T CELL SUPPRESSORS OF ANTITUMOR IMMUNITY The Production of Ly-1⁻,2⁺ Suppressors of Delayed Sensitivity Precedes the Production of Suppressors of Protective Immunity

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A central problem in tumor immunology has been to explain how immunogenic tumors escape destruction by the immune defenses of their immunocompetent hosts. The results of studies performed during the past few years in this laboratory (reviewed in 1) support the hypothesis that certain tumors with tumorspecific, transplantation antigens avoid destruction by the immune response of their hosts by evoking the production of a population of suppressor T cells that functions to downregulate the immune response before enough effector T cells are generated to cause tumor regression. These published studies (2, 3) show that growth of the immunogenic tumors under investigation evokes the generation of an underlying state of concomitant immunity, which develops progressively between days 6 and 9 of tumor growth, and which is mediated by Ly-2⁺ effector T cells. After day 9, however, there is progressive loss of effector T cells, and this is temporarily associated with the progressive acquisition of Ly- 1^+ , 2^- suppressor T cells that are functionally defined by their ability to inhibit, on passive transfer, the expression of adoptive immunity against an established tumor in immunodepressed test recipients. It was postulated, on the basis of these and other results (1), that the loss of effector T cells results from negative regulation by tumor-induced suppressor T cells.

However, other investigators have described the generation of a different type of suppressor T cell in mice bearing progressive immunogenic tumors (reviewed in 4). This second type of suppressor T cell displays the $Ly1^-,2^+$, $I-J^+$ membrane phenotype, is generated at a very early stage of tumor growth, and is defined by its ability to suppress, on passive transfer, a delayed-type hypersensitivity (DTH)¹ reaction to tumor antigens in tumor-immune recipients. Because these suppressors of DTH are generated progressively during the first 6–7 d of tumor growth (5, 6), but are then progressively lost (6), it seems unlikely that they would exert a meaningful suppressor function in those situations where a tumor evokes the generation of a state of concomitant antitumor immunity that does not peak until day 9 of tumor growth, and does not undergo decay until after days 9–12.

The purpose of the study reported here, therefore, was to determine whether both types of suppressor T cells are generated in response to growth of a

This work was supported by grants CA-16642 and CA-27794 from the National Cancer Institute and a grant from R. J. Reynolds Industries, Inc. Dr. DiGiacomo's current address is the Pathology Department, Monaldi Hospital, Via Leonardo Bianchi, 80131 Naples, Italy.

¹ Abbreviation used in this paper: DTH, delayed-type sensitivity.

J. EXP. MED. © The Rockefeller University Press · 0022-1007/86/10/1179/14 \$1.00 1179 Volume 164 October 1986 1179-1192

1180 T CELL SUPPRESSORS OF ANTITUMOR IMMUNITY

chemically induced tumor that can evoke the generation of concomitant immunity. It will show that $Ly-1^-,2^+$ T cells that can suppress a tumor-specific DTH reaction, and $Ly-1^+,2^-$ suppressor T cells that can suppress the expression of adoptive immunity, are generated in sequence in response to progressive growth of the immunogenic BALB/c Meth A fibrosarcoma. It will also show that the T cell suppressors that can suppress DTH fail to suppress the expression of adoptive immunity, and conversely, that T cells that suppress adoptive immunity fail to suppress DTH. It will be argued that because T cells that can suppress adoptive immunity are generated during the progressive decay of a concomitant immune response, it is they, rather than the earlier generated T cell suppressors of DTH, that serve best to explain the downregulation of concomitant immunity and failure of this form of immunity to cause tumor regression.

Materials and Methods

Mice. Specific pathogen-free CB6F1 (BALB/c \times C57BL/6) and BALB/c female mice were supplied by the Trudeau Institute Animal Breeding Facility. The mice were free of viral pathogens, as evidenced by the results of routine serological screening performed by the Diagnostic Testing Service of Microbiological Associates, Bethesda, MD.

