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(54) Title: RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS (57) Abstract Methods are described to obtain nucleic acid molecules that encode T cell receptors and their derivatives that are human HLA-restricted and which are specific for tumor-associated antigens found in human tumors. These nucleic acids are useful in preparing recombinant cells for diagnosis and therapy of human tumors.		

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RECOMBINANT CONSTRUCTS ENCODING T CELL RECEPTORS SPECIFIC
FOR HUMAN HLA-RESTRICTED TUMOR ANTIGENS

5 Technical Field

The invention is directed to recombinant T cell receptors and modified forms thereof that are useful in identifying displayed tumor antigens and in antitumor therapy.

Background Art

10 Cytotoxic T lymphocytes (CTLs) form an essential part of an immune response to infectious agents and to malignancies. Thus, CTLs which are directed to established tumors may be effective in destroying these targets. Greenberg, P.D. *Adv Immunol* (1991) 49:281-355. CTL may also be used to identify tumor-specific antigens such as MAGE, GP100, tyrosinase, and MART, as well as broadly expressed tumor-associated
15 antigens such as P53 (Yanuck, M. *et al. Cancer Res* (1993) 53:3257-3261) ; Houviers, J.G.A. *et al. Eur J Immunol* (1993) 23:2072-2077; Her-2/neu (Peoples, G.E. *et al. Proc Natl Acad Sci USA* (1995) 92:432-436; Fisk, B. *et al. J Exp Med* (1995) 181:2109-2177; as well as the tumor antigen Ras (Skipper, J. *et al. J Exp Med* (1993) 177:1493-1498).

20 It has been typical that such tumor-specific CTLs have been obtained from tumor infiltrating lymphocytes (TILs). However, this is subject to a number of disadvantages due to the complexity of the system and the endogenous mechanisms to counteract the effect of these CTLs. Importantly, the most effective CTLs may have been eliminated (Schwartz, R.H. *Cell* (1989) 57:1073-1081); the target tumors may
25 have become resistant (Browning, M.J. *et al. Curr Opin Immunol* (1992) 4:613-618); or the T cells may lose functional activity by down-regulating expression of the ζ chain of the CD3 complex or the p⁵⁶ LCK molecules (Mizoguchi, H. *et al. Science* (1992) 258:1795-1798).

 In order to overcome these disadvantages, the present applicants have used
30 transgenic mice as a source of CTLs that contain the desired nucleotide sequences

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encoding TCRs specific for tumor-associated antigens restricted by human HLAs. Both humans and HLA-A2 transgenic mice select the same A2-restricted antigenic epitopes from influenza (Vitiello, A. *et al. J Exp Med* (1991) 173:1007-1015). Also, the present applicants have shown that HLA-A2 transgenic mice can produce p53-specific, A2 restricted CTLs when immunized with certain p53 derived peptides. Theobald, M. *et al. Proc Natl Acad Sci USA* (1995) 92:11993-11997.

Of course, if murine-derived TCRs are to be used in a human context, humanization of such TCRs would be advantageous. In order to avoid competition for dimerization with endogenous $V\alpha/C\alpha$ or $V\beta/C\beta$ TCR, it may be advantageous to prepare chimeric TCRs using the ζ region of the CD3 receptor as the transmembrane and cytoplasmic domain. Such constructs could be prepared in either dimeric or single-chain form. Competition by $V\alpha/C\alpha$ or $V\beta/C\beta$ for each other or for the availability of CD3 chains has already been shown by Gorochoff, *International J Cancer* (1992) 8:53-57 and by Wegener, A.M.K. *et al. Cell* (1992) 68:83. Chimeric $V\alpha/\zeta + V\beta/\zeta$ chimeras were described by Engel, I. *et al. Science* (1992) 256:1318 who also showed that such chimeras could be activated by exposure to the appropriate antigen-MHC complex. In addition, Irving, B.A. *et al. Cell* (1991) 64:891 reported that chimeric molecules composed of the CD8/ ζ or CD16/ ζ and expressed in T cells had the capacity to transduce activation signals for IL-2 production and mediated specific cell lysis in a manner indistinguishable from those generated by the TCR/CD3 complex. In addition, Chung, S. *et al. Proc Natl Acad Sci USA* (1994) 91:12654-12658 constructed a single-chain TCR (scTCR) using the ζ -chain of CD3 and expressed it in T cells, thus conferring the T cells with the relevant specificity. These T cells further produce IL-2 on activation with the specific antigen. The present applicants have further confirmed this approach using clone 4 TCR as a model system.

However, there remains a need for a convenient source of nucleic acids encoding TCR molecules and their modified forms which are human HLA restricted and specific for common tumor-associated antigens. The present invention supplies this need.

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Disclosure of the Invention

The invention provides materials that are useful in tumor diagnosis and therapy by permitting altered T lymphocytes to recognize and destroy unwanted tumor tissue. T cell receptor-encoding nucleic acid molecules can be obtained by immunizing
5 transgenic mice which produce human HLA with tumor-associated antigens and recovering the nucleic acids encoding the T cell receptors from the cytotoxic T lymphocytes (CTL).

Thus, in one aspect, the invention relates to a method to prepare an isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one of the
10 variable regions of the α and β chains of a non-human TCR which TCR is human HLA-restricted and specific for a tumor-associated antigen, which method comprises cloning or amplifying a nucleic acid molecule containing said encoding nucleotide sequence from the CTL prepared by a method which comprises immunizing a transgenic non-human vertebrate which is modified so as to express at least one human
15 HLA antigen with said tumor-associated antigen (TAA) so as to effect the production in said mouse of cytotoxic T lymphocytes which display human HLA-restricted TCR specific for said TAA and which contain nucleic acid molecules comprising nucleotide sequences encoding the α and β chain of said TCR and recovering the CTL.

In other aspects, the invention relates to nucleic acid molecules obtained by the
20 foregoing method and to constructs employing their variable regions, to cells displaying TCRs or derivatives encoded by said nucleic acids or their modified forms, and use of these materials in diagnosis and therapy of human tumors.

Brief Description of the Drawings

25 Figure 1 shows the structure of several derivatives of effective T cell receptors wherein the ζ region is substituted as a chimeric transmembrane and cytoplasmic region.

Figure 2 shows, in more detail, the construction of the nucleotide sequence encoding such derivatives.

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Figure 3 shows the complete nucleotide sequence and deduced amino acid sequence of a single chain TCR derivative which contains variable α and β specific for HA linked through a short peptide linker and then fused through a CD8 hinge to the ζ chain.

5 Figure 4 shows the ability of cells transfected with various modified TCR forms specific for HA to produce IL2 in response to stimulation with HA.

Figure 5 shows the ability of CTL's generated in mice in response to Her 2/neu-peptides H3 and H7 to mice H7 or H3 bearing targets. CTLs from both A2.1xK^bxCD8

10 and from A2.1 transgenic mice were comparable in result.

Figure 6 shows the sequence of various primers useful in cloning or amplifying the nucleotide sequences in coding during variable regions of α and β TCR chains.

Figures 7A and 7B show the nucleotide sequence and deduced amino acid sequence of the variable regions of the α and β chains of H7-specific TCR respectively.

15 Figure 8 shows a diagram of an expression vector suitable for producing the modified TCRs of the invention.

Figure 9 shows the ability of H7 specific modified TCR forms transfected in the 27J cells to effect IL2 production in said cells in response to the H7 peptide when the H7 peptide is presented in the presence of JA2 cells.

20 Figure 10 shows the ability of the various modified H7 specific TCR constructs to stimulate IL2 production in 27J cells in response to tissues bearing Her2/neu-peptides.

Modes of Carrying Out the Invention

25 The invention provides a convenient source for desirable recombinant materials that are useful in therapeutic and diagnostic procedures related to human tumors. Specifically, the materials of the invention provide a means whereby enhanced populations of cells that display appropriate TCRs for identifying and destroying tumor tissue may be obtained, as well as providing cells that are useful in evaluating the

30 tumor-associated antigen that could usefully be targeted.

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Briefly, the recombinant materials are obtained from CTL produced by immunizing nonhuman subjects with tumor-associated antigens associated with human tumors, where the nonhuman subject has been modified so as to be capable of expressing a human HLA. Thus, the relevant TCRs are not only specific for the human tumor-associated antigen, but also restricted by a human HLA. While murine subjects are clearly the most convenient at the present time, further developments in the construction of transgenic animals may permit alternative nonhuman subjects to be used equally conveniently in the near future. Such additional nonhuman subjects may include rats, avian subjects, larger mammals, or any appropriate vertebrate system that can be manipulated to provide it with human HLA and which can mount an immune response to provide CTLs with the appropriate T cell receptors.

Further, while the human HLA illustrated herein is A2, there is no theoretical reason why other HLA domains such as A1, A3, and B7 could not be used as well. Because transgenic mice are readily available which produce this antigen, the use of A2 as the restrictive antigen is simply a matter of convenience. In addition, if murine subjects are used, and the MHC region is entirely human, it is preferred to use mice transgenic so as to express human CD8 as well as human Class MHC antigen. This is due to the inability of murine CD8 to interact effectively with human A2.1. Thus, expression of human CD8 on the murine cells facilitates lysis of target antigen presenting cells. On the other hand, for mice transgenic for MHC human/mouse chimeras, such as A2K^b mice also exemplified below, the presence of human CD8 is not necessary

The recombinant materials relevant to the invention include those associated with the TCR produced by the nonhuman subject *per se*, and also derivatives of this TCR which retain their HLA restriction and specificity characteristics. Such derivatives contain the variable regions of the α and β chains either as dimers or in single chain form and are more advantageous than the nonhuman TCR *per se* for a number of reasons. First, if the desired TCR can be "humanized," less unwanted side-reactions can be expected. Second, economies of production can be effected if shorter peptides can be substituted for the TCR *per se*. Third, if the TCR is produced as a

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single chain, rather than in its customary dimeric form, economies of production and ease of association of the relevant variable units are achieved. In all cases, substituting a derivative for one or both of the α and β chains or a single-chain form containing variable regions of both α and β precludes the formation of hybrid TCRs wherein for example the desired TCR α chain is coupled with an endogenous TCR β . Thus, the recovery of cells which produce the desired derivative is greater.

