IMMUNOLOGICALLY MEDIATED REGRESSION OF A MURINE LYMPHOMA AFTER TREATMENT WITH ANTI-L3T4 ANTIBODY

A Consequence of Removing L3T4$^+$ Suppressor T Cells from a Host Generating Predominantly Lyt-2$^+$ T Cell-Mediated Immunity

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It is known (1, 2) that certain immunogenic tumors evoke the generation of a state of T cell-mediated antitumor immunity in their syngeneic hosts. Nonetheless, these tumors continue to grow progressively to kill the hosts, because host immunity undergoes progressive decay after the tumor grows beyond a certain size. There is evidence that the decay of host concomitant immunity is caused by the negative regulatory influence of tumor-induced suppressor T cells. It has been shown (3, 4), for example, that the progressive decay of concomitant immunity after a certain stage of tumor growth is associated with the progressive acquisition of T cells capable, on passive transfer, of suppressing the expression of adoptive immunity against an established tumor in appropriate test recipients. Causal evidence that these suppressor T cells are responsible for the inadequacy of antitumor immunity was provided by the demonstrations (5, 6) that their preferential destruction by exposing the tumor-bearing host to a sublethal dose of ionizing radiation can result in the generation of an increased level of immunity and in spontaneous tumor regression.

In the case of several of the tumors under study in this laboratory (4, 5), including the L5178Y lymphoma (7), tumor-induced suppressor T cells have been shown to display the CD4$^+$ CD8$^-$ surface phenotype. It might be expected, therefore, that treating tumor-bearing mice with an anti-CD4 mAb capable of depleting the CD4$^+$ T cell subset (8) would result in tumor regression. This expectation could only be realized, however, if the T cell effectors of antitumor immunity did not also belong to the CD4$^+$ T cell subset. In other words, treatment with anti-CD4 mAb would only cause tumor regression if antitumor immunity were expressed exclusively or predominantly by CD8$^+$ T cells.

The purpose of this paper is to show that treatment of mice bearing the L5178Y lymphoma with anti-L3T4 mAb results in the elimination of CD4$^+$ T cells and in complete, immunologically mediated regression of the tumor by CD8$^+$ T cells. The results provide an additional demonstration that removal of CD4$^+$ suppressor T cells can result in complete regression of an established immunogenic tumor.

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Materials and Methods

Mice. Female B6D2F1 (C57BL/6 × DBA/2) adult mice (10–12 wk of age) were obtained from the Trudeau Institute Animal Breeding Facility (Saranac Lake, NY). They were reared under barrier-sustained conditions and were shown to be free of most common viral pathogens according to tests routinely performed by the diagnostic testing services of Charles River Professional Services, Wilmington, MA.

Tumors. The L5178Y lymphoma and P815 mastocytoma, both syngeneic in DBA/2 mice, were passaged in DBA/2 mice as ascites, harvested, cryopreserved over liquid nitrogen, and prepared for implantation as described previously (9). The origin of these tumors has also been described in previous publications (9). For experiments, 10^6 tumor cells in 0.05 ml of PBS were implanted intradermally in the belly region, just below the sternum, and their growth or regression was monitored by measuring changes against time in the mean of two diameters measured at right angles.

Passive Transfer of Immunity. Donor mice were immunized against the P815 mastocytoma or L5178Y lymphoma as described previously (10). Briefly 2×10^6 tumor cells admixed with 100 μg formalin-fixed *Propionibacterium acnes* (supplied by the Trudeau Institute) were implanted intradermally in the belly region. This method of immunization results in 9 d of progressive tumor growth followed by complete tumor regression (10). Donor mice were used either on day 9 of tumor growth at the onset of regression (active immune donors) or 30 d later (memory immune donors), well after the tumor had been rejected (11). Spleens of immunized mice were diced into small pieces, gently pushed through stainless screen into PBS containing 1% FCS, triturated with a pasteur pipette to break up clumps, and filtered through sterile gauze to remove debris. The cells were then washed twice in PBS and resuspended in PBS and injected via a tail vein.

Recipient mice were made T cell deficient (TXB), in order to remove a T cell suppressor barrier to adoptive immunization as described previously (12). Briefly, mice were thymectomized at 4 wk of age and exposed 1 wk later to 900 rad of whole-body γ irradiation generated by a ^{137}Cs irradiator (3M Company, St. Paul, MN) that delivered a mid-phantom dose rate of 30 rad/min. They were infused intravenously with 10^7 syngeneic bone marrow cells 1 h after irradiation and used in experiments no sooner than 4–6 wk later. For convenience, in some experiments the recipients were treated with cyclophosphamide (Cy)[1] (Mead-Johnson, Evansville, IN) to remove the suppressor barrier to adoptive immunity (7, 13). The Cy was dissolved in physiological saline and injected intravenously in a dose of 150 mg/kg. It has been shown that the L5178Y lymphoma is Cy resistant (7).

