. S. & Melnick, J. L. (1972) *Adv. Cancer Res.* **15**, 1–55.
2. Eddy, B. E. (1964) *Prog. Exp. Tumor Res.* **4**, 1–26.
3. Lewis, A. M., Jr. & Cook, J. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2886–2889.
4. Lohmann-Matthes, M. (1976) in *Immunobiology of the Macrophage*, ed. Nelson, D. S. (Academic, New York), pp. 463–486.
5. Hibbs, J. B., Jr., Lambert, L. H., Jr. & Remington, J. S. (1972) *Nature (London) New Biol.* **235**, 48–50.
6. Ruskin, J. & Remington, J. S. (1968) *Science* **160**, 72–74.
7. Weinberg, J. B., Chapman, H. A. & Hibbs, J. B., Jr. (1978) *J. Immunol.* **121**, 72–80.
8. Black, P. & Rowe, W. P. (1963) *Proc. Soc. Exp. Biol. Med.* **114**, 721–727.
9. Aaronson, S. A. & Todaro, G. J. (1968) *Science* **162**, 1024–1026.
10. Cook, J. L. & Lewis, A. M., Jr. (1979) *Cancer Res.* **39**, 1455–1461.
11. Black, P. H. & Rowe, W. P. (1963) *Proc. Natl. Acad. Sci. USA* **50**, 606–613.
12. Meltzer, M. S., Tucker, R. W., Sanford, K. K. & Leonard, E. J. (1975) *J. Natl. Cancer Inst.* **54**, 1177–1184.
13. Weinberg, J. B. & Hibbs, J. B., Jr. (1979) *J. Reticuloendothelial Soc.* **26**, 283–293.
14. Fidler, I. J., Roblin, R. O. & Poste, G. (1978) *Cell. Immunol.* **38**, 131–146.
15. Hibbs, J. B., Jr., Taintor, R. R., Chapman, H. A. & Weinberg, J. B. (1977) *Science* **197**, 279–282.
16. Kit, S., Kurimura, T. & Dubbs, D. R. (1969) *Int. J. Cancer* **4**, 384–392.
17. Takemoto, K. K., Ting, R. C. Y., Ozer, H. L. & Fabisch, P. (1968) *J. Natl. Cancer Inst.* **41**, 1401–1409.
18. Tevethia, S. S., Zarling, J. M. & Flax, M. H. (1976) in *Immunobiology of the Macrophage*, ed. Nelson, D. S. (Academic, New York), pp. 509–533.
19. Tevethia, S. S. (1980) in *Viral Oncology*, ed. Klein, G. (Raven, New York), pp. 581–602.
20. Glaser, M. (1979) *J. Exp. Med.* **149**, 774–779.
21. Chen, H., Quan, C. P., Zuinghedau, J., de Vaux Saint Cyr, C. & Lespinats, G. (1979) *Eur. J. Immunol.* **9**, 80–84.
22. Hibbs, J. B., Jr., Remington, J. S. & Stewart, C. C. (1980) *Pharmac. Ther.* **8**, 37–69.
23. Houston, K. J. & Blasecki, J. W. (1979) *J. Natl. Cancer Inst.* **63**, 665–673.