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(54) **ANTIGENE ANTICANCEREUX SOUS FORME DE PHOSPHORYLASE AGISSANT SUR LE GLYCOGENE CEREBRAL**

(54) **BRAIN GLYCOGEN PHOSPHORYLASE CANCER ANTIGEN**

(57) L'invention concerne des précurseurs d'antigènes de rejet de tumeur sous forme de phosphorylase agissant sur le glycogène cérébral, y compris les acides nucléiques codant ces précurseurs, les peptides antigéniques de rejet de tumeur ou leurs précurseurs et les anticorps apparentés. On décrit des procédés et des produits pour le diagnostic et le traitement des états caractérisés par l'expression d'un antigène de rejet de tumeur, sous la forme d'une phosphorylase agissant sur le glycogène cérébral, ou de son précurseur.

(57) The invention describes brain glycogen phosphorylase tumor rejection antigen precursors, including nucleic acids encoding such tumor rejection antigen precursors, tumor rejection antigen peptides or precursors thereof and antibodies relating thereto. Methods and products also are provided for diagnosing and treating conditions characterized by expression of a brain glycogen phosphorylase tumor rejection antigen or precursor thereof.

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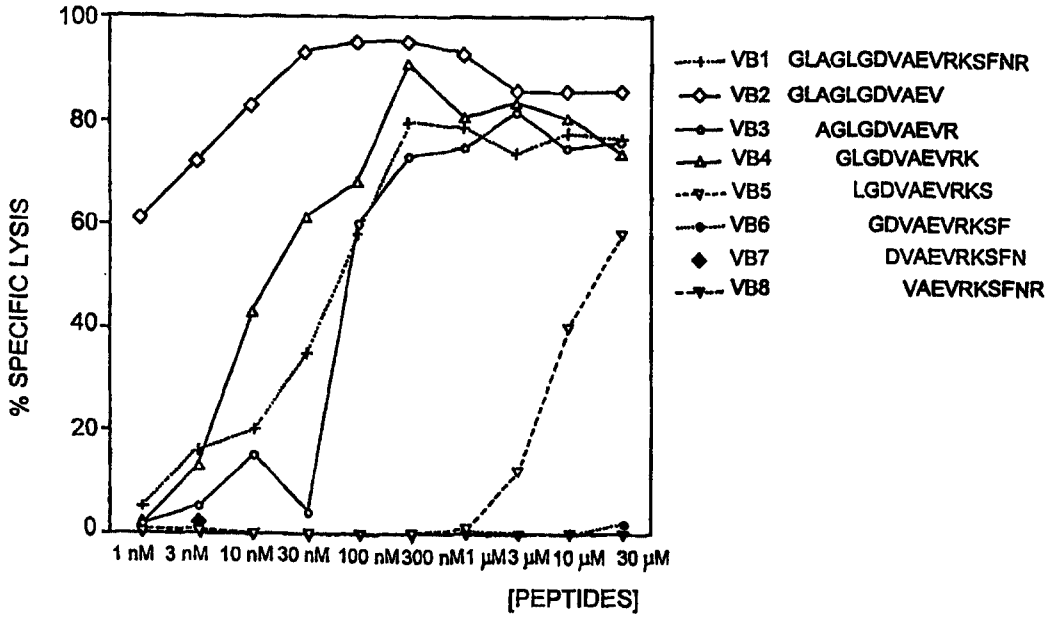

(57) Abstract

The invention describes brain glycogen phosphorylase tumor rejection antigen precursors, including nucleic acids encoding such tumor rejection antigen precursors, tumor rejection antigen peptides or precursors thereof and antibodies relating thereto. Methods and products also are provided for diagnosing and treating conditions characterized by expression of a brain glycogen phosphorylase tumor rejection antigen or precursor thereof.

BRAIN GLYCOGEN PHOSPHORYLASE CANCER ANTIGEN

Field of the Invention

This invention relates to tumor rejection antigens and precursors thereof. The tumor rejection antigen precursors are processed, *inter alia*, into at least one tumor rejection antigen that is presented by HLA molecules. The invention also relates to nucleic acid molecules which code for tumor rejection antigens and precursors thereof. The nucleic acid molecules, proteins coded for by such molecules and peptides derived therefrom, as well as related antibodies and cytotoxic lymphocytes, are useful, *inter alia*, in diagnostic and therapeutic contexts.

Background of the Invention

The process by which the mammalian immune system recognizes and reacts to foreign or alien materials is complex. An important facet of the system is the T cell response. T cells can recognize and interact with other cells via cell surface complexes on the other cells of peptides and molecules referred to as human leukocyte antigens ("HLA") or major histocompatibility complexes ("MHCs"). The peptides are derived from larger molecules which are processed by the cells which also present the HLA/MHC molecule. See Male et al., Advanced Immunology (J.P. Lipincott Company, 1987), especially chapters 6-10. The interaction of T cells and complexes of HLA/peptide is restricted, requiring a specific T cell for a specific complex of an HLA molecule and a peptide. If a specific T cell is not present, there is no T cell response even if its partner complex is present. Similarly, there is no response if the specific complex is absent, but the T cell is present. The mechanism is involved in the immune system's response to foreign materials, in autoimmune pathologies, and in responses to cellular abnormalities.

The mechanism by which T cells recognize alien materials also has been implicated in cancer. A number of cytolytic T lymphocyte (CTL) clones directed against autologous melanoma have been described. In some instances, the antigens recognized by these clones have been characterized. In PCT application PCT/US92/04354, published on November 26, 1992, the "MAGE" family, a tumor specific family of genes, is disclosed. The expression products of these genes are processed into peptides which, in turn, are expressed on cell surfaces. This can lead to lysis of the tumor cells by specific CTLs. The genes are said to code for "tumor rejection antigen precursors" or "TRAP" molecules, and the peptides derived therefrom are referred to as "tumor rejection antigens" or "TRAs". See Traversari et al., Immunogenetics 35: 145 (1992); van der Bruggen et al., Science 254: 1643 (1991), for further information on this family of genes. Also,

see U.S. Patent No. 5,342,774.