Preparation of Antibodies. Hybridoma GK1.5 (Dr. Frank Fitch, University of Chicago, Chicago, IL) producing anti-L3T4 mAb, and hybridomas 30-H12 and TIB-210 (American Type Culture Collection, Rockville, MD) producing anti-Thy-1.2 mAb and anti-Ly-2.2 mAb, respectively, were grown as ascites in the pristane-primed peritoneal cavities of sublethally irradiated (500 rad) BALB/c mice. The ascites fluid was harvested in heparinized syringes, transferred to tubes, and centrifuged to remove cells. The content of rat IgG in the cell-free fluid was quantified by radial immunodiffusion. Anti-L3T4 IgG was present at 6 mg/ml, whereas anti-Lyt-2.2 IgG and anti-Thy-1.2 IgG were present at 10 and 10.8 mg/ml, respectively. Antibody-containing ascites fluid was stored at −20°C. To obtain rat anti-Lyt-2 (nonallelic), and mouse anti-I-A^d and anti-H-2D^d mAb, hybridomas TIB-105, HB-3, and HB-102 (American Type Culture Collection), respectively, were grown to 10^6 cell/ml in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 10% FCS, 2 mM glutamine, and antibiotics. The antibody-containing supernatants were separated from cells by centrifugation and stored at −20°C.

Depletion of T Cell Subsets. In vitro depletion of T cell subsets was achieved by incubating 2×10^7 spleen cells/ml with the appropriate mAb (from ascites) at 5 μg/ml in RPMI-FCS for 20 min at 4°C. The cells were then treated for 1 h at 37°C with an equal volume of a 1:10 dilution of rabbit serum as a source of complement.

To achieve in vivo depletion of a T cell subset, mice that were thymectomized at 7 wk of age were infused intravenously 3–5 wk later (while bearing a L5178Y lymphoma) with

[1] *Abbreviation used in this paper:* Cy, Cyclophosphamide.

a single 1-mg dose of the appropriate mAb. The extent of depletion was determined by flow cytofluorometry (FACScan; Becton Dickinson & Co., Sunnyvale, CA) of spleen cells treated with FITC-conjugated anti-Thy-1.2 IgG, anti-Lyt-2.2 IgG, or anti-L3T4 IgG. Conjugation of antibody and FITC (Sigma Chemical Co., St. Louis, MO) was performed according to a method described by Hudson and Hay (14). Spleen cells from antibody-treated or untreated mice were stained by incubating them at 10^7/ml with the appropriate FITC-conjugated mAb at a concentration of 15 μg/ml in PBS containing 1% BSA and 0.02% sodium azide (PBS-BSA-Azide), for 40 min at 4°C. The cells were then washed twice with sheath buffer (Clay Adams, Parsippany, NJ), and subjected to flow cytofluorometric analysis. The results were recorded as $\log_{10}$ fluorescence against the number of cells stained.

To determine the surface phenotype of the L5178Y lymphoma, tumor cells from a peritoneal ascites were stained directly with FITC-conjugated anti-Thy-1.2 IgG or anti-L3T4 IgG as described for spleen cells above. To test for the presence of Lyt-2.1, the cells were incubated with anti-Lyt-2 (hybridoma TIB-105) for 40 min at 4°C, washed, and then incubated with FITC-conjugated goat anti-rat IgG (Cappel Laboratories, Cochranville, PA) for 40 min at 4°C. They were also examined for surface I-A and H-2D by incubating them with anti-I-A^d mAb or mouse anti-H-$2D^d$ mAb, respectively, for 40 min at 4°C, and then with FITC-conjugated goat anti-mouse IgG Ab (Cappel Laboratories) for 40 min at 4°C. The mAb supernatants were used at a dilution of 1:5, and the FITC-conjugated second antibodies were used at 20 μg/ml. The cells were then washed twice in sheath buffer and subjected to flow cytofluorometric analysis.

Results

Depletion of L3T4$^+$ T Cells with Anti-L3T4 mAb Results in Spontaneous Tumor Regression. Previous studies with the L5178Y lymphoma (5) and other tumors (7) showed that progressive tumor growth results in the generation of L3T4$^+$ suppressor T cells capable of suppressing antitumor immunity in an adoptive immunity assay. They showed, in addition, that the preferential elimination of these suppressor cells by exposing the host to sublethal ionizing radiation can result in spontaneous tumor regression (5, 6). Moreover, because pilot studies had shown (see later), in the case of the L5178Y lymphoma, that host immunity is mediated and expressed predominantly by Lyt-2$^+$ T cells, it was anticipated that in vivo removal of all L3T4$^+$ T cells from mice bearing this tumor with anti-L3T4 mAb should result in the removal of suppressor T cells and consequently in the generation of a large enough number of tumor-sensitized Lyt-2$^+$ T cells to cause complete tumor regression.

Fig. 1 shows the results of an experiment that tested the effect on the L5178Y lymphoma growing in thymectomized mice of an intravenous infusion of 1 mg of anti-L3T4 mAb, 1 mg of anti-Lyt-2 mAb, or 1 mg of both antibodies, on day 9 of tumor growth. It can be seen that treatment with anti-L3T4 mAb resulted in complete tumor regression and in long-term (>90 d) host survival. In contrast, treatment with anti-Lyt-2 mAb, or with both mAbs, resulted in a striking enhancement of tumor growth and in greatly decreased host survival time.

That treatment with mAbs eliminated the appropriate T cell subset is evidenced by the results of flow cytofluorometric analysis of the spleens of treated mice as shown in Fig. 2. At the time of onset of tumor regression, >98% of L3T4$^+$ T cells and >98% of Lyt-2$^+$ T cells were removed by treatment with anti-L3T4 mAb and anti-Lyt-2 mAb, respectively.

