

EXPRESSION OF PASSIVELY TRANSFERRED IMMUNITY
AGAINST AN ESTABLISHED TUMOR DEPENDS ON
GENERATION OF CYTOLYTIC T CELLS IN RECIPIENT

Inhibition by Suppressor T Cells*

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Recent publications from this laboratory (1, 2) have shown that it is possible to cause the regression of established immunogenic murine tumors by the passive transfer of tumor-sensitized T cells from immunized donors, provided the tumor-bearing recipients have been made T cell-deficient by thymectomy and irradiation. The need for T cell-deficient tumor-bearing recipients to demonstrate successful adoptive immunotherapy suggested the existence in normal tumor bearers of a T cell-dependent mechanism that prevents intravenously infused sensitized T cells from expressing their antitumor function. Evidence that this obstacle to adoptive immunotherapy is a tumor-induced, T cell-mediated mechanism of immunosuppression was supplied by the demonstration (3, 4) that the passive transfer of splenic T cells from normal tumor bearers prevents passively transferred tumor-sensitized T cells from causing tumors to regress in T cell-deficient recipients. It was hypothesized, on the basis of this and other evidence (5) that the growth of an immunogenic tumor results in the generation of a state of T cell-mediated immunosuppression that functions to "down-regulate" a preceding concomitant immune response. Hence, the explanation for the paradoxical growth of immunogenic tumors in their immunocompetent hosts, and the reason why these tumors develop refractoriness to active and adoptive immunotherapy.

In designing experiments to investigate the mode of action of suppressor T cells, two aspects of this model of adoptive immunotherapy need to be considered. The first is that the sensitized T cells routinely used to passively transfer anti-tumor immunity are obtained from donor mice that are immunized by causing their tumor to regress 2-3 wk earlier by intralesional therapy with *Corynebacterium parvum*. The sensitized T cells are harvested, therefore, after the cytolytic T cell response to the immunizing tumor has decayed (6) and when the donors possess a state of immunological memory. Thus, the sensitized T cells infused intravenously have no detectable cytolytic activity of their own. The second important aspect of the model is that the passive transfer of tumor-sensitized T cells does not result in an immediate onset of tumor regression in T cell-deficient recipients. Instead, there is invariably a 6-8-d delay before regression commences. It can be postulated, therefore, that the intravenously infused sensitized T cells do not possess the capacity themselves to immediately destroy the tumor, but

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impart to the tumor-bearing recipient the ability to generate a new population of effector T cells in response to tumor antigens over a 6–8-d period.

This paper will show that the passive transfer of noncytolytic, tumor-sensitized T cells does not cause the regression of tumors growing in T cell-deficient recipients until an adequate number of cytolytic T cells are generated in the recipients' draining lymph nodes. It will show, in addition, that a cytolytic T cell response of much lower magnitude is generated in normal tumor-bearing recipients of immune T cells, and that a similar low magnitude response is generated in T cell-deficient recipients of immune T cells that are also infused with suppressor T cells from tumor-bearing donors. The results indicate that suppressor T cells inhibit adoptive immunotherapy by inhibiting the production of cytolytic T cells in the tumor-bearing recipient.

Materials and Methods

Mice. B6D2F₁ (C57BL/6 × DBA/2) mice, 9–14 wk old, were supplied by the Trudeau Institute Animal Breeding Facility. The mice were free of known viral pathogens according to the results of routine serological screening performed by the Animal Diagnostic Testing Service of Microbiological Associates, Walkersville, MD.

Tumors. The P815 mastocytoma, syngeneic in DBA/2 mice, was originally obtained from Dr. Virginia Evans, Tissue Culture Section, National Cancer Institute. The P815 tumor is passaged weekly as an intraperitoneal ascites, and a new passage is initiated every 3 mo from tumor stocks that are cryopreserved over liquid nitrogen in RPMI 1640 (Gibco Laboratories, Grand Island, NY) containing 20% fetal bovine serum (FBS)¹ (Sterile Systems, Logan, UT) and 10% dimethylsulfoxide (Mallinckrodt, Inc., St. Louis, MD). The L5178Y and P388 lymphomas syngeneic in DBA/2 mice were obtained from Dr. E. F. Wheelock, Thomas Jefferson University, Philadelphia, PA and the EL-4 thymoma, syngeneic in C57BL/6 mice, was obtained from Dr. Virginia Evans. These tumors were passaged as an ascites and cryopreserved in a similar manner to the P815 tumor. All of the tumors were free of known viral pathogens according to the results of serological screening performed by Microbiological Associates.