Figures 1 and 2 describe some typical derivatives of TCRs useful in the invention. As shown in Figure 1, a dimeric form may be constructed wherein the variable regions of both α and β chains are directly coupled to the ζ regions of various CD receptors such as CD3, CD8 and CD16. These ζ regions substitute for the transmembrane and cytoplasmic regions normally associated with the TCR. In these examples, the constant region, as it is unnecessary, is eliminated in any case.

Further, in Figure 1, an alternative construction includes a CD8 hinge region between the variable region and the transmembrane portion of the ζ chain. This spacer may assist in appropriate folding of the receptor. Similarly, in Figure 1, construction of a single chain TCR wherein the variable regions of the α and β chains are fused through a linker and then fused to the ζ region is shown with and without the CD8 hinge.

Figure 2 shows a pattern for construction of the relevant plasmids containing the nucleotide sequences encoding the derivatives shown in Figure 1. As shown hereinbelow, a model system wherein clone 4 TCR directed against hemagglutinin antigen (HA) was used to supply the variable region verified the operability of these approaches.

It is important to recognize that the critical feature of the nucleic acid encoding the TCR derivative is the presence of the variable regions from the α and β chains, and that additional sequence, perhaps for added stability, including some or all of the constant region may be present. In addition, alternative transmembrane and signalling regions other than the ζ regions exemplified above may be substituted. Thus, the recombinant materials encoding the TAA-specific, human MHC restricted TCR derivatives of the invention need only include the variable α and β regions of the

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relevant TCR along with some additional transmembrane and signalling sequence and may further include additional non-interfering amino acid sequence.

The desired CTLs will be specific for TAAs associated with human cancers. Typical among these is Her-2/neu since this proto-oncogene is overexpressed in many human cancers and associated with aggressive disease and malignant transformation (Press, M.S. *et al. Cancer Res* (1994) 54:5675-5682; Slamon, D. *et al. Science* (1987) 235:177-182). Other suitable tumor-associated antigens include Ras, p53, tyranase, MART, Gp100, MAGE, BAGE and MUC-1. Any desired antigen which is associated with human tumors can readily be used.

The availability of nucleic acid molecules encoding the desired TCR permits of both diagnostic and therapeutic uses. Cells displaying the TCR at their surfaces can be used as diagnostic for the TAA that is actually expressed by the tumor. In order to conduct such assays, the tumor or a portion thereof or cells derived therefrom are exposed to cells transfected to contain an expression system for the TCR or derivative and the ability of the recombinant CTLs to lyse the tumor cells is assessed. The procedure described in Theobald, M., *et al. (1995) supra*, may, for example, be used. In addition, an expression for the appropriate TCR may be used therapeutically by transducing such an expression system into the peripheral blood lymphocytes (PBL) CD8⁺ T cells from a tumor-bearing host via, for example, retroviral-mediated gene transfer. Such transfer techniques are known in the art. See, for example, Kasid, A. *et al. Proc Natl Acad Sci USA* (1990) 87:473, Rosenberg, S.A. *et al. New England Journal of Medicine* (1990) 323:570. The altered CD8⁺ cells then provide a passive form of immunotherapy. Of course, humanized forms of the TCR as the appropriate derivatives are most helpful in this application.

The following examples are intended to illustrate but not to limit the invention.

Preparation A

Model System for TCR Derivatives

Clone 4 TCR (reference) is specific for the hemagglutinin antigen (HA). As the nucleotide sequences encoding the α and β chains of this TCR are available, constructs

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were made to mimic the intended derivatives of the TAA-specific, HLA-restricted TCR of the invention.

Briefly, four types of chimeric molecules were constructed: two are the dimers obtained as α/ζ + the β/ζ and two are single-chain TCR/ ζ chimeric molecules analogous to those shown in Figure 1 herein. The complete nucleotide sequence encoding the single chain form with the CD8 hinge is shown in Figures 3A-3B. These four constructs were transfected into the T cell hybridoma MD.45-27 and the transformants were grown under neomycin selection and screened for IL-2 secretion upon stimulation with either spleen cells from Balb/c or P815(H-2^d) cells pulsed with the HA-specific peptide or RENCA tumor cell line transfected with the HA gene. The results showing the levels of IL-2 produced are shown in Figure 4. As shown, none of the transfectants showed appreciable production of IL-2 in the absence of HA. Only the transfectants containing the clone 4 derivatives showed stimulation of IL-2 production when HA was present. Both single-chain forms, with and without the CD8 hinge and both dimeric forms, both with and without the CD8 hinge showed appreciable stimulation of IL-2 production when treated either with Balb/c spleen cells plus HA peptide, P815 cells plus HA peptide, or RENCA cells expressing HA at their surfaces.

20

Example 1

Selection of Her-2/neu Immunogenic Peptides

Eighteen peptides were synthesized based on the sequence of the human Her-2/neu protein wherein each sequence contained the anchor motif for HLA A2.1, that is, L, I, M, V, A, T at position 2 and position 8/9/10 (Rupert, J. *et al. Cell* (1993) 74:929-937). The binding efficiency of these peptides to A2 was determined using a competition assay as described by Morrison, J. *et al. Eur J Immunol* (1992) 22:903-907. Briefly, each test peptide (10 μ g) was incubated with radiolabeled target cells (T2-A2.1/K^b, 10⁶ target cells labeled with 150 μ g ⁵¹Cr at 37° for 1.5 hours) in the presence of an influenza virus matrix protein (0.1 μ g). The ability of these peptides to inhibit the binding of the influenza matrix protein peptide M1 (58-66) to A2.1 was

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measured by inhibition of lysis by an M1 (58-66) specific, A2.1 restricted CTL clone. As shown in Table 1, many of the tested peptides were able to inhibit binding of the M1 peptide.

Table 1. Her-2/neu peptides used for immunization				
PEPTIDE	SEQUENCE #	SEQUENCE	IMMUNOGENICITY	% INHIBITION
H3	369-377	KIFGSLAFL	+	38
H6	444-453	TLQGLGISWL	-	56
H7	773-782	VMAGVGSPYV	+	55
H8	546-555	VLQGLPREYV	-	43
H12	48-56	HLVQGOQW	-	15
H13	689-697	RLLOETELV	-	56
H14	747-755	KIPVAIKVL	-	35
H15	789-797	CLTSTVQLV	-	33
H16	799-807	QLMPYGCLL	-	50
H17	851-859	VLVKSPNHV	-	12
H18	871-879	DIDETEHYA	-	37
H19	933-941	DLLEKGERL	-	36
H20	971-979	ELVSEFSRM	-	5
H21	971-980	ELVSEFSRMA	-	25
H22	972-980	LVSEFSRMA	-	14
H23	1016-1024	DLVDAEEYL	-	35
H24	1172-1180	TLSPGKNGV	-	57
HIV-9K	POL	KLVGKLNWA	+	80

5

The peptides were then tested for their ability to elicit an immune response *in vivo*. The peptides were administered either to A2.1/K^bxCD8 or A2.1 transgenic mice and primary cultures of CTLs were generated. Mice were immunized with a mixture of 100µg of the Her-2/neu peptide with 120µg 'helper' peptide (the helper peptide is a I-A^b restricted peptide derived from Hepatitis B virus core protein comprising amino acid residues 128 to 140, that induces a strong CD4 helper response) in 100µl Incomplete Freund's adjuvant. A2.1/K^bxCD8 lipopolysaccharide (LPS)-blasts were prepared as stimulators for *in vitro* restimulation of spleen cells from immunized mice. These were prepared by incubating splenocytes in complete RPMI containing 25 µg/ml LPS and 7 µg/ml dextran sulfate at 1.5x10⁶ cells/ml in a total volume of 30 ml for 3

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days. Murine spleen cells, collected 10 days after immunization, were restimulated *in vitro* with the irradiated (3000rads) blasts which had bound Her-2/neu specific peptides. Six days following *in vitro* restimulation, the CTL populations were assayed for lytic activity against T2-A2.1/K^b target cells preincubated with the peptide used for stimulation (15μM). The resultant Her-2/neu peptide-specific CTL populations were maintained *in vitro* by weekly restimulation. CTL populations were restimulated in 2ml cultures by incubating with 0.1-0.2 x 10⁶ irradiated Jurkat-A2.1 cells (20,000 rad) preincubated with Her-2/neu peptide (15μM) and 5x10⁵ irradiated C57BL/6 spleen cells (3000 rad) as fillers in complete RPMI media containing 2% (v/v) supernatant from concanavalin A stimulated spleen cells (TCGF).

The cultured cells were assayed for cytotoxicity against T2A2.1/K^b target cells pulsed with the priming peptide. In the cytotoxicity assay, 10⁶ target cells were incubated at 37°C with 150 μCi of sodium ⁵¹Cr chromate for 90 minutes, in the presence or absence of specific peptide. Cells were washed three times and resuspended in 5% RPMI. For the assay, 10⁴ ⁵¹Cr-labeled target cells were incubated with different concentrations of effector cells in a final volume of 200μl in U-bottomed 96 well plates. Supernatants were removed after 4-7 hrs. at 37°C, and the percent specific lysis was determined by the formula: percent specific lysis = 100 x (experimental release-spontaneous release)/(maximum release-spontaneous release). As shown in Table 1, only the H3 and H7 peptides were able to stimulate a CTL response. (The HIV-9K peptide, known to be immunogenic, was used as a control.)