In U.S. Patent 5,405,940, MAGE nonapeptides are taught which are presented by the HLA-A1 molecule. Given the known specificity of particular peptides for particular HLA molecules, one should expect a particular peptide to bind one HLA molecule, but not others. This is important, because different individuals possess different HLA phenotypes. As a result, while identification of a particular peptide as being a partner for a specific HLA molecule has diagnostic and therapeutic ramifications, these are only relevant for individuals with that particular HLA phenotype. There is a need for further work in the area, because cellular abnormalities are not restricted to one particular HLA phenotype, and targeted therapy requires some knowledge of the phenotype of the abnormal cells at issue.

It also was discovered that a MAGE expression product is processed to a second TRA. This second TRA is presented by HLA-C clone 10 molecules. Therefore, a given TRAP can yield a plurality of TRAs.

In PCT WO94/14459, published July 7, 1994, tyrosinase is described as a tumor rejection antigen precursor. This reference discloses that a molecule which is produced by some normal cells (e.g., melanocytes), is processed in tumor cells to yield a tumor rejection antigen that is presented by HLA-A2 molecules.

In PCT WO94/21126, published September 29, 1994, a second TRA, not derived from tyrosinase is taught to be presented by HLA-A2 molecules. The TRA is derived from a TRAP, but is coded for by a non-MAGE gene. It is called Melan-A. This disclosure shows that a particular HLA molecule may present TRAs derived from different sources.

In PCT WO95/00159, published January 5, 1995, an unrelated tumor rejection antigen precursor, the so-called "BAGE" precursor, is described. TRAs are derived from the TRAP and also are described. They form complexes with MHC molecule HLA-C-Clone 10.

In PCT WO95/03422, published February 2, 1995, another unrelated tumor rejection antigen precursor, the so-called "GAGE" precursor, is described. The GAGE precursor is not related to the BAGE or the MAGE family.

The work which is presented by the papers, patents and patent applications described above deal, for the most part, with the MAGE family of genes, the BAGE gene and the GAGE gene. These genes are expressed in a number of tumors but are completely silent in normal tissues except testis. None is expressed in renal carcinoma.

Recently another unrelated tumor rejection antigen precursor, the "RAGE" precursor,

was discovered. It is distinguished, *inter alia*, by its expression in certain renal carcinomas. The RAGE precursor is not related to the GAGE, BAGE or MAGE family.

The brain glycogen phosphorylase gene is normally expressed in the adult in brain and retinal pigment epithelium. It previously was reported that this gene was over-expressed in certain renal, hepatoma and stomach cancers. It was not reported, however, that the brain glycogen phosphorylase gene was capable of provoking autologous CTL proliferation with specificity for brain glycogen phosphorylase peptides complexed with HLA. In other words, brain glycogen phosphorylase was not known as a TRAP.

It has now been discovered that the brain glycogen phosphorylase gene is expressed in melanoma tumor cells, and in certain other tumor cell types. It now has been discovered that , the brain glycogen phosphorylase gene, encodes tumor rejection antigens and precursors thereof. The brain glycogen phosphorylase gene does not show homology to the MAGE family of genes, to the BAGE gene, the GAGE gene or to the RAGE gene.

The invention is elaborated upon in the disclosure which follows.

Summary of the Invention

The invention provides isolated fragments of brain glycogen phosphorylase. The invention also provides isolated nucleic acid molecules, expression vectors containing those molecules and host cells transfected with those molecules. The foregoing, as well as brain glycogen phosphorylase itself, can be used in the diagnosis or treatment of conditions characterized by the expression of a brain glycogen phosphorylase TRA or TRAP.

According to one aspect of the invention, an isolated fragment of brain glycogen phosphorylase is provided. It includes at least the amino acid sequence of SEQ ID NO:15 and not more than 75% of the full length brain glycogen phosphorylase, SEQ ID NO:21. In some embodiments, the isolated fragment includes not more than 100 amino acids. In other embodiments the isolated fragment may consist essentially of a molecule between 7 and 100 amino acids, which molecule comprises the sequence of SEQ ID NO:15. The isolated fragment may also consist essentially of a molecule having the sequence of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, or SEQ ID NO:5. In some embodiments, the isolated fragment consists of a molecule having the sequence of SEQ ID NO:14, SEQ ID NO:13, or SEQ ID NO:12.

According to another aspect of the invention, an isolated nucleic acid molecule is

provided. The molecule encodes a polypeptide selected from the group consisting of the fragments of brain glycogen phosphorylase disclosed above. Preferably the nucleic acids encode a polypeptide which consists essentially of a molecule having the sequence of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, or SEQ ID NO:5. In some embodiments, the polypeptide has a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13 or SEQ ID NO:12.

According to another aspect of the invention, expression vectors and host cells containing those expression vectors are provided. The expression vectors include any one or more of the isolated nucleic acid molecules described above. In one embodiment, the expression vector comprises the isolated nucleic acid of SEQ ID NO:14, SEQ ID NO:13 or SEQ ID NO:12. Other expression vectors according to the invention include the isolated nucleic acids described above and a nucleic acid which codes for an HLA molecule which can present the brain glycogen phosphorylase tumor rejection antigens of the invention to cytolytic T cells. One example is HLA-A2. The host cells may endogenously express the HLA molecule such as HLA-A2.

According to another aspect of the invention, a method for enriching selectively a population of T cells with cytolytic T cells specific for a brain glycogen phosphorylase tumor rejection antigen is provided. The method involves contacting an isolated population of T cells with an agent presenting a complex of a brain glycogen phosphorylase tumor rejection antigen and an HLA presenting molecule. The T cells are contacted with the agent in an amount sufficient to selectively enrich the isolated population of T cells with the cytolytic T cells. In some embodiments, the HLA presenting molecule is HLA-A2 and the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15. In other embodiments, the peptide is between 7 and 100 consecutive amino acids of SEQ ID NO:21. In preferred embodiments, the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12 and SEQ ID NO:5. Most preferably, the peptide has the sequence consisting of SEQ ID NO:14, SEQ ID NO:13, or SEQ ID NO:12.