For tumor implantation, ascites tumor cells were harvested in phosphate-buffered saline (PBS) containing 10 U of heparin/ml, washed, and resuspended to an appropriate number in PBS. Intrafootpad tumors were initiated by implanting 10⁶ tumor cells in a volume of 0.05 ml PBS in the right hind footpad. Tumor growth was monitored by measuring changes in the dorsoventral thickness of the footpad with dial calipers.

Adoptive Immunization. Mice used as immune donors were immunized against the P815 tumor by injecting them intradermally with 2 × 10⁶ P815 tumor cells admixed with 100 μg of *Corynebacterium parvum* (Burroughs Wellcome, Greenville, NC). It is known that this immunization protocol results in a 9-d period of tumor growth followed by complete and permanent tumor regression by 3 wk in 80–90% of mice. Donor mice were used 3 wk after immunization. For cell transfer, spleens of immunized mice or of tumor-bearing mice were diced into small pieces and passed through a 60-mesh stainless steel screen using cold PBS containing 0.5% syngeneic mouse serum. The resulting cell suspension was washed and filtered through sterile surgical gauze to remove debris and cell clumps. The cells were infused intravenously in a volume of 1 ml.

Recipient mice were made T cell-deficient (TXB) by thymectomy at 5 wk of age, followed 7 d later by exposure to 900 rads of whole-body gamma irradiation from a ¹³⁷Cs source. They received an intravenous infusion of 10⁷ syngeneic bone marrow cells immediately after irradiation and were used in experiments after a further 4 wk.

⁵¹Cr-release Assay. The details of the assay system have been described previously (6). P815 tumor cells to be used as target cells in cytotoxicity assays were grown in RPMI 1640 containing 15% heat-inactivated horse serum (HS), 100 μg/ml streptomycin, and 100 U/ml penicillin.

¹ Abbreviations used in this paper: C', complement; FBS, fetal bovine serum; HS, horse serum; MOPS, mopholinopropane sulfonic acid; PBS, phosphate-buffered saline; TXB, T cell-deficient.

Unless indicated, these and other tissue culture reagents were purchased from Gibco Laboratories. Tumor cells were harvested during log phase growth, and 10^6 cells were labeled in 0.4 ml of the medium containing 100 μ Ci of $\text{Na}_2^{51}\text{CrO}_4$ (CJS.11; Amersham Corp., Arlington Heights, IL). The L5178Y and P388 lymphomas, and the EL-4 thymoma were grown in vitro and labeled with ^{51}Cr in the same manner as the P815 tumor.

Effector cells from the draining lymph nodes of adoptively immunized and control tumor-bearing recipients were harvested by pressing finely diced pieces of lymph nodes through a stainless steel screen using Hanks' balanced salt solution containing 1% FBS, antibiotics, and 10 mM morpholinopropane sulfonic acid (MOPS) (Sigma Chemical Co., St. Louis, MO). The lymph node cells were then centrifuged and resuspended to an appropriate concentration in assay medium that consisted of RPMI 1640 supplemented with 10% HS, antibiotics, and MOPS. The assay was performed in triplicate with plates containing 96 round-bottomed wells (Flow Laboratories, Inc., McLean, VA). Except where indicated, each well contained 5×10^5 effector cells and 10^4 ^{51}Cr -labeled target cells in a total volume of 200 μ l of medium. After a 6-h incubation at 37°C in an atmosphere of 7% CO_2 in air, 50 μ l of supernatant was removed from each well and counted in a Rack Gamma II gamma counter (LKB Instruments, Inc., Gaithersburg, MD). Total ^{51}Cr -release was the amount of ^{51}Cr released from the target cells by treatment with 0.5% Triton-X. Total release was >97%, and spontaneous release ranged from 8 to 12% of the total release. The percent specific ^{51}Cr -release was calculated as follows: $[(\text{experimental cpm} - \text{spontaneous cpm}) / (\text{total cpm} - \text{spontaneous cpm})] \times 100$.

Antibody Treatment. Hybridomas secreting monoclonal anti-Thy-1.2 (30-H12), anti-Lyt-1 (53-7.313), and anti-Lyt-2 (53-6.72) antibody (7) were obtained from the Salk Institute, La Jolla, CA. The cells were grown to 5×10^5 cells/ml in RPMI 1640 supplemented with 10% FBS and antibiotics. The cultures were subjected to centrifugation and the supernatants were collected and stored at -20°C until required. Rabbit sera used as a source of complement (C') were obtained from rabbits bred at the Trudeau Institute. The rabbits were selected on the basis of minimum toxicity of their sera for mouse leukocytes. Mouse anti-rat IgG serum was raised by injecting mice with 100 μ g of rat IgG (Cappell Laboratories, Cochranville, PA) in Freund's complete adjuvant. The mice were given two additional injections of rat IgG in 0.5 ml of Freund's incomplete adjuvant, intraperitoneally, and two injections of the antigen intravenously in PBS. Serum was collected 6 d later, heat inactivated, and stored at -20°C until required. The media used for antibody treatments consisted of RPMI 1640 supplemented with 1% FBS, antibiotics, and MOPS.

