

GENERATION AND DECAY OF THE IMMUNE RESPONSE  
TO A PROGRESSIVE FIBROSARCOMA

I. Ly-1<sup>+</sup>2<sup>-</sup> Suppressor T Cells Down-regulate the Generation of Ly-1<sup>-</sup>2<sup>+</sup>  
Effector T Cells

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A number of publications show that mice bearing progressive syngeneic tumors can acquire suppressor T cells, as measured by a variety of assays (reviewed in 1). In support of this evidence, a series of studies in this laboratory (2, 3) has shown that the acquisition of suppressor T cells by a tumor-bearing host is responsible for the failure of passively transferred, tumor-sensitized T cells to cause the regression of its tumor. First, it was shown with two different immunogenic tumors (2, 3) that in order for passively transferred sensitized T cells from immunized donors to cause the complete regression of an established tumor, the tumor-bearing recipient needs to have been made T cell deficient by thymectomy and lethal irradiation, and then restored with bone marrow (TXB mice)<sup>1</sup> or immunodepressed by treatment with cyclophosphamide (4) or exposure to sublethal irradiation (5). It was revealed next that tumor regression in TXB test recipients caused by the passive transfer of immune T cells can be inhibited by the transfer of T cells from immunocompetent tumor-bearing donors. It was hypothesized, on the basis of this and other evidence (6), that progressive growth of an immunogenic tumor eventually evokes the generation of a mechanism of T cell-mediated immunosuppression.

However, the demonstration that tumor-bearing mice acquire suppressor T cells does not in itself provide a satisfactory explanation of how tumors with transplantation rejection antigens escape destruction by the immune system. What is needed in addition is evidence that suppressor T cells either can prevent an antitumor immune response from developing or can down-regulate an immune response that already is in progress. In support of the latter possibility are numerous descriptions in the literature of the acquisition and subsequent loss by a host with a progressive tumor of a paradoxical state of concomitant immunity that enables the host to prevent the growth of an implant of cells of that tumor (reviewed in 7). It seems reasonable to suggest, therefore, that the function of suppressor T cells would be to down-regulate a concomitant antitumor immune

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<sup>1</sup>Abbreviations used in this paper: TXB, T cell-deficient; PBS, phosphate-buffered saline; FCS, fetal calf serum.

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response before it develops sufficiently to destroy the tumor.

This paper will show that early growth of the meth A fibrosarcoma evokes the generation in its syngeneic host of a mechanism of concomitant immunity that can be passively transferred to  $\gamma$ -irradiated recipients with Ly-1<sup>-</sup>2<sup>+</sup> T cells and is paradoxically expressed against an established tumor. It will show, in addition, that concomitant immunity and the Ly-1<sup>-</sup>2<sup>+</sup> effector T cells that mediate it are progressively lost after day 9 of tumor growth, and that this is associated with the progressive acquisition of Ly-1<sup>+</sup>2<sup>-</sup> suppressor T cells capable of suppressing the expression of passively transferred immunity against an established tumor in TXB recipients.

### Materials and Methods

*Mice.* Specific-pathogen-free BALB/c and CB6F<sub>1</sub> (BALB/c × C57BL/6) mice were supplied by the Trudeau Institute Animal Breeding Facility. They were known to be free of viral pathogens as evidenced by the results of routine screening performed by the Diagnostic Testing Service of Microbiological Associates, Bethesda, MD.

*Tumors.* The methylcholanthrene-induced meth A fibrosarcoma, syngeneic in BALB/c mice, was originally obtained from Dr. Lloyd Old of the Memorial Sloan-Kettering Cancer Center, New York. To ensure that all of the experiments in a given study were performed with the same stock of tumor cells, the tumor was grown as an ascites in the peritoneal cavities of a large number of BALB/c mice, harvested in heparinized (5 IU/ml) phosphate-buffered saline (PBS), pooled, washed in PBS, resuspended in Fisher's medium (Gibco Laboratories, Grand Island, NY) containing 20% fetal calf serum (FCS) and 10% dimethyl sulfoxide, and cryopreserved in small volumes over liquid nitrogen. For each experiment a vial was thawed, the cells washed in PBS, and  $2 \times 10^6$  of them used to initiate ascites tumors in CB6F<sub>1</sub> mice. After 6 d of intraperitoneal growth, the tumor cells were harvested in heparinized PBS, washed in PBS, and resuspended appropriately in PBS for implanting in experimental animals. Primary tumors were initiated in the midline of the belly region by intradermal injection of  $10^6$  meth A cells. For challenge experiments, the same number of tumor cells were implanted in the right hind footpad. In the case of intradermal tumors, tumor growth was followed by measuring changes against time in the means of two perpendicular diameters. Footpad tumors were followed by measuring changes against time in the dorsoventral thickness of the foot with dial calipers.

*Immunization.* Mice were immunized against the meth A fibrosarcoma by injecting them intradermally with an admixture of  $10^6$  meth A cells and 100  $\mu$ g of formalin-killed *Propionibacterium acnes* (*C. parvum* from Burroughs Wellcome Co., Research Triangle Park, NC). It has been shown (8) that this results in a 9-d period of progressive tumor growth and then complete tumor regression. Regression is associated with the acquisition of immunity to an implant and of T cells capable of passively transferring immunity to normal recipients.