The recognition that brain glycogen phosphorylase peptides are presented by HLA molecules and recognized by CTLs permits diagnosis of certain disorders. Thus, according to still another aspect of the invention, a method for diagnosis of a disorder characterized by expression of a brain glycogen phosphorylase tumor rejection antigen is provided. The method involves contacting a biological sample isolated from a subject with an agent that is specific for

the brain glycogen phosphorylase tumor rejection antigen. The biological sample is isolated from non-brain, and non-retinal pigment epithelium, tissue. The method then provides by determining the interaction between the agent and the brain glycogen phosphorylase tumor rejection antigen as a determination of the disorder. In one embodiment, the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15. In other embodiments, the peptide is between 7 and 100 consecutive amino acids of SEQ. ID NO:21 and includes the amino acids of SEQ ID NO:15. Preferably, the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12 and SEQ ID NO:5. Most preferably, the sequence of the peptide consists of SEQ ID NO:14, SEQ ID NO:13, or SEQ ID NO:12.

The above-described method provides diagnosis of a disorder based on the presence of brain glycogen phosphorylase TRAs. Another aspect of the invention provides methods for diagnosing a disorder characterized by the expression of a brain glycogen phosphorylase tumor rejection antigen which forms a complex with HLA molecules. In some embodiments the complex is formed with HLA-A2. The method involves contacting a biological sample isolated from a subject with an agent that binds the complex and then determining binding between the complex and the agent as a determination of the disorder. In one embodiment, the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15. Preferably, the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12 and SEQ ID NO:5. In other preferred embodiments, the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

According to yet another aspect of the invention, methods for diagnosing a disorder characterized by expression of brain glycogen phosphorylase or nucleic acids which encode brain glycogen phosphorylase are provided. The methods involve isolating a biological sample from non-brain, non-retinal pigment epithelium, non-renal cell carcinoma, non-hepatoma and non-stomach adenocarcinoma tissue of a subject. In some embodiments, the methods involve contacting the biological sample with an agent that binds the brain glycogen phosphorylase and determining the binding between the brain glycogen phosphorylase and the agent as a determinant of the disorder. In other embodiments, the methods involve contacting the biological sample with an agent that is specific for the nucleic acid which encodes brain

glycogen phosphorylase or an expression product thereof. The interaction between the agent and the nucleic acid or the expression product thereof is measured as a determination of the disorder.

In addition to diagnosis of disorders, treatment of certain disorders is also desirable. According to another aspect of the invention, a method for treating a subject with a disorder characterized by expression of a brain glycogen phosphorylase tumor rejection antigen is provided. The method involves administering to the subject an amount of an agent which enriches selectively in the subject the presence of complexes of HLA and brain glycogen phosphorylase tumor rejection antigen sufficient to ameliorate the disorder. Preferably the complexes are formed of HLA-A2 and a brain glycogen phosphorylase tumor rejection antigen. Preferably, the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12 and SEQ ID NO:5. In other preferred embodiments, the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12. Another method involves administering to a subject in need of such treatment an amount of autologous cytolytic T cells sufficient to ameliorate the disorder, wherein the autologous cytolytic T cells are specific for complexes of an HLA molecule and a brain glycogen phosphorylase tumor rejection antigen. Preferably the complexes are formed of HLA-A2 and certain brain glycogen phosphorylase peptides as described above.

According to another aspect of the invention, the use of an agent comprising an isolated nucleic acid molecule which encodes a brain glycogen phosphorylase tumor rejection antigen or a polypeptide which includes a brain glycogen phosphorylase tumor rejection antigen in the preparation of a medicament is provided. The agent enriches selectively in the subject the presence of complexes of HLA and the brain glycogen phosphorylase tumor rejection antigen. In certain embodiments, the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15. Preferably, the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:5. More preferably, the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12. Optionally, the agent include an HLA presenting molecule that forms a complex with the brain glycogen phosphorylase tumor rejection antigen or a nucleic acid which encodes such a molecule.

According to still another aspect of the invention, the use of autologous cytolytic T cells

specific for complexes of an HLA molecule and a brain glycogen phosphorylase tumor rejection antigen in the preparation of a medicament is provided. Preferably the HLA presenting molecule is HLA-A2 and the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising a molecule having the amino acids of SEQ ID NO:15. In certain embodiments, the peptide is between 7 and 100 consecutive amino acids of SEQ ID NO:21. Preferably the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:5. More preferably the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

The invention in another aspect also provides pharmaceutical preparations containing the agents and/or cells of the preceding paragraph. In one embodiment, the preparation contains a pharmaceutically effective amount of brain glycogen phosphorylase or a fragment thereof that binds an HLA molecule along with pharmaceutically acceptable diluents, carriers or excipients. In some embodiments the HLA molecule is HLA-A2. Preferably, the brain glycogen phosphorylase or fragment thereof comprises a peptide having the amino acid sequence of SEQ ID NO:15. In another embodiment, the preparation contains a pharmaceutically effective amount of isolated autologous cytolytic T cells specific for complexes of an HLA molecule and a brain glycogen phosphorylase tumor rejection antigen.

According to another aspect of the invention, the use of isolated brain glycogen phosphorylase or fragments thereof in the manufacture of a medicament is provided. The fragments comprise the sequence of SEQ ID NO:15. Preferred fragments of the brain glycogen phosphorylase molecules are described above. In certain embodiments, the medicament is an oral medicament, an inhalable medicament, or an injectable medicament.

According to another aspect of the invention, the use of brain glycogen phosphorylase or fragments thereof in the manufacture of a medicament for the treatment of cancer is provided.

According to another aspect of the invention, kits are provided. Such kits include at least separate portions of at least two of the previously discussed materials. Other components may be added, as desired. In some embodiments, kits comprising a separate portion of an isolated nucleic acid molecule which codes for a brain glycogen phosphorylase TRAP or a molecule including a brain glycogen phosphorylase TRA, and an HLA presenting molecule that forms a complex with that TRA and that stimulates a cytolytic T cell response. One such kit includes a nucleic acid which codes for the peptide of SEQ ID NO:12, SEQ ID NO:13 or SEQ ID NO:14

and a nucleic acid molecule which codes for HLA-A2. Another kit according to the invention is an expression kit comprising a separate portion of the isolated nucleic acid molecule which codes for a brain glycogen phosphorylase TRAP or TRA, or an expression vector including a brain glycogen phosphorylase TRAP or TRA encoding nucleic acid and a nucleic acid molecule which codes for HLA-A2. In certain embodiments the kits include host cells which express an HLA molecule which presents a brain glycogen phosphorylase TRAP or TRA.