For T cell depletion, lymph node cells (4×10^7 /ml) were incubated at 4°C for 40 min in a 1:5 dilution of the anti-Thy-1.2 culture supernatant. An equal volume of a 1:5 dilution of rabbit C' was then added, and the cells were incubated at 37°C for a further 60 min. They were then washed, counted, and resuspended to the appropriate concentration for use in the ^{51}Cr -release assay. Preliminary studies showed that treatment with anti-Thy-1.2 + C' killed >96% of thymocytes and decreased the ability of normal spleen cells to respond to concanavalin A or phytohemagglutinin by >99%. For depletion of Ly T cell subsets, lymph node cells (2×10^7 /ml) were incubated at 4°C for 40 min in a 1:5 dilution of the anti-Ly-1 or the anti-Ly-2 culture supernatants. The preparation was then centrifuged and the cells were resuspended at 4×10^7 /ml in a 1:50 dilution of mouse anti-rat IgG serum and incubated at 4°C for a further 40 min. The cells were then treated with rabbit serum as above.

Winn Assay. Lymph node or spleen cells to be tested for their ability to inhibit tumor growth in vivo (8) were admixed at 4°C either at a 10:1 or a 3:1 ratio with 5×10^6 P815 tumor cells. Immediately thereafter, the admixtures were injected in a volume of 0.05 ml into a hind footpad of mice that had been irradiated (700 rad) earlier the same day. Tumor growth was measured with dial calipers.

T Cell Enrichment. Lymphoid cell suspensions were enriched for T cells using anti-mouse IgG-coated plates by a previously described procedure (6).

Results

Onset of Tumor Regression is Preceded by Cytolytic T Cell Production in Recipients. A previous study showed (2) that intravenous infusion of P815-sensitized T cells causes

P815 tumors to regress in T cell-deficient mice, but not in normal mice. It also showed that tumor regression does not commence in TXB recipients until after a 6–8-d delay. It was necessary to determine, therefore, whether the delay before tumor regression commences in T cell-deficient recipients represents the time needed for the recipients to generate an adequate number of cytolytic T cells of their own, and whether failure of tumors to regress in normal recipients is associated with an inadequate production of cytolytic T cells.

It can be seen in Fig. 1 that, in agreement with previous studies (1, 2), intravenous infusion of 2×10^8 spleen cells from tumor-immune donors caused tumors to regress in T cell-deficient, but not in normal recipients. It can be seen, in addition, that tumor regression in T cell-deficient recipients did not commence until cytolytic T cells were generated in the lymph node draining the tumor. The cytolytic response did not begin until 3 d after the passive transfer, and peaked 4 d later at the time of onset of tumor regression. The cytolytic response then underwent rapid decline. In contrast, and as would be expected, tumor-bearing T cell-deficient mice made no detectable cytolytic T cell response. Fig. 1 also shows that the failure of tumors to regress in T cell-deficient recipients infused with normal spleen cells was associated with a cytolytic T cell response of much lower magnitude. Finally, it can be seen that passively transferred immune spleen cells gave no greater cytolytic response in normal tumor-bearing

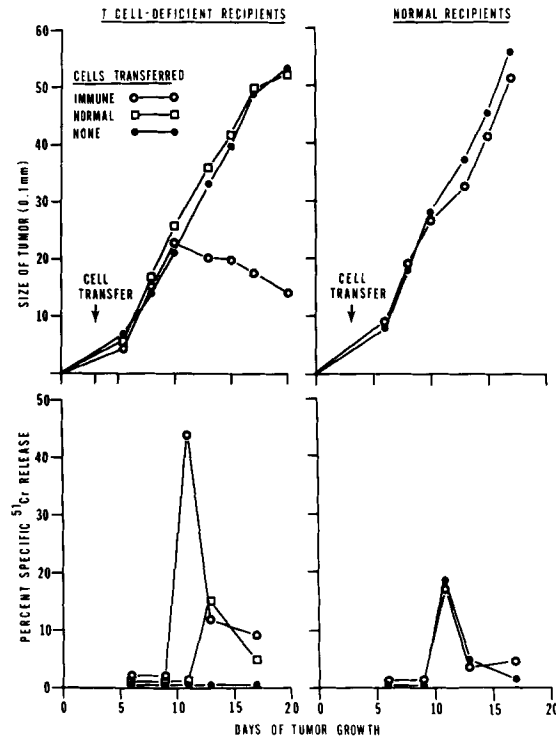


FIG. 1. Evidence that the onset of tumor regression in T cell-deficient recipients of 2×10^8 spleen cells from immune donors is preceded by the production in the recipients' draining lymph node of T cells cytolytic for P815 tumor cells in vitro. Failure of TXB recipients of normal spleen cells and of normal recipients of immune cells to destroy their tumors was associated with a cytolytic response of much lower magnitude.