CTL populations that were specific for H3 and H7 were established from either murine strain and maintained *in vitro* by weekly restimulation. The results of testing these established cell cultures for their ability to lyse T2-labeled targets at a ratio of 1:1 in a four-hour assay in the presence of peptide H3 or H7 are shown in Figure 5. As shown, the CTLs from either murine subject were comparably effective at comparable peptide concentrations.

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Example 2Lysis of Human Tumors by H3- and H7-Specific CTL

Various tumor cell lines were characterized by FACS analysis for surface expression of A2 and Her-2/neu peptides. These tumor cells and other control tumors were preincubated or not for 24 hours in media supplemented with 20 ng/ml γ -IFN and 3 ng/ml TNF- α , as such pretreatment increases expression of MHC-1 and adhesion molecules thus enhancing their sensitivity to lysis (Fady, C. *et al. Cancer Immun Immunother* (1993) 37:329-336; Fisk, B. *et al. Lympho and Cytokine Res* (1994) 13:125-131). In the assay, the tumor cells were mixed with the H3- or H7-specific CTL for 6 hours and lysis was measured. HIV-9K-specific CTL were used as a control. The results are shown in Table 2.

TUMOR	TYPE	A2	Her-2	H7	H7 + CYT	H3	H3 + CYT	HIV-9K	HIV-9K + CYT
MDA.MB231	BREAST	+	+	26	89	34	85	3	14
MCF-7	BREAST	+	+	7	40	7	54	3	7
BT549	BREAST	+	+	2	36	2	40	2	15
SAOS.175	OSTEOSARCOMA	+	+	27	35	27	33	18	11
U2-OS	OSTEOSARCOMA	+	+	30	62	32	91	18	24
SW480	COLON	+	+	2	17	6	50	1	4
OVCAR-5	OVARIAN	+	+	13	23	25	29	10	12
T98G	GLIOBLASTOMA	+	+	29	93	20	99	9	13
MALME-3M	MELANOMA	+	+	4	14	28	57	2	1
SKMEL-5	MELANOMA	+	+	16	40	6	38	5	4
NCI.H1355	LUNG	+	+	13	62	11	38	7	25
Hep-G2	HEPATOMA	+	+	4	29	4	20	1	8
CASKI	CERVIX	+	+	9	20	13	30	8	11
U87G	GLIOBLASTOMA	+	-	1	1	2	1	5	1
ST486	LYMPHOMA	+	-	5	8	1	1	1	1
LG-2	EBV-TRANS.	+	-	1	3	2	4	1	1
SV80	FIBROBLAST	+	-	2	2	4	8	2	2
JY	LYMPHOMA	+	-	4	2	2	1	2	1
MDA.MB435	BREAST	-	+	1	1	3	2	4	3

As shown, the CTLs were able to lyse effectively only those tumors expressing both A2 and Her-2 peptides. Further, repeating the experiment in the presence of an anti-A2 antibody significantly decreased lysis, and H3 and H7 could be extracted from the tumors using standard techniques.

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In a manner similar to that set forth above with respect to H3 and H7, A2-restricted CTLs specific for p53 have been generated. Theobald, M. *et al.* (1995) (*supra*).

Example 3

5 Recovery of Genes Encoding Her-2/neu and p53 TCRs

The genes encoding the relevant α and β chains of the TCR specific for H3, H7, and p53 are cloned according to the method of Zisman, B. *et al. Eur J Immunol* (1994) 24:2497-2505. Primers for the PCR amplification according to these methods are derived from V α or V β families paired with C α or C β primer. Suitable primers for
10 use in this process are shown in Figure 6. The amplified PCR products are cloned into Bluescript vectors and sequenced. Figure 7 shows the sequences of the variable regions of the α and β chains of the TCRs recovered from CTLs recovered in mice that had been administered the H7 peptide.

Chimeric molecules similar to those described hereinabove for clone 4 and as
15 set forth in Figures 1 and 2 were prepared from the amplified sequences of the H7-specific RR functionality is assayed by transfecting MD45.27 and testing for the production of IL-2 as described hereinabove.

A preferred vector for the insertion of the modified sequences, pBJ1Neo with a polylinker insertion site is shown in Figure 8. The host vector, pBJ1Neo is described in
20 _____, *Mol Cell Biol* (1988) 8:466; the polylinker is described by _____, Science (1990) 249:677.

The dimer and single chain constructs were transfected into 27J cells and the cells measured for production of IL-2 in the presence of JA² cells plus H7 peptide. As shown in Figure 9, all transfectants produced with the H7 specific TCR derivatives
25 produced IL-2. 27J cells without these constructs did not produce IL-2 in response to the JA2 cells and peptide, and none of the cells produced IL-2 in response to JA2 cells alone.

Finally, Figure 10 shows the production of IL-2 by these four constructs transfected into 27J cells in response to HER 2/neu derived peptides and cells

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presenting such peptides. Again, all four constructs rendered the transfected cells responsive.

Example 4

5 Preparation of T cells Expressing TCR and its Derivatives

Human PBL that are CD8⁺ are transduced with the chimeric constructs described above using the LXS_N and LXSH retroviral vectors (Hock, R.A. *et al. Nature* (1986) 320:275) and the technique of Anderson, W.F. *Science* (1992) 256:808. The β chimeric gene is inserted into the LXSH retroviral vector which confers

10 Hygromycin B resistance and α chimeric gene in LXS_N retroviral vector which confers neomycin resistance; thus selection of T lymphocytes expressing both the Vα/ζ and Vβ/ζ can be recovered. Recombinant retrovirus-producing cell lines are generated by transfection of the vectors into the Ecotropic packaging cell line GP+E86 and the

15 ecotropic virus produced by these cells is used to infect the amphotropic packaging cell line PA317. PA317 clones that produce helper virus free from amphotropic L(Vα/ζ)_{SN} and L(Vβ/ζ)_{SH} virus are obtained by selection in G418 or Hygromycin B-containing medium. Clones yielding the highest titer of virus are used to transduce T lymphocytes that have been incubated with anti-CD3 and recombinant IL-2. Similarly, the single-chain TCR is inserted into LXS_N retroviral vector and introduced similarly.

20 The resulting transformed human CD8⁺-PBL are tested for cytotoxic activity *in vitro* against tumor cells and then *in vivo* in SCID mice that have received tumor cells displaying the relevant TAA.

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Claims

1. A method to prepare an isolated nucleic acid molecule comprising a nucleotide sequence encoding at least one of the variable regions of the α and β chains
5 of a non-human TCR which TCR is human HLA-restricted and specific for a tumor-associated antigen, which method comprises
cloning or amplifying a nucleic acid molecule containing said encoding nucleotide sequence from cytotoxic T lymphocytes (CTL) prepared by a method which comprises
10 immunizing a transgenic non-human vertebrate which is modified so as to express at least one human HLA antigen with said tumor-associated antigen (TAA) so as to effect the production in said mouse of cytotoxic T lymphocytes which display human HLA-restricted TCR specific for said TAA and which contain nucleic acid molecules comprising nucleotide sequences encoding said variable regions of the α and
15 β chains of said TCR, and
recovering said CTL.
2. The method of claim 1 wherein said HLA antigen is a A2.
- 20 3. The method of claim 1 wherein said non-human vertebrate is a mouse.
4. The method of claim 3 wherein said amplifying is effected by a polymerase chain reaction using primers derived from murine TCR.
- 25 5. The method of claim 4 wherein said primers are essentially as set forth in Figure 6.
6. An isolated nucleic acid molecule which comprises a nucleotide sequence encoding a variable region of a non-human TCR α or β peptide wherein said
30 TCR is human HLA-restricted and specific for a tumor-associated antigen.

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7. The nucleic acid molecule of claim 6 which comprises the α or β variable region of the said TCR fused to the ζ region of CD3, CD8 or CD16.

5 8. The nucleic acid molecule of claim 7 wherein said ζ region is that of human CD3, CD8 or CD16.

9. The nucleic acid molecule wherein said non-human TCR is murine.

10 10. The nucleic acid molecule of claim 6 wherein said nucleotide sequence encodes a single-chain TCR.

11. The nucleic acid molecule of claim 10 wherein said single-chain TCR consists of the variable α region fused to variable β region by a flexible linker and said
15 β region is fused to a ζ region.

12. The nucleic acid molecule of claim 11 wherein said flexible linker is of the formula $(\text{Gly}_4\text{Ser}_3)_3$.

20 13. The nucleic acid molecule of claim 11 wherein said ζ chain is that of CD3, CD8 or CD16.

14. The nucleic acid molecule of claim 13 wherein the ζ chain is derived from human CD3, CD8 or CD16.

25

15. A recombinant expression system which expression system comprises the nucleotide sequence of claim 6 operatively linked to control sequences for effecting its expression in a host cell.

- 16 -

16. A recombinant host cell modified to contain the expression system of claim 15.

17. The recombinant cells of claim 16 which are T cells.

5

18. A method to obtain cells which display TCR or a functional derivative thereof at their surface, said TCR or derivative being human HLA-restricted and specific for a tumor-associated antigen, which method comprises culturing the cells of claim 16 under conditions wherein said nucleotide sequence is expressed and said TCR or derivative is displayed at the surface.

10

19. Recombinant cells displaying a TCR receptor or derivative thereof at their surface wherein said TCR or derivative is human HLA-restricted and specific for a tumor-associated antigen prepared by the method of claim 18.