In connection with any of the isolated nucleic acids encoding a brain glycogen phosphorylase tumor rejection antigen as described above, the invention also embraces degenerate nucleic acids that differ from the isolated nucleic acid in codon sequence only due to the degeneracy of the genetic code or complements of any of the foregoing nucleic acids.

The invention also embraces functional variants and equivalents of all of the molecules described above.

These and other objects of the invention will be described in further detail in connection with the detailed description of the invention.

Brief Description of the Drawings

Fig. 1 CTL lysis of T2 cells pulsed with peptides derived from brain glycogen phosphorylase.

Fig. 2 CTL lysis of T2 cells pulsed with peptides derived from brain glycogen phosphorylase.

Fig. 3 Expression of brain glycogen phosphorylase in normal and tumor tissues.

Fig. 4 CTL lysis of T2 cells pulsed with homologous peptides derived from brain, muscle and liver glycogen phosphorylase isoforms.

Fig. 5 CTL lysis experiments with various cell lines pulsed with the peptide of SEQ ID NO:14.

Brief Description of the Sequences

SEQ ID NO:1 The shortest fragment of brain glycogen phosphorylase prepared by exonuclease III digestion which was able to confer expression of the antigen recognized by CTL 246/7.

SEQ ID NO:2 A fragment of brain glycogen phosphorylase ending at position 161.

SEQ ID NO:3 A fragment of brain glycogen phosphorylase ending at position 140.

SEQ ID NO:4. A 17mer peptide (brain glycogen phosphorylase aa 18-33).

SEQ ID NO:5 An 11mer peptide (aa 1-11) derived from SEQ ID NO:4.

SEQ ID NO:6 A 10mer peptide (aa 3-12) derived from SEQ ID NO:4.

SEQ ID NO:7 A 10mer peptide (aa 4-13) derived from SEQ ID NO:4.

SEQ ID NO:8 A 10mer peptide (aa 5-14) derived from SEQ ID NO:4.

SEQ ID NO:9 A 10mer peptide (aa 6-15) derived from SEQ ID NO:4.

SEQ ID NO:10 A 10mer peptide (aa 7-16) derived from SEQ ID NO:4.

SEQ ID NO:11 A 10mer peptide (aa 8-17) derived from SEQ ID NO:4.

SEQ ID NO:12 A 10mer peptide (aa 2-11) derived from SEQ ID NO:5.

SEQ ID NO:13 A 9mer peptide (aa 3-11) derived from SEQ ID NO:5.

SEQ ID NO:14 A 8mer peptide (aa 4-11) derived from SEQ ID NO:5.

SEQ ID NO:15 A 7mer peptide (aa 5-11) derived from SEQ ID NO:5.

SEQ ID NO:16 A 6mer peptide (aa 6-11) derived from SEQ ID NO:5.

SEQ ID NO:17 A sense primer for specific PCR amplification of brain glycogen phosphorylase.

SEQ ID NO:18 An antisense primer for specific PCR amplification of brain glycogen phosphorylase.

SEQ ID NO:19 An 11mer peptide of the liver glycogen phosphorylase.

SEQ ID NO:20 An 11mer peptide of the muscle glycogen phosphorylase.

SEQ ID NO:21 The full length sequence of the brain glycogen phosphorylase cDNA.

SEQ ID NO:22 The translation product of SEQ ID NO:21.

SEQ ID NO:23 The nucleic acid encoding SEQ ID NO:14.

SEQ ID NO:24 The nucleic acid encoding SEQ ID NO:13.

SEQ ID NO:25 The nucleic acid encoding SEQ ID NO:12.

Detailed Description of the Invention

An antigen recognized on a melanoma by autologous CTL restricted by HLA-A2 is encoded by a previously known gene, brain glycogen phosphorylase. This gene is silent by PCR analysis in all normal tissues examined, except for brain and retinal pigment epithelium, and it is expressed in several tumor samples.

EXAMPLE 1: Description of an anti-melanoma CTL clone from patient LB373

Tumor line LB373-MEL is a melanoma cell line derived from a tumor sample of a patient named LB373. A sample thereof was irradiated, so as to render it non-proliferative. These irradiated cells were then used to isolate cytolytic T cell clones ("CTLs") specific thereto.

A sample of peripheral blood lymphocytes ("PBLs") was taken from patient LB373, and contacted to the irradiated melanoma cells. After 14 days, the mixture was observed for lysis of the melanoma cells, which indicated that CTLs specific for a complex of peptide and HLA molecule presented by the carcinoma cells were present in the sample.

The lysis assay employed was a chromium release assay following Herin et al., Int. J. Cancer 39:390-396 (1987). The assay, however, is briefly described herein. The target melanoma cells were grown *in vitro*, and then resuspended at 10^7 cells/ml in Dulbecco's Modified Eagles Medium (DMEM), supplemented with 30% FCS, and incubated for 45 minutes at 37°C with 200 μCi/ml of $Na(^{51}Cr)O_4$. Labeled cells were washed three times with DMEM. These were then resuspended in DMEM supplemented with 10 mM Hepes and 10% fetal calf serum (FCS), after which 100 μl aliquots containing 10^3 cells were distributed into 96 well microplates. Samples of lymphocytes were added in 100 μl of the same medium, and assays were carried out in duplicate. Plates were centrifuged for 4 minutes at 100g and incubated for four hours at 37°C in a 8% CO_2 atmosphere.

Plates were centrifuged again, and 100 μl aliquots of supernatant were collected and counted. Percentage of ^{51}Cr release was calculated as follows:

$$\%\ ^{51}Cr\ release = \frac{(ER-SR)}{(MR-SR)} \times 100$$

where ER is observed, experimental ^{51}Cr release, SR is spontaneous release measured by incubating 10^3 labeled cells in 200 μl of medium alone, and MR is maximum release, obtained by adding 100 μl 0.3% Triton X-100 to target cells.

Those mononuclear blood samples which showed high CTL activity were expanded and cloned via limiting dilution, and were screened again, using the same methodology. A first CTL clone was then isolated. The clone is referred to as 246/76 hereafter.