recipients than that generated by normal mice in response to progressive tumor growth. It is apparent, therefore, that an established tumor does not commence to regress in an adoptively immunized recipient until a large enough number of cytolytic effector T cells are generated: a number that can be generated in T cell-deficient recipients, but not in normal recipients of sensitized T cells.

The apparent need for the generation of an adequate number of cytolytic T cells to cause tumor regression in TXB recipients is illustrated by the results of an additional experiment that tested the effect of infusing graded numbers of sensitized T cells on cytolytic T cell production and tumor growth. Fig. 2 shows that increasing the number of sensitized T cells infused resulted in corresponding increases in the magnitude of the recipients' cytolytic T cell response and the extent to which the tumors regressed. It is apparent from Fig. 2 that passive transfer of $>10^8$ immune spleen cells into tumor-bearing TXB mice is necessary to generate a cytolytic response of sufficient magnitude to cause complete tumor regression in a majority of recipient mice.

Cytolytic Cells are Tumor-specific Ly-2⁺ T Cells. Some of the properties of cytolytic T cells generated at peak response in T cell-deficient tumor-bearing recipients of tumor-sensitized T cells are shown in Fig. 3. It can be seen that the cytolytic cells were T

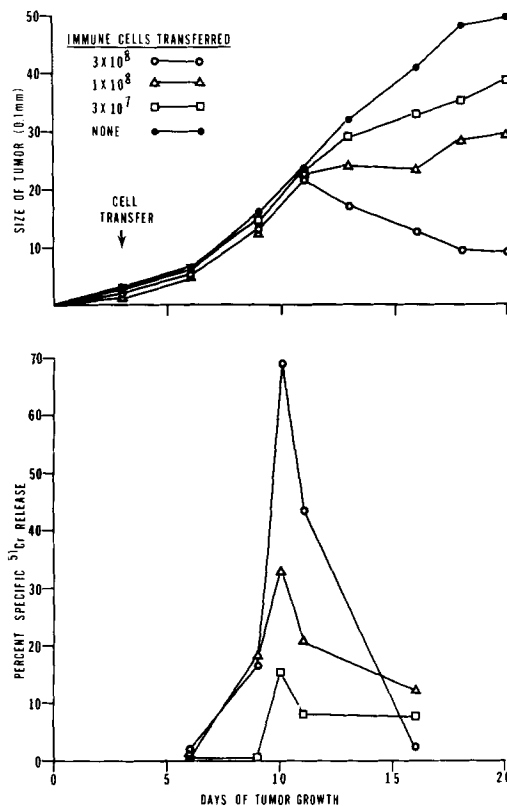


FIG. 2. Effect on tumor regression and the cytolytic T cell response of infusing different numbers of immune spleen cells into tumor-bearing TXB recipients. Increasing the number of immune spleen cells infused resulted in an increase in the magnitude of the cytolytic response and the degree to which tumors regressed.