15

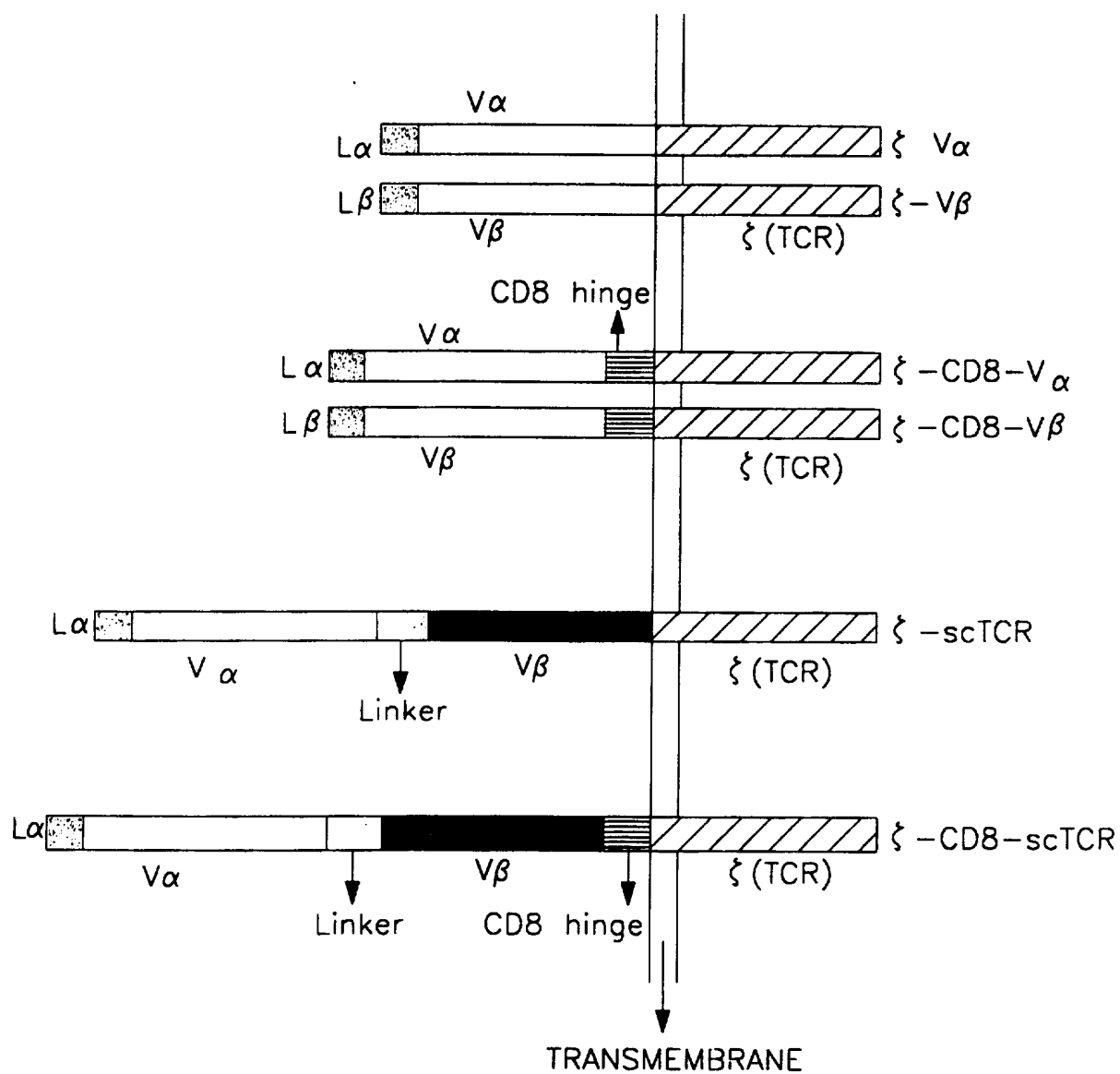
20. A method to identify antigens associated with a tumor which method comprises contacting said tumor or a fraction thereof with the cells of claim 19 under conditions wherein said tumor or fraction is lysed only if said tumor displays the TAA for which said TCR or derivative is specific.

20

21. A method to effect treatment of a tumor in a human, wherein said tumor is characterized by a specific tumor-associated antigen (TAA) which method comprises administering to said human subject peripheral blood cells from said subject which have been modified to contain an expression system for a nucleotide sequence which encodes a TCR or derivative thereof which is human HLA-restricted and specific for said TAA.

25

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**FIG. 1**

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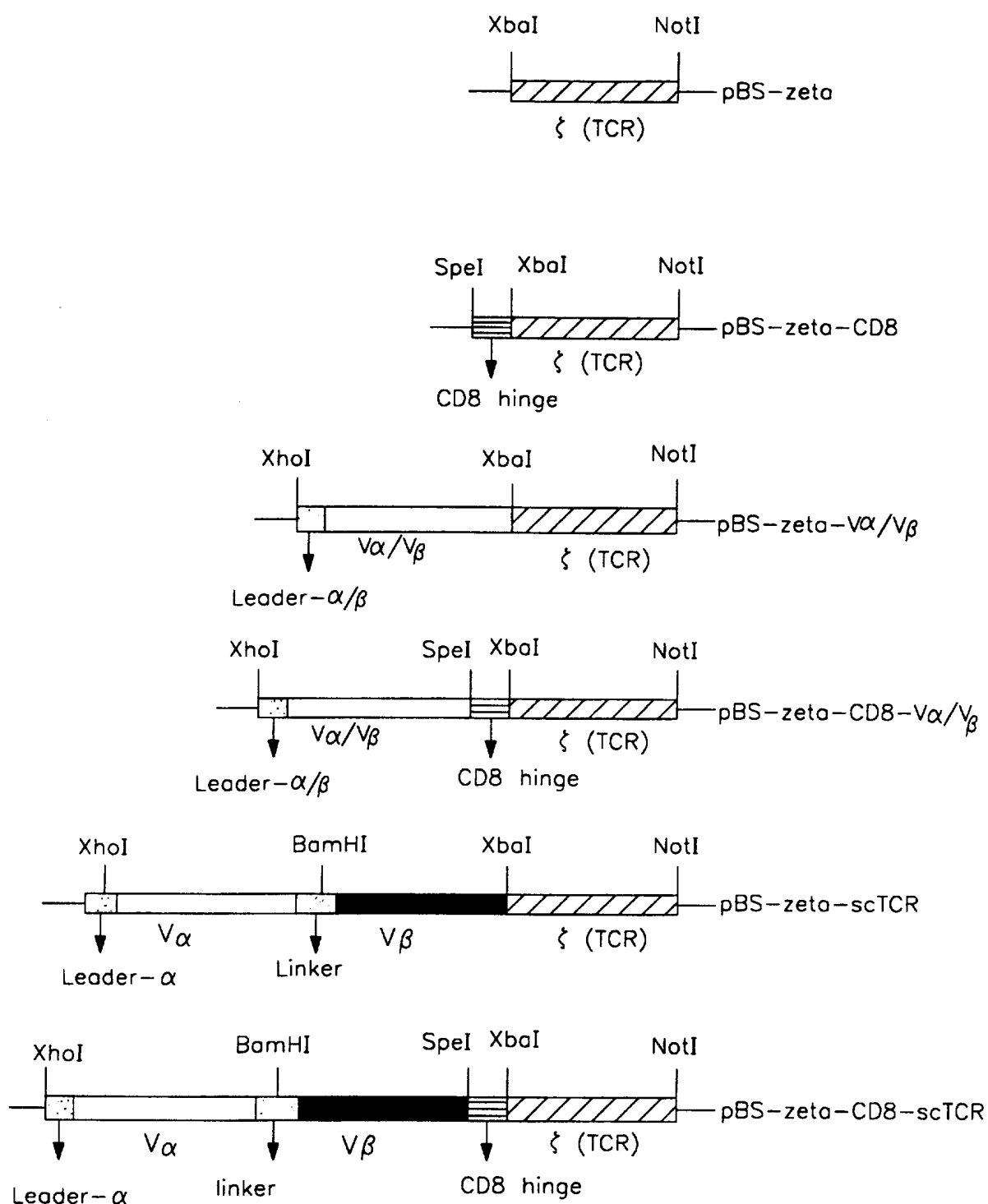


FIG. 2

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L α																	
XhoI	9	18	27	36	45	54											
CTC	GAG	ATG	CAG	AGG	AAC	CTG	GGA	GCT	GTG	CTG	GGG	ATT	CTG	TGG	GTG	CAG	ATT
L	E	M	Q	R	N	L	G	A	V	L	G	I	L	W	V	Q	I
		63	72	81	90	99	108										
TGC	TGG	CTG	AAA	GAA	CAG	CAA	GTG	CAG	CAG	AGT	CCC	GCA	TCC	TTG	GTT	CTG	CAG
C	W	L	K	E	Q	Q	V	Q	Q	S	P	A	S	L	V	L	Q
		117	126	135	144	153	162										
GAG	GGG	GAG	AAC	GCA	GAG	CTC	CAG	TGT	AGC	TTT	TCC	ATC	TTT	ACA	AAC	CAG	GTG
E	G	E	N	A	E	L	Q	C	S	F	S	I	F	T	N	Q	V
		171	180	189	198	207	216										
CAG	TGG	TTT	TAC	CAA	CGT	CCT	GGG	GGA	AGA	CTC	GTC	AGC	CTG	TTG	TAC	AAT	CCT
Q	W	F	Y	Q	R	P	G	G	R	L	V	S	L	L	Y	N	P
		225	234	243	252	261	270										
TCT	GGG	ACA	AAG	CAG	AGT	GGG	AGA	CTG	ACA	TCC	ACA	ACA	GTC	ATT	AAA	GAA	CGT
S	G	T	K	Q	S	G	R	L	T	S	T	T	V	I	K	E	R
		279	288	297	306	315	324										
CGC	AGC	TCT	TTG	CAC	ATT	TCC	TCC	TCC	CAG	ATC	ACA	GAC	TCA	GGC	ACT	TAT	CTC
R	S	S	L	H	I	S	S	S	Q	I	T	D	S	G	T	Y	L
		333	342	351	360	369	378										
TGT	GCC	TCA	AAT	TCT	GGA	GGA	AGC	AAT	GCA	AAG	CTA	ACC	TTC	GGG	AAA	GGC	ACT
C	A	S	N	S	G	G	S	N	A	K	L	T	F	G	K	G	T
		387	396	405	414	423	432										
AAA	CTC	TCT	GTT	AAA	TCA	GGT	GGC	GGA	GGG	TCT	GGC	GGG	GGT	GGG	TCC	GGG	GGT
K	L	S	V	K	S	G	G	G	G	S	G	G	G	G	S	G	G
		441	450	459	468	477	486										
GGA	GGC	TCA	GAG	GCT	GCA	GTC	ACC	CAA	AGC	CCA	AGA	AAC	AAG	GTG	GCA	GTA	ACA
G	G	S	E	A	A	V	T	Q	S	P	R	N	K	V	A	V	T
		495	504	513	522	531	540										
GGA	GGA	AAG	GTG	ACA	TTG	AGC	TGT	AAT	CAG	ACT	AAT	AAC	CAC	AAC	AAC	ATG	TAC
G	G	K	V	T	L	S	C	N	Q	T	N	N	H	N	N	M	Y
		549	558	567	576	585	594										
TGG	TAT	CGG	CAG	GAC	ACG	GGG	CAT	GGG	CTG	AGG	CTG	ATC	CAT	TAT	TCA	TAT	GGT
W	Y	R	Q	D	T	G	H	G	L	R	L	I	H	Y	S	Y	G