Because the L5178Y tumor is a T cell lymphoma, it is possible that the therapeutic effect of anti-L3T4 mAb was caused by direct destruction of the tumor by the mAb. However, a flow cytofluorometric examination of appropriately stained L5178Y cells

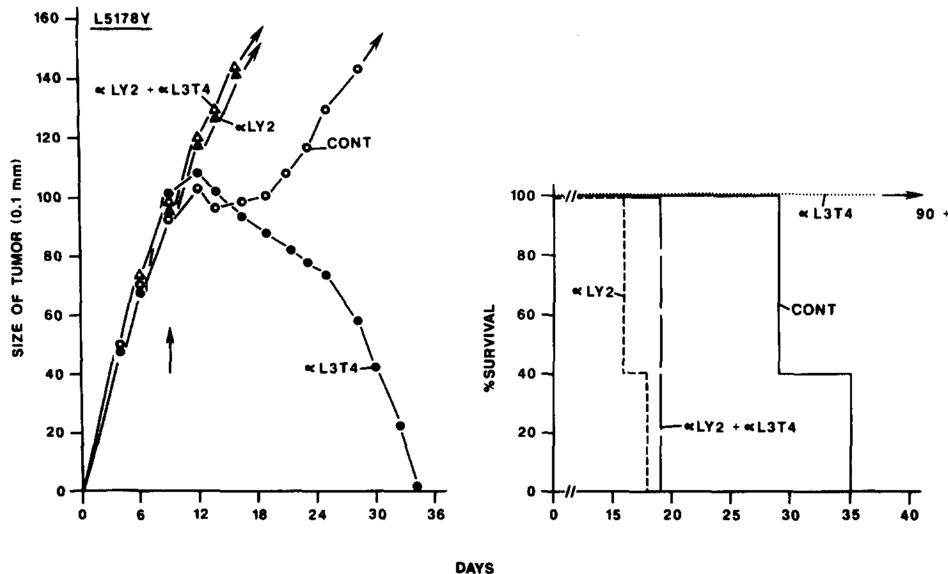

FIGURE 1. An intravenous injection (*arrow*) of 1 mg of rat anti-L3T4 mAb (αL3T4) into B6D2 mice bearing a day 9 L5178Y lymphoma results in complete tumor regression (*left*), and in long-term host survival (*right*). In contrast, intravenous injection of 1 mg anti-Lyt-2.2 mAb (αLy2), or both antibodies (αLy2 + αL3T4) resulted in a striking enhancement of tumor growth (*left*) and in a significant reduction in host survival time (*right*). Means of five mice per group.

(Fig. 3) showed that L5178Y lymphoma cells are Thy-1.2$^+$, H-2D^d, L3T4$^-$, Lyt-2$^-$, and I-A^{d-}. This evidence, plus that presented below, leaves no doubt that anti-L3T4 mAb has no direct effect on L5178Y cells in vivo.

Anti-L3T4 mAb Is not Therapeutic Against the L5178Y Lymphoma Growing in an Irradiated Host. If, as indicated by the foregoing results, the therapeutic action of anti-L3T4 mAb is mediated indirectly by an augmented level of host antitumor immunity, mAb treatment should not be therapeutic against the L5178Y lymphoma growing in a host whose immune system has been ablated by whole-body ionizing radiation. Fig. 4 shows that a 9-d L5178Y lymphoma growing in mice exposed to 900 rad of γ radiation 1 d before tumor implantion failed to undergo regression in response to treatment with anti-L3T4 mAb on day 9. There can be no doubt, therefore, that anti-L3T4 mAb does not directly affect growth of the L5178Y lymphoma.

Anti-L3T4 mAb-induced Regression Is Associated with Presence of Lyt-2$^+$ T Cells Capable of Passively Transferring Antitumor Immunity. The foregoing results show that anti-L3T4 mAb treatment of mice bearing a well-established L5178Y lymphoma results in complete tumor regression, whereas treatment with anti-Lyt-2 mAb results in a striking enhancement of tumor growth. It was logical to anticipate, therefore, that tumor regression would prove to be associated with the possession by the host of tumor-sensitized Lyt-2$^+$ T cells. This was investigated by testing the ability of T cells from mice in the process of causing regression of their L5178Y tumors in response to anti-L3T4 mAb to cause, on passive transfer, regression of a L5178Y tumor in appropriate recipient mice. The experiment involved giving anti-L3T4 mAb on day 9