CTL clone 246/76 produced TNF when stimulated with the autologous tumor cells. Melanoma cell lines showing at least one class I molecule with the melanoma cell line LB373-MEL were tested for recognition by CTL clone 246/76. Cell lines sharing the HLA-A2 molecule

were recognized by the CTL. The conclusion was that CTL 246/76 recognized an antigen presented by HLA-A2.

EXAMPLE 2: Isolation of a cDNA clone that directs the expression of the antigen recognized by CTL 246/76

A. cDNA library

RNA was isolated from LB373-MEL, and poly-A^+ RNA was purified by oligo-dT binding. cDNA was prepared by reverse transcription with an oligo-dT primer containing a *Not* I site, followed by second strand synthesis (Superscript Choice System, BRL, Life Technologies). The cDNA was then ligated to a *BstX* I adaptor, digested with *Not* I, size-fractionated (Sephacryl S-500 HR columns, BRL, Life Technologies) and cloned unidirectionally into the *BstX* I and *Not* I sites of pcDNA-I-Amp (Invitrogen). The recombinant plasmid was then electroporated into TOP10F' *E. coli* bacteria. 700 pools of 100 recombinant bacteria were amplified and plasmid DNA of each pool was extracted by alkaline lysis, potassium acetate precipitation and phenol extraction.

B. Transfection of cells and identification of cDNA

Most autologous CTL recognized COS cell transfected with HLA-A2 alone. Thus other cells were prepared to present the peptide recognized by CTL 246/76.

Two cell systems were used for isolation of cDNAs encoding the peptide recognized by CTL 246/76. HeLa cells expressing the BK virus large T antigen (hereinafter HOB cells) were able to present the peptide encoded by a control cDNA at a level similar to that observed for COS cells: a tyrosinase cDNA diluted in 200 unrelated cDNAs was recognized by anti-tyrosinase CTLs. The second cell system was purchased from Invitrogen (San Diego, CA). 293-EBNA-1 cells were able to present a peptide derived from tyrosinase cDNA cloned in pCEP4 even when diluted 1:800 with unrelated cDNAs. This second system was used to confirm the identity of the cDNA isolated using the HOB cell system.

The transfection of HOB cells was made in duplicate wells. Briefly, samples of HOB cells were seeded, at 15.000 cells/well into tissue culture flat bottom microwells, in DMEM supplemented with 10% fetal calf serum. The cells were incubated overnight at 37°C, medium was removed and then replaced by 100 μl/well total volume of DMEM medium containing 20% Nu-Serum (Collaborative Research, Bedford, MA), 300μg/ml DEAE-dextran, and 200 μM chloroquine, plus 100 ng of the LB373-MEL cDNA library cloned in pcDNAI/Amp and 50 ng of

HLA-A2 cloned in pcDNAI/Amp. Following four hours of incubation at 37°C, the medium was removed, and replaced by 50 µl of PBS containing 10% dimethyl sulfoxide (DMSO). This medium was removed after two minutes and replaced by 200 µl of DMEM supplemented with 10% FCS.

Following this change in medium, HOB cells were incubated for 48 hours at 37°C. The transfectants then were screened with CTL 246/76. After first removing the medium, 2000 CTL 246/76 cells were added to each well in 100 µl of medium containing 25 U/ml IL-2. The amount of TNF present in the supernatant was then measured by testing its cytotoxicity for WEHI 164.13 cells. Most pools gave a TNF concentration below 5 pg/ml. cDNAs from pools which gave higher concentrations in both of the duplicate wells were cloned in bacteria. Their plasmid DNA was extracted and transfected into HOB cells with HLA-A2. The transfectants were screened with CTL 246/76. One cDNA clone gave a high TNF production by CTL 246/76. The cDNA was sequenced, compared with DNA sequence databases and determined to encode brain glycogen phosphorylase.

EXAMPLE 3: Identification of the portion of brain glycogen phosphorylase encoding a tumor rejection antigen

Fragments of the brain glycogen phosphorylase cDNA were prepared by exonuclease III digestion from the 3' end of the cDNA according to art standard procedures, were cloned into an expression vector and transfected into HOB cells with HLA-A2 as described above. As a positive control, the brain glycogen phosphorylase cDNA was cotransfected with HLA-A2 into HOB cells. These transfectants were used to provoke release of TNF from CTL 246/76 cells. The shortest fragment prepared by exonuclease III digestion which was able to confer expression of the antigen recognized by CTL 246/76 ended 100 bp after the start codon (SEQ ID NO:1).

Shorter fragments were generated by PCR. A fragment ending at position 161 (SEQ ID NO:2) did confer expression of the antigen. A shorter fragment ending at position 140 (SEQ ID NO:3) did not confer expression of the antigen. Thus, at least the valine residue encoded at nucleotides 141, 142 and 143 of brain glycogen phosphorylase was important for efficient recognition of the brain glycogen phosphorylase tumor rejection antigen by CTL 246/76.

EXAMPLE 4: Identification of brain glycogen phosphorylase tumor rejection antigen peptide

Synthetic peptides corresponding to the 3' end of SEQ ID NO:2 were synthesized and

tested for lysis of HLA-A2 expressing cells. For these assays, T2 cells were used. T2 cells are HLA-A2$^-$ cells which have an antigen-processing defect resulting in an increased capacity to present exogenous peptides. T2 cells were mixed with a synthetic peptide corresponding to a 3' portion of SEQ ID NO:3. CTL 246/76 cells were added and lysis was measured after 4 hours (Fig. 1). Peptide VB1 (GLAGLGDVAEVRKSFNR, SEQ ID NO:4) efficiently stimulated the lysis of T2 cells bearing HLA-A2. To detemine the boundaries of the brain glycogen phosphorylase tumor rejection antigen, we tested a series of 10mer and 11mer peptides (SEQ ID NOs:5-11), i.e., peptides of 10 or 11 amino acids, derived from the 17mer peptide (SEQ ID NO:4) previously used to stimulate lysis by CTL 246/76 cells (Fig. 1). One of these peptides (LGDVAEVRKS, SEQ ID NO:8) was recognized by CTL 246/76, but to a far lesser extent than the VB1 peptide (SEQ ID NO:4), which suggested that the nonamer (SEQ ID NO:8) was lacking an amino acid important for efficient recognition by CTL 246/76. The 10mer peptide which includes the glycine immediately to the amino terminal side of SEQ ID NO:8 (GLGDVAEVRK, SEQ ID NO:7) was efficiently recognized by CTL 246/76, as were the peptides of SEQ ID NO:5 and SEQ ID NO:9.