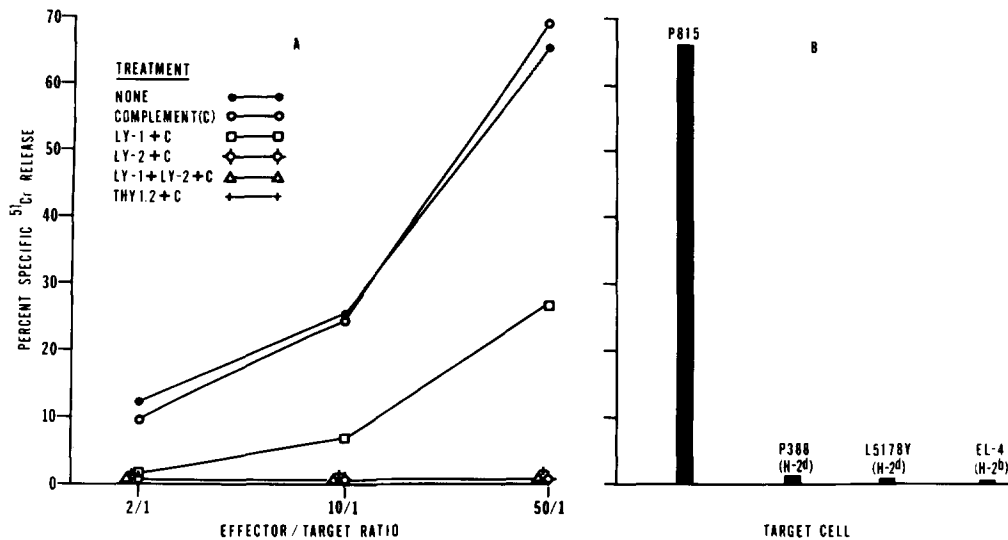


FIG. 3. Properties of cytolytic cells harvested at peak response from the draining lymph node of tumor-bearing TXB recipients of immune T cells. Cytolytic activity was totally eliminated by treating the lymph node cells with anti-Thy-1.2 antibody and C', or with anti-Ly-2-antibody and C'. (A) Cytolytic function was partially eliminated with anti-Ly-1 antibody and C' treatment. (B) T cell cytolytic activity was directed at P815 targets, but not against two other DBA/2 tumors, the P388 and L5178Y lymphomas, or a C57BL/6 tumor, the EL-4 lymphoma.

cells as evidenced by the finding that their ability to lyse ⁵¹Cr-labeled targets was completely ablated by incubation with monoclonal anti-Thy-1.2 antibody and C'. Their cytolytic activity also was totally eliminated by treatment with monoclonal anti-Ly-2 antibody and C' and was partially eliminated with monoclonal anti-Ly-1 and C'. Therefore, all of the cytolytic T cells display the Ly-2 antigen and a proportion of them also display enough Ly-1 antigen to make them susceptible to complement mediated lysis by the particular anti-Ly-1 monoclonal antibody used.

The specificity of the cytolytic T cells for the P815 mastocytoma is shown in Fig. 3 where it can be seen that they were not capable of lysing two other DBA/2 tumors, the L5178Y and P388 lymphomas, or a C57BL/6 tumor, the EL-4 thymoma. These three tumors are known to release ⁵¹Cr after lysis by appropriate effector T cells.

To verify that the ability of cytolytic T cells to cause ⁵¹Cr release from P815 tumor targets in vitro was a measure of their capacity to prevent tumor growth in vivo, cytolytic T cells were harvested at peak response from adoptively immunized TXB recipients and tested in a Winn assay (8) in secondary recipients. It can be seen in Fig. 4 that cytolytic T cells harvested from the popliteal lymph nodes of tumor-bearing T cell-deficient recipients that had been infused 8 d earlier with tumor-immune spleen cells completely prevented the growth of P815 tumor cells in the footpads of secondary recipients at a 10:1 or a 3:1 effector cell to tumor cell ratio. As was found in the ⁵¹Cr-release assay, the ability of cytolytic T cells to prevent P815 tumor growth in the Winn assay was abolished by treatment with anti-Ly-2 antibody and C', and was partially eliminated by treatment with anti-Ly-1 antibody and C'. In contrast to the results obtained with cytolytic T cells generated as part of a secondary response in tumor-bearing recipients, the spleen cells from tumor-immune donors used to adoptively immunize these recipients possessed no capacity to prevent the growth of tumor

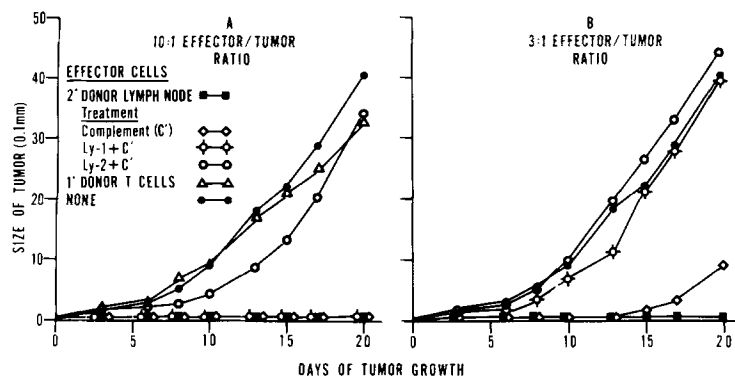


FIG. 4. Demonstration that lymph node cells harvested at peak cytolytic response and at the time of onset of tumor regression in adoptively immunized tumor-bearing TXB recipients were capable of inhibiting tumor growth in vivo. When 5×10^6 P815 cells were admixed with (A) 5×10^6 or (B) 1.67×10^6 lymph node cells and the admixture injected into the left hind footpad of irradiated recipients, the tumor cells failed to grow. The ability of lymph node cells to inhibit tumor growth was abolished by treating them with anti-Ly-2 antibody and C' treatment (A, B), and was partially abolished by treatment with anti-Ly-1 antibody and complement treatment (B). Spleen cells of the primary donor used to adoptively immunize TXB recipients did not inhibit tumor growth, even though they were enriched for T cells by incubation in anti-mouse IgG coated plates (A).

cells according to the Winn assay, even though the spleen cells were enriched for T cells by panning on anti-mouse IgG-coated plates (Fig. 4).