*T Cell-deficient Mice.* Mice were made T cell deficient (TXB) at 6 wk of age by thymectomy followed 7 d later by lethal (900 rad)  $\gamma$  radiation from a <sup>137</sup>Cs source that delivered a midphantom dose rate of 30 rad/min. The mice were given  $5 \times 10^6$  syngeneic bone marrow cells immediately after irradiation and were used in experiments after a further 4–6 wk.

*Passive Transfer of Immunity.* Immunized or tumor-bearing donor mice were killed by cervical dislocation and a single-cell suspension of spleen cells was prepared by dicing their spleens into small pieces and pushing the pieces gently through a 60-mesh stainless screen into PBS. The resulting cell suspension was triturated with a pasteur pipette to break up clumps and passed through six layers of sterilized surgical gauze to remove debris. The cells were then washed in PBS and resuspended in PBS for intravenous infusion via a lateral tail vein into TXB or sublethally (500 rad)  $\gamma$ -irradiated, tumor-bearing recipients.

*Antibodies.* Hybridomas secreting monoclonal anti-Thy-1.2 (30-H12), anti-Ly-1 (53-

7.313), and anti-Ly-2 (53-6.72) antibody (9) were obtained from the Salk Institute, La Jolla, CA. They were grown to  $5 \times 10^6$ /ml in RPMI medium (Gibco Laboratories) with 10% FCS and antibiotics and the cultures were subjected to centrifugation in order to pellet the cells and debris. The supernatants were dispensed in small volumes, frozen, and stored at  $-20^\circ\text{C}$  until required. Rabbit serum was used as a source of complement. It was obtained from rabbits bred at the Trudeau Institute and selected on the basis of minimal toxicity of their sera for mouse thymocytes. Mouse anti-rat IgG serum was raised by injecting mice with 100  $\mu\text{g}$  of rat IgG (Cappell Laboratories, Cochranville, PA) in Freund's complete adjuvant. The mice were given two additional injections of 100  $\mu\text{g}$  of rat IgG in incomplete adjuvant and were bled 6 d later. Their sera were pooled and stored at  $-20^\circ\text{C}$  until required.

For deletion of T cells, donor spleen cells were incubated at  $2 \times 10^7$ /ml in a 1:5 dilution of the anti-Thy-1.2 supernatant at  $4^\circ\text{C}$  for 30 min and then in a 1:10 dilution of rabbit serum at  $37^\circ\text{C}$  for 30 min. The cells were then washed and resuspended in PBS for intravenous infusion. For depletion of Ly T cell subsets, spleen cells were incubated at  $2 \times 10^7$ /ml in a 1:5 dilution of anti-Ly-1 or anti-Ly-2 supernatant at  $4^\circ\text{C}$  for 30 min. The cells were then washed in PBS and resuspended in a 1:50 dilution of mouse anti-rat IgG and incubated at  $4^\circ\text{C}$  for a further 30 min. They were then washed and incubated in rabbit complement as above and washed and resuspended for intravenous infusion. All reagents were diluted in RPMI medium containing 1% FCS.

## Results

*Acquisition and Loss of Immunity to a Challenge Implant.* Concomitant immunity is defined as the acquired ability by a host with a progressive tumor to inhibit the growth of a challenge implant of cells of the same tumor given at another site. The fact that progressive growth of the Meth A fibrosarcoma results in the generation and subsequent loss of concomitant immunity is evident from the results of an experiment that measured growth of a challenge implant of  $10^6$  tumor cells injected in the right hind footpad of mice bearing 3, 6, 9, 16, and 20 d intradermal tumors. It can be seen in Fig. 1 that immunity to the growth of the challenge implant was acquired by day 6 of growth of the primary tumor, was present on day 9, and had almost completely decayed by day 16.

*Concomitant Immunity Is Associated with Acquisition of Ly-1<sup>-</sup>2<sup>+</sup> T Cells Capable of Adoptively Immunizing Against an Established Tumor.* Although the preceding results leave no doubt that concomitant immunity is acquired and subsequently lost by mice bearing the Meth A fibrosarcoma, the identification and characterization of the cells that mediate this immunity required that it be passively transferred to appropriate recipients, preferably against an established tumor. However, past attempts in this laboratory to passively transfer concomitant immunity systemically, even against the growth of tumor implant, have failed. It was for this reason that the Winn neutralization assay (10) was used to show that concomitant immunity is T cell mediated (11). Recent publications (5, 6) from this laboratory have revealed the reason for past failures to demonstrate adoptive immunization against established tumors: To passively transfer immunity against an established tumor with T cells from preimmunized donors, it is necessary to use immunodepressed recipients that are incapable of generating suppressor T cells that prevent passively transferred immunity from being expressed. It was reasoned, therefore, that it would be possible to adoptively immunize against an established tumor with lymphoid cells from a concomitantly immune tumor

