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Direct transfer of a foreign MHC gene into human melanoma alters T cell receptor Vβ usage by tumor-infiltrating lymphocytes

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Abstract The direct introduction of foreign genes into tumors shows promise as a therapeutic modality to enhance tumor immunogenicity. Hence, melanoma nodules were directly injected with a vector encoding an allogeneic MHC class I molecule, HLA-B7. Tumor-infiltrating lymphocytes (TIL) were isolated from cutaneous melanoma biopsies before and after HLA-B7 gene transfer. TIL were expanded in interleukin-2 (IL-2) by standard techniques for approximately 4 weeks, then analyzed for T cell receptor Vβ usage by quantitative reverse transcriptase polymerase chain reaction (RT-PCR). Prior to gene transfer, TIL Vβ usage was found to be highly restricted, the only one to four Vβ families being expressed and one or two of these families representing more than 90% of the repertoire. As anticipated, TIL Vβ usage varied among patients expressing different HLA types. However, Vβ13 was over-represented in that six of eight patients utilized Vβ13 as a dominant family, regardless of HLA type. Following HLA-B7 gene transfer, TIL Vβ usage was markedly altered: (1) Vβ families that dominated following gene transfer differed from the Vβ families utilized by TIL prior to treatment, and (2) introduction of the HLA-B7 gene resulted in a more diverse repertoire with an increase in the number of Vβ families represented. In two patients, TIL were evaluated before treatment and from multiple, distinct melanoma nodules following gene transfer. In these two patients, a comparison was made between TIL Vβ profiles obtained after treatment from nodules that had been injected with the HLA-B7 gene or left untreated. Interestingly, the Vβ repertoires of TIL from uninjected nodules following gene transfer were similar to that of TIL from injected nodules, rather than pretreatment TIL. These data demonstrate a direct immunological effect of foreign MHC gene transfer into human melanoma, and suggest that local expression of an allogeneic MHC molecule generates systemic alterations in the antitumor immune response.

Key words Human · T Lymphocytes · Tumor immunity · T cell receptors · Gene therapy

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Introduction

The introduction of recombinant genes into tumors represents a potential mechanism to enhance the antitumor immune response. This immunopotentiating strategy includes the introduction of genes encoding cytokines [4, 13–16, 23, 26, 39, 43], costimulatory molecules [9], and foreign major histocompatibility (MHC) proteins [28, 34]. In many gene-transfer models, tumor cells are cultured and transfected in vitro, then reintroduced into the host. Disadvantages of this approach include the need to establish an autologous tumor cell line for each patient, and the potential risk of altering the tumor by in vitro manipulation. The direct in situ transfer of an immunostimulatory gene into

the tumor provides an alternative therapeutic approach, which is not subject to these limitations. In addition, direct gene transfer may be performed immediately, thereby avoiding any delay in treatment posed by the need to establish and transfect tumor lines in vitro. Thus, in the current study, human melanoma nodules were directly injected with a vector encoding the allogeneic HLA-B7 class I molecule and its associated β2 microglobulin.

Tumor-infiltrating lymphocytes (TIL), which are thought to be enriched for T cells specific for autologous tumor, may be isolated by culturing tumor biopsy material in interleukin-2 (IL-2) [21, 45]. The repertoire of T cells present in freshly isolated tumor biopsies may be quite diverse [5, 10], owing to nonspecific recruitment of circulating cells with distinct antigen specificities. However, the presence of IL-2 and tumor antigens in TIL cultures provides a selective advantage for the outgrowth of T cells that are responsive to the tumor and have been stimulated and amplified in situ [11, 17, 24, 36, 47]. Hence, characterizing TIL should provide insight into the mechanisms by which immunopotentiating gene therapies augment the antitumor immune response. TIL generated from melanoma lesions are primarily CD3+ T cells that express an α/β T cell receptor (TCR) [35]. Antigen specificity is imparted by rearrangement of the variable (V), diversity (D), and joining (J) segments for the β locus and of V and J segments of the α locus (reviewed in [27]). Random combinations of these segments as well as imprecise V(D)J joining and the addition of N-region nucleotides generate TCR diversity. Several reports have documented restricted usage of Vβ elements by TIL in melanoma [11, 36, 40, 44] and other carcinomas, including renal, lung, and ovarian [3, 17, 33]. The purpose of this study was to evaluate the effect of HLA-B7 gene transfer on TIL TCR Vβ usage. This report provides the first demonstration that introduction of a foreign MHC gene alters the repertoire of T cells infiltrating human melanoma.

Materials and methods

Patients and HLA-B7 gene therapy

Eight HLA-B7-negative patients with stage IV melanoma refractory to conventional therapies were enrolled on the basis of the guidelines of the clinical protocol [29]. A eukaryotic expression vector plasmid, pHLA-B7, was prepared by insertion of β2 microglobulin and HLA-B7 cDNA into the Rous sarcoma virus β-globin plasmid. DNA-liposome complexes were prepared immediately prior to injection by adding lactated Ringer's solution to pHLA-B7, then combining with 1, 2-dimyristyloxpropyl-3-dimethylhydroxyethyl ammonium bromide (DMRIE) and dioleoyl glycerophosphethanolamine (DOPE). Each patient received a treatment series that consisted of three injections of 3 μg DNA-liposome complex into a single subcutaneous melanoma nodule. DNA-liposome injections were spaced 2 weeks apart, and injected nodules were biopsied 48 h following the third injection. HLA-B7 gene expression was verified by the reverse transcriptase polymerase chain reaction (RT-PCR), and immunohistochemistry revealed that 1%–10% of tumor cells near the injection sites expressed HLA-B7 protein [30].