EXAMPLE 5: Activity of brain glycogen phosphorylase tumor rejection antigen peptides

This example shows the ability of the brain glycogen phosphorylase TRA peptides to induce lysis of HLA-A2-expressing cells pulsed with such peptides and the relative efficiencies of the 6mer, 7mer, 8mer, 9mer, 10mer and 11mer peptides.

Brain glycogen phosphorylase peptides of decreasing size were synthesized based on the 11mer peptide, VB2 (SEQ ID NO:5), by successively removing one amino acid from the amino terminal end of the peptide. These peptides, of 10, 9, 8, 7, and 6 amino acids, are represented as SEQ ID NOs:12-16 respectively (see Fig. 2). These peptides were tested for the ability to induce cell lysis of HLA-A2$^+$ T2 cells by CTL 246/76 cells in a dose response assay. Lyophilized peptides were dissolved at 20 mg/ml in DMSO, then diluted to 2 mg/ml in 10mM acetic acid and stored at -80°C. Target cells, HLA-A2$^+$ T2 cells, were labeled with ^{51}Cr, as described above, for 1 hour at 37°C followed by extensive washing to remove unincorporated label. T2 cells were pretreated (Fig. 2A) or not pretreated (Fig. 2B) with anti-HLA-A2 antibody, MA2.1 (Wölfel et al., European Journal of Immunology **24:** 759-764, 1994), and then incubated in 96-well microplates in the presence of various concentrations of peptides for 30 minutes at 37°C. CTL 246/76 were then added in an equal volume of medium at an effector:target ratio of 30:1.

Chromium-51 release was measured after 4 hours. Fig. 2 shows the results of the dose response assay. The 8mer, 9mer and 10mer peptides (SEQ ID NOs:12-14) most efficiently stimulated the lysis of T2 cells bearing HLA-A2. The 11mer peptide (VB1, SEQ ID NO:5) was about 1 log less active than the optimal peptides. The 7mer peptide (SEQ ID NO:15) was about 2 logs less active than the optimal peptides. The 6mer peptide (SEQ ID NO:16) exhibited little or no activity.

EXAMPLE 6: Expression of brain glycogen phosphorylase gene

The expression of brain glycogen phosphorylase was tested by PCR using the following primers:

SEQ ID NO:17 --

5'- TGC CAG GCA CAG GTG GAC CA -3' (sense primer. nucleotides 2369-2388)

SEQ ID NO:18 --

5'- CAG ACC CCA GAA TCC AGA GGC -3' (antisense primer. nucleotides 2890-2910)

First, total RNA was taken from the particular sample, using art recognized techniques. This RNA was used to prepare cDNA. The protocol used to make the cDNA involved combining 4 μl of 5x reverse transcriptase buffer, 1 μl of each dNTP (10mM), 2 μl of dithiothreitol (100mM), 2 μl of dT-15 primer (20 μM), 0.5 µl of RNasin (40 units/μl), and 1 μl of M-MLV reverse transcriptase (200 units/μl). Next, 6.5 μl of template RNA (1 μg/3.25 μl water, or 2 μg total template RNA) was added. The total volume of the mixture was 20 μl. This was mixed and incubated at 42°C for 60 minutes, after which it was chilled on ice. A total of 80 μl of water was then added, to 100 μl total. This mixture was stored at -20°C until used in PCR.

The reagents for PCR included:

- 5 microliters of 10x DynaZyme buffer
- 20 pmoles of each primer
- 5 nanomoles of each dNTP
- 1 unit of polymerizing enzyme "Dynazyme" (2 units/μl)
- 5 μl of cDNA (corresponding to 100 ng total RNA)
- water to a final volume of 50 μl

The mixture was combined, and layered with one drop of mineral oil. The mixture was transferred to a thermocycler block, preheated to 94°C, and amplification was carried out for one

cycle of 15 min at 94°C, followed by 25 cycles of:

- 1 min. at 94°C
- 30 sec. at 65°C
- 2 min. at 72°C

A final extension step of 15 min. was then performed at 72°C. The PCR product was visualized on an agarose gel (1.5%) containing ethidium bromide.

The brain glycogen phosphorylase gene demonstrated a pattern of over-expression in tumors. The gene was expressed at levels lower that the level of expression found in LB373-MEL cells in all normal tissues tested (Fig. 3). In particular, the gene was expressed weakly in normal adrenals, bladder, breast, colon, endometrium, heart, kidney, liver, myometrium, ovary, retina, spleen, stomach, and testis. The gene, however, was found to be expressed in a variety of tumor tissue samples (Fig. 3). A 10- to 40-fold greater expression of brain glycogen phosphorylase was observed in 15% of melanomas and a similar proportion of colon, ovarian and renal carcinomas. These results were confirmed by staining tumor samples with an antiserum specific for the brain glycogen phosphorylase (Ignacio et al., Brain Res. **529:** 42-49, 1990): tumor tissue demonstrated a high level of staining but the surrounding normal tissue was negative for staining.

EXAMPLE 7: Homologous peptides of liver and muscle isoforms are not recognized by CTL 246/76

The muscle, liver and brain isoforms of glycogen phosphorylase display about an 80% amino acid identity (Newgard et al., J. Biol. Chem. **263:** 3850-3857, 1988). To demonstrate that the tumor rejection antigen was specific for the brain isoform, 11mer peptides of the liver (GIVGVENVAEL, SEQ ID NO:19) and muscle (GLAGVENVIEL, SEQ ID NO:20) isoforms were synthesized and used in a dose response-chromium release assay as described above in Example 5. As shown in Fig. 4, the liver and muscle peptides do not provoke lysis, whereas the brain peptide, VB1 (SEQ ID NO:5), induces specific lysis.