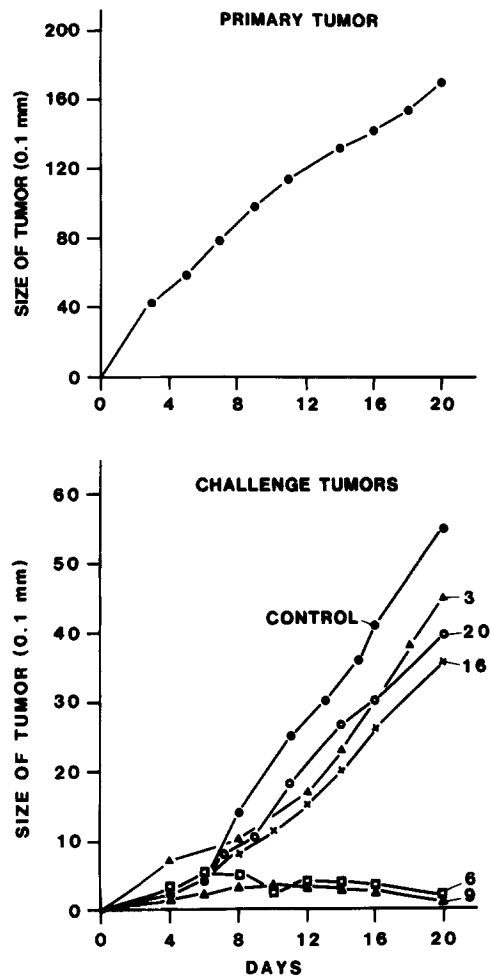


FIGURE 1. The generation and subsequent decay of concomitant immunity to growth of a tumor implant in mice bearing a progressive meth A tumor. (*Top*) Growth of the primary meth A tumor growing from an intradermal implant in the belly region. (*Bottom*) Growth of an intra-footpad implant of  $10^6$  meth A cells given to control mice and to tumor-bearing mice on day 3, 6, 9, 16, or 20 of tumor growth (numbers on individual graphs). Means of five mice per group.

bearer, provided that the recipient is immunodepressed and has a smaller tumor than the donor.

Results supporting this reasoning are shown in Fig. 2, in which the passive transfer of one organ equivalent ( $1.5 \times 10^8$ ) of spleen cells from donors with a 9-d intradermal tumor caused, after a 2–3-d delay, complete regression of a 3-d tumor in recipients, provided the recipients were given 500 rad of  $\gamma$  radiation 1 h before they received spleen cells. At the time of passive transfer, the donor tumor had an ~8-mm diam, whereas the diameter of recipient tumor was ~2 mm. Fig. 2 shows, in addition, that the spleen cells that passively transferred immunity to irradiated recipients were T cells, as evidenced by their functional elimination by treatment with anti-Thy-1.2 antibody and complement.

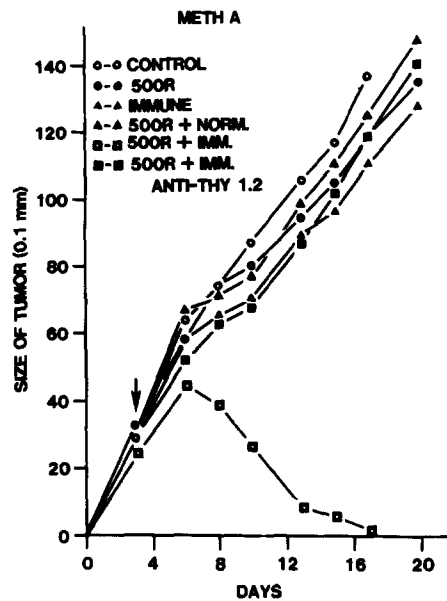


FIGURE 2. Evidence that concomitant immunity can be passively transferred with T cells and paradoxically expressed against an established tumor in recipients given 500 rad of  $\gamma$  radiation 1 h before passive transfer. An intravenous infusion (arrow) of  $1.5 \times 10^8$  spleen cells from donors bearing a 9-d tumor was given to nonirradiated (IMMUNE) and irradiated (500 R + IMM) recipients with a 3-d tumor. Spleen cells caused regression of the recipient's tumor only if the recipient was irradiated. The spleen cells that transferred immunity were T cells, as evidenced by their susceptibility to treatment with anti-Thy-1.2 antibody and complement.

The Ly phenotype of the T cells that transfer concomitant immunity also was investigated. The results in Fig. 3 show that they were Ly-1<sup>-</sup>2<sup>+</sup> T cells, in that their capacity to passively transfer immunity against an established tumor was completely ablated by treatment with anti-Ly-2 antibody and complement, but was not affected at all by treatment with anti-Ly-1 antibody and complement. In fact, treatment with the anti-Ly-1 reagent, more often than not, enhanced the level of concomitant immunity transferred.

*Kinetics of Generation and Loss of T Cells that Transfer Concomitant Immunity.* The foregoing results show that growth of the meth A fibrosarcoma evokes the generation in its host of a state of concomitant immunity to the growth of a tumor implant. They also show that the concomitant immunity can be passively transferred with Ly-1<sup>-</sup>2<sup>+</sup> T cells and expressed against an established tumor in  $\gamma$ -irradiated recipients. They show again that concomitant immunity, as measured by resistance to the growth of a tumor implant, is lost after day 9 of growth of the primary tumor. We predicted, therefore, that T cells capable of passively transferring immunity also would be lost after day 9. We measured changes in the capacity of one organ equivalent ( $\sim 1.5 \times 10^8$ ) of spleen cells from mice bearing a 3, 6, 9, 12, 15, or 18 d tumor to cause the regression of a standard 3-D tumor growing in recipients given 500 rad of  $\gamma$  radiation 1 h before receiving spleen cells. Passive transfer was performed at a single time with recipients bearing a standard-sized tumor.