Generation of TIL

Tumor specimens were dissected free from normal tissue, cut into pieces and digested in 40 ml RPMI-1640 medium (Gibco, Grand Island, New York) containing 2.5 units/ml of hyaluronidase type V, 0.5 mg/ml collagenase type IV, and 0.05 mg/ml deoxyribonuclease type I (all from Sigma Chemical Co., St Louis, Mo.) at room temperature overnight. The resulting cell suspension was filtered through a 100 μm nylon mesh (Tetko Inc., Briarcliff Mannor, N.Y.), washed three times with RPMI-1640 medium, and placed in X-Vivo-15 medium (Bio Whittaker, Walkersville, MD,) supplemented with 10% human AB serum (Sigma) and 1000 units IL-2/ml (Cetus Oncology, Emeryville, Calif.) at 2.5×10^5 nucleated cells in Lifecell tissue-culture bags (Baxter Health Care Corp., Fenwall Division, Deerfield, Ill.). As TIL proliferation became evident the cultures were split 1:2 every 2 or 3 days with X-Vivo-15 medium supplemented with IL-2 without serum. TIL were harvested 4 weeks following initiation of the culture.

RNA isolation

Total cellular RNA was isolated using RNAzol B (Tel-Test Inc., Friendswood, Tex.) which utilizes guanidinium thiocyanate. A 1/10 volume of chloroform isoamyl alcohol (24:1) was added to samples, incubated on ice for 5 min, then centrifuged at 14 000 rpm for 15 min at 4 °C. RNA contained in the upper aqueous phase was collected, precipitated with an equal volume of isopropanol, and washed twice in 70% ethanol. RNA pellets were dried under vacuum then resuspended in 0.5% sodium dodecyl sulfate. RNA was evaluated and quantified using a Beckman DU-64 spectrophotometer and by ethidium bromide visualization in an agarose gel. RNA samples were stored at −70 °C upon addition of 1/20 volume of 4 M NaCl and 2 volumes of 100% ethanol.

cDNA synthesis

Aliquots of 3 μg total RNA, or less when necessary, were reversed-transcribed using a cDNA cycle kit (Invitrogen, San Diego, Calif.). The Invitrogen cDNA cycle kit uses random primers and avian myeloblastosis virus reverse transcriptase to generate high yields of full-length, first-strand cDNA from total RNA for use in PCR. Mock cDNA synthesis, leaving out reverse transcriptase, was performed and used as a control for DNA contamination in PCR analysis.

Polymerase chain reaction

PCR was performed in a Hybaid OmniGene thermal cycler in 50 μl volumes, and primers were based on those described by Hall and Finn [18, 19]. PCR reaction mixtures consisted of 100 μM dNTP (Gibco), 5 μCi [^{32}P]dCTP (>3000 Ci/mmol; Amersham, Arlington Heights, Ill.), 2.5 units *Taq* enzyme (Perkin-Elmer), and primers at 500 nM in Perkin-Elmer PCR buffer (10 mM TRIS HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin). To facilitate quantification, a master mix was prepared which contained cDNA reverse-transcribed from 3 μg total RNA and all reagents except for primers specific for the 5′Vβ family, which were added separately to each of 22 individual microcentrifuge tubes. An equal volume of light mineral oil (Sigma Chemical, St. Louis, Mo.) was overlaid and PCR was carried out with an initial 3-min denaturation at 94 °C, followed by 30 cycles of the sequence: 30 s at 94 °C, 30 s at 60 °C, and 1 min at 72 °C. Each sample was analyzed by 5% polyacrylamide gel electrophoresis. Gels were completely dried and exposed to film (Hyperfilm-MP) after which bands were located and quantified using a Molecular Dynamics scanning densitometer.

Quantitative PCR analysis

Quantitative PCR analysis of Vβ gene usage by T cell populations is described by Hall and Finn [18, 19]. Each Vβ signal detected by

autoradiography was corrected for product incorporation of radiolabled dCTP. Each correction factor represented the actual number of cytosine residues in the product, excluding primers, assuming an average contribution of 35 cytosines by DJ gene segments. Data are expressed as the relative percentage of Vβ usage calculated as:

Vβ usage (%)=100×(specific signal/sum of all Vβ signals)

This technique allows for distinctions to be made for the percentage usage of Vβ families that are sufficiently separated (0–1%, 3–5%, 10%, 30%, >50%).

Flow cytometry

TIL were stained with fluorescein-isothiocyanate (FITC)-conjugated anti-CD3 or dual-stained with FITC-conjugated anti-CD4 and phycoerythrin-conjugated anti-CD8 (Becton-Dickinson, San Jose, Calif.). TIL phenotypic analysis was performed using a Becton-Dickinson FACScane flow cytometer.

Results

Gene transfer and isolation of TIL

Cutaneous melanoma nodule biopsies were obtained prior to treatment and at 48 h after the third intratumor injection of pHLA-B7 plasmid DNA-liposome complex for eight patients. As previously reported (28, 30], HLA-B7 gene expression was verified at both the mRNA and the protein level by RT-PCR and immunohistochemistry respectively. TIL were derived by culturing biopsy samples in 1000 U/ml IL-2 for approximately 4 weeks. All pretreatment TIL grew proficiently, while post-treatment TIL cultures were established for five of eight patients. For two patients, post-treatment TIL were derived from multiple, distinct nodules. TIL TCR Vβ usage was evaluated by quantitative RT-PCR and the results are presented as the relative percentage Vβ usage.