V α V β

FIG. 3A

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GCT	GGC	603 AGC	ACT	GAG	612 AAA	GGA	GAT	621 ATC	CCT	GAT	630 GGA	TAC	AAG	639 GCC	TCC	AGA	648 CCA
A	G	S	T	E	K	G	D	I	P	D	G	Y	K	A	S	R	P
AGC	CAA	657 GAG	AAC	TTC	666 TCC	CTC	ATT	675 CTG	GAG	TTG	684 GCT	ACC	CCC	693 TCT	CAG	ACA	702 TCA
S	Q	E	N	F	S	L	I	L	E	L	A	T	P	S	Q	T	S
GTG	TAC	711 TTC	TGT	GCC	720 AGC	GGT	GAG	729 ACA	GGG	ACC	738 AAC	GAA	AGA	747 TTA	TTT	TTC	756 GGT
V	Y	F	C	A	S	G	E	T	G	T	N	E	R	L	F	F	G
CAT	GGA	765 ACC	AAG	CTG	774 TCT	GTC	CTG	783 ACT	AGT	AAC	792 TCC	ATC	ATG	801 TAC	TTC	AGC	810 CAC
H	G	T	K	L	S	V	L	T	S	N	S	I	M	Y	F	S	H
TTC	GTG	819 CCG	GTC	TTC	828 CTG	CCA	GCG	837 AAG	CCC	ACC	846 ACG	ACG	CCA	855 GCG	CCG	CGA	864 CCA
F	V	P	V	F	L	P	A	K	P	T	T	T	P	A	P	R	P
CCA	ACA	873 CCG	GCG	CCC	882 ACC	ATC	GCG	891 TCG	CAG	CCC	900 CTG	TCC	CTG	909 CGC	CCA	TCT	918 AGT
P	T	P	A	P	T	I	A	S	Q	P	L	S	L	R	P	S	S
<u>AbaI</u> TCT	AGA	927 GAT	CCC	AAA	936 CTC	TGC	TAC	945 CTG	CTG	GAT	954 GGA	ATC	CTC	963 TTC	ATC	TAT	972 GGT
S	R	D	P	K	L	C	Y	L	L	D	G	I	L	F	I	Y	G
GTC	ATT	981 CTC	ACT	GCC	990 TTG	TTC	CTG	999 AGA	GTG	AAG	1008 TTC	AGC	AGG	1017 AGC	GCA	GAC	1026 GCC
V	I	L	T	A	L	F	L	R	V	K	F	S	R	S	A	D	A
CCC	GCG	1035 TAC	CAG	CAG	1044 GGC	CAG	AAC	1053 CAG	CTC	TAT	1062 AAC	GAG	CTC	1071 AAT	CTA	GGA	1080 CGA
P	A	Y	Q	Q	G	Q	N	Q	L	Y	N	E	L	N	L	G	R
AGA	GAG	1089 GAG	TAC	GAT	1098 GTT	TTG	GAC	1107 AAG	AGA	CGT	1116 GGC	CGG	GAC	1125 CCT	GAG	ATG	1134 GGG
R	E	E	Y	D	V	L	D	K	R	R	G	R	D	P	E	M	G

V β CD8
HINGEZ
CHAIN

FIG. 3B

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GGA	AAG	¹¹⁴³ CCG	AGA	AGG	¹¹⁵² AAG	AAC	CCT	¹¹⁶¹ CAG	GAA	GGC	¹¹⁷⁰ CTG	TAC	AAT	¹¹⁷⁹ GAA	CTG	¹¹⁸⁸ CAG	AAA
G	K	P	R	R	K	N	P	Q	E	G	L	Y	N	E	L	Q	K
GAT	AAG	¹¹⁹⁷ ATG	GCG	GAG	¹²⁰⁶ GCC	TAC	AGT	¹²¹⁵ GAG	ATT	GGG	¹²²⁴ ATG	AAA	GGC	¹²³³ GAG	CGC	¹²⁴² CGG	AGG
D	K	M	A	E	A	Y	S	E	I	G	M	K	G	E	R	R	R
GGC	AAG	¹²⁵¹ GGG	CAC	GAT	¹²⁶⁰ GGC	CTT	TAC	¹²⁶⁹ CAG	GGT	CTC	¹²⁷⁸ AGT	ACA	GCC	¹²⁸⁷ ACC	AAG	¹²⁹⁶ GAC	ACC
G	K	G	H	D	G	L	Y	Q	G	L	S	T	A	T	K	D	T
TAC	GAC	¹³⁰⁵ GCC	CTT	CAC	¹³¹⁴ ATG	CAG	GCC	¹³²³ CTG	CCC	CCT	¹³³² CGC	TAA	^{NOT I} ¹³⁴¹ GCG	GCC	GCC	ACC	¹³⁵⁰ GCG
Y	D	A	L	H	M	Q	A	L	P	P	R	*	A	A	A	T	A