EXAMPLE 8: Normal cells are not lysed by CTL246/76

This example describes CTL lysis experiments with various cell lines with or without incubation with the peptide of SEQ ID NO:14. LB373-MEL cells, normal B cells from patient LB373 transformed with EBV (LB373-EBV) and normal peripheral blood lymphocytes from the

same patient (LB373-PBL) were tested for lysis by CTL 246/76 cells in a dose response assay. These cells were incubated with CTL246/76 at the effector/target ratios shown in Fig. 5A and assayed for lysis as described above. Only the LB373-MEL cells were lysed by the CTL246/76, demonstrating that LB373-EBV and LB373-PBL cells were not recognized by the CTL because such cells do not normally express the brain glycogen phosphorylase tumor rejection antigen.

It was next determined whether these cells would be lysed by CTL if pulsed with a brain glycogen phosphorylase peptide. The peptide of SEQ ID NO: 14 was tested for the ability to induce cell lysis of LB373-MEL cells, LB373-EBV cells, and HLA-$A2^+$ T2 cells by CTL 246/76 cells in a dose response assay as in previous examples. Fig. 5B shows the results of the dose response assay. LB373-EBV and LB373-PBL were not lysed by CTL 246/76, but a non-autologous cell line, T2, was lysed by CTL 246/76.

The invention pertains to the abnormal expression of human brain glycogen phosphorylase. A gene encoding human brain glycogen phosphorylase is presented in SEQ ID NO:21. Alleles are also a part of the invention. Alleles share >95% homology with SEQ ID NO:21 and code for a brain glycogen phosphorylase tumor rejection antigen precursor. They hybridize to a nucleic acid molecule consisting of SEQ ID NO:21, under stringent conditions. The term "stringent conditions" as used herein refers to parameters with which the art is familiar. Nucleic acid hybridization parameters may be found in references which compile such methods, e.g. *Molecular Cloning: A Laboratory Manual*, J. Sambrook, et al., eds., Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989, or *Current Protocols in Molecular Biology*, F.M. Ausubel, et al., eds., John Wiley & Sons, Inc., New York. More specifically, stringent conditions, as used herein, refers to hybridization at 65°C in hybridization buffer (3.5 x SSC, 0.02% Ficoll, 0.02% Polyvinyl pyrolidone, 0.02% Bovine Serum Albumin, 25mM NaH_2PO_4 (pH 7), 0.5% SDS, 2mM EDTA). SSC is 0.15M Sodium Chloride/0.15M Sodium Citrate, pH 7; SDS is Sodium Dodecyl Sulfate; and EDTA is ethylenediaminetetraacetic acid. After hybridization, the membrane upon which the DNA is transferred is washed at 2xSSC at room temperature and then at 0.1xSSC/0.1xSDS at 65°C.

There are other conditions, reagents, and so forth which can be used, which result in the same degree of stringency. The skilled artisan will be familiar with such conditions, and thus they are not given here. It will be understood, however, that the skilled artisan will be able to manipulate the conditions in a manner to permit the clear identification of homologs and alleles

of the nucleic acids of the invention. The skilled artisan also is familiar with the methodology for screening cells, preferably cancer cells, and libraries for expression of such molecules which then are routinely isolated, followed by isolation of the pertinent nucleic acid molecule and sequencing of the nucleic acid molecule.

Abnormal expression of brain glycogen phosphorylase can be detected by a variety of technologies. For example, antibodies specific for brain glycogen phosphorylase have been described in the literature and can be prepared by routine procedures, some of which are described in greater detail below. More preferably, expression (and relative expression levels in various tissues) can be detected by measuring mRNA. For example, the expression of brain glycogen phosphorylase in tumor cells or tissues can be compared to control cells or tissues of like origin. PCR and other techniques can be used for this purpose. For any pair of PCR primers constructed and arranged to selectively amplify the brain glycogen phosphorylase gene, a brain glycogen phosphorylase specific primer may be used. Such a specific primer would fully hybridize to a contiguous stretch of nucleotides only in brain glycogen phosphorylase, but would hybridize only in part to non-brain glycogen phosphorylase genes. For efficient PCR priming and brain glycogen phosphorylase identification, the brain glycogen phosphorylase specific primer should be constructed and arranged so it does not hybridize efficiently at its 3' end to glycogen phosphorylase genes other than brain glycogen phosphorylase. The mismatch generated at the 3' end of the primer when hybridized to glycogen phosphorylase genes, other than brain glycogen phosphorylase, would preclude efficient amplification of those genes. Primers can be chosen by one of ordinary skill in the art based on the published sequences of the brain, liver and muscle isoforms of glycogen phosphorylase (see, e.g. Newgard et al., J. Biol. Chem. 263: 3850-3857, 1988). Additional methods which can distinguish nucleotide sequences of substantial homology, such as ligase chain rection ("LCR") and other methods, will be apparent to skilled artisans.

The invention also includes the use of nucleic acid sequences which include alternative codons that encode the same amino acid residues as encoded by the brain glycogen phosphorylase genes. For example, as disclosed above in Example 5, a decameric peptide LAGLGDVAEV (SEQ ID NO:12) is a brain glycogen phosphorylase tumor rejection antigen. The leucine residues (amino acids No. 1 and 4 of SEQ ID NO:12) for example, are encoded by the codons CTG and CTA, respectively. In addition to CTG and CTA, leucine amino acid residues may also be encoded by the codons CTC, CTT, TTA and TTG. Each of the six codons

is equivalent for the purposes of encoding a leucine residue. Thus, it will be apparent to one of ordinary skill in the art that any of the leucine-encoding nucleotide triplets may be employed to direct the protein synthesis apparatus, *in vitro* or *in vivo*, to incorporate a leucine residue. Similarly, nucleotide sequence triplets which encode other amino acid residues comprising a brain glycogen phosphorylase tumor rejection antigen include:CGA, CGC, CGG, CGT, AGA and AGG (arginine codons); GGA, GGC, GGG, and GGT (glycine codons); GCA, GCC, GCG, and GCT (alanine codons); GAC and GAU (aspartic acid codons); and CGA, CGC, CGG, CGT, AGA, and AGG (arginine codons). Other amino acid residues may be encoded similarly by multiple nucleotide sequences. Thus, the invention embraces degenerate nucleic acids that differ from the biologically isolated nucleic acids in codon sequence due to the degeneracy of the genetic code.