Inhibition of Adoptive Cytolytic T Cell Production by Suppressor Cells from Tumor-bearing Donors. The foregoing results serve to confirm previous findings (1, 2) that it is possible to cause tumors to regress by the passive transfer of tumor-sensitized T cells, but only if the tumors are growing in T cell-deficient recipients. More importantly, they serve to indicate the reason why passively transferred sensitized T cells cause tumors to regress in T cell-deficient, but not in normal recipients. They show that it is only in the former that intravenously infused immune T cells are able to give rise to the production of an adequate number of cytolytic effector T cells. On the basis of results of previous studies (3, 4), which showed normal tumor bearers possess T cells that can suppress the expression of adoptive immunity in TXB recipients, it was postulated that failure of passively transferred immune T cells to cause tumor regression and give rise to the generation of an adequate number of cytolytic T cells in normal tumor-bearing recipients is due to the presence in these recipients of suppressor T cells. This was investigated by determining whether spleen cells from donors with established P815 tumors are capable, on passive transfer, of inhibiting the generation of cytolytic T cells in T cell-deficient tumor-bearing recipients of immune spleen cells.

It can be seen in Fig. 5, in agreement with the foregoing results, that intravenous infusion of T cell-deficient tumor-bearers with immune spleen cells resulted, after a delay, in the generation of cytolytic T cells in the draining node and progressive tumor regression. It can be seen in addition, however, that if the recipients of immune spleen cells were infused 1 h later with spleen cells from donors with 15-d progressive tumors, a cytolytic T cell response of much lower magnitude was generated, and the tumors underwent only partial regression and then regrew. In contrast, an infusion of normal spleen cells had no such inhibitory effect on cytolytic T cell production or tumor regression.

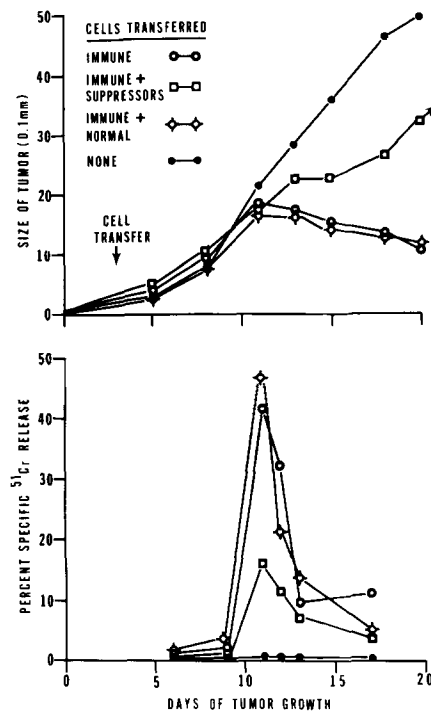


FIG. 5. Evidence that suppressor T cells from mice with progressive P815 tumors inhibit adoptive immunotherapy of an established tumor in TXB recipients by suppressing an adoptive cytolytic response in the recipients. Tumor regression failed to occur in TXB recipients of immune T cells if the recipients were also infused with suppressor T cells (A) and this was associated with a greatly reduced production of cytolytic T cells (B) in the draining lymph node. Infusion of normal T cells, instead of suppressor T cells, had no inhibitory effect on tumor regression or cytolytic T cell production.

Discussion

The results of this study show that the expression of passively transferred, T cell-mediated immunity against established P815 tumors growing in TXB recipient mice does not begin until a secondary cytolytic T cell response is generated in the recipients. It is apparent, therefore, that the 6–8 d delay that invariably occurs before tumor regression commences represents the time needed for the intravenously infused, tumor-sensitized T cells to give rise to the production of effector or mediator T cells. This is not surprising, in view of the fact that the tumor-sensitized T cells routinely used for adoptive immunity have no cytolytic activity of their own. They are harvested from immunized donors well after the production of cytolytic T cells to immunizing tumor antigens has decayed, and when the donors possess a state of immunological memory (6). It should be pointed out, moreover, that the donors themselves need to generate a secondary cytolytic T cell response before they can reject a challenge implant of the immunizing tumor (C. D. Mills and R. J. North, manuscript in preparation). Therefore, the sensitized T cells infused intravenously were memory or helper T cells.