STOP

FIG. 3C

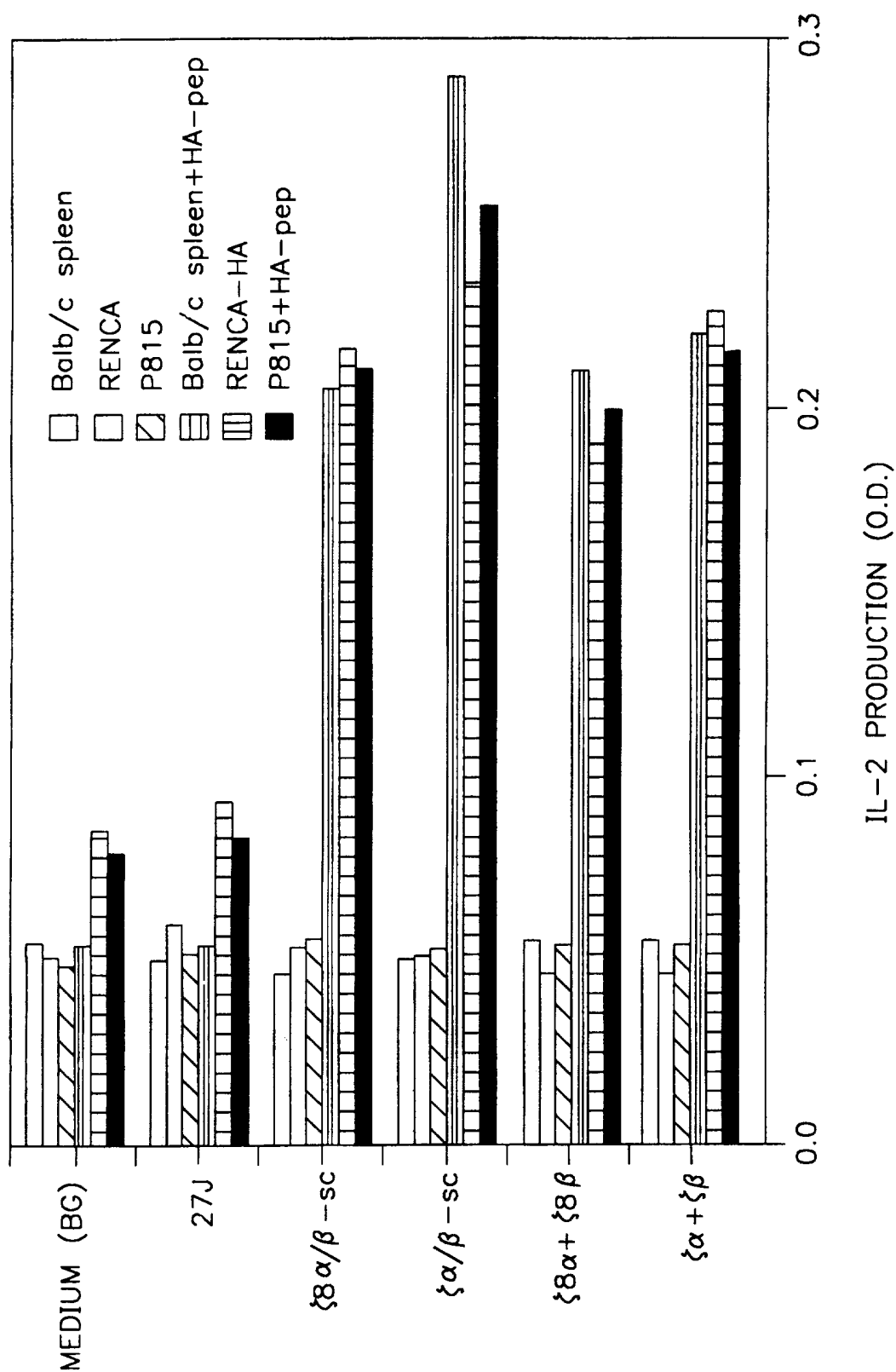


FIG. 4

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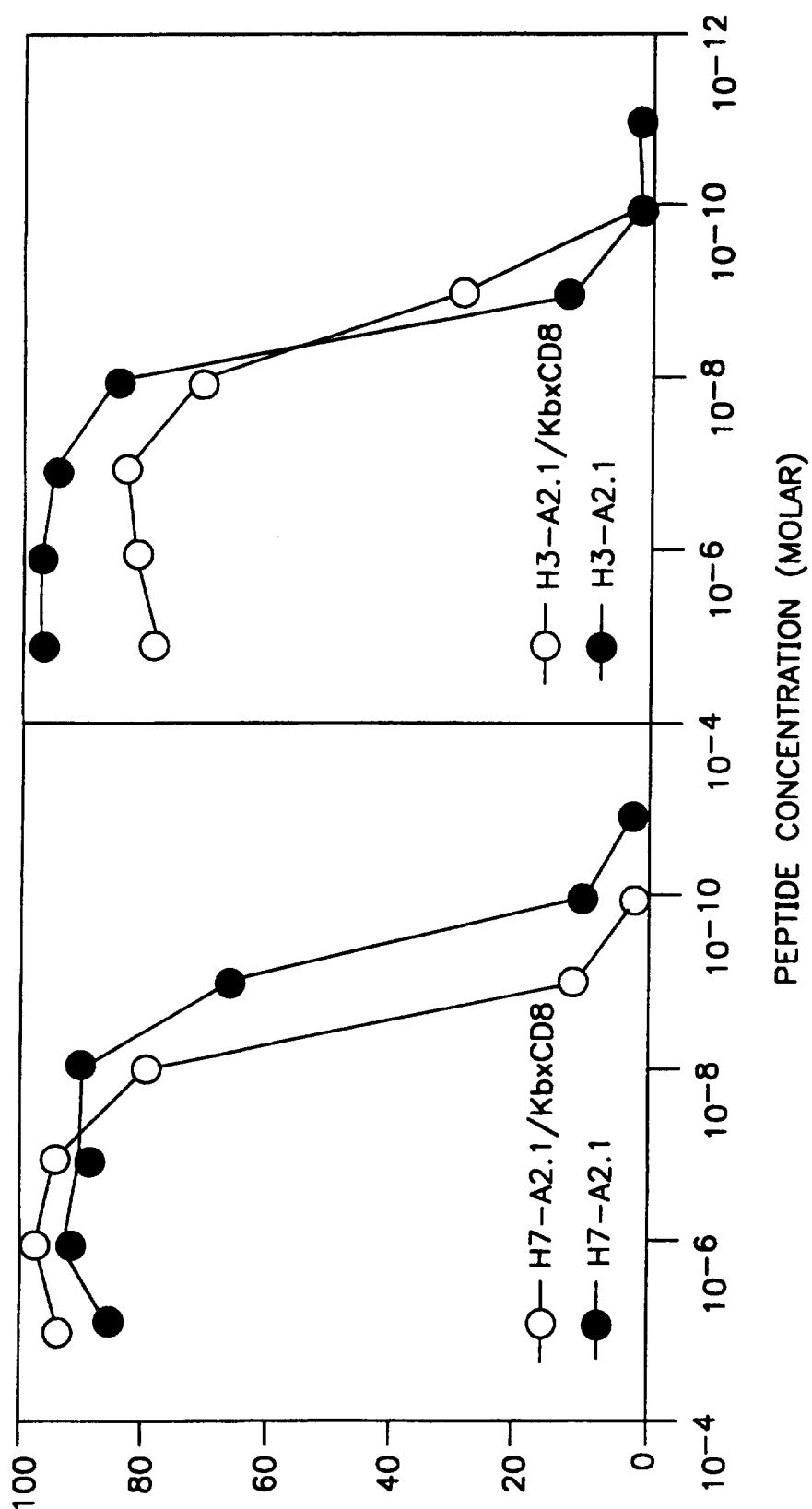


FIG. 5

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Alpha Groups

1.										
V α 1	CCC	AAG	GCA	CTG	ATG	TTC	ATC	TTC		
V α 2	TGA	GAC	AAA	GTC	CCC	AAT	CTC	TGA	CAG	
V α 3	CTG	CAG	CTG	CTC	CTC	AAG	TAC	TAT	TC	
V α 4.1.2.3	TCC	CGG	AGA	AGG	TCC	ACA	GTT	CCT	CTT	T
V α 4.4	GAA	GCA	GCA	GAG	GGT	TTG	AAG	CCA	CAT	AC
2.										
V α 5	GGC	AGG	TCT	TCA	GTT	GCT	TAT	GAA	GGT	
V α 6	GGT	TCC	TCT	TCA	GGG	TCC	AGA	ATA	TGT	
V α 7	GCG	AAG	AAC	TCA	CCC	TGG	ACT	GTT	CAT	
V α 8	GAG	CTC	CAC	AGA	CAA	CAA	GAG	GAC	CGA	GCA
V α 9	GAG	CTG	CGA	CGT	TCC	TTA	GTG	ACT	GTG	
3.										
V α 10	CCT	CGT	CAG	CCT	GTT	GTC	CAA	TCC	TTC	TGG
V α 11	CAG	CCT	CAT	CAA	TCT	GTT	CTA	CTT	GGC	T
V α 12	CCA	CCA	GGG	ACC	ACA	GTT	TAT	CAT	TCA	A
V α 14	ACC	TGG	AGA	GAA	TCC	TAA	GCT	CAT	CAT	
V α 15	AGG	TCT	TGT	GTC	CCT	GAC	AGT	CCT	GGT	T
4.										
V α 16	CAA	GCA	AAC	ACT	GTA	GTG	CAG	AGC	CCT	TCC
V α 17	CAA	GAC	ATC	CAT	AAC	TGC	CCT	ACA	G	
V α 18	GTG	TAT	GAA	ACC	CAG	GAC	AGT	TCT	TAC	
V α 19	CCG	TAT	TTC	TTT	CTT	ATG	TTG	TTT	TGG	AT
V α 20	CAA	AGC	TCT	CCA	TCG	CTG	ACT	GTT	CAA	G

Beta Groups

1.										
V β 1	ATC	TAA	TCC	TGG	GAA	GAG	CAA	AT		
V β 2	GGC	GTC	TGG	TAC	CAC	GTG	GTC	AA		
V β 3	GTG	AAA	GGG	CAA	GGA	CAA	AAA	GC		
V β 4	GAT	ATG	CGA	ACA	GTA	TCT	AGG	C		
V β 5.1	ACA	TAA	TCA	AAG	GAA	AGG	GAG	AA		
2.										
V β 6	TCC	TGA	TTG	GTC	AGG	AAG	GGC	AA		
V β 7	TAC	CTG	ATC	AAA	AGA	ATG	GGA	GA		
V β 8.1	ATA	ACC	ATG	ACA	ATA	TGT	ACT	GG		
V β 8.2	ATA	ACC	ACA	ACA	ACA	TGT	ACT	GG		
V β 8.3	ATA	GCC	ACA	ACT	ACA	TGT	ACT	GG		
3.										
V β 9	AGC	TTG	CAA	GAG	TTG	GAA	AAC	CA		
V β 10	GAT	TAT	GTT	TAG	CTA	CAA	TAA	TA		
V β 11	ACA	AGG	TGA	CAG	GGA	AGG	GAC	AA		
V β 12	ACC	TAC	AGA	ACC	CAA	GGA	CTC	AG		
V β 13	CAG	TTG	CCC	TCG	GAT	CGA	TTT	TC		
4.										
V β 14	GCC	GAG	ATC	AAG	GCT	GTG	GGC	AG		
V β 15	AGA	ACC	ATC	TGT	AAG	AGT	GGA	AC		
V β 16	CAT	CAA	ATA	ATA	GAT	ATG	GGG	CA		
V β 17	GTA	GTC	CTG	AAA	AAG	GGC	ACA	CT		
V β 18	CAT	CTG	TCA	AAG	TGG	CAC	TTT	A		