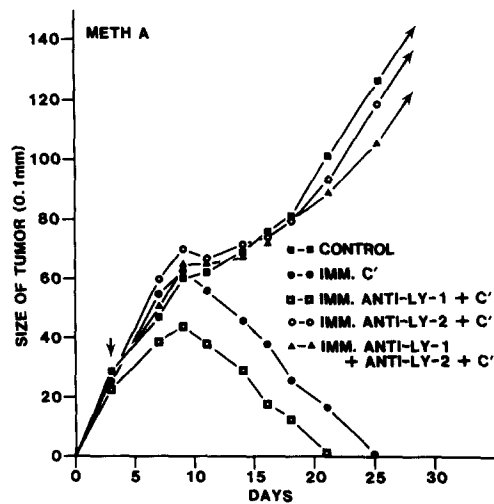


FIGURE 3. The T cells that passively transferred concomitant immunity and caused regression of an established tumor in  $\gamma$ -irradiated recipients were functionally eliminated by treatment with anti-Ly-2 antibody and complement, but not by anti-Ly-1 antibody and complement. In this experiment, all recipients were given 500 rad of  $\gamma$  radiation 1 h before receiving spleen cells on day 3 (arrows). Means of five mice per group.

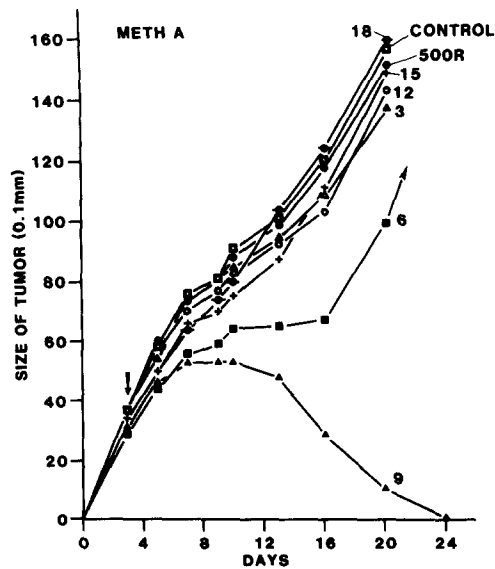


FIGURE 4. Kinetics of generation, during progressive tumor growth, of T cells capable of passively transferring immunity against an established tumor in irradiated recipients. Recipients bearing a 3-d tumor were given 500 rad of  $\gamma$  radiation and infused 1 h later (arrow) with one organ equivalent ( $\sim 1.5 \times 10^8$ ) of spleen cells from donor mice with a 3 ( $\Delta$ ), 6 ( $\blacksquare$ ), 9 ( $\blacktriangle$ ), 12 ( $\circ$ ), 15 ( $+$ ), or 18 ( $\bullet$ ) d tumor. T cells capable of passively transferring immunity were already generated by day 6, reached peak numbers on day 9 (complete regression), and were rapidly lost after day 9. Means of five mice per group.

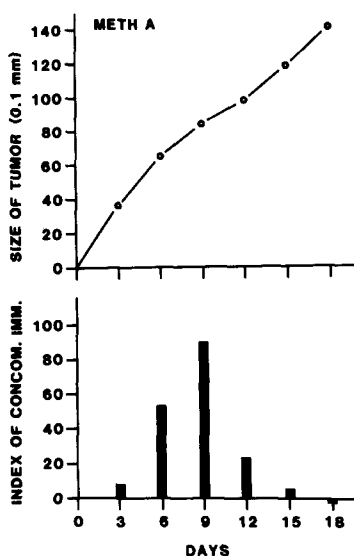


FIGURE 5. Results in Fig. 4 expressed as changes in the index of concomitant immunity (*bottom*) which was calculated as the difference between the growth of the tumor in control recipients and its growth in recipients of donor T cells on day 16 of the experiment. (*Top*) Growth of the donor tumor.

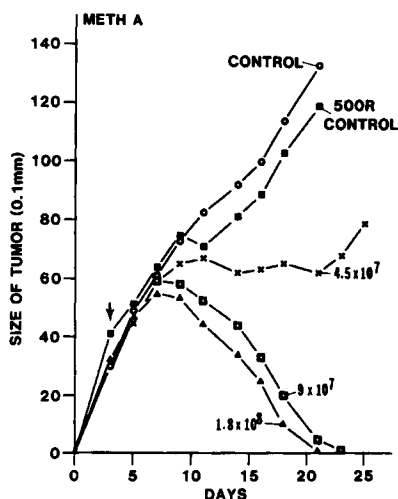


FIGURE 6. Evidence that the extent to which the  $\gamma$ -irradiated recipient's tumor underwent regression was dependent on the number of donor spleen cells infused intravenously. The recipients were given 500 rad of  $\gamma$  radiation and were infused 1 h later on day 3 of tumor growth (arrow) with  $1 (1.8 \times 10^8)$ ,  $0.5 (9 \times 10^7)$ , or  $0.25 (4.5 \times 10^7)$  organ equivalents of spleen cells from donor mice with a 9-d tumor. Means of five mice per group.

The results in Fig. 4 show that splenic T cells capable of adoptively immunizing against an established tumor were acquired between days 3 and 6 of tumor growth, reached peak numbers on day 9, and were rapidly lost over the next 3 d. It was only on day 9 that the spleen contained enough sensitized T cells to cause complete regression of the recipient tumor. Fig. 5 shows the same results

expressed as the difference between the growth of tumors in control recipients and their growth in recipients of immune cells on day 16. This enabled concomitant immunity to be plotted as an index of immunity against time of tumor growth, and serves to show more clearly the kinetics of acquisition and loss of immunity.