There are three main findings that justify postulating that cytolytic T cells are required for tumor regression. The first is that tumor regression was preceded, in every case, by peak production of cytolytic T cells in the draining node. Second, the larger

the number of cytolytic T cells generated in recipients at peak response, the greater was the extent of tumor regression in the recipients. Third, tumor regression failed to occur unless cytolytic T cell production exceeded a certain minimal level. These findings, together with the knowledge that the T cells generated in the recipient's lymph nodes, in contrast to donor T cells, lysed P815 tumor targets specifically *in vitro* and were capable of neutralizing the growth of a P815 implant *in vivo*, represent strong circumstantial evidence that cytolytic T cells are involved in the rejection process. The additional finding that the cytolytic T cells are of the Ly-2⁺ phenotype, but that some of them also display Ly-1 antigen, is consistent with evidence published by others about cytolytic T cells in general (9–12) and with the knowledge (13) that most T cells express the Ly-1 antigen to some degree.

Even so, evidence has been published by others (14) that has been interpreted as showing that cellular mechanisms other than, or in addition to, cytolytic T cells may be responsible for allograft or tumor rejection. It has been demonstrated, for example, that the rejection of skin or tumor allografts in TXB recipient mice can be achieved by the passive transfer of Ly-1⁺ helper T cells, to the exclusion of Ly-2⁺ T cells that were eliminated by treatment with anti-Ly-2 antibody and C'. On the basis of the view that Ly-1⁺2⁻ T cells cannot change into Ly-2⁺ T cells (10, 15), coupled with the belief that the TXB recipients are essentially devoid of Ly-2⁺ cytolytic T cell precursors, this evidence was interpreted (16–18) as meaning that allosensitized, noncytolytic Ly-1⁺ T cells are capable by themselves of mediating graft rejection, presumably by mediating a delayed-type hypersensitivity reaction in the graft (14). Again, there is published evidence showing (19–21) that the rejection of virus- or carcinogen-induced tumors in sublethally irradiated syngeneic rats can be achieved by infusing helper T cells, rather than cytolytic T cells, generated *in vitro* by primed spleen cells in response to mitomycin C-treated tumor stimulator cells. The authors of these papers have considered the possibility that other cells besides helper T cells may be responsible for the ultimate destruction of tumor. In support of the importance of helper T cells is a more recent publication (22), which shows that the rejection of skin allografts in TXB rats can be achieved by the infusion of W3/25-positive helper T cells that are depleted of cytolytic T cell precursors by treatment with MRC-OX-8 monoclonal antibody and C'. All of these studies of the passive transfer of T cell-mediated immunity could be interpreted as indicating, therefore, that helper T cells, rather than cytolytic T cells, are responsible for mediating graft rejection. If so, then, the functional significance of the results presented in this paper is doubtful.

It would seem premature at this stage, however, to discount the role of cytolytic T cells in allograft or tumor rejection on the basis of the type of evidence that has been published thus far. The main reason for suggesting caution is that TXB animals that are routinely used as recipients of sensitized helper T cells as a means of avoiding a contribution of cytolytic T cells by the recipient, may not be suitable for this purpose. On the contrary, it is known that TXB animals are well endowed with cytolytic T cell precursors that can give rise to functional cytolytic T cells under appropriate stimulation. It has been shown that spleen cells from TXB mice (23) and even nude mice (24, 25) can generate appreciable numbers of cytolytic T cells *in vitro* and *in vivo*, provided interleukin 2 is made available. It is surely significant, moreover, that Ly-1⁺ helper T cells (26) produce interleukin 2 when stimulated with specific antigen: a situation that would exist in a tumor- or allograft-bearing TXB recipient infused with

specifically sensitized Ly-1⁺ helper T cells. Therefore, before cytolytic T cells are discounted as being involved in graft rejection, it would seem imperative to show that cytolytic T cells are not being generated from cytolytic precursors of the TXB recipient at the time the graft is being rejected, because it is only at this time that one would expect effector T cells to be produced (6, 27–29). It is surely relevant, in this connection, that in one study (22) of adoptive anti-allograft immunity with helper T cells, it was revealed that an appreciable number of lymphocytes displaying the phenotype of cytolytic T cells were present in the graft at the time of its rejection in TXB recipients. It should also be kept in mind that a striking feature common to all of the published studies of adoptive immunity discussed above, including our own, is the long delay, sometimes of >2 wk, before graft rejection commences. This surely means that the passively transferred helper T cells had no immediate capacity of their own to initiate graft rejection, but needed an appreciable amount of time, either to acquire this function themselves, or to recruit recipient cells into the response. The long delay before graft rejection commences is certainly not in keeping with what is known about passively transferred delayed-type hypersensitivity, the expression of which can be evoked routinely by injecting specific antigen immediately after the passive transfer of sensitized T cells. It would be difficult to explain, therefore, why Ly-1⁺ T cells with the capacity to initiate delayed-type hypersensitivity reactions immediately after passive transfer take so long to express this function in a target graft. T cell depletion studies using anti-Ly-1 and anti-Ly-2 monoclonal antibody plus C' currently are in progress in this laboratory. The results obtained thus far (Mills, C. D., and R. J. North, manuscript in preparation) indicate that the T cells that can adoptively immunize against an established P815 tumor (memory T cells) are partly eliminated by treatment with either antibody, and are almost totally eliminated by treatment with both. Experiments designed to determine whether the T cells that remain after treatment with anti-Ly-2 antibody and complement can cause by themselves the regression of P81 tumors in T cell-deficient recipients if used in sufficient numbers, are being planned. However, the published knowledge (13) that the efficiency of elimination of Ly T cell subsets by complement-mediated lysis is far from absolute, indicates that a knowledge of the number of Ly-2⁺ T cells that remain after treatment will be essential for proper interpretation of the results.

