the test of further experimentation. The availability of pure enzyme may facilitate the discovery of the reaction mechanism, some 55 years after the discovery of penicillin (*10*).

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Differential NK Cell and Macrophage Killing of Hamster Cells Infected with Nononcogenic or Oncogenic Adenovirus

Abstract. *Hamster cells infected with highly oncogenic human adenovirus type 12 (Ad12) were resistant to lysis by natural killer cells and macrophages, compared to cells infected with nononcogenic adenovirus type 2 (Ad2). The data suggest that early adenovirus gene expression in hamster cells results in preferential survival of Ad12, compared to Ad2, infected cells in vivo, thus providing an explanation for the differences in the oncogenicities of these two transforming viruses.*

Adenoviruses (Ad's) are common human pathogens and were the first human viruses observed to be oncogenic in rodents (*1*). On the basis of their oncogenicities for newborn hamsters, human Ad's were classified as highly oncogenic, weakly oncogenic, and nononcogenic (*2*). In spite of the marked differences in the tumor-inducing capacities of the viruses, Ad serotypes from both highly oncogenic and nononcogenic subgroups transform hamster and rat cells to a neoplastic state in vitro with approximately equal efficiency (*3*). Cell lines transformed in vitro by highly oncogenic Ad's (for example, Ad12) efficiently induce tumors in syngeneic adult hamsters, whereas cell lines transformed by nononcogenic Ad's (for example, Ad2) are either nontumorigenic or induce tumors only in immunodeficient hosts (*4*). All such Ad-transformed cells are highly immunogenic (*3*). Therefore, neither the transforming efficiencies of these viruses nor the immunogenicities of the cells they transform explains the differences in oncogenicities of highly oncogenic compared to nononcogenic Ad's. Earlier we showed that hamster cells transformed by Ad12 are more resistent to the cytolytic effects of hamster natural killer (NK) cells than are Ad2-transformed cells (*5*). On the basis of the results of histopathological studies, such host cells appear to be one of the key factors in the primary hamster defense against tumor induction by Ad2-transformed cells (*6*). Lytic infection by adenoviruses has not been shown to induce increased cellular susceptibility to lysis by NK cells or other host effector cells. We now report an increased cytolytic susceptibility in hamster cells infected with Ad2 compared to Ad12-infected cells, a phenomenon that is linked to the cellular accumulation of Ad2-encoded early polypeptides (T antigens). This suggestion of functional differences in the early gene products of Ad2 and Ad12 in the context of the host cellular immune response provides a new explanation for the differences in the tumor-inducing capacities of these two Ad serotypes.

To determine the cytolytic susceptibilities of cells infected with nononcogenic Ad2 and highly oncogenic Ad12, we used quantitative assays of target cell lysis by normal (nonimmune) hamster spleen cells (the functional hamster analog of the NK activity observed in other species) and bacillus Calmette-Guerin (BCG)–activated hamster macrophages in which the cytolytic susceptible cell line Ad2HE1 was used as a sensitive control (*5, 7*). The cytolytic activity of these preparations of spleen cells from unsensitized hamsters exhibits several of the properties of NK cells from other species, such as the lack of a requirement for specific sensitization for expression of lytic activity, the lack of genetic restriction, and the persistence of activity after depletion of adherent cells (*5*). Secondary LSH hamster embryo cells (HEC's) prepared from the inbred LSH strain of Syrian hamsters (*4*) were relatively resistant to activated macrophage-mediated lysis compared to Ad2HE1, but they expressed progressively increasing cytolytic susceptibility when infected with increasing multiplicities of Ad2 for 18 to 24 hours (Fig. 1). No significant viral replication appeared [less than 0.2 $\log_{10}$ plaque-forming units (PFU) per milliliter above the inoculum] as detected by production of viral progeny in one-step growth curve assays until approximately 30 hours after infection. The viral specificity of the induction of cytolytic susceptibility was confirmed by the abrogation of the effect with Ad2-specific neutralizing antiserum (Table 1) (*8*). In this same series of experiments the macrophage-resistant, SV40-transformed LSH hamster cell line SV40HE1 (*5, 9*) also exhibited increased susceptibility to macrophage-mediated lysis 18 to 24 hours after infection with Ad2.

In kinetic studies with a multiplicity of infection of 10 PFU per cell, an association between the induction of cytolytic susceptibility in Ad2-infected HEC's and the accumulation of Ad2 T antigens in target cells was shown (Fig. 2). A second method to evaluate the role of early Ad2 polypeptides in the induction of cytolytic susceptibility was to block viral DNA synthesis and thus the expression of late viral polypeptides by infecting cells and performing cytolysis assays in the pres-

ence of arabinosyl cytosine (AraC) (Table 1) (*10*). The increase in cytolytic susceptibility of HEC's after Ad2 infection was not effected by this drug. These same observations hold for an Ad2-induced increase in susceptibility to lysis by hamster NK cells (Table 1). These results complement those of our earlier studies of somatic cell hybrids formed between Ad2- and SV40-transformed cells in which Ad2 T antigens were implicated as the transformed cell factors responsible for increased susceptibility to lysis by hamster NK cells and activated macrophages (*7*). Thus, the expression of early Ad2 gene products in infected hamster cells is associated with increased susceptibility to lysis by two different types of immunologically nonspecific host effector cells, one of which (NK cells) is not specifically activated and the other of which (BCG-activated macrophages) is stimulated to a high level of tumoricidal activity.

We used these two effector cell populations to compare directly the abilities of nononcogenic Ad2 and highly oncogenic Ad12 to induce increased susceptibility to lysis in HEC's (Fig. 3 and Table 1). To compare the effects of infection with Ad2 or Ad12 on HEC susceptibility to NK cell–mediated lysis, a constant number of mock-infected or virus-infected (100 PFU per cell) HEC's was incubated in the presence of various numbers of normal spleen cells with the Ad2HE1 cell line being used as a sensitive control (Fig. 3). At spleen cell to target cell ratios greater than or equal to 50 to 1, Ad2-infected HEC's were significantly more susceptible to lysis than were Ad12-infected HEC's ($P < 0.01$ to 0.05). These differences in cytolytic susceptibility between Ad2- and Ad12-infected HEC's were not due to differences in the numbers of target cells expressing the respective Ad T antigens [mean ± standard error of the mean (S.E.M.) percent of cells containing viral T antigens in five consecutive experiments: Ad2 infection, 80.6 ± 2.7; Ad12 infection, 74.0 ± 5.4]. Most estimates indicate that NK cells comprise approximately 5 percent of the spleen cell population (*11*). If similar proportions of hamster spleen cells expressed NK activity in this experimental model, hamster NK cells present in effector cell to target cell ratios as low as 2.5 to 1 may be able to discriminate between Ad2-infected HEC's (more susceptible to lysis) and Ad12-infected HEC's (relatively resistant to lysis) (Fig. 3).

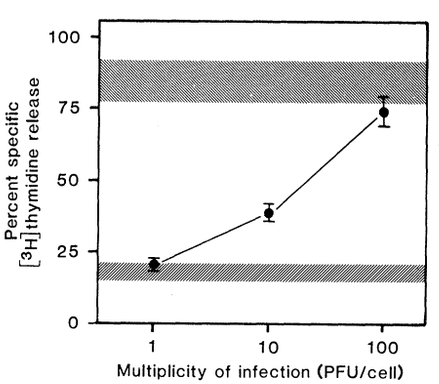

Fig. 1. Effect of varying the multiplicity of infection (PFU per cell) of Ad2 on the susceptibility of secondary LSH hamster embryo cells (HEC's) to lysis by activated macrophages. Adenovirus type 2 was grown and titered in the human cell line A549. The titer of the virus pool used in these studies was 6.6×10^9 PFU/ml. Similar results were obtained with an Ad2 pool grown and titered in primary human embryonic kidney cells (not shown). The HEC's were labeled with [^{3}H]thymidine, infected for 3 hours in suspension, and assayed in triplicate after 18 to 24 hours of infection for their susceptibility to lysis (as evidenced by release of the label after 48 hours) on monolayers of BCG-activated hamster peritoneal macrophages at effector cell to target cell ratios of 33 to 1 under optimal conditions (*9*). The lower hatched area represents the mean ± S.E.M. (17.9 ± 3.5) percent lysis of mock-infected HEC's. The upper hatched area represents the mean ± S.E.M. (84.8 ± 7.2) percent lysis of the cytolytic sensitive Ad2HE1 control cell line (*5*). Each point represents the mean ± S.E.M. of the results of three experiments with different HEC preparations.

A similar comparison of the induction of cytolytic susceptibility in HEC's infected with nononcogenic Ad2 and highly oncogenic Ad12 was performed with activated macrophages as effector cells under optimal conditions (Table 1). In contrast to the small differences observed in cytolytic susceptibility between Ad12-infected and uninfected HEC's with NK cells (Fig. 3), Ad12-infected HEC's were more susceptible to macrophage-mediated lysis. Therefore, highly activated effector cell populations, such as BCG-activated macrophages, can differentiate Ad12-infected cells from uninfected HEC's. However, the increased cytolytic susceptibility of Ad2-infected HEC's compared to Ad12-infected HEC's observed in NK assays was also observed with activated macrophages used as effector cells. No significant differences were observed in lysis of cytolytic-resistant HEC's or cytolytic-susceptible Ad2HE1 in macrophage and NK cytolysis assays performed in medium conditioned for 48 hours by Ad2-infected HEC's (100 PFU per cell at a cell to volume ratio identical to that used in cytolysis assays; data not shown), which indicates that neither the presence of residual virus nor the presence of mediators that might be elaborated during these target cell infections appeared to have a significant effect on the cytolytic activities of the effector cells or on the cytolytic-resistant and -susceptible phenotypes of target cells. Therefore, these data support the conclusion that the increased cytolytic susceptibility observed in HEC's infected with Ad2 is due to a direct effect of the early viral gene products expressed in such infected cells.

Hamster and rat cells transformed by both Ad2 and Ad12 are highly immunogenic in vivo as evidenced by their ability to induce virus-specific transplantation immunity (*3*). Such specific immunity is usually ascribed to specifically sensitized cytotoxic T lymphocytes (CTL's). Raska and Gallimore reported that rat cells transformed by both adenovirus serotypes are equally sensitive to lysis by CTL's from syngeneic rats immunized with relevant Ad-transformed cells (*12*), which suggests that the relatively late appearing, immunologically specific host cellular immune defenses directed at adenovirus-specific cell surface antigens may not discriminate between cells transformed by highly oncogenic and nononcogenic Ad serotypes in the immunologically unsensitized host. Apparently conflicting results have been reported by Bernards *et al.* (*13*), who observed that transformed rat cells ex-

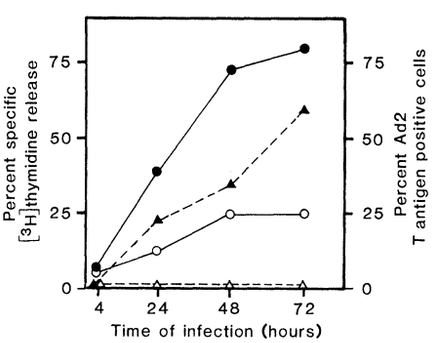

Fig. 2. Representative experiment showing the correlation between susceptibility of a population of Ad2-infected HEC's to lysis by BCG-activated macrophages (expressed as specific release of target cell label) and the percent of cells in the infected population expressing detectable Ad2 T antigens. The HEC's were infected for 4 hours in suspension with 10 PFU per cell of Ad2 and then assayed immediately or at 24, 48, or 72 hours of infection for susceptibility to lysis on monolayers of BCG-activated hamster peritoneal macrophages at effector cell to target cell ratios of 33 to 1 under optimal conditions (*9*). All HEC's were from the same lot of cells. Closed and open circles represent the percent lysis, and closed and open triangles represent the percent of the cells staining for Ad2 T antigens by indirect immunofluorescence with specific antiserums (*15*). Open symbols, mock-infected cells; closed symbols, Ad2-infected cells. Similar correlations were obtained in four other experiments.

pressing the early region 1a of Ad12 were less sensitive than cells expressing the analogous region of nononcogenic Ad5 to lysis by allogeneic CTL's sensitized in vitro to the relevant alloantigen. Direct comparisons of the cytolytic activities of these two types of CTL's against Ad12- and Ad2- or Ad5-transformed cells may be necessary to clarify the role of such immunologically specific effector cells in differentiating cells transformed by highly oncogenic and nononcogenic Ad serotypes. In contrast to the CTL's used in those two studies, neither NK cells nor macrophages appear to require specific sensitization to target cell antigen or recognition of class 1 major histocompatibility antigens on target cells to express cytolytic activity. Therefore, these immunologically nonspecific host effector cells may offer the host a first line of defense against neoplastic transformation that may function during the lag phase required for the induction of specific cellular immunity. If transformed cells avoid destruction by such early nonspecific host cellular defenses and establish early tumors, the host immune-mediated rejection of the incipient malignancy may be subverted by a variety of local and systemic tumor-related factors (*14*). These complex interactions usually result in tumor progression in spite of the host cellular immune response. Therefore, the effect of the first line of host defense against Ad-transformed cells provided by nonspecific effector cells may be a pivotal factor in eventual tumor progression or rejection.

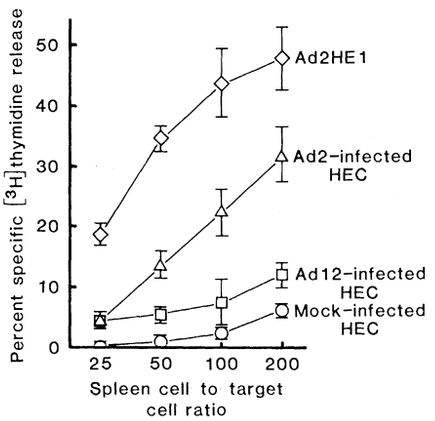

Fig. 3. Comparison of the susceptibilities of HEC's to lysis by normal hamster spleen cells after HEC infection with Ad2 or Ad12 (100 PFU per cell). Adenovirus type 2 was prepared as described (see Fig. 1); Ad12 virus was grown and titered in primary human embryonic kidney cells. The titer of the Ad12 virus pool used was 5.4×10^8 PFU/ml. The HEC's were infected with the indicated virus or cultured without virus (mock-infected) for 3 hours in suspension and were assayed in triplicate after 18 to 24 hours of infection for susceptibility to lysis by normal spleen cells with the same culture conditions as for macrophage cytolysis assays (*5*). A fixed number of target cells (6×10^4 cells per 2 ml of culture in 16-mm wells) was cocultured with varying numbers of spleen cells (1.5, 3.0, 6.0, or 12×10^6 cells per well). The Ad2HE1 cell line was used as a cytolytic sensitive control (*5*) in these assays. Each point represents the mean ± S.E.M. of the results of five experiments with different HEC preparations. The differences between the lysis of Ad2- and Ad12-infected HEC's were significant ($P < 0.01$ to 0.05) at spleen cell to target cell ratios of 50 to 1 or greater, as estimated by Student's *t*-test.

Table 1. Susceptibilities to macrophage- or NK cell–mediated lysis of target cells infected with Ad2 or Ad12.

Target cell	Viral inoculum* (PFU per cell)	Assay conditions†	Target cell lysis‡ (%)
	AMϕ effector cell§		
Ad2HE1	None	DMM	88.2 ± 6.3
HEC	None	DMM	18.5 ± 3.5
HEC	Ad2(50)	DMM	52.7 ± 1.5‖
HEC	Ad2(50) + Ab	DMM	18.2 ± 2.5
SV40HE1	None	DMM	28.6 ± 2.0
SV40HE1	Ad2(50)	DMM	72.9 ± 0.7‖
Ad2HE1	None	DMM	84.3 ± 3.2
Ad2HE1	None	DMM + AraC	87.0 ± 5.4
HEC	None	DMM	15.0 ± 3.8
HEC	Ad2(50)	DMM	49.3 ± 5.5‖
HEC	None + AraC	DMM + AraC	23.4 ± 4.2
HEC	Ad2(50) + AraC	DMM + AraC	55.6 ± 4.8‖
Ad2HE1	None	DMM	78.1 ± 6.0
HEC	None	DMM	20.7 ± 2.5
HEC	Ad2(50)	DMM	69.3 ± 8.8¶
HEC	Ad12(50)	DMM	43.4 ± 6.1¶
	NK effector cell#		
Ad2HE1	None	DMM	47.5 ± 1.7
Ad2HE1	None	DMM + AraC	55.1 ± 7.3
HEC	None	DMM	13.1 ± 2.6
HEC	Ad2(100)	DMM	31.7 ± 4.5‖
HEC	None + AraC	DMM + AraC	21.4 ± 0.8
HEC	Ad2(100) + AraC	DMM + AraC	37.9 ± 2.7‖

*Virus infection was initiated with cells in suspension at a low shaker speed at 37°C for 3 hours. Cells were infected with Ad2 or Ad12 at 50 or 100 PFU per cell, as indicated in parentheses, and used in cytolysis assays after 18 to 24 hours of infection. In the first series of experiments, Ad2 was neutralized with specific antibody (Ab) for 30 minutes at 20°C as described (*8*). †In the second and fourth series, Ad2 infections and cytolysis assays were performed in the presence of arabinosyl cytosine (AraC, 10 μg/ml) to block viral DNA synthesis (*10*). ‡Expressed as mean ± S.E.M. percent specific release of radioactivity, calculated as described (*5, 9*). The mean ± S.E.M. total radioactivities of [^{3}H]thymidine for each 500-μl sample of lysed target cells was between 8,560 ± 839 disintegrations per minute for HEC's infected with 100 PFU of Ad2 per cell and 22,729 ± 2,351 disintegrations per minute for Ad2HE1; the mean ± S.E.M. percent spontaneous release of radioactivity was between 8.5 ± 0.6 percent for SV40HE1 and 21.1 ± 1.5 percent for Ad-2HE1. §AMϕ, BCG-activated hamster peritoneal macrophage monolayers at a ratio of effector cell to target cell (E:T) of approximately 33 to 1. Assays were performed as described (*5, 9*) in Dulbecco's modified Eagle medium (DMM) containing Hepes buffer (20 m*M*), penicillin G (100 unit/ml), and streptomycin (100 μg/ml) and supplemented with glucose (final concentration, 15 m*M*) and 10 percent endotoxin-free (<1 ng/ml) fetal bovine serum. Four experiments were performed in the first series, five were performed in the second series, and seven were performed in the third series. ‖These virus-infected target cells were more sensitive to cytolysis than were the relevant, uninfected control cells ($P < 0.05$ to 0.001) as estimated by Student's *t*-test. ¶These Ad2-infected HEC's were more sensitive to lysis than Ad12-infected HEC's ($P < 0.05$). #NK, spleen cells from unsensitized adult hamsters at an E:T ratio of 100 to 1. Assays were performed as described above for AMϕ. Four experiments were performed in this series.

This discovery of differences in susceptibilities of Ad2-infected compared to Ad12-infected hamster cells to destruction by host effector cells provides one explanation for the differences in the oncogenicities of these two Ad serotypes in hamsters. As a working hypothesis, we propose that functional differences in the Ad gene products (T antigens) encoded in hamster cells by different Ad serotypes at early times after infection are responsible for the induction of different levels of susceptibility of infected cells to destruction by early appearing, immunologically nonspecific host effector cells. We reported earlier that hamster cells transformed by Ad2 are significantly more sensitive to destruction by hamster NK cells than are Ad12-transformed cells (*15*). This discovery is augmented by the results of studies in the adenovirus–rat tumor model (*12*) in which Ad2-transformed rat cells were also found to be more susceptible to lysis by rat NK cells than were Ad12-transformed rat cells. Together with the data presented here, this suggests an explanation for the differences in the tumor-inducing capacities of nononcogenic versus highly oncogenic Ad serotypes in hamsters on the basis of differences in the interactions that occur at the interface between virus-infected or -transformed cells and the host response. Thus, most hamster cells infected with

nononcogenic adenoviruses such as Ad2 may be destroyed efficiently in vivo by early appearing, nonspecific host effector cells, whereas cells infected with highly oncogenic adenoviruses such as Ad12 may be relatively resistant to such host defenses. This differential susceptibility of Ad-infected hamster cells to host cell–mediated lysis would result in an increase in the number of cells infected with a highly oncogenic adenovirus serotype that could become stably transformed. A further selection in vivo against tumor induction by nononcogenic Ad serotypes and for tumor induction by highly oncogenic Ad serotypes might be exerted by NK's cells, activated macrophages, and CTL after the establishment of transformation.

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Immunologic Inhibition of Ultraviolet Radiation–Induced Tumor Suppressor Cell Activity

Abstract. *Long-term exposure of C3H mice to ultraviolet radiation resulted in the formation of suppressor T cells that recognize ultraviolet radiation–induced regressor skin cancers as a class before the appearance of overt tumors. Administration of monoclonal antibodies to the product of the I-J^k subregion of the major histocompatibility complex or low doses of cyclophosphamide in vivo inhibited the development or activity of these cells. This activity of the monoclonal antibody was eliminated by adsorption on B10.BR (I-J^k) but not B10.D2 (I-J^d) splenocytes. These findings provide evidence that elements expressing the I-J determinant are important in regulating the host response prior to the overt development of ultraviolet radiation–induced skin cancers and suggest novel therapeutic approaches to malignancies or other diseases involving suppressor T cells in their pathogenesis.*

Long-term exposure of C3H mice to ultraviolet (UV) radiation results in the appearance of cutaneous fibrosarcomas and squamous cell carcinomas, most of which regress on transplantation to naïve syngeneic recipients (*1*). This tumor system has several unusual characteristics, among which is the appearance of T suppressor (Ts) cells that abrogate T cell–mediated immune rejection of most UV radiation–induced regressor tumors (*2–4*). These Ts cells appear before the development of overt tumors and have been shown to be crucial to the development and growth of UV radiation–induced skin tumors (*4*). Thus, this system offers the opportunity to study the influence of immunotherapeutic strategies designed to eliminate or modify Ts cells early in the process of carcinogenesis.

In other tumor systems, interference with Ts cell generation or function by various modalities has been shown to result in retarded primary tumor growth

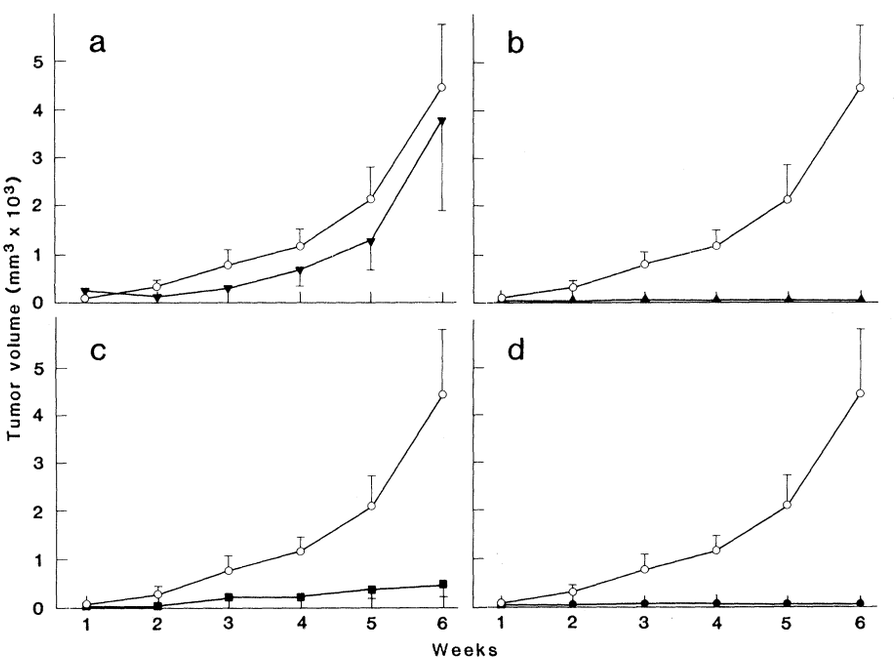

Fig. 1. Results of experiment 1. Eight mice in each group received 10^8 spleen cells intravenously from each category of donor and 2×10^6 cells of UV radiation–induced regressor tumor 2240 subcutaneously. Tumor volume was scored weekly by measuring three dimensions. Mean tumor size in animals that received cells from mice treated with UV radiation alone (positive control) (○) is compared with that in the other groups. (a) Ultraviolet radiation plus 0.2 ml of medium given intraperitoneally twice weekly (▼). (b) Ultraviolet radiation plus 500 ng of monoclonal antibody to I-J^k (WF8.C12.8) given intraperitoneally twice weekly (▲). (c) Ultraviolet radiation plus 20 mg of CY per kilogram of body weight given intraperitoneally twice weekly (■). (d) No treatment of donors (negative control) (●). Tumors regressed with time in some individual animals even in groups in which the mean tumor volume increased progressively. At 6 weeks the percentage of tumor-bearing animals was 75.0 percent (○), 75.0 percent (▼), 0 percent (▲), 25.0 percent (■), and 12.5 percent (●) (N = 8 in all groups). Error bars are ± standard error of the mean (S.E.M.). At 6 weeks there was no significant difference between (○) and (▼) groups (P = 0.360 by rank-sum method). Other groups compared to composite of (○) and (▼) groups as: P = 0.002 (▲), P = 0.012 (■), and P = 0.002 (●). Because of multiple comparisons, the 95 percent significance level for these is P = 0.017.