Tumor. The methylcholanthrene-induced Meth A fibrosarcoma syngeneic in BALB/c mice was used in this study. The tumor, grown as ascites in the peritoneal cavities of syngeneic hosts, was harvested in heparinized (5 U/ml) PBS, the cells were pooled, washed in PBS, resuspended in Fisher's medium (Grand Island Biological Co., Grand Island, NY) containing 20% FCS and 10% DMSO, and were cryopreserved in small volumes over liquid nitrogen to serve as stock tumor. For each experiment a vial was thawed, the cells were washed in PBS and 2×10^6 of them used to initiate peritoneal ascites tumors in semisyngeneic F1 hybrid mice. After 6 d of growth, the tumor cells were harvested in heparinized PBS, washed, and resuspended in PBS. In all experiments, tumors were initiated by injecting 10^6 tumor cells in a volume of 50 μ l of PBS.

Generation and Passive Transfer of DTH and Its Suppression. To induce a state of DTH, mice were immunized by intradermal injection of an admixture of 3×10^6 Meth A cells and 50 µg of Propionibacterium acnes (formal in-killed Corynebacterium parvum from Burroughs Wellcome Co., Research Triangle Park, NC). This is known to result in a tumor that grows for 8–9 d then undergoes regression, leaving the animals immune to growth of a subsequent implant of tumor cells (7) and capable of mounting a DTH reaction to intrafootpad injection of Meth A cells (see later section). For passive transfer of DTH, the spleens of immunized mice were diced into small pieces, and the pieces were gently pushed through a 50-mesh stainless steel screen into PBS containing 1% FCS. The resulting cell suspension was triturated with a pasteur pipette to break up clumps, and passed through six layers of sterile surgical gauze to remove cell debris. The cells were washed twice in PBS, suspended in PBS containing 1% syngeneic mouse serum, and 1 ml of the cell suspension containing 1.5 spleen equivalents (about 1.5×10^8 cells) was infused into appropriate recipients via a lateral vein.

Mice bearing a progressive Meth A tumor were used as donors of suppressor cells. Spleen cells were harvested from these mice at different times after initiating the tumor intradermally in the belly region with 10^6 Meth A cells in 50 μ l of PBS. To test for the presence of suppressor T cells, 1.5 organ equivalents ($\sim 1.5 \times 10^8$) of spleen cells from tumor-bearing donors were infused into recipients that had been infused 1 h earlier with 1.5 organ equivalents ($\sim 1.5 \times 10^8$) of sensitized spleen cells from immunized donors. An eliciting intrafootpad injection of 2×10^6 mitomycin c-treated Meth A cells in 50 μ l of PBS was then given, and DTH was monitored over the next 48 h by measuring increases in footpad thickness with dial calipers (Schnelltaster H.C. Droplin, Hessen, Federal Republic of Germany).

Passive Transfer of Immunity and Its Suppression. The assay for determining the

presence of tumor-induced suppressor T cells that suppress protective immunity has been described elsewhere (2, 3). It involves showing that splenic T cells from tumor-bearing donors can, on passive transfer, inhibit the ability of passively transferred immune T cells to cause regression of a palpable 4-d tumor in T cell-deficient recipients. Briefly, donors of immune T cells were immunized by injecting them with an admixture of 3×10^6 living tumor cells and *C. parvum* as described for DTH above. T cell-deficient, tumor-bearing test recipients received one organ equivalent $(1.5-2.0 \times 10^8)$ of immune spleen cells, followed 3 h later by one organ equivalent $(1.5-2.0 \times 10^8)$ of suppressor spleen cells from mice bearing a progressive tumor. The test recipients were made T cell-deficient at 3 wk of age by thymectomy followed 1 wk later by lethal (950 rad) γ -irradiation. They were infused with 10^7 syngeneic bone marrow cells immediately after irradiation, and used in experiments no sooner than 4 wk later.

Antibody Treatment. A hybridoma secreting anti-Ly-1.2 mAb (CP30) was a gift from Dr. Jan Klein, Max Planck Institute, Tubingen, Federal Republic of Germany. Anti-Ly-2.2 mAb was generated by a hybridoma (TIB-150) obtained from American Type Culture Collection (Rockville, MD). Anti-L3T4a mAb (8) produced by hybridoma GK-1.5 was obtained from Dr. M. J. Bevan, Scripps Clinic, La Jolla, CA. All hybridomas were grown to 5×10^5 cells/ml in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) containing 10% FCS and antibiotics. The cultures were centrifuged to pellet the cells and debris, and the supernatants were dispensed in small volumes and stored at -20° C until needed. We used rabbit serum as a source of complement. It was obtained from rabbits bred at the Trudeau Institute and selected on the basis of minimal cytotoxicity of their serums for mouse thymocytes. For deletion of T cell subsets, spleen cells were incubated at $2 \times 10^7/ml$ in a 1:5 dilution of the appropriate antibody supernatant at 4°C for 45 min, and then in a 1:10 dilution of rabbit serum at 37°C for 60 min, as described elsewhere (2, 3).