Another feature that the examples of adoptive immunity discussed above have in common is that they are all performed with mice or rats made T cell deficient by thymectomy and irradiation, and restored with bone marrow cells (TXB recipients), or with rats immunodepressed by sublethal whole-body irradiation. The reason for using TXB recipients for adoptively immunizing against allografts is obviously because normal recipients cannot serve as negative controls in such experiments, in that they vigorously reject allografts without the need for an infusion of sensitized donor T cells. In the case of tumor syngrafts, however, the situation is quite different. An immunogenic tumor not only grows progressively to kill its immunocompetent syngeneic or semisyngeneic host, but the passive transfer of tumor-sensitized T cells fails to alter this situation. On the other hand, the passive transfer of tumor-sensitized T cells causes complete and permanent regression of tumors growing in TXB recipients. The reason for the refractoriness of tumors growing in normal mice to adoptive immunotherapy with tumor-sensitized T cells was revealed in previous publications from this laboratory which showed (3, 4) that normal tumor bearers

acquire a tumor-induced state of T cell-mediated immunosuppression. It was demonstrated, for example, that splenic T cells from immunocompetent mice bearing established tumors are capable, on passive transfer, of preventing intravenously infused tumor-sensitized T cells from causing the regression of established tumors growing in TXB recipients. The experimental results presented in this paper serve to explain how suppressor T cells inhibit the expression of adoptive immunity. They show that passive transfer of tumor-immune T cells into normal tumor-bearing recipients results in a cytolytic T cell response of much lower magnitude than the cytolytic T cell response generated in TXB tumor-bearing recipients. More to the point, they show that an infusion of suppressor spleen cells from tumor-bearing normal mice causes a severe depression of the adoptive cytolytic T cell response generated in TXB tumor-bearing recipients of immune T cells. This evidence is consistent with the interpretation that suppressor T cells function in this model to inhibit the generation of cytolytic T cells from cytolytic T cell precursors. This could result either from a direct inhibitory action of suppressor cells on the replication of cytolytic precursors or from an inhibition of the function of helper T cells. The interpretation that suppressor T cells inhibit the generation rather than the function of cytolytic T cells is in keeping with the recent demonstration that tumor-induced suppressor T cells can inhibit the production of tumor-sensitized cytolytic T cells *in vitro* (30).

Summary

The results of this study with the P815 mastocytoma confirm the results of previous studies that showed that the passive transfer of tumor-sensitized T cells from immunized donors can cause the regression of tumors growing in T cell-deficient (TXB) recipients, but not in normal recipients. The key additional finding was that the expression of adoptive immunity against tumors growing in TXB recipients is immediately preceded by a substantial production of cytolytic T cells in the recipients' draining lymph node. On the other hand, failure of adoptive immunity to be expressed against tumors growing in normal recipients was associated with a cytolytic T cell response of much lower magnitude, and a similar low magnitude response was generated in TXB recipients infused with normal spleen cells and in tumor-bearing control mice. Because the passively transferred sensitized T cells possessed no cytolytic activity of their own, the results indicate that the 6–8-d delay before adoptive immunity is expressed represents the time needed for passively transferred helper or memory T cells to give rise to a cytolytic T cell response of sufficient magnitude to destroy the recipient's tumor. In support of this interpretation was the additional finding that inhibition of the expression of adoptive immunity by the passive transfer of suppressor T cells from tumor-bearing donors was associated with a substantially reduced cytolytic T cell response in the recipient's draining lymph node. The results serve to illustrate that interpretation of the results of adoptive immunization experiments requires a knowledge of the events that take place in the adoptively immunized recipient. They support the interpretation that suppressor T cells function in this model to "down-regulate" the production of cytolytic effector T cells.

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