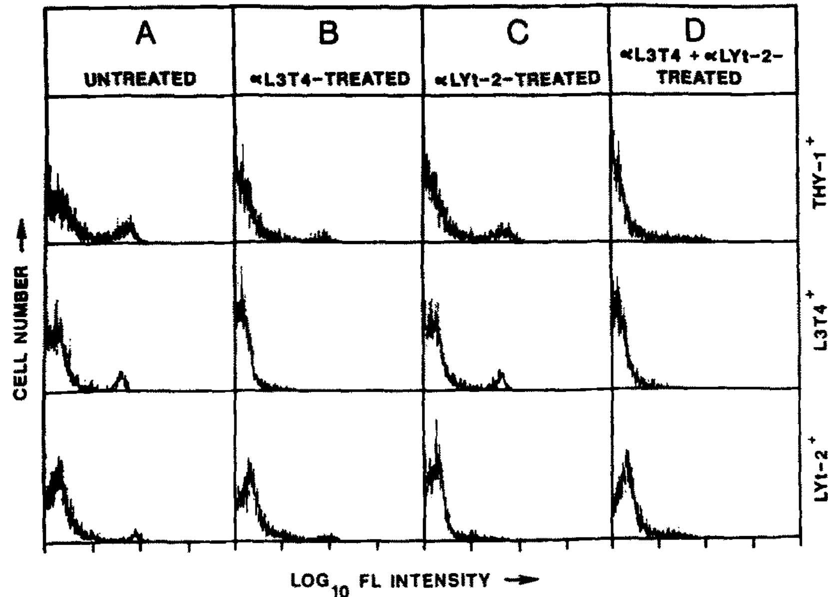

FIGURE 2. Evidence that treating thymectomized tumor bearers with 1 mg anti-L3T4 mAb resulted in depletion of L3T4⁺ T cells, and that treatment with 1 mg of anti-Lyt-2 mAb resulted in depletion of Lyt-2⁺ T cells. Shown are the results of a flow cytofluorometric examination of spleen cells from untreated tumor bearers (*A*), anti-L3T4-treated tumor bearers (*B*), and Lyt-2-treated tumor bearers (*C*), and anti-L3T4- and anti-Lyt-2-treated tumor bearers (*D*) stained with FITC-conjugated anti-Thy-1.2 IgG, anti-L3T4 IgG, or anti-Lyt-2.2 IgG. Before antibody treatment the spleen cell population contained 14–15% L3T4⁺ T cells and 8–9% Lyt-2⁺ T cells, but contained <0.5% L3T4⁺ T cells and Lyt-2⁺ T cells after treatment with the appropriate antibody.

of tumor growth and harvesting spleen cells for passive transfer 5 d later. The antitumor action of these spleen cells was compared with that of spleen cells harvested from control donors bearing a 14-d progressive tumor. Recipient mice were given a 150 mg/kg dose of Cy 1 h before receiving spleen cells, in order to remove a sup-

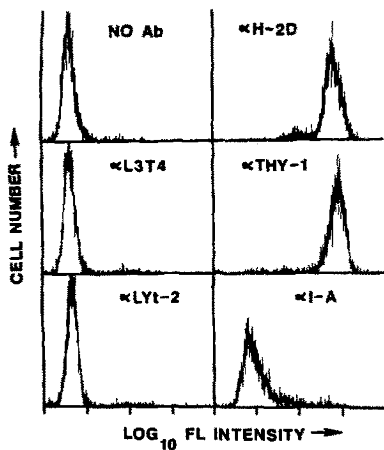

FIGURE 3. Results of a flow cytofluorometric examination of the surface phenotype of cells of the L5178Y lymphoma grown as a peritoneal ascites and stained with the indicated FITC-conjugated antibodies. It can be seen that the surface of L5178Y cells were H-2Dd⁺, Thy-1.2⁺, L3T4⁻, Lyt-2.1⁻, I-A^{d-}. The very small number of L3T4⁺ cells detected almost certainly were host peritoneal lymphocytes.

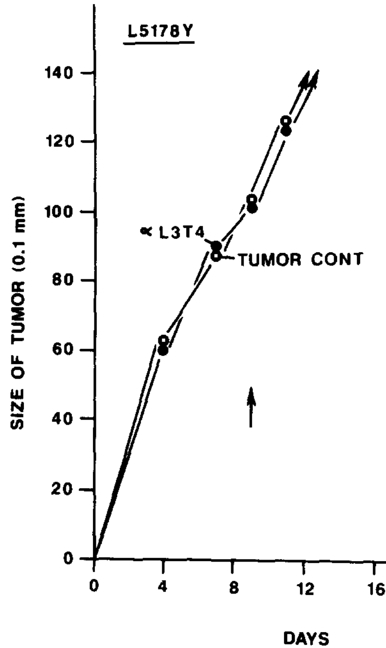

FIGURE 4. Anti-L3T4 mAb had no direct in vivo effect on the L5178Y lymphoma. An intravenous injection (*arrow*) of 1 mg of mAb failed to cause regression of the tumor growing in lethally irradiated (900 rad) mice. Means of five mice per group.

pressor T cell barrier to adoptive immunotherapy (7, 13). It was possible to use Cy treated, instead of TXB recipients, because the L5178Y is known to be a Cy-resistant tumor (7).

Fig. 5 shows that, whereas spleen cells from anti-L3T4 mAb-treated donors caused

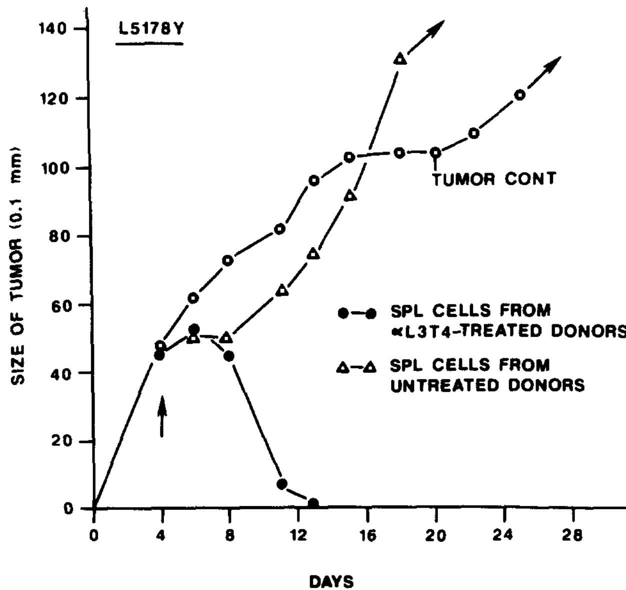

FIGURE 5. Spleen cells (one organ equivalent) from day 14 tumor bearers treated with 1 mg anti-L3T4 mAb 5 d earlier (on day 9 of tumor growth) were capable, on passive transfer (*arrow*), of causing complete regression of a 4-d tumor in recipients. In contrast, one organ equivalent of spleen cells from untreated day 14 tumor bearers caused only a temporary reduction in tumor growth. In these experiments the recipients were treated with a 150 mg/kg dose of Cy 1 h before receiving donor cells to remove a T cell barrier to adoptive immunization. Means of five mice per group.

complete regression of the recipient tumor, spleen from control donors had very little antitumor effect. As was expected, the spleen cells from anti-L3T4 mAb-treated donors that caused regression of the recipient tumor were Lyt-2^+ T cells, as evidenced by their functional elimination by treatment with anti-Lyt-2.2 mAb and complement, but not with anti-L3T4 mAb and complement (Fig. 6).