TIL TCR Vβ usage is restricted prior to HLA-B7 gene transfer

HLA types and the Vβ repertoires of TIL obtained from pretreatment nodules are presented for each patient in Fig. 1. For each patient, pretreatment TIL Vβ repertoires were restricted to between one and four families, and one or two of these families represented more than 90% of Vβ usage. As expected, Vβ usage varied from individual to individual with different HLA types. However, Vβ13 was over-represented, in that pretreatment TIL obtained from six of eight patients utilized Vβ13 as a dominant family. It should be noted that three of these patients (AH, DM, GM) expressed HLA-A2, supporting a previous report of increased Vβ13 usage by HLA-A2-restricted TIL [31]. However, the three additional patients who utilized Vβ13 as a dominant family (FB, GB, MS) did not express HLA-A2, and an association between HLA-A or -B loci and TCR usage was not apparent.

Direct gene transfer of HLA-B7 alters TIL TCR Vβ usage

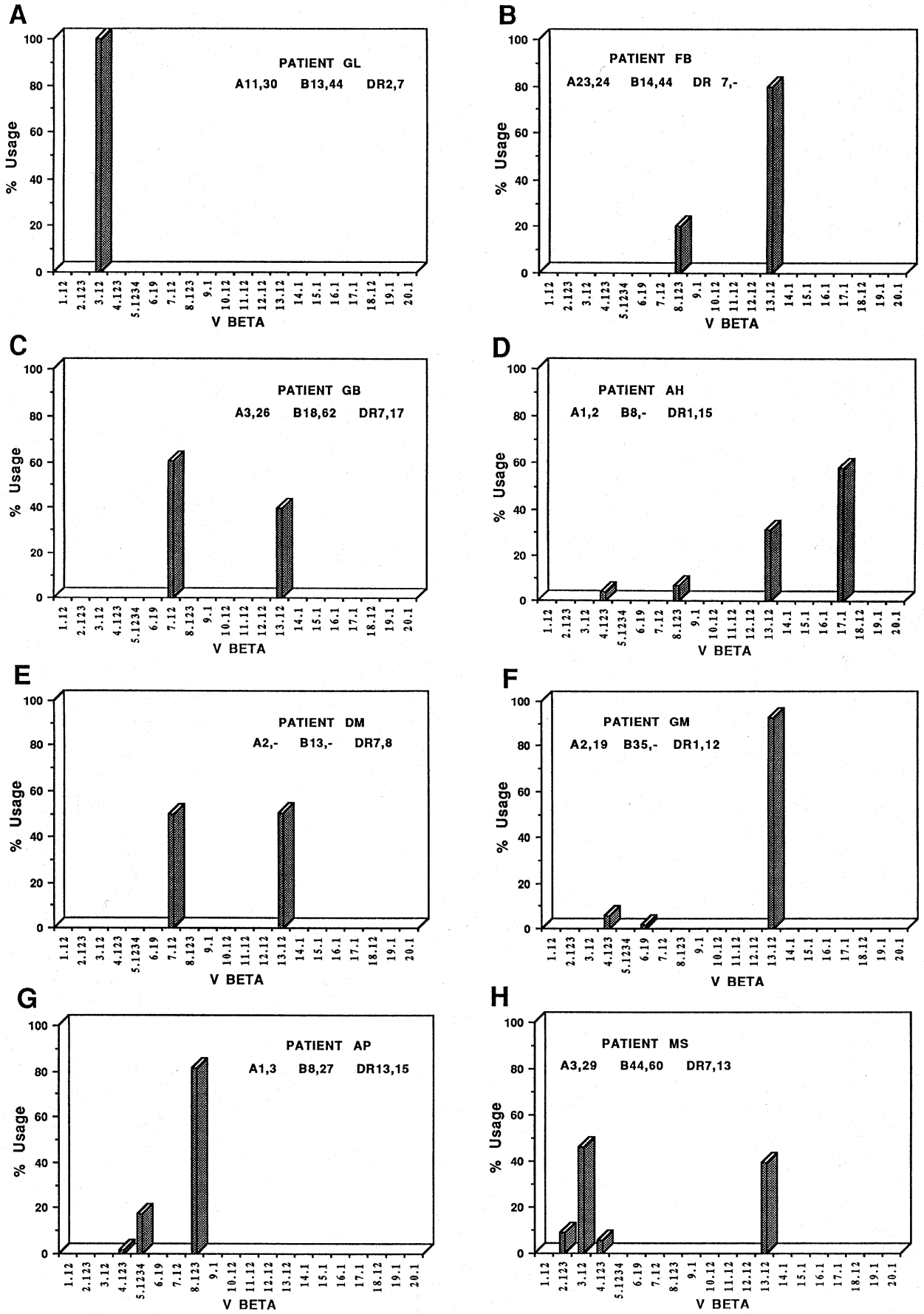
Figure 2 compares Vβ repertoires of TIL obtained prior to HLA-B7 gene transfer to that of TIL derived from tumor nodules that had been injected with pHLA-B7 (five patients). In all patients, introduction of the HLA-B7 gene resulted in a more heterogeneous Vβ repertoire, with up to nine families being represented in the case of patient AP (Fig. 2A). Further, Vβ families that dominated TIL populations after HLA-B7 gene transfer differed from those utilized by TIL prior to treatment. In patients AP (Fig. 2A), GM (Fig. 2B), and GB (Fig. 2C), Vβ families that comprised more than 80% of the pretreatment TIL repertoire contributed less than 5% to the post-treatment Vβ repertoire. In patient MS (Fig. 2D), the dominant pretreatment family, Vβ3, was lost and replaced by Vβ7 in post-treatment TIL. Likewise, pretreatment Vβ13 was lost in patient DM (Fig. 2E), yielding to the single dominant family, Vβ7. Hence, HLA-B7 gene transfer altered the repertoire of T cells that dominated TIL cultures.

TIL TCR Vβ usage is altered in both injected and uninjected nodules following HLA-B7 gene transfer

For two patients (GM and AP), TIL were isolated from multiple, distinct nodules following HLA-B7 therapy. Figure 3 compares pretreatment TIL Vβ usage (Fig. 3A) to that of a pHLA-B7-injected nodule (Fig. 3B) and a post-treatment uninjected nodule (Fig. 3C) for patient GM. Introduction of the HLA-B7 gene altered the TIL Vβ repertoire for patient GM with a marked decrease of Vβ13 and the emergence of distinct Vβ families in the pHLA-B7-injected nodule. Interestingly, the Vβ profile of TIL obtained from the uninjected post-treatment nodule resembled that of the pHLA-B7-injected nodule, rather than that of the pretreatment nodule. For both injected and uninjected post-treatment nodules, Vβ2 emerged as a dominant family, while Vβ13 contributed less than 7% to the repertoire. This finding contrasts with the pretreatment TIL repertoire, where Vβ13 comprised more than 90% and Vβ2 was not detectable.

This phenomenon is best illustrated by patient AP (Fig. 4). For this patient, post-treatment TIL were obtained from two distinct pHLA-B7-injected nodules located on the lateral thigh and the scapular regions (Fig. 4B, C) and one uninjected nodule located on the contralateral side of the patient overlying the iliac crest (Fig. 4D). Six Vβ families (Vβ2, 3, 4, 5, 12, and 13) composed more than 90% of the Vβ repertoire in both of the HLA-B7-injected nodules. Further, four of these families (Vβ2, 5, 12, and 13)

Fig. 1A–H Restricted Vβ usage by tumor-infiltrating lymphocytes (TIL) prior to HLA-B7 gene transfer. Quantitative reverse transcriptase polymerase chain reation for Vβ expression was performed on TIL populations derived from untreated melanoma nodules prior to initiation of HLA-B7 gene therapy. Data are presented as the relative percentage Vβ usage, and individual patient HLA types are shown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PATIENT GL
A11,30 B13,44 DR2,7
B
PATIENT FB
A23,24 B14,44 DR 7,-
C
PATIENT GB
A3,26 B18,62 DR7,17
D
PATIENT AH
A1,2 B8,- DR1,15
E
PATIENT DM
A2,- B13,- DR7,8
F
PATIENT GM
A2,19 B35,- DR1,12
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A1,3 B8,27 DR13,15
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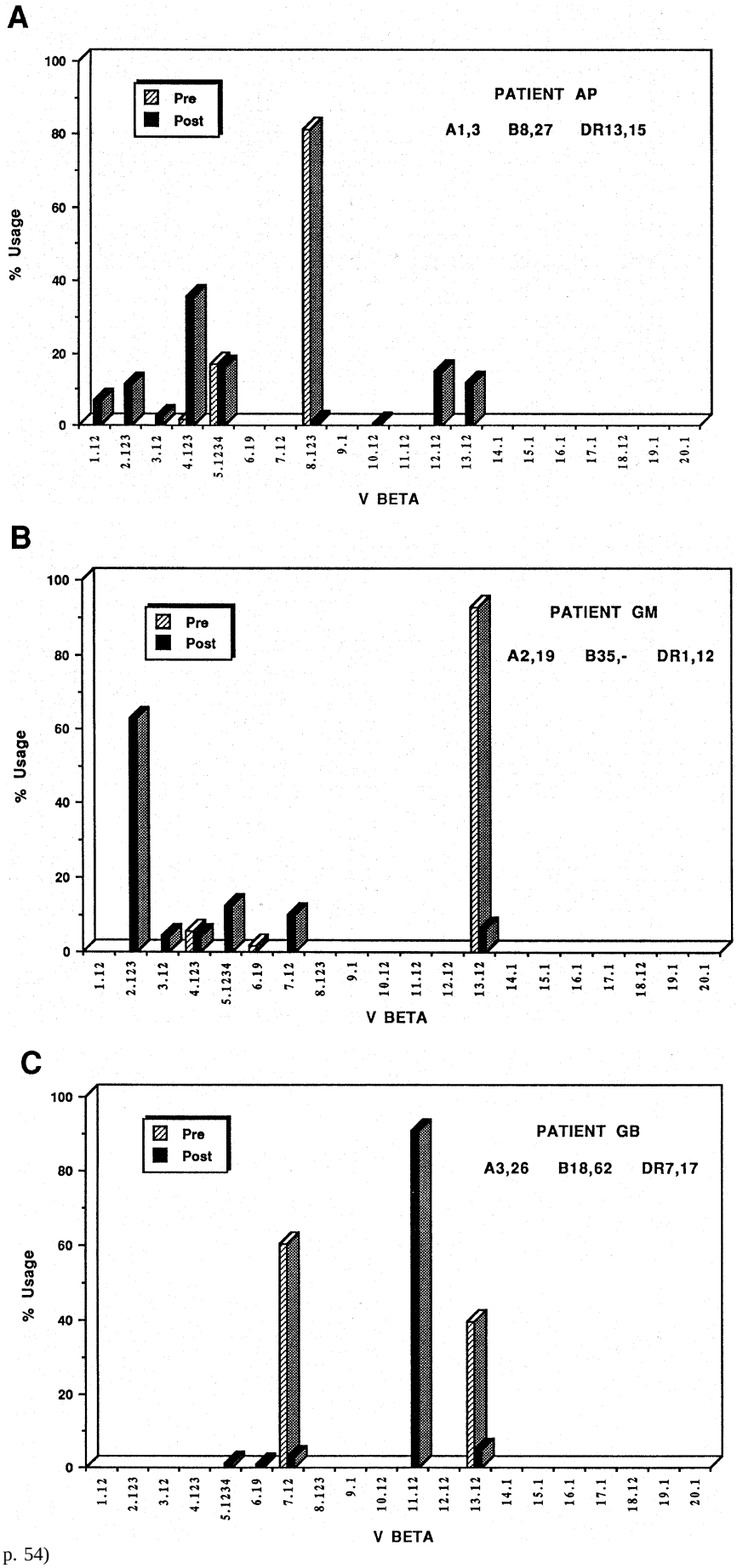

Fig. 2 (For legend see p. 54)

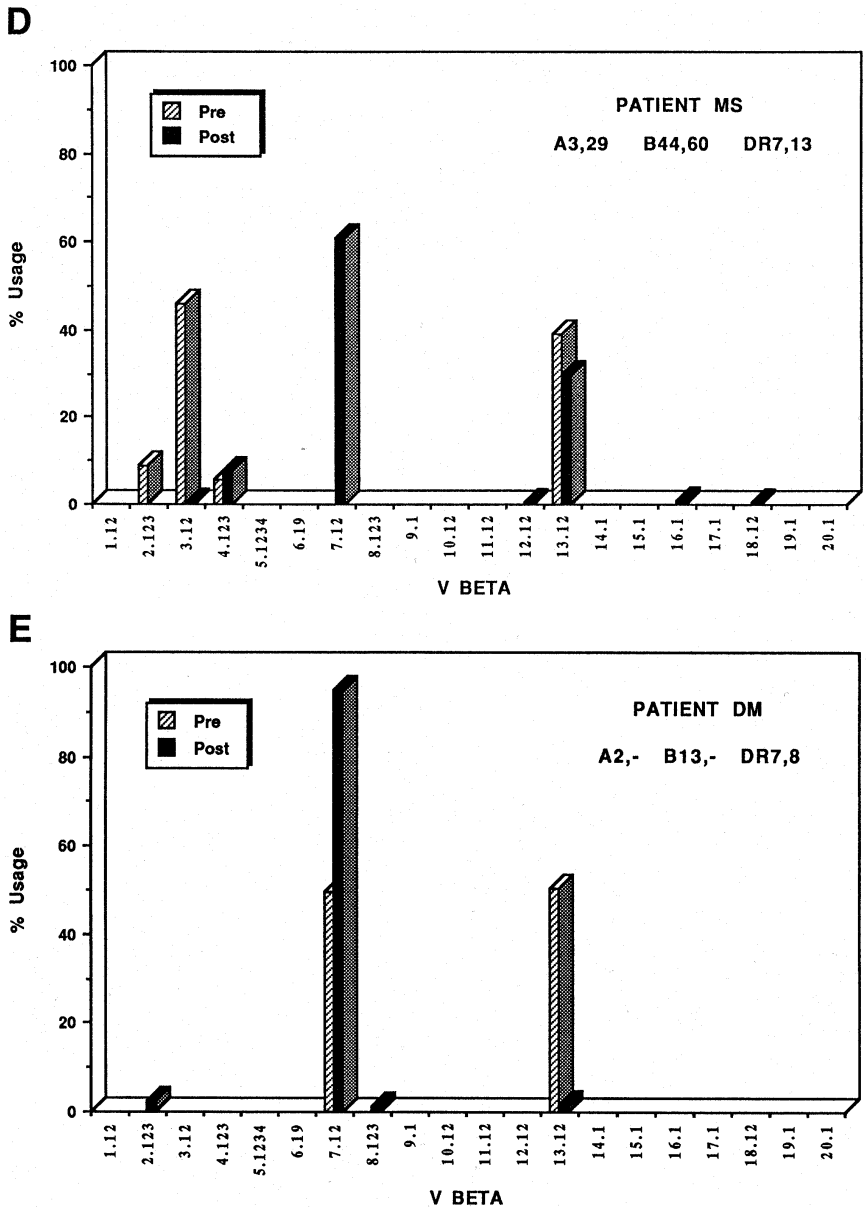

Fig. 2A–E Comparison of TIL Vβ usage before and after HLA-B7 gene transfer. TIL were isolated from cutaneous melanoma nodules prior to gene transfer and at 48 h following the third injection of pHLA-B7 DNA-liposome complexes. *Hatched* bars pretreatment TIL, *solid* bars post-treatment TIL

comprised the complete repertoire of TIL obtained from the uninjected post-treatment nodule. It should be noted that Vβ8, which accounted for more than 80% of the pretreatment TIL repertoire, represented less than 2% of Vβ usage by all three post-treatment TIL populations. Hence, HLA-B7 gene transfer reproducibly selected for T cells utilizing similar Vβ elements, and these alterations in Vβ usage were apparent in nodules that were not directly transduced to express the foreign MHC protein.

Increased Vβ heterogeneity following HLA-B7 gene transfer is associated with increased CD4 expression by TIL

While introduction of HLA-B7 increased the number of Vβ families utilized by TIL from all patients evaluated, the degree of Vβ heterogeneity of post-treatment TIL varied between patients, ranging from four to nine families (patients DM and AP respectively). Hence, CD4 and CD8 expression was determined for TIL obtained before and after gene transfer, and compared to the degree of Vβ heterogeneity for four patients (Table 1). Vβ heterogeneity is illustrated by the total number of Vβ families represented, as well as by the number of families required to compose more than 90% of the repertoire [20]. For the four patients evaluated, pretreatment TIL were highly restricted, with only one to two families comprising more than 90% of the Vβ repertoire, and these TIL were predominantly $CD8^+$ T cells. Though a greater number of Vβ families were represented in post-treatment TIL of patients DM and GB, one family still represented more than 90% of the repertoire, and these TIL were uniformly $CD8^+$. In contrast, post-treatment TIL from patients GM and AP were more heterogeneous, and this heterogeneity was present in TIL

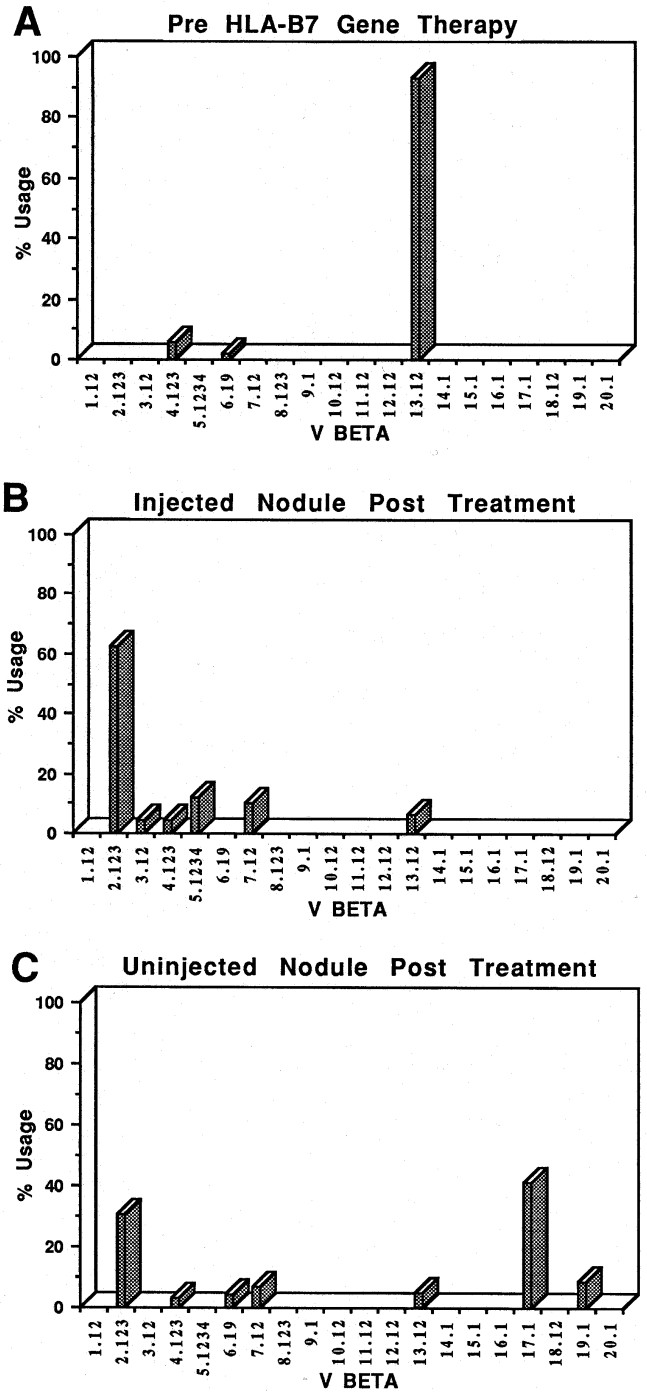

Fig. 3A–C HLA-B7 gene transfer alters Vβ usage by TIL derived from treated and untreated nodules. Various TIL populations were derived from patient GM before treatment (**A**), from an pHLA-B7-injected nodule (**B**), and from an uninjected nodule after treatment (**C**)

obtained from both pHLA-B7-injected and uninjected nodules. It is worth noting that the increased Vβ heterogeneity of these post-treatment TIL was associated with an increased number of $CD4^+$ T cells. For example, post-treatment TIL, obtained from pHLA-B7-injected and uninjected-nodules from patient GM, were 80% and 30% $CD4^+$, respectively, compared to 2.9% $CD4^+$ for pretreatment TIL. A similar, though less marked increase in CD4 expression was noted for post-treatment TIL from patient AP.

Discussion

Tumor cell growth can be directly related to their ability to evade the immune system. Tumors may actively inhibit the immune response by producing immunosuppressive factors [12, 32, 38], or by inducing the development of regulatory cells with suppressive activity [1, 2, 6]. Since tumors arise from self-tissue, they may be poorly immunogenic and, therefore, may be viewed as self by the immune system. Likewise, tumor cells may have a decreased capacity to present antigen in association with class I MHC, owing to deficiencies in β2 microglobulin, peptide transporter, or proteosome expression [22, 37, 46]. Genetic modification of tumors shows promise as a therapeutic modality to enhance the production of immunopotentiating cytokines, as well as increase the antigenicity of tumor cells [4, 9, 13–16, 23, 26, 28, 34, 39, 43]. In the current study, human melanoma nodules were genetically transduced to express a foreign MHC molecule, HLA-B7, and its associated β2 microglobulin. As a measure of immune responsiveness to allogeneic MHC gene transfer, we have evaluated the repertoire of TIL derived from transduced and nontransduced tumor nodules.

It has long been appreciated that the frequency of alloantigen-reactive T cells is quite high, even in naive animals that have not been previously immunized with alloantigens [25]. Since T cells with the potential to respond to foreign MHC molecules are dispersed throughout the immune system at frequencies 100–1000 times greater than that of T cells responsive to nominal antigen, alloantigens are potent immunogens. Alloantigen-reactive T cells may directly recognize intact MHC proteins (direct recognition), or may respond to allogeneic MHC peptides that have been processed and presented in the context of self-MHC proteins by autologous antigen-presenting cells (indirect recognition) (Reviewed in [41]). Direct recognition is likely the dominant pathway by which $CD8^+$ CTL respond to allogeneic class I MHC, while the indirect pathway may play an important role in stimulating regulatory $CD4^+$ helper T lymphocytes. Hence, gene transfer of a foreign class I MHC molecule has the potential of stimulating both effector and regulatory arms of the immune response.

Owing to the limited availability of biopsy material, this study evaluated Vβ usage by TIL expanded by in vitro culture. Hence, the influence of HLA-B7 gene transfer on the repertoire of T cells that are present in freshly isolated tumor biopsy material was not determined. However, published reports indicate that the repertoire of T cells that infiltrate tumors is diverse [5, 10], though certain clones may be expanded in situ [11, 17, 24, 36, 47]. Selection for dominant T cell clones occurs in TIL cultures, and the presence of IL-2 in these cultures favors expansion of T cells that were activated in vivo. Prior to HLA-B7 gene

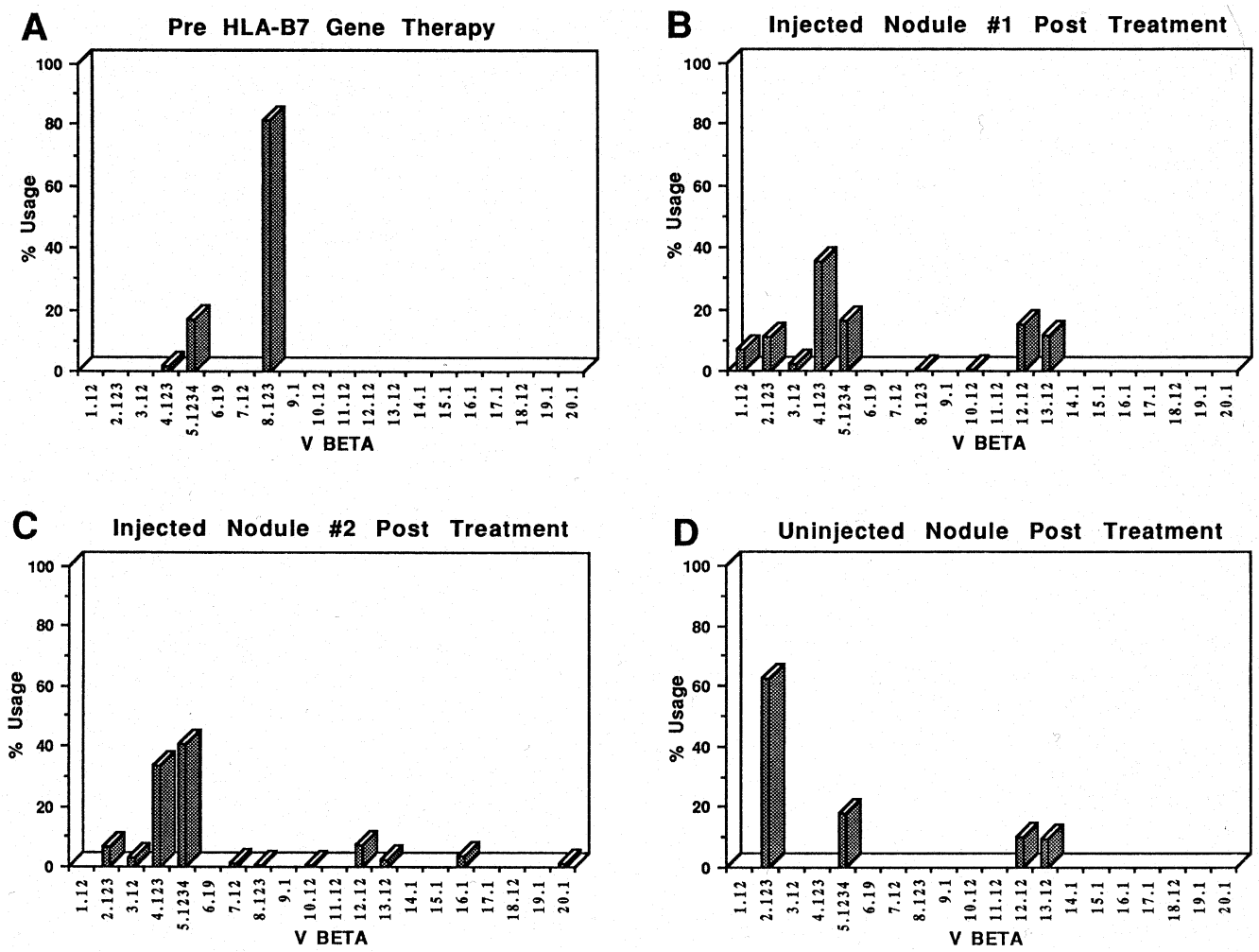

Fig. 4A–D Comparative analysis of Vβ usage by TIL derived from multiple melanoma nodules following HLA-B7 gene transfer. TIL were derived from patient AP before treatment (**A**), from two distinct pHLA-B7-injected nodules (**B, C**), and from an uninjected nodule after treatment (**D**)

Table 1 Vβ heterogeneity following HLA-B7 gene therapy is associated with increased CD4 expression by tumor-infiltrating lymphocytes (*TIL*)

Patient	*TIL* sample	CD3 (%)	CD4 (%)	CD8 (%)	Numbers of families	
					Vβ[a]	Vβ ≥90%[b]
GM	Before therapy	97.3	2.9	97.3	3	1
	After therapy	96.3	80.4	16.2	6	4
	After therapy, uninjected nodule	95.2	30.2	65.5	7	5
AP	Before therapy	99.7	1.2	97.6	3	2
	After therapy	95.7	10.6	86.4	9	6
	After therapy, uninjected nodule	98.2	8.9	89.5	4	3
DM	Before therapy	99.6	0.1	99.2	2	2
	After therapy, injected nodule	99.9	1.2	98.5	4	1
GB	Before therapy	99.5	0.1	97.7	2	2
	After therapy, injected nodule	99.5	0.1	99.6	5	1

TIL were assessed for CD3, CD4, and CD8 expression by flow cytometry
[a] Total number of Vβ families utilized by each TIL population
[b] Number of Vβ families required to comprise 90% of the total repertoire

transfer, Vβ usage by cultured TIL was highly restricted (Fig. 1). Similar restricted Vβ usage has been reported for TIL derived from a variety of tumor types [3, 11, 17, 33, 36, 40, 44], though the degree of Vβ restriction may vary. The highly restricted Vβ usage by pretreatment TIL in this study suggests that a limited number of immunodominant tumor antigens were recognized by pretreatment TIL. The critical point of this study is that HLA-B7 gene transfer altered the repertoire of T cells that surfaced in TIL cultures (Fig. 2), indicating that distinct T cell populations, likely with distinct antigen specificities, were activated in vivo. In most cases, the T cells that dominated pretreatment TIL cultures were replaced following gene transfer, giving rise to a more diverse population. This effect was systemic, in that TIL derived from uninjected nodules after treatment exhibited a similar altered Vβ profile (Figs. 3, 4). Hence, T cells induced by HLA-B7 gene transfer have a selective advantage over cells that were initially recovered from pretreatment tumor nodules. The mechanisms responsible for this altered dominance are currently not known, but may include in situ expansion or an enhanced state of activation of these T cells.

The antigen specificity of TIL obtained prior to and following HLA-B7 gene transfer has not been determined and is currently under investigation. Since TIL obtained from distinct, untreated nodules expressed the same altered Vβ profile as those obtained from nodules that had been injected with the HLA-B7 gene, it is unlikely that all cells in these TIL populations are reactive with HLA-B7. Further, HLA-B7 therapy increased the ability of TIL to lyse autologous, HLA-B7-negative tumor cells and to secrete proinflammatory cytokines upon stimulation with autologous, non-transduced tumor cells in some patients [30]. These observations support the idea that HLA-B7 therapy promotes the differentiation and/or expansion of T cells responsive to pre-existing tumor antigens. It is possible that HLA-B7 gene therapy produces an adjuvant effect, thus stimulating the development of additional tumor-reactive T cells expressing distinct TCR. One hypothesis is that HLA-B7-reactive T cells respond to the locally expressed alloantigen, which may be presented directly to $CD8^+$ cells, as well as indirectly by infiltrating macrophages to $CD4^+$ cells. Cytokines produced by these HLA-B7-reactive cells could potentiate and expand the antitumor immune response at several levels: (1) by providing help for the development of additional tumor-reactive T cell clones, (2) up-regulating MHC and therefore antigen expression by tumor cells [8, 42], and (3) by increasing antigen-presenting cell recruitment and function within the tumor [7]. This amplified antitumor response would likely be reflected by increased diversity in the composition of TIL. In support of this possibility, post-treatment TIL that expressed the most heterogeneous Vβ repertoires had an increased percentage of $CD4^+$ T cells (Table 1).

The biological consequence of altered Vβ expression by TIL following HLA-B7 transfer is not fully understood. Of the patients enrolled in this study, a clinically significant response to therapy was noted only in patient AP [30]. Following HLA-B7 gene transfer, both the pHLA-B7-injected and uninjected nodules of patient AP underwent partial regression. Patient AP subsequently received TIL therapy utilizing post-treatment TIL that expressed the heterogeneous Vβ repertoire depicted in Fig. 4. This TIL therapy resulted in complete regression of all melanoma nodules, suggesting potent in vivo antitumor activity of the TIL induced by HLA-B7 gene transfer. It should be noted that post-treatment TIL expressed the greatest degree of Vβ heterogeneity of those assessed in this study. Hence, a phase I clinical trial that employs treatment with TIL derived from pHLA-B7-injected nodules is being initiated at this institution.

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