FIG. 6

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ATG	AAA	⁹ TCC	TTG	AGT	¹⁸ GTT	TCC	CTA	²⁷ GTG	GTC	CTG	³⁶ TGG	CTC	CAG	⁴⁵ TTA	AAC	TGG	⁵⁴ GTG
Met	Lys	Ser	Leu	Ser	Val	Ser	Leu	Val	Val	Leu	Trp	Leu	Gln	Leu	Asn	Trp	Val
CAG	AGC	⁶³ CAG	CAG	AAG	⁷² GTG	CAG	CAG	⁸¹ AGC	CCA	GAA	⁹⁰ TCC	CTC	AGT	⁹⁹ GTC	CCA	GAG	¹⁰⁸ GGA
Gln	Ser	Gln	Gln	Lys	Val	Gln	Gln	Ser	Pro	Glu	Ser	Leu	Ser	Val	Pro	Glu	Gly
GGC	ATG	¹¹⁷ GCC	TCT	CTC	¹²⁶ AAC	TGC	ACT	¹³⁵ TCA	AGT	GAT	¹⁴⁴ CGC	AAT	TTT	¹⁵³ CAG	TAT	TTC	¹⁶² TGG
Gly	Met	Ala	Ser	Leu	Asn	Cys	Thr	Ser	Ser	Asp	Arg	Asn	Phe	Gln	Tyr	Phe	Trp
TGG	TAC	¹⁷¹ AGA	CAG	CAT	¹⁸⁰ TCT	GGA	GAA	¹⁸⁹ GGC	CCC	AAA	¹⁹⁸ GCA	CTG	ATG	²⁰⁷ TCC	ATC	TTC	²¹⁶ TCT
Trp	Tyr	Arg	Gln	His	Ser	Gly	Glu	Gly	Pro	Lys	Ala	Leu	Met	Ser	Ile	Phe	Ser
GAT	GGT	²²⁵ GAC	AAG	AAA	²³⁴ GAA	GGC	AGA	²⁴³ TTC	ACA	GCT	²⁵² CAC	CTC	AAT	²⁶¹ AAG	GCC	AGC	²⁷⁰ CTG
Asp	Gly	Asp	Lys	Lys	Glu	Gly	Arg	Phe	Thr	Ala	His	Leu	Asn	Lys	Ala	Ser	Leu
CAT	GTT	²⁷⁹ TCC	CTG	CAC	²⁸⁸ ATC	AGA	GAC	²⁹⁷ TCC	CAG	CCC	³⁰⁶ AGT	GAC	TCC	³¹⁵ GCT	CTC	TAC	³²⁴ TTC
His	Val	Ser	Leu	His	Ile	Arg	Asp	Ser	Gln	Pro	Ser	Asp	Ser	Ala	Leu	Tyr	Phe
TGT	GCA	³³³ GTT	ATG	GAT	³⁴² TAT	AAC	CAG	³⁵¹ GGG	AAG	CTT	³⁶⁰ ATC	TTT	GGG	³⁶⁹ CAG	GGT	ACC	³⁷⁸ AAG
Cys	Ala	Val	Met	Asp	Tyr	Asn	Gln	Gly	Lys	Leu	Ile	Phe	Gly	Gln	Gly	Thr	Lys
TTA	TCT	³⁸⁷ ATC	AAG	CCC	3'												
Leu	Ser	Ile	Lys	Pro													

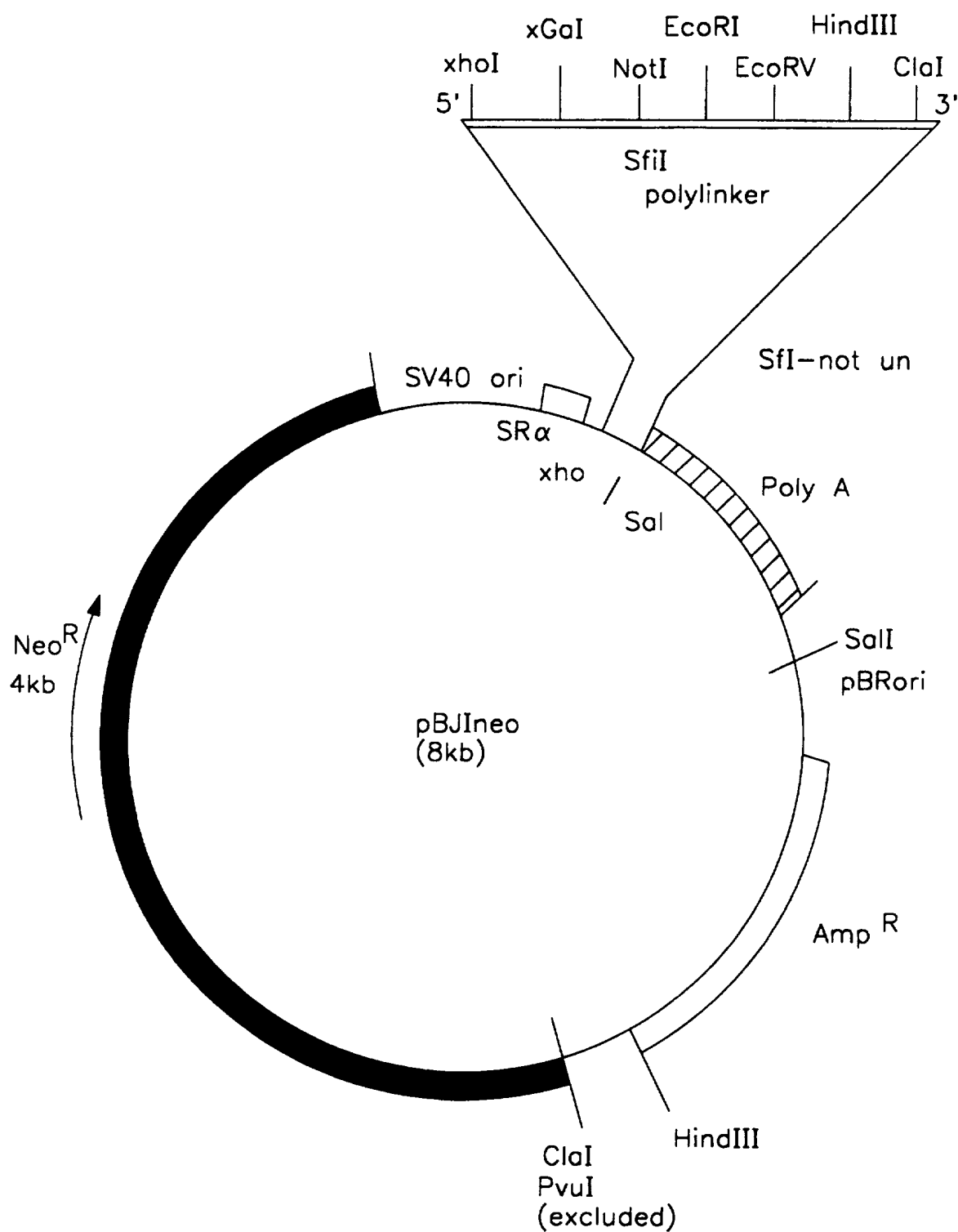
FIG. 7A

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ATG	GGC	⁹ TCC	AGA	CTC	¹⁸ TTC	TTT	GTG	²⁷ GTT	TTG	ATT	³⁶ CTC	CTG	TGT	⁴⁵ GCA	AAA	CAC	⁵⁴ ATG
Met	Gly	Ser	Arg	Leu	Phe	Phe	Val	Val	Leu	Ile	Leu	Leu	Cys	Ala	Lys	His	Met
GAG	GCT	⁶³ GCA	GTC	ACC	⁷² CAA	AGT	CCA	⁸¹ AGA	AGC	AAG	⁹⁰ GTG	GCA	GTA	⁹⁹ ACA	GGA	GGA	¹⁰⁸ AAG
Glu	Ala	Ala	Val	Thr	Gln	Ser	Pro	Arg	Ser	Lys	Val	Ala	Val	Thr	Gly	Gly	Lys
GTG	ACA	¹¹⁷ TTG	AGC	TGT	¹²⁶ CAC	CAG	ACT	¹³⁵ AAT	AAC	CAT	¹⁴⁴ GAC	TAT	ATG	¹⁵³ TAC	TGG	TAT	¹⁶² CGG
Val	Thr	Leu	Ser	Cys	His	Gln	Thr	Asn	Asn	His	Asp	Tyr	Met	Tyr	Trp	Tyr	Arg
CAG	GAC	¹⁷¹ ACG	GGG	CAT	¹⁸⁰ GGG	CTG	AGG	¹⁸⁹ CTG	ATC	CAT	¹⁹⁸ TAC	TCA	TAT	²⁰⁷ GTC	GCT	GAC	²¹⁶ AGC
Gln	Asp	Thr	Gly	His	Gly	Leu	Arg	Leu	Ile	His	Tyr	Ser	Tyr	Val	Ala	Asp	Ser
ACG	GAG	²²⁵ AAA	GGA	GAT	²³⁴ ATC	CCT	GAT	²⁴³ GGG	TAC	AAG	²⁵² GCC	TCC	AGA	²⁶¹ CCA	AGC	CAA	²⁷⁰ GAG
Thr	Glu	Lys	Gly	Asp	Ile	Pro	Asp	Gly	Tyr	Lys	Ala	Ser	Arg	Pro	Ser	Gln	Glu
AAT	TTC	²⁷⁹ TCT	CTC	ATT	²⁸⁸ CTG	GAG	TTG	²⁹⁷ GCT	TCC	CTT	³⁰⁶ TCT	CAG	TCA	³¹⁵ GCT	GTA	TAT	³²⁴ TTC
Asn	Phe	Ser	Leu	Ile	Leu	Glu	Leu	Ala	Ser	Leu	Ser	Gln	Ser	Ala	Val	Tyr	Phe
TGT	GCC	³³³ AGC	AGC	GAT	³⁴² TTC	GCC	GGG	³⁵¹ ACA	GGG	GGC	³⁶⁰ TTC	TAT	GAA	³⁶⁹ CAG	TAC	TTC	³⁷⁸ GGT
Cys	Ala	Ser	Ser	Asp	Phe	Ala	Gly	Thr	Gly	Gly	Phe	Tyr	Glu	Gln	Tyr	Phe	Gly
CCC	GGC	³⁸⁷ ACC	AGG	CTC	³⁹⁶ ACG	GTT	TCT	3'									
Pro	Gly	Thr	Arg	Leu	Thr	Val	Ser										