Anti-L3T4 mAb Treatment Is not Therapeutic Against the P815 Mastocytoma. It was considered important to determine next whether the above results with the L5178Y lymphoma could be obtained with another immunogenic tumor. The therapeutic action of anti-L3T4 mAb treatment against an established P815 mastocytoma was therefore tested. It was found (Fig. 7) that injecting 1 mg of anti-L3T4 mAb into mice bearing a 9-d P815 mastocytoma had no effect on tumor growth or on host survival time. A flow cytofluorometric analysis of spleen cells from these mice showed, in agreement with results obtained wtih spleen cells from mice bearing the L5178Y lymphoma, that the mAb removed >98% of the T cell subset against which it was directed (results not shown).

L3T4$^+$ T Cells Are Involved in Immunity to the P815 Mastocytoma, but not the L5178Y Lymphoma. The results show that the P815 mastocytoma, in contrast to the L5178Y lymphoma, is not responsive to therapy with anti-L3T4 mAb. The possibility that needed to be tested, therefore, was that immunity to the P815 mastocytoma, but not the L5178Y lymphoma, relies to a considerable degree on the participation of L3T4$^+$ T cells. This involved determining whether mice responding to immunization with the P815 mastocytoma, but not the L5178Y lymphoma, acquire tumor-sensitized L3T4$^+$ T cells, as well as tumor-sensitized Lyt-2^+ T cells capable of passively transferring immunity. In these experiments mice were immunized by intradermal implantation of an admixture of 2×10^6 living tumor cells and 100 μg of formalin-fixed *P. acnes*. It is known (10) that implanting this admixture results in 9–10 d of progressive tumor growth followed by complete tumor regression and in the acquisi-

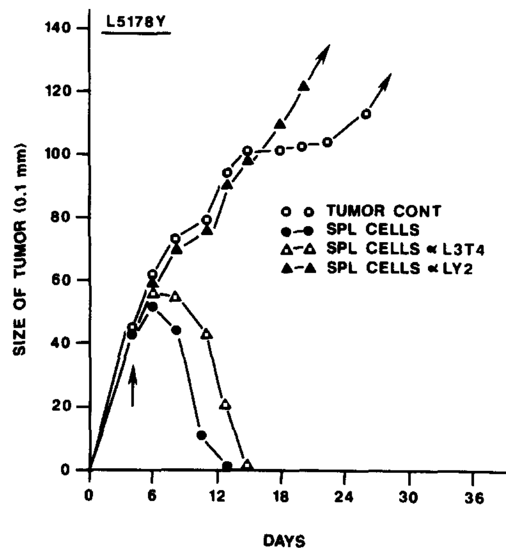

FIGURE 6. Evidence that the antitumor activity of spleen cells from anti-L3T4 mAb-treated tumor bearers resides in the Lyt-2^+ T cell subset. The ability of an infusion (*arrow*) of one organ equivalent of spleen cells harvested from anti-L3T4 mAb-treated mice during tumor regression to cause regression of a recipient tumor was eliminated by treating the spleen cells with anti-Lyt-2 mAb and C′, but not with anti-L3T4 mAb and C′. Means of five mice per group.

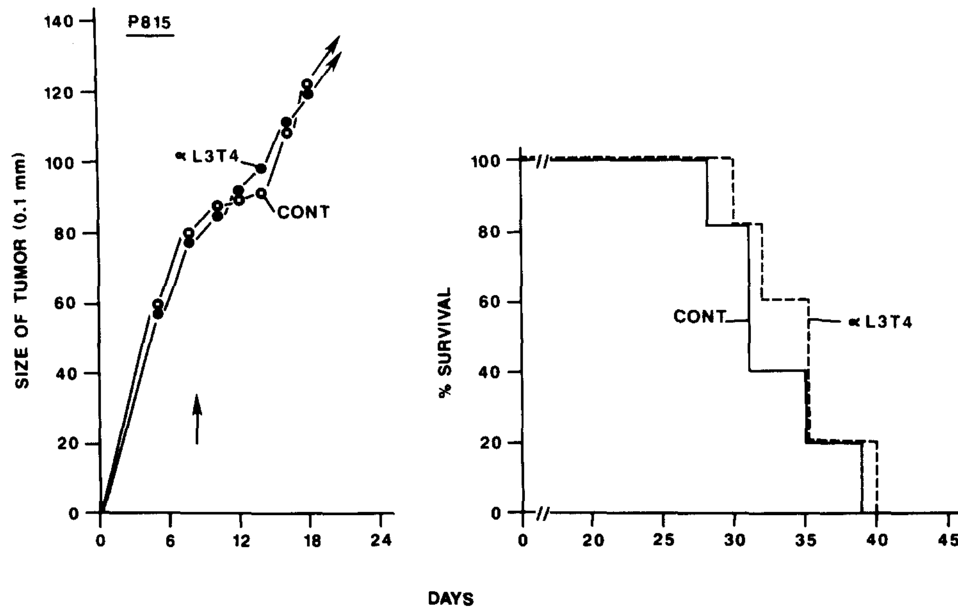

FIGURE 7. The P815 mastocytoma failed to undergo regression in response to treatment with anti-L3T4 mAb. Treatment of mice bearing a day 9 P815 mastocytoma with 1 mg of anti-L3T4 mAb (*arrow*) had no effect on tumor growth (*left*), or on host survival time (*right*). Means of five mice per group.

tion of immunity to a tumor implant. It is also known (9, 11) that the onset of tumor regression is temporally associated with the presence in the host of augmented numbers of activated immune T cells capable of passively transferring antitumor immunity, and that activated T cells are subsequently replaced by memory T cells capable of passively transferring immunity (11).

It can be seen in Fig. 8 that the ability of spleen cells from mice generating a therapeutic level of active immunity (immediately preceding tumor regression on day 9) to the P815 mastocytoma to cause regression of a 3-4-mm diam P815 tumor in appropriate recipients was eliminated by treating the cells with anti-L3T4 mAb and complement, or with anti-Lyt-2 mAb and complement. Fig. 8 also shows that when anti-L3T4 mAb-treated and anti-Lyt-2 mAb-treated spleen cells were added together and infused into tumor-bearing recipients, complete tumor regression ensued. Therefore, in mice generating active immunity to the P815 mastocytoma, T cells of both phenotypes are involved in immunity. Memory immunity to the P815 mastocytoma apparently was different, in that the T cells from memory-immune donors capable of causing regression of the recipient tumor were functionally eliminated by treatment with anti-L3T4 mAb and complement, but not by anti-Lyt-2 mAb and complement (Fig. 9). It will be noted, with regard to the difference between active and memory immune T cells that, whereas active immune T cells caused tumor regression to commence shortly after cell transfer, memory T cells caused tumor regression only after a delay of 6-8 d. This is in keeping with previously published results (11).

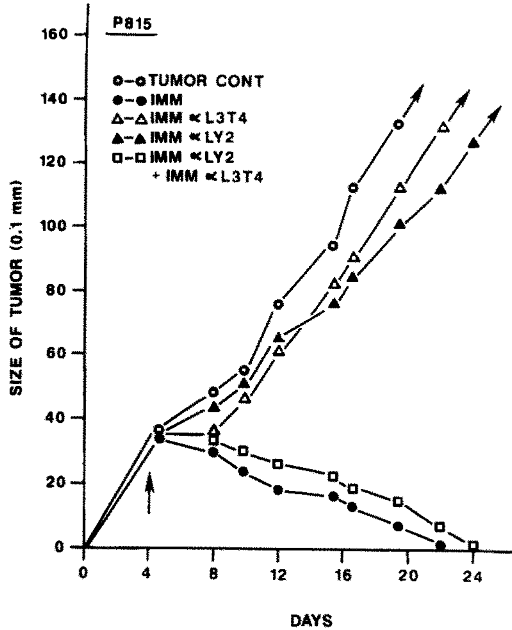

FIGURE 8. Active immunity, acquired by mice in the process of causing regression of their P815 mastocytoma in response to the adjuvant action of intralesional *P. acnes* is mediated by both T cell subsets. The ability of 1.5 organ equivalents (2×10^8) of spleen cells (IMM) from these mice to cause, on passive transfer (*arrow*), regression of a 4-d tumor in γ-irradiated recipients was eliminated by treatment with anti-L3T4 mAb and C′ (IMMαL3T4), or with anti-Lyt-2 mAb and C′ (IMMαLy2). Passive transfer of cells remaining after anti-Lyt-2 mAb treatment plus those remaining after anti-L3T4 mAb treatment (IMMαLy2 + IMMαL3T4) resulted in complete tumor regression. Means of five mice per mouse.

Figs. 10 and 11 show that *P. acnes*-augmented immunity to the L5178Y lymphoma was different from that generated against the P815 mastocytoma, in that it was passively transferred exclusively by Lyt-2^+ T cells in all cases tested. Thus, the ability of spleen cells harvested on day 9 of growth of a *P. acnes*-containing L5178Y lymphoma (just before the onset of tumor regression), as well as of spleen cells harvested

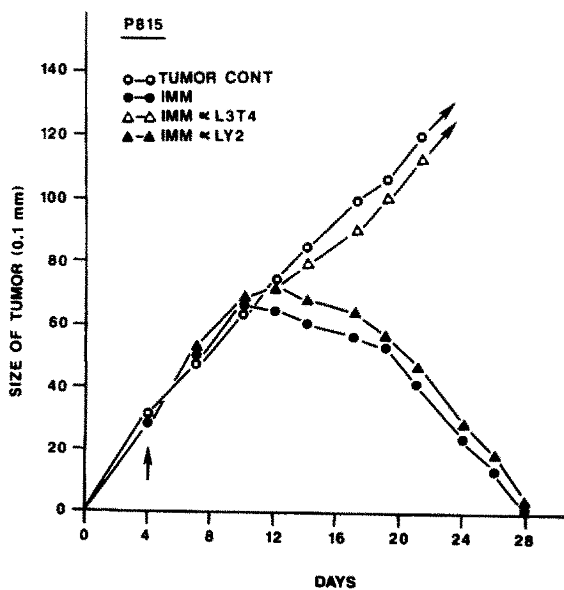

FIGURE 9. Evidence that regression of the P815 mastocytoma by spleen cells from memory-immune (30 d after *P. acnes*-induced tumor regression) donors is mediated mainly by L3T4$^+$ T cells. The capacity of 1.5 spleen equivalents (1.8×10^8) of cells from these donors to cause complete regression of a recipient tumor was eliminated by treatment with anti-L3T4 mAb, but not with anti-Lyt-2 mAb or C′. In this experiment the recipients were T cell-deficient (TXB) mice bearing a 4-d tumor. It should be noted that tumor regression did not commence until after a 6-d delay. Means of five mice per group.

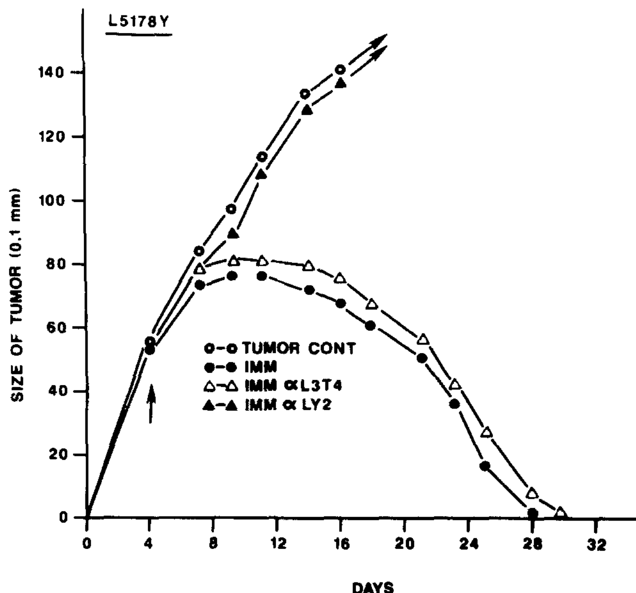

FIGURE 10. Active immunity, acquired by mice in the process of causing regression of their L5178Y lymphoma in response to intralesional *P. acnes*, could be passively transferred solely by Lyt-2$^+$ t cells. The ability of one organ equivalent (2×10^8) of spleen cells (IMM) from these mice to cause regression of a 4-d tumor in γ-irradiated recipient was eliminated by treating the cells with anti-Lyt-2 mAb (IMMαLy2), but not with anti-L3T4 mAb and C′ (IMMαL3T4). Means of five mice per group.

>30 d later (memory immunity), to cause regression of an L5178Y tumor in recipient mice was totally eliminated by treating the cells with anti-Lyt-2 mAb and complement, but not by treating them with anti-L3T4 mAb and complement (Figs. 10 and 11).

Discussion

This study confirms the results of previous investigations (2) showing that mice bearing certain immunogenic tumors generate suppressor T cells capable of down-regulating the antitumor immune response, and that preferential removal of these suppressor T cells can result in spontaneous tumor regression (5, 6). Previous studies (5, 6) showed that suppressor T cells are of the L3T4 phenotype and that they can be preferentially eliminated by subjecting the host to a sublethal dose of whole body γ radiation. The present study represents a more convincing example of the therapeutic consequences of selectively eliminating L3T4$^+$ suppressor T cells from a tumor-bearing host. It shows that intravenous injection of a single 1-mg dose of anti-L3T4 mAb on day 9 of growth of the L5178Y lymphoma (when the tumor was 8 mm in diameter) resulted, after a 2–3-d delay, in complete tumor regression and in long-term host survival. It shows, in addition, that tumor regression was not caused by the direct effect of anti-L3T4 mAb on the tumor, in that the L5178Y lymphoma provied to be L3T4$^-$, and tumor regression failed to occur in lethally irradiated tumor bearers treated with anti-L3T4 mAb. It is apparent, therefore, that anti-L3T4 mAb–induced tumor regression was immunologically mediated. Moreover, because the antibody removed practically all L3T4$^+$ T cells, without reducing the number of Lyt-2$^+$ T cells, it follows that immunity must have been exclusively mediated by Lyt-2$^+$ T cells.

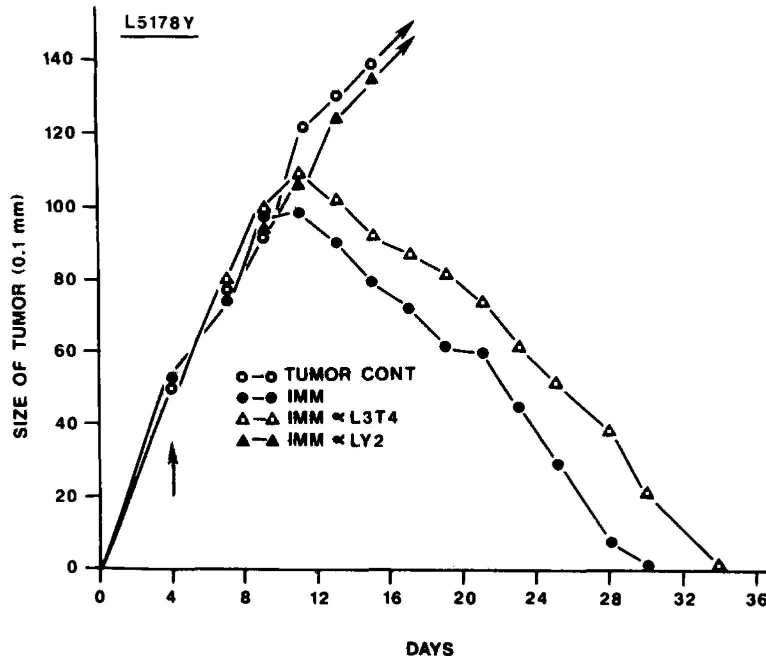

FIGURE 11. Memory immunity generated against the L5178Y lymphoma and possessed by mice 30 d after complete regression of their tumor in response to intralesional *P. acnes* is mediated exclusively by Lyt-2+ T cells. The ability of 1.5 organ equivalents (1.8 × 10^8) of spleen cells from these mice (IMM) to cause complete regression of a 4-d tumor in T cell-deficient recipient mice was eliminated by treatment with anti-Lyt-2 mAb (IMMαLy2), but not with anti-L3T4 mAb and C (IMMαL3T4). Means of five mice per group.

In fact, in contrast to findings published from other laboratories (15–19) showing that regression of certain other tumors in adoptively immunized recipients is achieved predominantly by L3T4+ T cells, regression of the L5178Y lymphoma was shown here to be mediated exclusively by Lyt-2+ T cells, regardless of the procedure used to evoke immunity. Thus, active immunity possessed by a host in the process of causing regression of its L5178Y lymphoma in response to the adjuvant action of intralesional *P. acnes*, as well as memory immunity possessed by a host long after the completion of regression, was passively transferred with T cells that were susceptible to treatment with anti-Lyt-2 mAb and complement, but resistant to treatment with anti-L3T4 mAb and complement.

This was not the case for immunity to the P815 mastocytoma, however, which failed to undergo regression after treatment with anti-L3T4 mAb. An investigation of the T cells that mediate *P. acnes*-augmented active immunity to this tumor, and of the T cells that carry immunologic memory, revealed that L3T4+ T cells, as well as with Lyt-2+ T cells, needed to be infused in order for immunity to be passively transferred. In this regard immunity to the P815 mastocytoma is similar to that generated against other tumors (15–19). This does not mean, however, that the L5178Y lymphoma is unique in terms of the immunity it evokes, although it may prove representative of only a small proportion of immunogenic tumors. Even if this proved

to be the case, it would not take away from the importance of the L5178Y lymphoma model for showing that immunogenic tumors can evoke the generation of L3T4$^+$ suppressor T cells, on the one hand, and that removal of these suppressor T cells can result in spontaneous tumor regression on the other.

To this point in time, in vivo treatment with anti-L3T4 mAb apparently has not been used to eliminate suppressor T cells. Instead, it has been used to remove L3T4$^+$ effector T cells with the aim of preventing the development of spontaneous (20, 21) or experimentally induced (22) autoimmune diseases, and of preventing the rejection of tissue and organ allografts (23, 24). A likely exception to this use is seen with murine leishmaniasis, where it has been demonstrated (25, 26) that treatment of highly susceptible BALB/c mice with anti-L3T4 mAb enables these mice to resolve infection with *Leishmania major*. Because there is evidence (27) that BALB/c mice are susceptible to leishmaniasis, and because they generate Lyt-1$^+$2$^-$ suppressor T cells in response to infection, it seems highly likely that the therapeutic effect of anti-L3T4 mAb is based on its ability to remove L3T4$^+$ suppressor T cells. According to the interpretation given above, this would allow Lyt-2$^+$ T cells to expand in number and express immunity.

Regardless of whether this proves to be the reason for susceptibility to leishmaniasis, it can be stated in conclusion that the evidence presented here makes it difficult to ignore a possible role for suppressor T cells in determining the level of immunity generated in response to certain tumors and other replicating antigens.

Summary

This study shows that intravenous injection of 1 mg of anti-L3T4 mAb (GK1.5) into thymectomized mice bearing the syngeneic L5178Y lymphoma results, after a delay of 2–3 d, in complete regression of this tumor and in long-term host survival. A flow cytofluorometric examination of the spleen cells of mAb-treated mice revealed that antibody treatment resulted in the elimination of >98% of L3T4$^+$ T cells, but had no effect on the Lyt-2$^+$ T cells subset. Tumor regression was immunologically mediated, because L5178Y lymphoma cells were shown to be L3T4$^-$, and regression of the tumor failed to occur in mice that had been lethally irradiated before anti-L3T4 mAb was given. Tumor regression was mediated by tumor-sensitized Lyt-2$^+$ T cells, as evidenced by the finding that treatment of tumor-bearing mice with anti-Lyt-2 mAb alone, or in combination with anti-L3T4 mAb, resulted in enhancement of tumor growth and a significant decrease in host survival time. Moreover, the spleens of mice whose tumors were undergoing regression in response to anti-L3T4 mAb treatment contained Lyt-2$^+$ T cells capable, on passive transfer, of causing regression of a tumor in recipient mice. These results can be interpreted as showing that removal of tumor-induced L3T4$^+$ suppressor T cells results in the release of Lyt-2$^+$ effector T cells from suppression, and consequently in the generation of enough Lyt-2$^+$ T cell–mediated immunity to cause tumor regression. This can only be achieved, however, if immunity to the tumor is mediated exclusively by Lyt-2$^+$ T cells, as is the case for the L5178Y lymphoma. In the case of the P815 mastocytoma, treatment with anti-L3T4 mAb was without a therapeutic effect, and this was in keeping with the finding that immunity to this tumor is mediated by L3T4$^+$, as well by Lyt-2$^+$ T cells.

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