It is assumed in interpreting these differences, however, that the level of passively transferred concomitant immunity expressed by the recipients is proportional to the number of tumor-sensitized T cells infused. The results of an experiment that measured the immunity transferred against an established recipient tumor with graded numbers of spleen cells from donors with a 9-d tumor are shown in Fig. 6. They reveal that both the speed and the degree to which the recipients' tumor underwent regression depended on the number of donor cells infused, but that even as few as 0.25 organ equivalents ( $5 \times 10^7$ ) of donor spleen cells caused appreciable tumor regression to occur. Therefore, the small degree of tumor regression caused by the passive transfer of day 15 T cells, as shown in Fig. 4, indicates that by this stage of tumor growth the donor possessed <25% of the sensitized T cells that it possessed at peak response on day 9.

*Kinetics of the Generation of Suppressor T Cells.* Previous publications from this laboratory (2, 3) show that progressive growth of immunogenic tumors eventually results in the generation of splenic suppressor T cells that are capable, on passive transfer, of preventing the regression of established tumors in TXB recipients infused with T cells from preimmunized donors. Another of our publications (12) revealed that the suppression of adoptive immunity depends on the ability of infused suppressor T cells to inhibit the generation of cytolytic effector T cells in the adoptively immunized TXB recipients. However, all previous studies in this laboratory of tumor-induced immunosuppression by suppressor T cells used suppressor T cells that were harvested from donor mice bearing a 14-d tumor. We needed to determine whether suppressor T cells are acquired before day 14, to explain the rapid loss of effector T cells after day 9. This involved measuring the ability of one organ equivalent ( $1.5 \times 10^8$ ) of spleen cells from mice with a 3, 6, 9, 12, 15, or 18 d tumor to inhibit the capacity of  $1.5 \times 10^8$  spleen cells from preimmunized donors to cause the regression of a 4-d tumor in TXB recipients. Suppressor T cells were infused 3 h after immune spleen cells. This assay is different, therefore, from the adoptive concomitant immunity assay, both in terms of the donors of immune T cells and the type of recipients.

It can be seen in Fig. 7 that the capacity of immune spleen cells to cause regression of an established tumor in TXB recipients was inhibited by passive transfer of spleen cells from tumor-bearing donors, provided that spleen cells were harvested from the donors on day 9 of tumor growth or later. There was a progressive increase in the number of suppressor T cells between days 9 and 15. By subtracting the size of tumors in recipients of immune cells from the sizes in recipients of immune cells plus suppressor T cells on day 21 of the experiment, a suppressor index was obtained that we plotted against the growth of tumors in donors of suppressors (Fig. 8). Suppressor T cells were present in appreciable numbers on day 9 of tumor growth (Fig. 8) at the same time that effector T cells



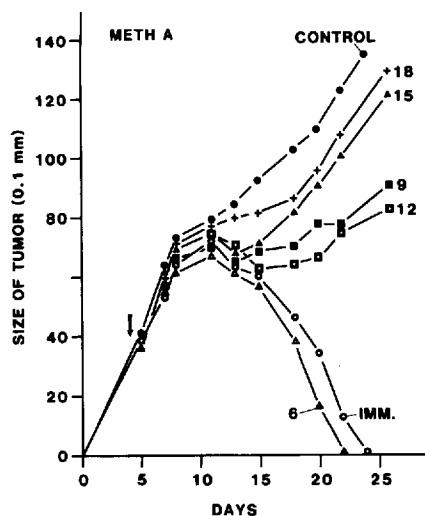


FIGURE 7. Changes against time in capacity of one organ equivalent ( $1.5 \times 10^8$ ) of spleen cells from donor mice bearing a progressive tumor to suppress the expression of adoptive immunity in TXB tumor-bearing recipients. The test tumor in TXB recipients underwent complete regression after infusion (arrow) of  $1.5 \times 10^8$  spleen cells from preimmunized donors (IMM). Shown are changes in the ability of one organ equivalent ( $1.5 \times 10^8$ ) of spleen cells from mice with a 6, 9, 12, 15, or 18 d tumor (numbers on individual graphs) to inhibit the antitumor function of the passively transferred immune spleen cells. The spleen cells of tumor-bearing donors were infused 3 h after immune spleen cells. Means of five mice per group.

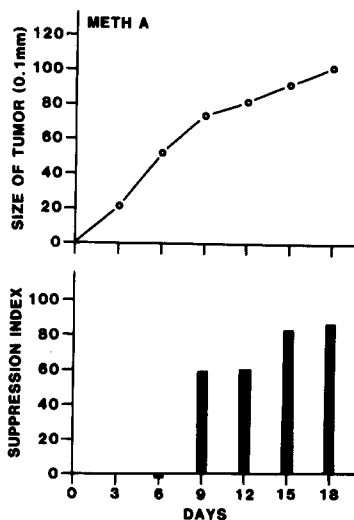


FIGURE 8. The results in Fig. 7 expressed as a suppressor index that was obtained by subtracting the size of the tumors in recipients of immune cells from their sizes in the recipients of immune plus suppressor cells on day 21 of the experiment (bottom). (Top) Growth of the tumor that caused the generation of suppressor cells.

