T-Cell Tumor Elimination as a Result of T-Cell Receptor–Mediated Activation

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It has recently been shown that activation of murine T-cell hybridomas with antigen inhibits their growth in vitro. The "suicide" of these neoplastic T cells upon stimulation with antigen suggested the possibility that activation via the antigen-specific receptor could also inhibit the growth of neoplastic T cells in vivo. To test this, mice were subcutaneously inoculated with antigen-specific T-cell hybridomas and then treated intraperitoneally with antigen. Administration of the appropriate antigen immediately after inoculation with the T-cell hybridoma abrogated tumor formation; antigen administered after tumors had become established decreased the tumor burden and, in a substantial fraction of animals, led to long-term survival. The efficacy of antigen therapy was due to both a direct inhibitory effect on tumor growth and the induction of host immunity. These studies demonstrate the utility of cellular activation as a means of inhibiting neoplastic T-cell growth in vivo and provide a rationale for studying the use of less selective reagents that can mimic the activating properties of antigen, such as monoclonal antibodies, in the treatment of T-cell neoplasms of unknown antigen specificity.

ACTIVATION OF NORMAL RESTING T lymphocytes leads to a cascade of events that culminates in entry into the cell growth cycle (*1*). In contrast, stimulation of spontaneously dividing murine T-cell hybridomas with the antigen for which they are specific, presented in the context of the appropriate major histocompatibility complex (MHC) gene products, results in an apparently irreversible block of the cell cycle at the G_1/S interface (*2*). Inhibition of growth was a direct result of cellular activation and was not due to the secretion and action of "toxic" lymphokines. These observations raised the intriguing possibility that antigen receptor–mediated activation of neoplastic T cells in vivo might inhibit the progression of their malignant growth. In vivo, lymphocyte hybridomas behave like typical malignancies in that they generate a progressive tumor burden, metastasize, and kill the inoculated animal (*3*). Since T-cell hybridomas bear antigen receptors of known specificity, it should be possible to activate them in vivo with the appropriate antigen in a highly selective manner. Therefore, antigen-specific T-cell hybridomas, produced by fusing antigen-primed B10.A T cells with the AKR thymoma BW5147, were injected subcutaneously into (AKR × B10.A)F_1 mice. Treatment with the appropriate antigen prevented the growth of these neoplastic cells by two distinct mechanisms, one involving a direct inhibitory effect upon T-cell hybridoma growth, as demonstrated in nude mice, and the other involving the induction of host T cell–dependent immunity. Furthermore, treatment with antigen caused the host to reject even established solid T-cell tumors within several days. These observations suggest that the cellular events that occur after activation via the antigen-specific receptor may be exploited in a novel approach to the treatment of some lymphocytic neoplasms.

From 1×10^6 to 2.5×10^6 of the 2B4.11 murine T hybridoma cells, which are specific for the antigen pigeon cytochrome c, were injected in the flank of (AKR × B10.A)F_1 mice, that is, mice that were syngeneic to the hybridoma (Table 1). Animals in the control groups received either no antigen or an irrelevant protein antigen, while mice in the treated group received pigeon cytochrome c intraperitoneally, beginning on the day of tumor inoculation. All 20 animals that did not receive pigeon cytochrome c developed progressive tumors at the site of injection, eventually resulting in death. In contrast, none of the 21 animals treated with pigeon cytochrome c developed tumors. A different T-cell hybridoma, C10.9, which is specific for hen egg lysozyme (HEL), was tested in a similar fashion. After inoculation with 1×10^6 to 5×10^6 C10.9 T hybridoma cells, tumors grew in 23 of 24 animals in the control group. After the initial appearance of a tumor at the site of injection, the C10.9 cells were spontaneously rejected in a substantial fraction of the animals. However, 15 (63%) of these mice developed a progressive tumor burden and eventually died (mean survival, 31 days). In contrast, only 1 of 24 mice (4%) treated with the antigen HEL developed a tumor, and the appearance of tumor in that animal was markedly delayed. Therefore, despite the complication of spontaneous rejection in a subset of the inoculated animals, it was clear that treatment with specific antigen resulted in the rejection of the C10.9 T-cell hybridoma ($P < 0.001$).

To determine if the recipient's immune response contributed to the efficacy of this therapy, we rechallenged 13 mice that had survived a first inoculation with 2B4.11 T hybridoma cells (Table 1, experiments 1, 2, and 3; Table 2). None of these 13 animals developed tumor at the site of the second inoculation despite the fact that no further specific antigen was administered. It was possible that sufficient pigeon cytochrome c remained after the first round of therapy to cause rejection of the 2B4.11 T cells upon rechallenge, although this would seem unlikely after a delay of up to 2 months. This possibility, however, could not account for the observation that 2B4.11 T cells injected into mice that had survived injection of C10.9 after treatment with HEL (that is, had never received pigeon cytochrome c) also failed to form tumors (Table 2). These experiments showed that antigen activation of these T-cell hybridomas caused the recipient mice to develop immunity directed toward one or more shared antigen.

To further explore the role of host immunity in tumor rejection, we injected 2B4.11 T cells into C3H *nu/nu* mice. The antigen-presenting cells of these mice bear E^k_β:E^k_α, the allelic form of the Ia molecule required for the presentation of pigeon cytochrome c to 2B4.11 (Table 3). In experiment 1, 50% of the nude mice inoculated with 1×10^6 2B4.11 T cells did not develop tumors when treated with pigeon cytochrome c, whereas all of the control animals developed tumors at the site of inoculation. In other experiments in which we used an inoculum of either 1×10^6 or 2.5×10^6 2B4.11 T cells (experiments 2 and 3), tumors developed in all mice, whether they were treated with pigeon cytochrome c or not. In all animals treated with pigeon cytochrome c, however, the onset of tumor was substantially delayed, with a mean time of onset of 24 days in the treated group compared to 9 days in the control group. As expected, the therapeutic efficacy of antigen administration was dependent on the presence of the H-2^k MHC haplotype (encoding the E^k_β:E^k_α Ia molecule). This was demonstrated by the failure of pigeon cytochrome c to inhibit the appearance of tumors in BALB/c (H-2^d) mice (Table 3, experiment 4).

We removed 2B4.11 cells from five animals that had developed a mass at the site of inoculation despite treatment with pigeon cytochrome c. Flow cytometric analysis with

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Table 1. Inhibition of growth of T-cell hybridomas as tumors in (AKR × B10.A)F_1 mice by specific antigen. The T-cell hybridomas 2B4 [specific for the antigen pigeon cytochrome c (cyt. c) plus the E^k_β:E^k_α Ia molecule] and C10 (specific for HEL plus the A^k_β:A^k_α Ia molecule) were prepared by polyethylene glycol–mediated fusion of the AKR-derived thymoma BW5147 to antigen-primed T cells from B10.A mice (*23*), and the subclones 2B4.11 and C10.9 were derived in our laboratory by cloning by limiting dilution. These cells were maintained in logarithmic phase growth in RPMI 1640 supplemented with 10% fetal calf serum, 50 μ*M* 2-mercaptoethanol, 4 m*M* glutamine, penicillin, and gentamicin. The T hybridoma cells were washed three times in phosphate-buffered saline (PBS) and injected subcutaneously in the flank of syngeneic (AKR × B10.A)F_1 mice (Jackson Laboratory) in a volume of 100 μl. With 2B4.11, the treatment group received 1 mg of pigeon cytochrome c (Sigma) diluted in PBS and injected intraperitoneally every other day, from the day of tumor inoculation and continuing for 14 days (a total of 8 mg was administered). Animals in the control group received 1 mg of HEL (Sigma) or chicken ovalbumin (Sigma) according to the same treatment regimen or no antigen. With C10.9, the treatment group received 1 mg of HEL diluted in PBS intraperitoneally every other day as above, and the control animals received 1 mg of pigeon cytochrome c according to the same regimen. Mice were inspected daily for the appearance of a mass at the site of injection. Mean time of tumor onset for the control animals injected with 2B4.11 ranged from 4 to 6 days. Mean time of tumor onset for the control animals injected with C10.9 ranged from 4 to 7 days. Mean survival stated in days is described as mean ± standard error of the mean.

Experiment	T-cell hybridoma	No. of cells inoculated	Antigen	No. with tumors/no. inoculated	Deaths	Mean survival*
1	2B4.11	1×10^6	Ovalbumin	6/6	6/6	30 ± 2 days
			Pigeon cyt. c	0/6	0/6	>5 months
2	2B4.11	2.5×10^6	HEL	6/6	6/6	27 ± 1 days
			Pigeon cyt. c	0/6	0/6	>6 months
3	2B4.11	2.5×10^6	None	8/8	8/8	25 ± 1 days
			Pigeon cyt. c	0/9	0/9	>4 months
4	C10.9	1×10^6	Pigeon cyt. c	6/6	6/6	33 ± 6 days
			HEL	1/6†	1/6	>7 months‡
5	C10.9	2.5×10^6	Pigeon cyt. c	5/6	2/6	25 ± 2 days§
			HEL	0/6	0/6	>7 months
6	C10.9	2.5×10^6	Pigeon cyt. c	6/6	3/6	23 ± 2 days§
			HEL	0/6	0/6	>6 months
7	C10.9	5×10^6	Pigeon cyt. c	6/6	4/6	43 ± 3 days§
			HEL	0/6	0/6	>6 months

*The mean survival data include some animals that were used in the rechallenge experiments given in Table 2. †The time of tumor onset at the injection site was 44 days after inoculation. ‡Excludes the pigeon cytochrome c–treated animal that developed a tumor on day 44. §Mean survival only of those animals that died of disseminated tumor.

the anticlonotypic antibody A2B4-2 (*4*) revealed that all tumors had retained the antigen-specific receptor, and in vitro [^{3}H]thymidine incorporation assays indicated that they were just as sensitive to the growth inhibitory effect of pigeon cytochrome c as were 2B4.11 T cells that had been maintained continuously in vitro. Therefore, in these animals, treatment with specific antigen did not result in the selection of antigen-resistant variants. Three C3H nude mice that survived tumor inoculation after treatment with pigeon cytochrome c (Table 3, experiment 1) were rechallenged with 2B4.11 and not given any further treatment. All developed tumors at the site of inoculation, proving that they had not developed host immunity to the T-cell hybridoma (Table 3). These data suggested that specific antigen alone had a therapeutic effect on tumor growth, but that host T cell–dependent defenses contributed significantly to tumor rejection.

To determine whether treatment with antigen could cause the elimination of established tumors in normal animals, we injected 2B4.11 cells subcutaneously into (AKR × B10.A)F_1 mice and allowed them to grow for up to 11 days before treating the mice with pigeon cytochrome c (Table 4). Some mice were free of palpable tumor after only one injection of pigeon cytochrome c, and 24 of 25 mice were free of demonstrable tumor after four injections. In most cases this was associated with hair loss over the injection site, often accompanied by an area of central ulceration. Despite the resolution of most of the primary lesions, some mice in each group died of metastatic disease, usually of the liver or spleen or of the central nervous system. The survival of mice that died of metastatic disease was significantly prolonged by treatment with pigeon cytochrome c if the therapy was begun within 9 days of tumor inoculation (Table 4). Long-term survivors were followed for up to 26 weeks without evidence of tumor recurrence.

It was possible to recover cells from the primary site in one mouse, and from the livers of three mice that had metastatic disease. The antigen-specific receptor was absent on 2B4.11 cells from one of the mice with liver metastases but was expressed at normal levels on the other three tumor populations. The 2B4.11 T cells taken from the primary site and from one mouse with liver metastases were as sensitive to pigeon cytochrome c as were 2B4.11 T cells removed from the inoculation site of a control animal that had not been treated with pigeon cytochrome c (that is, these cells exhibited an 80 to 90% reduction in [^{3}H]thymidine incorporation when incubated with pigeon cytochrome c) (*2*). Cells from the second liver metastasis were relatively refractory to antigen-induced growth inhibition; at the plateau response there was only a 50% reduction in the amount of [^{3}H]thymidine incorporated. Finally, the antigen receptor–negative tumor cells from the third liver metastasis failed to respond at all to pigeon cytochrome c. In summary, treatment with specific antigen resulted in the elimination of established tumors at the primary site of inoculation in 24 out of 25 mice (96%), with long-term survival of 9 out of 25 mice (36%). In two of three cases in which T hybridoma cells were recovered from metastatic lesions and tested, they had lost sensi-

Table 2. Induction of immunity to the T-cell hybridoma by treatment with antigen in vivo. Mice in the antigen treatment groups that had survived an initial challenge with either 2B4.11 (Table 1, experiments 1, 2, and 3) or C10.9 (Table 1, experiments 5, 6, and 7) were challenged with the indicated number of 2B4.11 T cells subcutaneously in the flank opposite from that used for the primary inoculation. No further treatment with antigen was given. The control groups consisted of naïve (AKR × B10.A)F_1 mice that received the indicated number of 2B4.11 T cells subcutaneously and no therapy. The time between the final therapeutic injection of antigen (either pigeon cytochrome c or HEL) and rechallenge with 2B4.11 T cells was (described by experiment designations assigned in Table 1): experiment 1, 11 days; experiment 2, 62 days; experiment 3, 33 days; experiment 5, 66 days; experiment 6, 53 days; experiment 7, 39 days.

Survivors (experiments in Table 1)	Primary challenge	No. of 2B4.11 used for rechallenge	No. of tumors developed*	
			Control	Experimental
1	2B4.11	1×10^6	2/2	0/6
2, 3	2B4.11	2.5×10^6	3/3	0/7
5, 6, 7	C10.9	2.5×10^6	3/3	0/9

*All control animals developed tumors and died after a mean survival time of 26 ± 2 days. The mice in the experimental groups were observed for 4 months after challenge with 2B4.11 without any evidence of tumor.

Table 3. Antigen-induced tumor elimination is less efficient in nude mice. C3H *nu/nu* and BALB/c *nu/nu* mice (obtained from the Animal Program, Division of Cancer Treatment, National Cancer Institute) were inoculated with the indicated number of 2B4.11 T cells subcutaneously in the left flank. Mice in the experimental groups were treated with pigeon cytochrome c, from the time of tumor inoculation, as described in Table 1 (except experiment 2, in which a total of 14 injections were given). Mice in the control groups were treated with 1 mg of HEL intraperitoneally according to the same schedule. Day of tumor onset is described as mean ± standard error of the mean; NA, not applicable.

Experiment	No. of 2B4.11 T cells inoculated	Nude mouse strain inoculated	Day of tumor onset	
			Control (no. with tumors/ no. inoculated)	Experimental (no. with tumors/ no. inoculated)
1	1×10^6	C3H	10 ± 2 (5/5)	23 ± 3 (3/6)
2	1×10^6	C3H	10 ± 3 (5/5)	22 ± 1 (6/6)
3	2.5×10^6	C3H	8 ± 1 (5/5)	27 ± 2 (5/5)
4	2.5×10^6	BALB/c	6 ± 0.3 (6/6)	7 ± 0.4 (6/6)
Rechallenge*	1×10^6	C3H	NA	8 ± 0 (3/3)

*The three pigeon cytochrome *c*–treated mice that had survived the challenge with 2B4.11 T hybridoma cells (experiment 1) were reinoculated with 1×10^6 2B4.11 T cells in the opposite flank 15 days after the last therapeutic injection of pigeon cytochrome *c*. No further antigen was administered after reinoculation.

tivity to the growth inhibitory effects of stimulation with pigeon cytochrome c, in one of these cases as a result of the loss of the antigen-specific receptor.

Although T-cell tumors have received relatively little attention in this regard, a variety of immunological maneuvers have been used to treat model in vivo neoplasms of the B-lymphocyte lineage. In mice, the administration of anti-idiotypic antibodies and, in a few cases, the injection of the antigen for which the neoplastic cells were specific, have been used with limited success in suppressing the in vivo growth of TEPC-15 (*5, 6*), MOPC-315 (*5, 7*), BCL_1 (*8*), ABPC48 (*9*), MOPC-104E (*10, 11*), MOPC 406 (*10*), J558 (*11*), and MOPC-460 (*12*). Some, but not all (*13*), studies of murine B-cell tumors in vitro, such as S107 (*14*), MOPC-104E (*15*), BCL_1 (*8*), and WEHI 231 (*16*), showed that their growth was inhibited by treatment either with anti-idiotypic antibodies or with their specific antigen, and raised the possibility that the efficacy of tumor treatment in vivo with these agents was at least partially due to direct inhibition of neoplastic B-cell growth.

The basis for studying the effect of antigen treatment on neoplastic T-cell growth in vivo was the recent observation that engagement of the antigen-specific receptor on murine T-cell hybridomas in vitro resulted in interleukin-2 (IL-2) production and an irreversible block in the cell cycle at the G_1/S interface (*2*). In the present study the direct cytotoxic effect of antigenic stimulation was evident in vivo as well, as demonstrated by the ability of antigen to slow or abolish T-cell hybridoma tumor growth in nude mice, that is, in the absence of a normal immune response, and the rapid resolution of established primary tumors after antigen was administered. The failure of antigen therapy to completely eliminate the growth of tumors in nude mice, without the appearance of antigen-resistant variants, was probably due to an insufficient level of the T-cell antigen-specific receptor's ligand when 1 mg of antigen was given every other day. Activation of the T-cell hybridoma in vivo is dependent on both the concentration of antigen and the number of class II–bearing antigen-presenting cells at the site of the tumor; it is possible that one or both of these was limiting in the protocol used. A second mechanism of tumor elimination in normal mice was the generation of T cell–dependent immunity, a phenomenon that is probably related to the process by which, after immunization with tumor-specific antigens, nonviable tumor cells, or tumor cells at subtumorigenic doses, mice are capable of rejecting a challenge with the same or a related tumor cell (*16*). An additional factor that may have played a role in the therapeutic efficacy of treatment with specific antigen is that antigen-stimulated T-cell hybridomas secrete IL-2, possibly enhancing the host's tumor-specific T-cell response.

This study demonstrates that activation of neoplastic cells via a physiologically normal receptor can cause the regression of at least some lymphoid tumors. The potential therapeutic usefulness of the approach described here for de novo T-cell tumors will depend on the development of antigen surrogates that "stimulate" neoplastic lymphocytes whose appropriate antigen is unknown. It may be possible to accomplish this with antibodies directed against the cell surface receptors. For example, cross-linking of immunoglobulin on immature B-cell lymphomas in vitro, a signal that causes resting B cells to enter the cell cycle (*17*), causes a G_1/S cell cycle block in immature B-cell lymphomas (*18*). In humans, the administration of anti-idiotypic antibodies to some patients with B-cell lymphomas has resulted in a number of partial remissions and, in one case, apparent cure (*19*). Although mechanisms such as complement-mediated lysis, opsonization, or antibody-mediated cellular cytotoxicity may have contributed to this effect, we would suggest that the direct cytotoxic effect of antigen-receptor cross-linking may have played a substantial role. In the case of transformed T cells, we have shown that cross-linked monoclonal antibodies directed against the antigen-specific receptor arrested the in vitro growth of

Table 4. Regression of established T-cell tumors after treatment with antigen. (AKR × B10.A)F_1 mice were inoculated subcutaneously in the flank with 2.5×10^6 2B4.11 T hybridoma cells. Therapy consisted of a 14-day course of 1 mg of pigeon cytochrome c intraperitoneally as described in Table 1, and was begun at various times after the tumor had been injected. Mice in the control group received no antigen. Palpable tumor size at the inoculation site was determined by measuring two perpendicular diameters, and the area was estimated by multiplying these values. Area of tumor and survival are described as mean ± standard error of the mean; NA, not applicable.

No. of days before initiation of treatment	Treatment with pigeon cyt. c	Area of tumor at time therapy begun (cm^2)	Resolution of primary tumor*	No. of long-term survivors†	Survival of mice that died with metastatic tumor (days)
		Experiment 1			
0	No	NA	0/4	0/4	27 ± 3
0	Yes	Not detectable	NA	5/5	NA
7	Yes	1.02 ± 0.14	5/5	4/5	40
9	Yes	2.16 ± 0.26	4/4	0/4	41 ± 3
11	Yes	2.58 ± 0.34	5/5	2/5	32 ± 4
		Experiment 2			
0	No	NA	0/5	0/5	25 ± 2
0	Yes	Not detectable	NA	11/11	NA
7	Yes	1.16 ± 0.14	6/6	1/6	36 ± 1
10	Yes	2.46 ± 0.29	4/5	2/5	23 ± 1

*Resolution of tumor was defined as complete loss of a palpable or visible mass. This generally occurred after two to four injections of pigeon cytochrome c. †Despite resolution of the primary tumor mass, some treated mice died as a result of metastatic spread. Metastatic lesions were found to be most prominent in the central nervous system, or the liver or spleen. Surviving mice have been observed for 26 (experiment 1) and 18 (experiment 2) weeks after the final therapeutic injection of pigeon cytochrome *c* without evidence of tumor recurrence.

2B4.11 (*2*). Furthermore, we have found that antibodies directed against CD3, which are mitogenic for normal T cells (*20*), induce both IL-2 production and growth inhibition in a murine chemically induced T-cell lymphoma, EL-4, and a human T-cell leukemia, Jurkat (*21*). These results demonstrate that activation-induced growth inhibition is not a property unique to T-cell hybridomas and suggest that mitogenic antibodies to CD3, or perhaps other common and nonclonally distributed T-cell surface structures such as CD2 (*22*), may prove useful in the treatment of T-cell neoplasms of unknown antigen specificity.

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Structurally Divergent Human T Cell Receptor γ Proteins Encoded by Distinct Cγ Genes

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The human T cell receptor (TCR) γ polypeptide occurs in structurally distinct forms on certain peripheral blood T lymphocytes. Complementary DNA clones representing the transcripts of functionally rearranged TCR γ genes in these cells have been analyzed. The expression of a disulfide-linked and a nondisulfide-linked form of TCR γ correlates with the use of the Cγ1 and Cγ2 constant-region gene segments, respectively. Variability in TCR γ polypeptide size and disulfide linkage is determined by the number of copies and the sequence of a repeated segment of the constant region. Thus, Cγ1 and Cγ2 are used to generate structurally distinct, yet functional, T3-associated receptor complexes on peripheral blood lymphocytes. Tryptic peptide mapping suggests that the T3-associated TCR γ and δ peptides in the nondisulfide-linked form are distinct.

THE T CELL RECEPTOR (TCR) γ gene, like those encoding the TCR α and β polypeptides, is composed of immunoglobulin-like gene segments, which are joined through somatic rearrangement during T cell differentiation (*1–3*). The human TCR γ locus consists of at least five functional variable (V) region, five joining (J) region, and two constant (C) region genes (*4–11*). Although the total number of functional V and J region genes is limited, significant diversity is introduced during the process of V–J joining (*3, 8, 12*). However, in the majority of functional T cell lines and tumors examined to date, the V–J joining process fails to maintain an open reading frame, and a functional TCR γ protein is not synthesized.

The use of antisera against TCR γ-specific peptides has led to the identification of T3-associated TCR γ on some peripheral blood and thymic T cells, as well as a leukemic T cell line (*13–17*). We have characterized two examples of peripheral blood TCR γ lymphocytes in detail (*13, 16*). The IDP2 cell line expresses a T3-associated TCR γ peptide of 55 kD (40 kD nonglycosylated) which immunoprecipitates along with a 40-kD peptide termed δ. The γ and δ polypeptides on the surface of IDP2 are not disulfide-linked. In contrast, PBL C1 cells express a T3-associated TCR γ peptide of 40 kD (31 kD nonglycosylated), which is disulfide-linked. Although a distinct partner chain has not yet been identified, indirect evidence suggests that this disulfide-linked TCR γ peptide is part of a heterodimer, and not a homodimer. Both IDP2 and PBL C1 appear to function as cytotoxic T lymphocytes (*16*).

The present study was conducted to elucidate the molecular bases for the structural differences among the TCR γ and δ peptides on IDP2 and PBL C1. In particular, we wished to investigate the dramatic differences in both the size and disulfide linkage of the TCR γ peptides on these cells and the structural relation between TCR γ and δ on IDP2.

Complementary DNA (cDNA) libraries were prepared from IDP2 and PBL C1 poly(A)$^+$ RNA in the vector λgt10 (*18*) and were screened by hybridization with ^{32}P-labeled human TCR γ cDNA clone pTγ-1 (*6*). Clones were selected for detailed analysis on the basis of both size and limited restriction enzyme mapping, which suggested that they represented the transcripts of rearranged TCR γ genes. Four PBL C1 clones and the one IDP2 clone so selected lacked Kpn I sites, suggesting rearrangement to Vγ9 (*7*). Nucleotide sequence analysis indicated that all PBL C1 clones arose from identical transcripts. The nucleotide sequence of the longest of these, PBLC1.15 (1.5 kb), is compared with that of IDP2.11 (1.4 kb) in Fig. 1. PBLC1.15 contains approximately 150 bp of 5′ noncoding region preceding a presumed initiator methionine codon and extends through a canonical poly(A) addition site at its 3′ end. IDP2.11 contains less 5′ sequence but also extends through a poly(A) addition site at its 3′ end.

The PBLC1.15 and IDP2.11 V regions are nearly identical to each other and to the coding region of a Vγ9 genomic sequence (*7*). Differences between PBLC1.15 and IDP2.11 at nucleotides 226, 230, and 234 are probably the result of reverse transcriptase errors in the region of the IDP2.11 hairpin loop. In addition, a difference at nucleotide 511 likely represents a reverse

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