Cyclophosphamide Treatment. Cytoxan was obtained from Mead Johnson & Co. (Evansville, IN) and dissolved in sterile saline (15 mg/ml). Freshly prepared solutions were injected intravenously into tumor-bearing suppressor donors 24 h before their lymphoid cells were harvested for passive transfer.

Results

Generation of DTH to Tumor Antigens and Its Passive Transfer. To test tumorbearers for cells that suppress DTH, it was first necessary to show that true DTH is generated in response to the Meth A tumor. This required showing that a Meth A-immunized host can mount an inflammatory reaction to an injection of Meth A cells and that the reaction has delayed kinetics. It also required showing that the ability to mount the reaction can be passively transferred to recipients with Ly-1⁺,2⁻, L3T4a⁺ T cells.

It can be seen in Fig. 1 that when mice were injected intradermally with an admixture of 3×10^6 living Meth A cells and *C. parvum*, they developed a palpable tumor which grew for 8–9 d and then underwent complete regression. It can also be seen that when these mice were challenged in a hind footpad with an eliciting dose of 2×10^6 mitomycin c-treated Meth A cells, they had an 18-h inflammatory reaction, the size of which depended on the time of tumor growth or regression that the eliciting injection of tumor cells was given. The ability to mount an inflammatory reaction was acquired by day 3 of immunization, peaked on day 8, and then progressively decayed until about day 20 when the mice were left with a relatively low, but stable state of sensitivity that lasted beyond 30 d of immunization.

Fig. 2 shows that the intrafootpad inflammatory reaction described above had the kinetics of a DTH reaction. It can be seen that when mice were given an



FIGURE 1. Immunization for DTH by intradermal injection of an admixture of 3×10^6 tumor cells and 100 µg of *C. parvum* resulted in 8–9 d of growth of the immunizing tumor that emerged, followed by complete tumor regression (*top*). The ability to mount an 18-h DTH reaction to an intrafootpad injection of 2×10^6 mitomycin c-treated tumor cells (*bottom*) was acquired progressively during the first 8 d of immunization, peaked immediately before a tumor regression, and then progressively decayed to a stable level from day 21 on. Means and SEM of five mice are shown.



HOURS AFTER CHALLENGE

FIGURE 2. Time course of development of the expression of a DTH reaction to an intrafootpad injection of 2×10^6 mitomycin c-treated tumor cells given on day 8 (peak) of immunization, as shown in Fig. 1. It is obvious that the reaction had delayed kinetics. Means and SEM of five mice are shown.

1182



FIGURE 3. The ability of immunized mice to express a DTH reaction could be passively transferred to normal recipients with donor lymphoid cells. The lymphocytes that transferred immunity were Ly-1⁺,2⁻, L3T4⁺ T cells, in that they were destroyed by treatment with anti-Ly-1 antibody, or anti-L3T4 antibody and complement, but not by anti-Ly-2 antibody and complement. In this experiment donor spleen cells were harvested on day 8 of immunization, and the recipients received the eliciting dose of mitomycin c-treated tumor cells 1 h after receiving donor cells. Means and SEM of five mice per group are shown.

intrafootpad injection of mitomycin c-treated tumor cells on day 8 of immunization there was minimal footpad swelling at 3 h, maximum swelling at 18 h, and then a progressive decrease in footpad thickness. Additional evidence that the reaction was true DTH is shown in Fig. 3, where it can be seen that the ability to mount the reaction could be passively transferred from immunized mice to normal recipient mice with spleen cells. Fig. 3 also shows that the responsible cells were of the Ly-1⁺,2⁻, L3T4⁺ helper phenotype, in that their ability to transfer DTH was eliminated by treatment with either anti-Ly-1.2 antibody and complement, or anti-L3T4a antibody and complement, but not by treatment with anti-Ly-2.2 antibody and complement.