FIG. 7B

11 / 13



Ref:
 pBJIneo— MCB 8: 466,1988
 polylinker— Science,249:677,1990

FIG. 8

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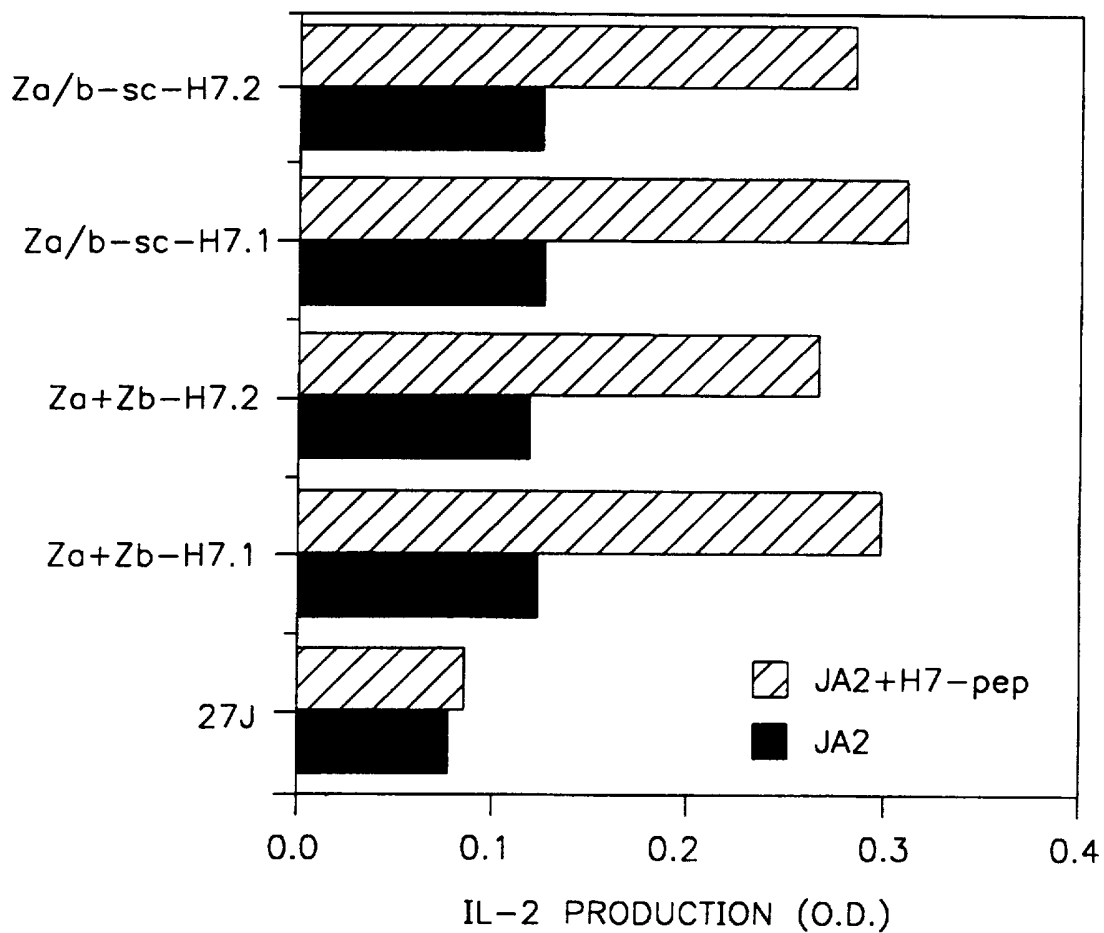


FIG. 9

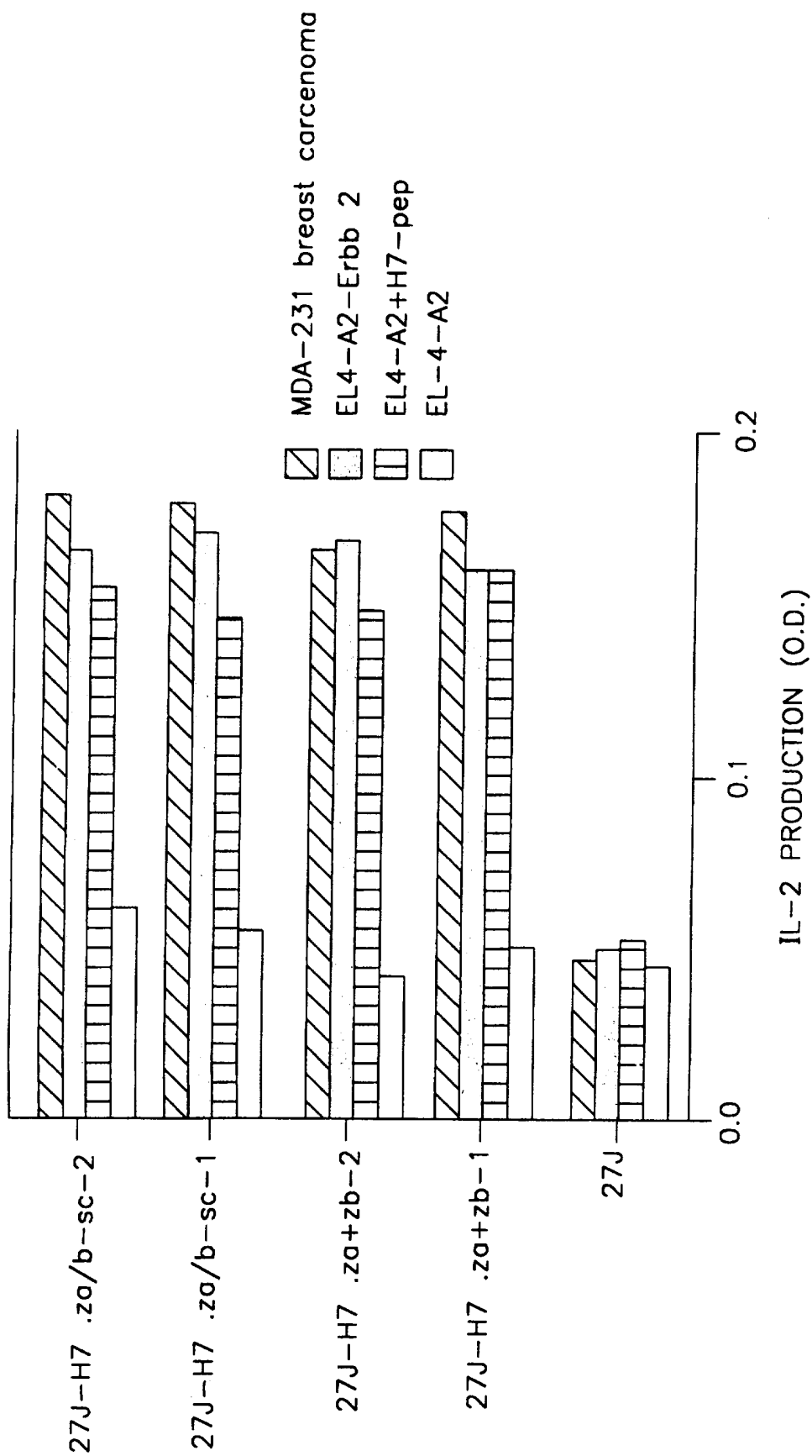


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03611

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 536/23.4, 23.5; 435/6, 91.41, 372.3; 424/277.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.4, 23.5; 435/6, 91.41, 372.3; 424/277.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, STN medline embase scisearch biosis lifesci cancerlit

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THEOBALD M. et al. Targeting p53 as a general tumor antigen. Proc. Natl. Acad. Sci. USA. December 1995, Vol. 92, pages 11993-11997, see entire document.	1-20
Y	LE A.T. et al. Cytotoxic T cell responses in HLA-A2.1 transgenic mice. J. Immunol. 15 February 1989, Vol. 142, No. 4, pages 1366-1371, see entire document.	1-20
Y	RESSING M.E. et al. Human CTL epitopes encoded by human papillomavirus type 16 E6 and E7 identified through in vivo and in vitro immunogenicity studies of HLA-A*0201-binding peptides. J. Immunol. 01 June 1995, Vol. 154, pages 5934-5943, see entire document.	1-6, 15-20

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

25 APRIL 1997

Date of mailing of the international search report

07 MAY 1997

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F. PIERRE VANDERVEGT

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03611

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y, P	HOCK R.A. et al. Retrovirus-mediated transfer and expression of drug resistance genes in human haematopoietic progenitor cells. Nature. 20 March 1996, Vol. 320, pages 275-277, see entire document.	6-19
Y	LaFACE D.M. et al. Human CD8 transgene regulation of HLA recognition by murine T cells. J. Exp. Med. November 1995, Vol. 182, pages 1315-1325, see entire document.	1-20
Y	WENTWORTH P.A. et al. Differences and similarities in the A2.1-restricted cytotoxic T cell repertoire in humans and human leukocyte antigen-transgenic mice. Eur. J. Immunol. February 1996, Vol. 26, pages 97-101, especially pages 97-98.	1-20
Y	MAN S. et al. Diversity and dominance among TCR recognizing HLA-A2.1* influenza matrix peptide in human MHC Class I transgenic mice. J. Immunol. November 1994, Vol. 153, pages 4458-4467, especially page 4459 and Figure 5.	4-19
Y	ZISMAN E. et al. Dichotomy between the T and the B cell epitopes of the synthetic peptide (T,G)-A--L*. Eur. J. Immunol. November 1994, Vol. 24, pages 2497-2505, especially Tables 2 and 3.	4-19
A, P	LUSTGARTEN J. et al. Identification of Her-2/Neu CTL epitopes using double transgenic mice expressing HLA-A2.1 and human CD.8. Human Immunology. February 1997, Vol. 52, No. 2, pages 109-118.	1-20
A	MOLLER P. et al. The role of surface HLA-A,B,C molecules in tumour immunity. Cancer Surveys. 1992, Vol. 13, pages 101-127.	1-20

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US97/03611

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-20

Remark on Protest

☐

The additional search fees were accompanied by the applicant's protest.

☐

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/03611

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

A61K 45/05; C12N 5/10, 15/12; C07H 21/04; C12Q 1/68

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING.

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claims 1-20, drawn to an isolated TCR nucleic acid, recombinant expression system, recombinant cells with the TCR recombinant expression system and method of use thereof.

Group II, claim 21, drawn to treatment of a human tumor by gene therapy.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of treating a patient with a particular tumor using recombinant human cells in Group II is different from methods of diagnosing a tumor in Group I. Accordingly, the claims are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept.