IN VIVO T CELL TUMOR THERAPY WITH MONOCLONAL ANTIBODY DIRECTED TO THE Vβ CHAIN OF T CELL ANTIGEN RECEPTOR

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Several studies have demonstrated the effectiveness of mAbs specific for tumor cell surface molecules as therapeutic agents to inhibit tumor growth both experimentally (1–4) and in clinical trials (5–7). Since B cell lymphomas bear unique determinants (idiotopes) on their surface Ig molecules, and since only a small number of normal B cells share that same idiotype, antiidiotype mAb could be used as an antitumor agent without affecting a significant fraction of the normal B cell population (8, 9). There are, however, two problems with this approach: (*a*) the high rate of somatic mutation in Ig genes (10, 11), which can alter the epitope to be recognized by mAb; and (*b*) the necessity to establish a different antiidiotypic mAb for each individual B cell lymphoma.

For T cell tumors, the situation appears to be different. Recent analysis of TCR V gene segments has revealed very limited, if any, somatic mutation among the TCR genes isolated from various T cell clones, hybridomas, and T cell tumor lines (12). Furthermore, the number of different TCR V gene segments is relatively small both in man and mouse (13), and the evidence indicates that deletion of several V gene segments in certain strains of mice does not affect the general immune status of such mouse strains (14). These observations raise the intriguing possibility that mAbs directed to determinant of TCR V framework regions, rather than to unique idiotopes of the TCR, might be useful for treating T cell tumors without compromising host immunity. Moreover, because of the small number of TCR V genes, it seems feasible to produce a panel of mAbs specific for each of these V gene products. These mAbs may be effective in diagnosis or clinical protocols dealing with spontaneously arising T cell tumors expressing one of the V gene products.

Here we explore the feasibility of this approach in mice and show that mAbs specific for Vβ6 TCR products given four times over a 2-wk period prevent the growth of a $V\beta6^+$ syngeneic T cell tumor without compromising the immune status of these mice to other experimental antigens. To our knowledge, this is the first demonstration of the effectiveness of preventing tumor growth with a mAb having specificity for a particular subset of T cells expressing a defined Vβ TCR marker.

Materials and Methods

Mice. C57BL/6 and B10.BR mice (purchased from The Jackson Laboratories, Bar Harbor, ME) of both sexes, 6–10 wk old, were used.

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Cell Lines and mAbs. Two thymoma cell lines, C6VL and EL-4, derived from C57BL/6, and one AKR-derived thymoma, BW5147, were maintained in culture in 10% FCS DME. mAb directed to determinant on the TCR Vβ8 chain (KJ16-133, rat IgG2a) (15) and on the Vβ6 chain (RR4-7, rat IgG2b) (16) were partially purified from ascites fluids.

Immunofluorescent Staining. Two-step surface immunofluorescence staining was performed as described previously (16). Briefly, tumor cells or nylon wool nonadherent spleen cells were incubated with saturating doses of mAb (100 μl) for 30 min at 4°C, washed, and further incubated with FITC-conjugated goat anti-rat Ig (Caltag Laboratories, San Francisco, CA) for another 30 min at 4°C. Stained samples were analyzed on a FACScan flow cytometer (Becton Dickinson Immunocytometory Systems, Mountain View, CA).

T Cell Proliferation Assays. Proliferative responses of T cells to antigen and mitogen (Con A) were measured by procedures described previously (17). Briefly, mice were immunized with keyhole limpet hemocyanin (KLH, 100 μg/mouse) in CFA at the base of the tail. 7 d later, 10^5 cells, recovered from inguinal lymph nodes, were stimulated with KLH (10 μg/ml), Con A (2 μg/ml), or medium alone in flat-bottomed microtiter plates in a final volume of 200 μl of 5% FCS DME. The proliferative response was measured on day 3 following a 6-h pulse with [^{3}H]thymidine.

Generation of Alloantigen-specific CTL. Cytolytic T cell responses were measured by culturing 5×10^6 C57BL/6 spleen cells and an equal number of irradiated (2,000 rad) B10.BR spleen cells in 24-well tissue culture plates in a final volume of 2 ml 5% FCS DME. 5 d later, cells were recovered and tested for cytolytic activity using ^{51}Cr-labeled BW5147 (H-2^k) target cells in standard Cr-release assays. Lytic units (LU) were calculated from plots of titrated cytolytic activities. 1 LU is defined as the number of effector cells required to lyse 50% of 10^4 target cells under the specific conditions of this assay system.

Results

Treatment of T Cell Tumor In Vivo. To test the possible therapeutic efficacy of mAbs specific for Vβ6 on T cell tumor growth in vivo, a large panel of T cell tumors derived from C57BL/6 mice were screened for the expression of Vβ6 or Vβ8 TCR using a mAb specific for Vβ6 (RR4-7) and another specific for Vβ8 (KJ-16.133).

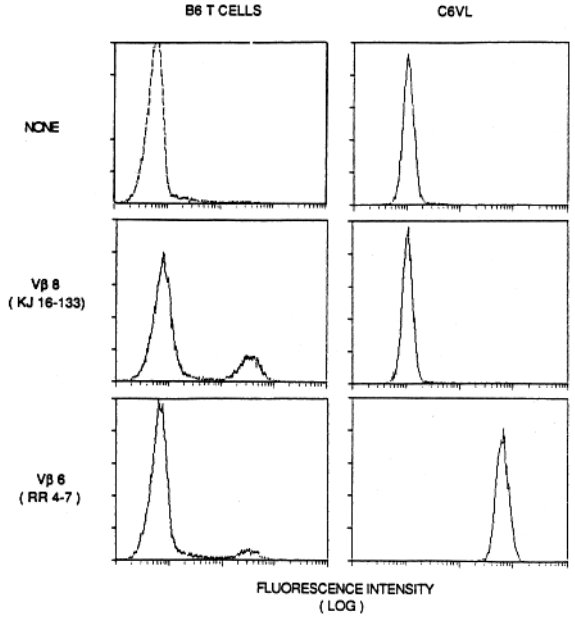

FIGURE 1. Surface expression of TCR by C57BL/6 splenic T cells and C6VL tumor cells. Nylon wool-purified splenic T cells and C6VL tumor cells were analyzed by indirect immunofluorescence flow cytometry for cell surface expression of TCR using KJ 16-133 (anti-Vβ 8.1 and 8.1 antibody; kindly provided by Dr. J. Kappler and P. Marrack, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO) and RR4-7 (anti-Vβ6 antibody). Control staining was determined with the fluoresceinated goat anti-rat Ig alone.

TABLE I
Treatment of C57BL/6 Mice Bearing C6VL Tumor Cells with Anti-Vβ6 mAb

Exp.	T cell tumor	Treatment	Death	Mean survival
				d ± SD
A I	C6VL	Anti-Vβ6	2/5	>60
II	C6VL	Anti-Vβ8	5/5	30 ± 3
III	C6VL	None	5/5	30 ± 2
IV	EL-4	Anti-Vβ6	5/5	51 ± 4
V	EL-4	None	5/5	53 ± 5
B I	C6VL	Anti-Vβ6	1/5	>60
II	C6VL	None	5/5	29 ± 2
III	EL-4	Anti-Vβ6	5/5	30 ± 2
IV	EL-4	None	5/5	30 ± 3

Groups of 5 C57BL/6 mice were injected with either C6VL or EL-4 tumor cells intravenously (2×10^4/mouse in a final volume of 500 μl). Mice were treated with either RR4-7 (anti-Vβ6, 150 μg/mouse), KJ16 (anti-Vβ8, 150 μg/mouse), or none. Treatment was initiated 24 h after the tumor inoculation and given every 3 d for 12 d.

These two antibodies stain 8 and 13%, respectively, of nylon wool nonadherent B6 spleen cells. One tumor, C6VL, was identified with anti-Vβ6 mAb staining and usage of this TCR gene was confirmed in mRNA analyses using a Vβ6 probe.

The results of the first two efficacy tests of anti-Vβ6 mAb on the growth of a $V\beta6^+$ tumor are shown in Table I. Panels of C57BL/6 mice were injected intravenously with lethal numbers (20×10^4) C6VL ($V\beta6^+$) or EL4 ($V\beta6^-$) tumor cells and then treated with anti-Vβ6 mAb, anti-Vβ8 mAb, or nothing (150 μg/injection, i.p., four times in 12 d). All mice given EL4 tumor cells died, regardless of which antibody they received in subsequent treatment. By comparison, however, of mice injected with $V\beta6^+$ tumor cells, only 3/10 animals died after treatment with anti-Vβ6 mAb, while all died (15/15) if treated with anti-Vβ8 mAb or nothing. Furthermore, use of isotype-matched control IgG2b antibody did not protect mice from tumor-related death. It should be noted that of the three mice that died with $V\beta6^+$ tumor cells and anti-Vβ6 treatment, these had significantly prolonged survival times (>60 d) compared with the other treatment groups (~30 d).

C6VL tumor cells were recovered from a liver mass in a mouse that developed a tumor despite treatment with anti-Vβ6 mAb. These cells were analyzed for surface expression of Vβ6 TCR by flow cytometric analysis using RR4-7 (anti-Vβ6). The data shown in Fig. 2 revealed that this tumor retained the original Vβ6 TCR on a majority of its cells, indicating that treatment of this $V\beta6^+$ tumor with anti-Vβ6 mAb does not result in the selection of significant number of TCR negative variants in vivo.

Effect of Anti-Vβ6 mAb on Host Immune Responses. Flow cytometric analysis of nylon wool nonadherent spleen cells from mice treated with anti-Vβ6 mAb, using anti-Vβ6 and anti-Vβ8 mAbs, indicated that the number of $V\beta6^+$ T cells was greatly reduced while number of $V\beta8^+$ T cells was normal in both treated and nontreated

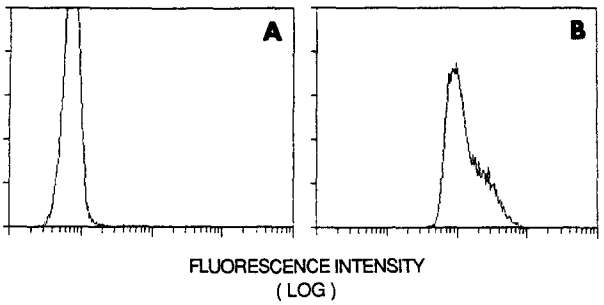

FIGURE 2. Vβ6 TCR expression on C6VL tumor cells recovered from mice treated with RR4-7 antibody. Tumor cells from liver in a mouse that developed a tumor despite treatment with RR4-7 Ab were cultured in vitro for 3 d. Cells were stained with medium alone (*A*) or RR4-7Ab (*B*) followed by fluoresceinated goat anti-rat Ig. Stained samples were analyzed by FACScan flow cytometry.

groups (Table II). These data demonstrate that anti-Vβ6 mAb can eliminate Vβ6$^+$ tumor cells as well as normal T cells that are Vβ6$^+$, which comprise ~8% of the peripheral T cell pool.

Despite elimination of T cells bearing Vβ6 TCR, this treatment seemed not to have significant effect on the immune status of these mice. T cell responses of mAb treated animals, measured by proliferative response to KLH and Con A, and the generation of allospecific CTL, were comparable to those of nontreated animals (Table II).

Immunity to the Tumor Cells after mAb Treatment. Mice that had been successfully treated with RR4-7 antibody to prevent C6VL tumor growth were rechallenged with the same tumor cells without further antibody treatment (Table III). All of these mice showed complete resistance to further tumor challenge.

Discussion

In this article we use an experimental murine model to show that a rat mAb of the IgG2b isotype specific for an epitope determined by the TCR Vβ6 gene is effective in preventing growth in vivo of a syngeneic T cell tumor expressing a Vβ6 TCR. This antibody appears to be effective in inhibiting tumor growth without any significant

TABLE II

Effect of In Vivo Anti-Vβ6 Antibody on the Host Immune Response

Mice	TCR expression		In vitro proliferative response*			CTL response in vitro
	Vβ8	Vβ6	KLH	Con A	None	
	%		*cpm* × 10^{-3}			*LU*‖
Control	14.8‡	7.5	65 ± 8.5	385 ± 12	3.4 ± 0.5	20
Treated	16.5	1.1	73 ± 22	384 ± 54	3.5 ± 0.5	17

C57BL/6 mice received an intraperitoneal injection of 150 μg of RR4-7 antibody in PBS. 3 d after injection, spleen cells were analyzed for the expression of Vβ6 TCR and tested for the generation of the CTL response against H-2k alloantigens, as described in Materials and Methods.

* 3 d after antibody treatment, mice were tested for the antigen-specific proliferative responses as described in Materials and Methods.

‡ Percent T cells stained; mean of three individual determinations.

§ Mean of triplicate cultures; ± SD.

‖ LU/10^6 recovered cells.

TABLE III
Acquisition of Immunity to the C6VL Tumor Cell in Mice Surviving after Antibody Treatment

Mice	Death	Mean survival
		d ± *SD*
Naive	5/5	32 ± 3
Survivors	0/4	>65

Mice that survived in the initial tumor challenge from the group I shown in Table I and naive age-matched control mice received intravenous injection of C6VL tumor cells (2×10^4/mouse). The time between the final RR4-7 antibody injection and the C6VL tumor rechallenge was >60 d.

compromise to the immune status of the treated host despite the fact that it also eliminates normal T cells of the Vβ6$^+$ subset that comprise nearly 10% of the peripheral T lymphocyte pool.

Two lines of evidence support the conclusion that the effectiveness of anti-Vβ6 in inhibiting tumor growth is dependent on expression of a target molecule on tumor cells that is some epitope of the Vβ6 gene product of the TCR. Mice treated with mAb specific for a different Vβ epitope, Vβ8, do not show inhibition of Vβ6 tumor growth, and mice treated with anti-Vβ6 show no growth inhibition of EL4, a different syngeneic T cell tumor.

How treatment with anti-Vβ antibodies inhibits growth of Vβ^+ tumor cells in vivo is not clear, but two general possibilities can be considered. First, like MHC/antigen, mAbs specific for T cell receptors cause activation and lymphokine production of T cell hybridomas, and subsequent inhibition of growth both in vivo and in vitro (18, 19). However, we could find no evidence of the capacity of C6VL tumor cells to respond in any way to stimulation with RR4-7 in vitro. Thus we consider it unlikely that elimination of Vβ6$^+$ tumor cells with anti-Vβ6 antibody requires their activation in vivo. Secondary, anti-Vβ antibodies might participate in some cellular mechanism involving binding to Fc receptors of effector cells to cause inhibition of tumor growth. In favor of this possibility is the fact that antibodies of the rat IgG2b subclass have been found to be the most effective in eliminating target cells in vivo (20). In addition, in not yet complete studies, we have found that anti-Vβ6 together with murine spleen cells bearing Fc receptor inhibit the growth of C6VL in vitro, and that treatment of mice in vivo with F(ab')$_2$ fragment of anti-Vβ6 mAb is not as effective as treatment with the whole molecule.

The findings that anti-Vβ6-treated mice that survived initial tumor challenge are resistant to further challenge without additional treatment with antibody is particularly striking. In these experiments, the number of normal peripheral Vβ6$^+$ T cells had returned to normal, thus it seems unlikely that residual levels of anti-Vβ6 antibody from the first treatment can account for subsequent tumor resistance. Active immunization of mice with irradiated syngeneic tumor cells also causes resistance to subsequent challenge. Thus, our provisional interpretation of the effect of these antibodies is that they retard tumor growth by some cell-mediated, Fc-dependent mechanism to a degree sufficient to permit the opportunity for host immune antitumor responses that would otherwise be overwhelmed by rapid tumor cell growth.

The findings that antitumor treatment with anti-Vβ antibody does not compromise the immune response capacity to other test antigens despite the fact that a significant portion of the peripheral T cell pool is also eliminated do not rule out the possibility that lack of T cells bearing one particular V gene segment may cause a deficit in the response to certain antigens. However, these findings are comparable to the prior studies, in that nearly one-third of Vβ chains are genetically deleted in certain strains of mice, causing a significant deficit in the expressed repertoire of TCR without jeopardizing their immune response potential.

While the mechanisms of anti-Vβ antibody protection against T cell tumor growth clearly need to be investigated further, the important point to emphasize here is that an epitope expressed on a TCR V gene product of normal cells can be used as a target molecule for mAb therapy of a T cell neoplasm. Since the number of genes encoding known TCR Vβ segment is only 21 in mouse and 35 in man (13), it seems a reasonable approach to establish panels of mAb specific for each Vβ gene segment for use both diagnostically and therapeutically in the treatment of T cell tumor expressing clonally distributed TCR (21).

Summary

To test whether antibodies directed to TCR affect T cell tumor growth in vivo, mice were inoculated intravenously with C6VL tumor cells expressing Vβ6 TCR and then treated intraperitoneally with mAb specific for Vβ6 TCR. Administration of anti-Vβ6 antibody prolonged survival of mice bearing Vβ6-expressing tumor cells and it led to the induction of host immunity to the tumor cells in surviving animals. This treatment eliminated not only tumor cells bearing Vβ6 TCR but also normal host T cells expressing Vβ6 T cell receptors. However, the lack of Vβ6-expressing T cells in such treated mice did not result in generalized immune disfunction. These data demonstrate the utility of anti-TCR V segment antibody in the treatment of T cell tumors. Most importantly, since the number of V genes for the T cell antigen receptor is limited, both in man and in mouse, it should be possible to establish a panel of mAbs directed to each V gene product and use such antibodies in the treatment of T cell neoplasms.

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