Spleen Cells from Mice Bearing a 6-d Tumor, But Not a 16-d Tumor, Suppress Expression of Passively Transferred DTH. According to the results of others, lymphoid cells obtained from mice bearing the syngeneic S1509a sarcoma (4, 5) or Meth A fibrosarcoma (6) can, upon passive transfer, suppress DTH reactions to these tumors in appropriately immunized recipients. To determine whether similar suppressor cells are generated in response to the Meth A tumor under study in this laboratory, an attempt was made to suppress passively transferred Meth A-specific DTH with spleen cells from donors bearing a progressive Meth A tumor. This involved infusing spleen cells from suppressor donors bearing either a 6-d, or 16-d, progressive Meth A tumor into recipients that had received spleen cells from immunized donors 1 h earlier. The recipients were then challenged in a hind footpad with mitomycin c-treated Meth A cells. The sensitized T cells for transferring DTH were harvested from donors with peak DTH on day 8 of immunization with the C. parvum-tumor cell admixture, as shown in Fig. 1.

It can be seen in Fig. 4 that spleen cells from mice bearing a 6-d Meth A tumor could, on passive transfer, suppress the expression of passively transferred DTH

SUPPRESSION OF TRANSFERRED DTH





FIGURE 4. The expression of passively transferred DTH was suppressed by passive transfer of spleen cells from donor mice bearing a day 6 (5 mm diameter) tumor, but not from donors bearing a day 16 (12 mm diameter) tumor. Suppressor cells were transferred 1 h after transferring sensitized T cells, and 2 h after challenging the recipients with antigen. Means and SEM of five mice per group are shown. Asterisk indicates p < 0.05. Supp. suppressor cells.

in naive recipients. On the other hand, spleen cells from donors bearing a 16-d Meth A tumor failed to significantly suppress the expression of passively transferred DTH in a similar group of recipients. Therefore suppressors of DTH are acquired during early stages of growth of the Meth A fibrosarcoma, but are not present in significant numbers at later stages of tumor growth when Ly-1⁺,2⁻ suppressor T cells that can suppress the expression of adoptive immunity are acquired (2, 3).

The consequences of infusing suppressor cells on the kinetics of development of the DTH reaction is clearly illustrated by the results of an additional experiment shown in Fig. 5. These results leave no doubt that spleen cells from donors with a 6-d tumor, but not a 16-d tumor, had a profound suppressive effect on the DTH reaction from an early stage of its development.

Membrane Phenotype and Cyclophosphamide Sensitivity of Suppressors of DTH. Fig. 6 shows that when cells from 6-d tumor-bearing donors were treated with anti-Ly-2.2 antibody and complement, their ability, on passive transfer, to suppress a DTH reaction in recipients of sensitized T cells was completely abolished. In contrast, treatment with anti-Ly-1.2 mAb and complement had no effect on the ability of the cells to suppress DTH. Thus, in keeping with the findings of others (4-6), spleen cells from a 6-d tumor bearer that suppress the expression of passively transferred DTH bear the Ly1⁻,2⁺ membrane phenotype.

These suppressors also proved highly sensitive to cyclophosphamide, in that a single dose of 20 or 100 mg/kg given intravenously to mice bearing a 6-d Meth A tumor caused complete elimination of T cells that could, upon passive transfer, suppress the expression of passively transferred DTH (Fig. 7).

Kinetics of Generation of T Cells that Suppress DTH and T Cells that Suppress Adoptive Immunity. By using the assay for suppression of DTH described above, it was possible to follow the kinetics of generation of T cells that suppress DTH against time of tumor growth. Fig. 8 shows that cells that can, upon passive transfer, suppress the expression of passively transferred DTH were first detected

1184



FIGURE 5. Kinetics of development of an intrafootpad DTH reaction in adoptively immunized recipients, and in adoptively immunized recipients that received spleen cells from donors bearing a day 6 or day 16 tumor. Suppressor cells from donors with a day 6 tumor blocked the DTH reaction at any early stage of its development. In contrast, spleen cells from donors with a day 16 tumor had no suppressive effect. Means of five mice per group are shown. Cells transferred: (O) immune, (Δ) immune + 6-d suppression, (\bullet) immune + 16-d suppression.



FIGURE 6. Time course of generation of DTH suppressors in the spleen during tumor growth. Cells that could suppress a DTH reaction in adoptively immunized recipients upon passive transfer were generated progressively during the first 6 d of tumor growth, and were then progressively lost. Means of five mice per group are shown.

in the spleens of tumor-bearing donors around day 3 of tumor growth, reached maximum production on about day 6, and declined progressively in number thereafter.

In contrast, the generation of suppressor T cells that could suppress adoptive immunity against an established tumor in T cell-deficient recipients occurred much later. Fig. 9 serves to confirm results previously published (2, 3) by showing that spleen cells that can, upon intravenous infusion, inhibit the ability of



FIGURE 7. The suppressor spleen cells from a day 6 tumor bearer that could suppress the expression of DTH in an adoptively immunized recipient, proved to be Ly-1⁻,2⁺ T cells. Their ability to passively transfer suppression was eliminated by treatment with anti-Ly-2 antibody plus complement, but not by treatment with anti-Ly-1 antibody and complement. Means and SEM of five mice per group are shown. Asterisk indicates p < 0.05, double asterisk indicates p < 0.01. CY, cyclophosphamide.



FIGURE 8. Suppressor T cells in donors bearing a day 6 tumor were eliminated by treating the donors with 100 or 20 mg/kg of cyclophosphamide 24 h before harvesting their spleen cells. Means and SEM of five mice per group are shown. Asterisk indicates p < 0.05.

intravenously infused immune T cells to cause regression of an established tumor in T cell-deficient recipients were not acquired progressively in the spleens of tumor bearers until after 9–12 d of tumor growth. Therefore, at a time when the host possesses maximal numbers of T cells that can suppress DTH (Fig. 8), it does not possess T cells that can suppress the expression of adoptive immunity in a T cell-deficient recipient.

T Cells that Suppress DTH Do Not Suppress Immunity in the Same Recipients. It was shown above that T cells from day-6 Meth A bearers can convincingly suppress, on passive transfer, the expression of passively transferred DTH, but not the expression of passively transferred immunity against an established tumor in T cell-deficient recipients. This indicated the distinct possibility that suppression of DTH is not the same as suppression of protective immunity. Therefore, it was important to determine, with the Meth A tumor under study in this





FIGURE 9. Time course of generation of T cells that suppresses the expression of adoptive immunity against an established tumor in T cell-deficient recipients. The *left panel* shows the results of infusing T cell-deficient recipient mice bearing a 4-d tumor with one organ equivalent (1.5×10^8) of spleen cells from immune donors (*IMM. CONT.*), or with one organ equivalent of spleen cells from immunized donors plus one organ equivalent of spleen cells from immunized donors plus one organ equivalent of spleen cells from immune donors (*IMM. CONT.*), or with one organ equivalent of spleen cells from immunized donors plus one organ equivalent of spleen cells from donors bearing a 6-, 9-, 12-, 15-, or 18-d tumor (numbers on individual graphs). It can be seen that T cells that could suppress the function of immune T cells were not acquired progressively until after day 9 of growth of the donor tumor. The *right panel* shows the same results expressed in terms of changes against time of tumor growth in an index of suppression that was obtained by subtracting the size of the time in recipients of immune cells alone from its size in the recipients of immune cells plus suppressor cells on day 23 of the experiment. Means of five mice per group are shown.

laboratory, whether T cell suppressors of DTH can suppress immunity to growth of a tumor implant. It can be seen in Fig. 10 that injection of 2×10^6 living tumor cells into the hind footpad of immunized mice resulted in the emergence of a tumor that grew briefly and then underwent complete regression. It can be seen, in addition, however, that growth of the implant involved a substantial increase in footpad thickness during the first 18 h of challenge, which did not occur in unimmunized control mice. Therefore, the swelling at 18 h was not due to tumor growth, but to a DTH reaction to the challenge implant. This was evidenced by the additional finding that the same 18-h swelling reaction occurred in response to injection of the same number of mitomycin c-treated tumor cells. Indeed, if immunized mice were infused with suppressor cells from a day 6 tumor bearer immediately before being given the living challenge implant, the 18-h DTH reaction was essentially suppressed. However, this suppression of DTH in no way interfered with the expression of immunity to growth of the same implant. Therefore, under the conditions used, suppression of DTH did not result in the suppression of the host's ability to express antitumor immunity at the same site.

Discussion

Two models of T cell-mediated suppression of antitumor immunity have been studied in some detail. One is based on the capacity of T cells from tumor-



FIGURE 10. Evidence that suppression of DTH did not result in suppression of immunity. Passive transfer of suppressor cells from donors with a day 6 tumor failed to suppress the ability of immunized mice to express immunity against an intrafootpad implant of 2×10^6 living tumor cells, even though the 18-h DTH reaction to the living implant was severely suppressed. The 18-h increase in footpad thickness that resulted from implanting living tumor cells was identical to the increase in footpad thickness that resulted from implanting the same number of mitomycin c-treated tumor cells (*DTH CONTROL*). Regardless of whether or not they received T cell suppressors of DTH, the immunized recipients could cause complete regression of the tumor that emerged from the implant. In contrast, the tumor implant grew progressively in normal mice. Means of five mice per group are shown. Supp, suppressor cells.

bearing donors to suppress, upon passive transfer, the expression of a tumorspecific DTH reaction to an injection of tumor cells in tumor-immunized recipients. The other is based on the capacity of T cells from tumor bearers to suppress, upon passive transfer, the expression of adoptive immunity against an established tumor in T cell-deficient recipients. The tumor on which the first mentioned model is based is the S1509a sarcoma syngeneic in A/J mice (reviewed in 4), although similar results have been published more recently with the BALB/c Meth A fibrosarcoma (6, 9). According to studies with these two tumors, T cells that can suppress a tumor-specific DTH reaction in immunized recipients are produced very early (24–48 h) after implanting tumor cells subcutaneously, increase in number to peak around days 6–7 of tumor growth, and are then progressively lost as the tumor grows larger in size. These suppressors display the Ly-1⁻,2⁺, 1-J⁺ membrane phenotype and are functionally eliminated by giving the host as little as 20 mg/kg of cyclophosphamide.

The second major model of tumor-induced suppression is based on the ability of T cells from tumor-bearing donors to inhibit, on passive transfer, the expression of passively transferred immunity against a relatively large palpable tumor in T cell-deficient recipient mice. This model of suppression of immunity developed from the initial finding in this laboratory (10) that tumor-sensitized T cells from immunized donor mice failed, on passive transfer, to cause regression of a recipient tumor, unless the recipient had been made T cell-deficient by

1188

DIGIACOMO AND NORTH

thymectomy and irradiation. It was reasoned that sensitized T cells express their function in T cell-deficient recipients, but not in immunocompetent recipients, because the latter recipients acquired suppressor T cells in response to progressive growth of their tumor. Evidence consistent with this interpretation was supplied by the demonstration that the obstacle to adoptive immunotherapy could be passively transferred. In other words, it was shown (10, 11) that an infusion of T cells from tumor-bearing donors, but not from normal donors, could suppress the ability of T cells from immunized donors to cause regression of a palpable tumor in T cell-deficient recipients. It was shown later (12) that the suppressor T cells detected by this assay are specific for the tumor that evokes their generation. Moreover, they are different in a number of ways from the suppressor T cells that function to suppress tumor-specific DTH. For example, unlike the suppressor of DTH, the suppressors of adoptive immunotherapy bear the Ly-1⁺,2⁻ membrane phenotype, and are not generated until late in tumor growth, when the tumor grows beyond about 8 mm in diameter (2, 3). More recent studies show (A. DiGiacomo and R. J. North, manuscript submitted for publication) that these suppressor T cells also display the L3T4 antigen. Again, the suppressors of adoptive immunotherapy used against an established tumor are not as sensitive to cyclophosphamide as the suppressors of DTH. Whereas, as shown here and elsewhere (9), suppressors of Meth A-specific DTH are destroyed by a 20 mg/kg dose of the drug, it requires a 100 mg/kg dose to destroy the suppressors of adoptive immunity (13).

However, in spite of the differences between these two types of suppressor T cells, this study leaves no doubt that both types are produced in response to the Meth A fibrosarcoma under study in this laboratory. The results show that Ly- $1^-,2^+$ suppressor T cells defined by their ability to suppress DTH are generated progressively during the first 6 d of tumor growth, whereas suppressor T cells that can suppress the expression of adoptive immunity are generated later in tumor growth when the production of the suppressors of DTH is on the wane. There is a need, therefore, to discuss the functional significance of each type of suppressor T cell, with a view to deciding how each type might explain downregulation of the antitumor immune response.