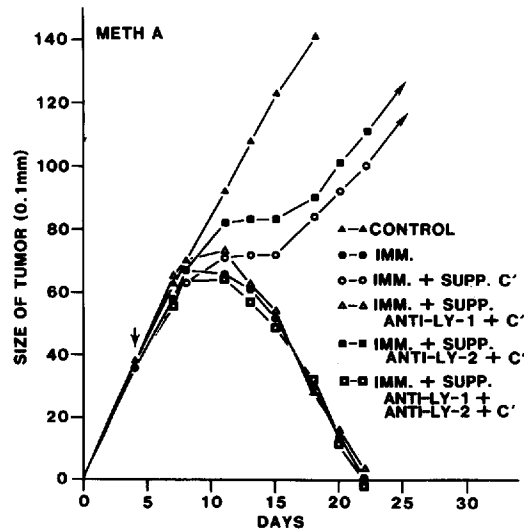


FIGURE 9. The cells from donors with a 15-d tumor that were capable, on passive transfer, of inhibiting the regression of an established tumor in TXB recipients were Ly-1<sup>+</sup>2<sup>-</sup> T cells. Their suppressor function was not eliminated by treatment with anti-Ly-2 plus complement, but was totally eliminated by treatment with anti-Ly-1 antibody and complement. Immune and suppressor T cells were infused 3 h apart on day 4 of tumor growth. Means of five mice per group.

were present in peak numbers, as shown in a preceding section. This paradox will be explained later.

*Suppressor Cells Are Ly-1<sup>+</sup>2<sup>-</sup> T Cells.* The standard suppressor assay was used to determine the Ly phenotype of suppressor T cells in 15-d tumor bearers. The results in Fig. 9 show unequivocally that, unlike effector T cells capable of passively transferring concomitant immunity, the T cells that transferred suppression were Ly-1<sup>+</sup>2<sup>-</sup> cells, as evidenced by their elimination by treatment with anti-Ly-1 antibody and complement, but not with anti-Ly-2 antibody and complement.

### Discussion

A central problem in tumor immunology has been to explain why tumors with transplantation rejection antigens, capable of specifically immunizing their immunocompetent hosts against growth of a tumor implant, are not destroyed by an antitumor immune response. Similar to previous findings with the meth A fibrosarcoma (2) and P815 mastocytoma (3), this paper shows that progressive growth of an immunogenic tumor eventually evokes the generation of suppressor T cells whose function can be measured by their ability to suppress, on passive transfer, the expression of adoptive immunity against an established tumor in TXB recipients infused with T cells from preimmunized donors. It shows, in addition, that the suppressor T cells were of the Ly-1<sup>+</sup>2<sup>-</sup> phenotype, in that they were functionally eliminated by treatment with anti-Ly-1 antibody and complement, but not by an anti-Ly-2 antibody capable of completely eliminating effector T cells (see below). Suppressor T cells were first generated on about day 9 of

tumor growth when the tumor was 7–8 mm in diameter, and increased in number as the tumor increased in size. These findings appear more significant in light of the additional information that the onset of production of Ly-1<sup>+</sup>2<sup>-</sup> suppressor T cells was associated with the onset of progressive decay of a preceding concomitant antitumor immune response that was measured in terms of both the acquisition of immunity to growth of an implant of tumor cells and the acquisition of T cells capable of adoptively immunizing against an established tumor in irradiated recipients. The concomitant immune response was generated after day 6 of tumor growth, peaked on day 9, and then rapidly decayed. Moreover, the T cells that transferred concomitant immunity were Ly-1<sup>-</sup>2<sup>+</sup>, in that they were totally eliminated by treatment with anti-Ly-2 antibody and complement but not with the same anti-Ly-1 reagent that eliminated suppressor T cells.

This apparently clear-cut absence of Ly-1 antigen on effector T cells might seem surprising, in view of the demonstration by others (13) that all T cells display Ly-1 antigen to some degree. However, it remains to be determined whether the expression of Ly-1 antigen on all T cells depends on the strain of mouse used and on whether the T cells are harvested from normal mice or from mice generating an active immune response. In any case, it is apparent that not enough Ly-1 antigen was present on the effector T cells generated in response to the meth A to make them susceptible to complement-mediated lysis after treatment with a particular anti-Ly-1 reagent.

The immune response to the progressive growth of the meth A fibrosarcoma is represented diagrammatically in Fig. 10. It is meant to show that a tumor of a certain minimal critical size is required to evoke the generation of Ly-1<sup>-</sup>2<sup>+</sup> effector T cells and that a tumor of a larger critical size is required to evoke the generation of Ly-1<sup>+</sup>2<sup>-</sup> suppressor T cells that down-regulated the production of effector T cells. It also shows that immunosuppression is not a sudden, all-or-nothing event but involves a progressive increase over time in the ratio of suppressor T cells to effector T cells, meaning that effector and suppressor T cells can coexist. This is probably the reason why treatment with anti-Ly-2 antibody and complement always resulted in a slight increase in the level of suppression passively transferred and why treatment of day 9–10 spleen cells with anti-Ly-1 antibody and complement resulted in an increase in the level of concomitant immunity transferred. Taken as a whole, the results in Fig. 10 are consistent with the hypothesis that immunogenic tumors are not rejected by their immunocompetent hosts, because the immune response they evoke is down-regulated by suppressor T cells before an adequate number of effector T cells is generated to destroy the tumor. In the case of the meth A fibrosarcoma, there is only ~3–4 d from when the tumor becomes large enough to trigger the generation of effector T cells to when it is large enough to provide enough antigen to trigger the generation of suppressor T cells. This narrow window for generating effector T cells is the result of the syngeneic tumor being a weak replicating antigen. It is likely that other replicating antigens, such as those represented by bacteria and protozoa that cause chronic infections, can give rise to the same sequence of immunological events as the meth A. Experiments with BALB/c mice infected with *Leishmania tropica* (14) show that progressive growth

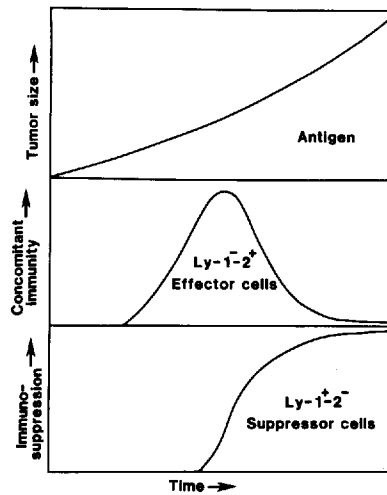


FIGURE 10. Diagrammatic representation of the immune response to a progressive immunogenic tumor of the type represented by the meth A fibrosarcoma. After the tumor reaches a critical minimum size it provides enough antigen to evoke the generation of  $Ly-1^{-2^{+}}$  effector T cells. However, a short period of additional tumor growth provides antigenic conditions that favor the generation of  $Ly-1^{+2^{-}}$  suppressor T cells that function to down-regulate the production of  $Ly-1^{-2^{+}}$  effector T cells. Consequently, not enough effector T cells are made to destroy the tumor.

of this parasite engenders the production of  $Ly-1^{+2^{-}}$  suppressor T cells that are capable, on passive transfer, of suppressing the expression of immunity against a *Leishmania* challenge inoculum in immunized recipients. For several reasons, the suppression of anti-*Leishmania* immunity is similar to the suppression of antitumor immunity described here. First, both models are based on the response to a replicating antigen that needs to expand to a certain critical quantity before suppressor T cells are generated. Second, the assay for suppressor T cells, in both cases, is an in vivo one that relies on the ability of suppressor T cells to function after they are passively transferred. Third, because in both cases the T cells that passively transfer suppression are of the  $Ly-1^{+2^{-}}$  phenotype, they are different from the T cells that have been shown to suppress antibody responses (15, 16), delayed-type hypersensitivity, and contact sensitivity (17). This does not make them unique, however, because there are other descriptions of  $Ly-1^{+2^{-}}$  suppressor T cells in the literature. For example, the in vivo suppression of acute graft-vs.-host disease is mediated by  $Ly-1^{+2^{-}}$  T cells (18, 19) as is the suppression in vitro of the cytolytic T cell responses to major (20) and minor (21) histocompatibility antigens. In addition, it has been shown more recently (22) that ultraviolet light-facilitated induction of the suppression of sensitivity to dinitrofluorobenzene is associated with the generation of  $Ly-1^{+2^{-}}$  cells that can transfer afferent suppression to irradiated recipients. Again,  $Ly-1^{+2^{-}}$  T cells have been shown to exclusively suppress the H-2-restricted interaction of helper T cells with B cells (23).

This is not to say that the passively transferred  $Ly-1^{+2^{-}}$  suppressor cells that suppress immunity to the meth A fibrosarcoma are the ultimate mediators of suppression. It remains possible, until shown otherwise, that these are inducer

suppressors that give rise to functional Ly-1<sup>-</sup>2<sup>+</sup> suppressor T cells, as has been postulated to occur in other models of suppression (17, 24). Even so, the failure of treatment with anti-Ly-2 antibody and complement to have any effect at all on the T cells that passively transfer suppression certainly does not support a role for Ly-1<sup>-</sup>2<sup>+</sup> suppressor T cells, particularly when one considers that the suppressor T cells were harvested from tumor-bearing donors in which suppression was being actively expressed.

Ly-1<sup>+</sup>2<sup>-</sup> T cell suppressors of concomitant immunity to the meth A fibrosarcoma appear to have little resemblance to the Ly-1<sup>-</sup>2<sup>+</sup> T cells that have been shown to suppress immunity to the S1509a sarcoma syngeneic in A/J mice. This well-studied model of tumor-induced suppression (25, 26) is based on the demonstration that T cells from mice with an established S1509a tumor are capable, on passive transfer, of inhibiting to a certain extent the capacity of immunized mice to prevent the growth of an implant of tumor cells. The finding that these Ly-1<sup>-</sup>2<sup>+</sup> suppressor T cells are generated as early as 24 h after implanting 10<sup>6</sup> S1509a cells subcutaneously (26) makes them quite different from meth A-induced suppressor T cells, which are not generated until 9 d of tumor growth. Presumably, suppression of anti-S1509a immunity is an example of T cell-mediated unresponsiveness (passive suppression), in contrast to active T cell-mediated suppression of an already ongoing mechanism of concomitant immunity, as seen with the meth A fibrosarcoma (2) and P815 mastocytoma (3).

The specificity of active suppression of anti-meth A immunity was not investigated in this study. However, the same type of suppression was recently investigated (27) in this laboratory with two DBA/2 tumors, the P815 mastocytoma and P388 lymphoma. It was shown by reciprocal passive transfer experiments that the T cells that transfer immunity and the T cells that suppress it are specific for the tumor that evokes their generation. This is in keeping with the knowledge that meth A-induced immunosuppression does not cause the host to display a state of generalized immunosuppression, as evidenced by retention of normal capacities to reject a tumor allograft (2) and to generate T cell-mediated immunity to infection with viral and bacterial pathogens (28).

Regarding the type of antitumor immunity generated against the meth A fibrosarcoma, it has been demonstrated repeatedly over a number of years (7) that progressive tumor growth can evoke the generation of a paradoxical, short-lived state of concomitant immunity, as measured by the acquisition and subsequent loss of immunity to the growth of a challenge implant. Even more paradoxical is the demonstration here that this immunity can be passively transferred and expressed against an established tumor in  $\gamma$ -irradiated recipients. However, the paradox becomes more apparent than real when one considers that the donor's 9-d tumor was twice the size of the recipient's 3-d tumor at the time of transfer. It is not unreasonable to suggest, therefore, that the donor would have been able to reject its own tumor if its tumor on day 9 had been as small as the recipient's 4-d tumor.

One might also question the finding that the effector T cells that passively transfer concomitant immunity to the meth A fibrosarcoma display the Ly-1<sup>-</sup>2<sup>+</sup> phenotype of cytolytic T cells on the grounds that recent publications (29) show that Ly-1<sup>+</sup>2<sup>-</sup> T cells passively transfer immunity to tumor allografts at the

exclusion of Ly-1<sup>-</sup>2<sup>+</sup> T cells. This has been interpreted to mean that allografts are not rejected by cytolytic T cells but by a delayed-type hypersensitivity reaction mediated by Ly-1<sup>+</sup>2<sup>-</sup> T cells in the graft. As was pointed out recently (30), however, the interpretation that Ly-1<sup>+</sup>2<sup>-</sup> T cells are the mediators of graft rejection does not take into account the knowledge that the TXB mice which were routinely used as recipients in the experiments are able to contribute their own Ly-2<sup>+</sup> cytolytic T cells to the rejection process (30). Neither does it consider that the immunized donors of allosensitized T cells might possess a state of immunological memory rather than active immunity. It was shown recently (12) that the passive transfer of tumor-sensitized memory T cells does not cause the regression of a target tumor in TXB recipient mice until the recipients generate a cytolytic T cell response of their own. The need for the generation of cytolytic T cells would explain the delay of 1–2 wk after the passive transfer of sensitized T cells before the expression of adoptive antitumor and antiallograft immunity. Presumably, the failure to show that Ly-2<sup>+</sup> T cells are able, on passive transfer, to reject a target graft is the result of the use of memory donors that have lost the sensitized Ly-2<sup>+</sup> cytolytic T cells generated as part of the active immune response. Studies show that Ly-2<sup>+</sup> T cells can passively transfer immunity to allografts (31) and tumor syngrafts (32). It even has been demonstrated that *in vitro* generated, interleukin 2-expanded cytolytic T cells can passively transfer immunity locally (33) and systemically (34) against a tumor syngraft.

The question of cytolytic T cells versus memory T cells as effectors of cell-mediated immunity leads to a comparison of the assays used in the present study to measure immunity and suppression. This is necessary to explain the contradiction of finding peak numbers of immune T cells on day 9 of tumor growth, according to one assay, but at the same time, appreciable numbers of suppressor T cells according to the other. This can be explained by taking into account the fact that the assay for suppression is based on the ability of suppressor T cells from tumor-bearing donors to inhibit, on passive transfer, the expression of passively transferred memory immunity against an established tumor in TXB recipients. Because passively transferred immunity is not expressed in this assay for 6–8 d, any functionally immature suppressor T cells infused 3 h after immune T cells would have plenty of time to mature into functional suppressor T cells. It seems reasonable to suggest, therefore, that suppressor T cells detected by the assay in donor spleens at the height of concomitant immunity on day 9 probably were not functionally mature at that time. Nevertheless, they would need to mature very soon after day 9 to explain the rapid progressive decay of concomitant immunity and loss of immune T cells after this time.

The assay for concomitant immunity was different in that the recipients were given 500 rad of  $\gamma$  radiation 1 h before passive transfer. This is an important difference, because recent results (to be published) show that this dose of irradiation given on days 3–4 of tumor growth did not suppress the concomitant immune response of the recipient. Consequently the regression of an established tumor in  $\gamma$ -irradiated recipients of Ly-1<sup>-</sup>2<sup>+</sup> T cells from tumor-bearing donors is mediated not only by donor T cells but also by the recipient's own T cells. The dose of irradiation does, however, greatly delay the generation of suppressor

T cells. These findings need to be considered in interpreting the results of any adoptive immunization experiment with irradiated recipients.

### Summary

It was shown that the progressive growth of the immunogenic meth A fibrosarcoma in its semisyngeneic host results in the generation of concomitant immunity to the growth of a tumor implant. The generation of immunity occurred between days 6 and 9 of tumor growth and was associated with the generation of sensitized T cells that were capable, on passive transfer, of causing regression of a 3-d tumor in  $\gamma$ -irradiated recipients. After day 9 of tumor growth, concomitant immunity and the T cells able to passively transfer it were progressively lost, and this was associated with the generation of splenic suppressor T cells able to suppress the expression of adoptive immunity against an established tumor in T cell-deficient (TXB) recipients. The T cells that passively transferred concomitant immunity were shown to be of the Ly-1<sup>-</sup>2<sup>+</sup> phenotype, in contrast to the T cells that transferred suppression, which were shown with the same reagents to be Ly-1<sup>+</sup>2<sup>-</sup>. The results are consistent with the hypothesis that the progressive growth of an immunogenic tumor results in the generation of Ly-1<sup>-</sup>2<sup>+</sup>-sensitized effector T cells that fail to reach a number sufficient to destroy the tumor because their generation is down-regulated by tumor-induced Ly-1<sup>+</sup>2<sup>-</sup> suppressor T cells.

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## 1310 CONCOMITANT ANTITUMOR IMMUNITY AND IMMUNOSUPPRESSION

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