In this regard, it can be stated first, that because the reason for postulating the existence of suppressor T cells in tumor-bearing mice is to explain how tumors avoid being destroyed by an antitumor immune response, suppressor cells that can suppress a proven antitumor effector mechanism in vivo are likely to be more important than those that suppress the expression of a correlate of immunity, like DTH. It should be mentioned, however, that the T cell suppressors of DTH induced by the S1509a sarcoma were originally defined in terms of their ability to partly suppress the expression of immunity to growth of a tumor implant in immunized recipients (14). More recently published findings (6) with the Meth A fibrosarcoma indicate, nevertheless, that suppression of a DTH reaction to this tumor by passively transferred Ly-1⁻,2⁺ suppressors may not necessarily be associated with suppression of immunity. The results presented here serve to show that this is the case. They reveal that, although T cell suppressors of DTH can efficiently suppress a DTH reaction to an implant of living tumor cells in immunized recipients, they fail to suppress, to any extent,

1190 T CELL SUPPRESSORS OF ANTITUMOR IMMUNITY

the expression of immunity to growth of the same implant. At this stage of the investigation, therefore, it is difficult to see a role for the T cell suppressors of tumor-specific DTH in the escape of the immunogenic Meth A fibrosarcoma from immunity.

Even so, the fact that suppressor T cells that can suppress tumor-specific DTH reaction in immunized recipients are generated in response to tumor growth, means that these suppressors must play some part in the antitumor immune response. There can be little doubt that the sensitivity reaction that these suppressors suppress is true DTH, because it was shown here that it develops with delayed kinetics and is mediated by Ly-1⁺,2⁻, L3T4a⁺ T cells. However, it needs to be appreciated that the DTH described here and elsewhere (4-6, 9)was induced by immunization, rather than by progressive tumor growth. To understand the meaning of suppression of DTH, we need to first determine whether tumor-specific DTH represents a mechanism of tumor rejection, and second, whether it can develop in a tumor-bearing animal in the absence of suppressors of DTH. Presumably such evidence will be forthcoming. In the meantime, an explanation of tumor escape based on suppression of concomitant immunity by $Ly-1^+, 2^-$ suppressor T cells seems relatively easy to accept, because these suppressors have the capacity to suppress a mechanism of immunity that can cause the regression of a relatively large tumor mass. They have the same surface phenotype (2, 3) as the T cells that can passively transfer immunosuppression in other in vivo models of antitumor immunity (13, 14), and that can passively transfer tolerance to allografts in rats (15). The possibility that these suppressor T cells are "inducer suppressors," that recruit Ly-1⁻,2⁺ "effector suppressors" that ultimately express suppression is worth considering, but has been questioned (1, 17). In any case, it would not explain why suppression of DTH by Ly-1⁻,2⁺ suppressor T cells is not associated with suppression of immunity.

Summary

The results of this study show that during growth of the immunogenic Meth A fibrosarcoma, two different types of suppressor T lymphocytes are generated in sequence. One type is generated during early tumor growth, reaches peak number around day 6, and is progressively lost thereafter. It is defined by its ability, upon passive transfer, to suppress the expression of a DTH reaction to tumor antigens in tumor-immunized recipients. It bears the $Ly1^-,2^+$ membrane phenotype and is sensitive to relatively low doses of cyclophosphamide. In contrast, the second type of suppressor cell is not detected until after day 9 of tumor growth, and is defined by its ability to inhibit, upon passive transfer, the expression of adoptive immunity against an established tumor in T cell-deficient recipients. According to previous studies it bears the Ly1⁺,2⁻, L3T4a⁺ membrane phenotype and is less sensitive to cyclophosphamide than the T cell suppressor of DTH. It is argued that this second type of suppressor T cell seems likely to be responsible for the escape of immunogenic tumors from antitumor immunity, because it can suppress the expression of a powerful mechanism of antitumor immunity in recipient mice, and is generated progressively as the tumor-bearing host loses concomitant immunity. In contrast, although the Ly-1⁻,2⁺ T cell suppressors of DTH can efficiently suppress a DTH reaction to an implant of living tumor cells, they fail to suppress the expression of immunity to the same implant.

Received for publication 29 May 1986 and in revised form 26 June 1986.

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1192 T CELL SUPPRESSORS OF ANTITUMOR IMMUNITY

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