The examples above also show the isolation of peptides which are brain glycogen phosphorylase TRAs. These exemplary peptides are processed translation products of the nucleic acids of brain glycogen phosphorylase (SEQ ID NO:21). As such, it will be appreciated by one of ordinary skill in the art that the translation products from which a brain glycogen phosphorylase TRA is processed to a final form for presentation may be of any length or sequence so long as they encompass the "core" brain glycogen phosphorylase TRA represented by SEQ ID NO:15. As demonstrated in the examples above, peptides or proteins as small as 7, 8, 9, 10, or 11 amino acids and as large as the amino acid sequence encoded by the brain glycogen phosphorylase cDNA are appropriately processed if necessary, presented by HLA-A2 and recognized by CTL246/76. The peptide of SEQ ID NO:15 may have one, two, three, four, five, six, seven, eight, nine, ten, or more amino acids added to either or both ends. Thus the tumor rejection antigen can consist essentially of seven consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, eight consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, nine consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, ten consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, eleven consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, twelve consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, thirteen consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, fourteen consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, fifteen consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, sixteen consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, seventeen consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, eighteen consecutive

amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, nineteen consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15, and/or up to 100 consecutive amino acids of SEQ ID NO:21 inclusive of SEQ ID NO:15. The antigenic portion of such peptides is cleaved out under physiological conditions for presentation by HLA class I molecules.

The amino acid sequence of proteins and peptides from which brain glycogen phosphorylase TRAs are derived may be of natural or non-natural origin, that is, they may comprise a natural brain glycogen phosphorylase TRAP molecule or may comprise a modified sequence as long as the amino acid sequence retains the tumor rejection antigen sequence recognized by the CTL when presented on the surface of a cell. For example, brain glycogen phosphorylase tumor rejection antigens in this context may be fusion proteins of a brain glycogen phosphorylase tumor rejection antigen and unrelated amino acid sequences, synthetic peptides of amino acid sequences shown in SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12 or SEQ ID NO:5, labeled peptides, peptides isolated from patients with melanoma, peptides isolated from cultured cells which express brain glycogen phosphorylase, peptides coupled to nonpeptide molecules, for example, in certain drug delivery systems, and other molecules which include the amino acid sequence of SEQ ID NO:15.

It will also be seen from the examples that the invention embraces the use of the sequences in expression vectors, as well as to transfect host cells and cell lines, be these prokaryotic (e.g., *E. coli*), or eukaryotic (e.g., CHO cells, COS cells, HeLa cells, yeast expression systems and recombinant baculovirus expression in insect cells). The expression vectors require that the pertinent sequence, i.e., those described *supra*, be operably linked to a promoter. As it has been found that human HLA-A2 presents a TRA derived from these genes, the expression vector may also include a nucleic acid sequence coding for an HLA molecule, especially HLA-A2. In a situation where the vector contains both coding sequences, it can be used to transfect a cell which does not normally express either one. The TRAP or TRA coding sequence may be used alone, when, e.g. the host cell already expresses HLA-A2. Of course, there is no limit on the particular host cell which can be used. As the vectors which contain the two coding sequences may be used in HLA-A2 presenting cells if desired, and the nucleic acid coding for the TRAP or TRA can be used in host cells which do not express HLA-A2.

The invention also embraces so-called expression kits, which allow the artisan to prepare a desired expression vector or vectors. Such expression kits include at least separate portions of at least two of the previously discussed materials. Other components may be added, as desired.

The invention as described herein has a number of uses, some of which are described herein. First, the invention permits the artisan to diagnose a disorder characterized by expression of the TRAP. These methods involve determining expression of the TRAP gene, and/or TRAs derived therefrom, such as a TRA presented by HLA-A2. In the former situation, such determinations can be carried out via any standard nucleic acid determination assay, including the polymerase chain reaction, or assaying with labeled hybridization probes. In the latter situation, assaying with binding partners for complexes of TRA and HLA, such as antibodies, is especially preferred. An alternate method for determination is a TNF release assay, of the type described *supra*.

Other TRAPs or TRAs encoded by brain glycogen phosphorylase and recognized by other CTL clones and/or presented by other HLA molecules may be isolated by the procedures detailed herein. (There are numerous HLA molecules known to those skilled in the art, including but not limited to, those encoded by HLA-A, HLA-B, HLA-C, HLA-E, HLA-F and HLA-G genes.) A variety of methodologies well-known to the skilled practitioner can be utilized to obtain isolated TRAP molecules, and/or TRAs derived therefrom. The protein may be purified from cells which naturally produce the protein. Alternatively, an expression vector may be introduced into cells to cause production of the protein. In another method, mRNA transcripts may be microinjected or otherwise introduced into cells to cause production of the encoded protein. Translation of mRNA in cell-free extracts such as the reticulocyte lysate system also may be used to produce protein. Peptides comprising TRAs of the invention may also be synthesized *in vitro*. Those skilled in the art also can readily follow known methods for isolating proteins in order to obtain isolated TRAP and/or TRAs derived therefrom. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography and immune-affinity chromatography. These isolated molecules when processed and presented as the TRA, or as complexes of TRA and HLA, such as HLA-A2, may be combined with materials such as adjuvants to produce vaccines useful in treating disorders characterized by expression of the TRAP molecule. In addition, vaccines can be prepared from cells which present the TRA/HLA complexes on their surface, such as non-proliferative cancer cells, non-proliferative transfectants, etcetera. In all cases where cells are used as a vaccine, these can be cells transfected with coding sequences for one or both of the components necessary to provoke a CTL response, or be cells which already express both molecules without the need for transfection. Vaccines also encompass naked DNA or RNA, encoding a brain glycogen

phosphorylase TRA or precursor thereof, which may be produced *in vitro* and administered via injection, particle bombardment, nasal aspiration and other methods. Vaccines of the "naked nucleic acid" type have been demonstrated to provoke an immunological response including generation of CTLs specific for the peptide encoded by the naked nucleic acid (Science 259:1745-1748, 1993).

The TRAP molecule, its associated TRAs, as well as complexes of TRA and HLA, may be used to produce antibodies, using standard techniques well known to the art. Standard reference works setting forth the general principles of antibody production include Catty, D., Antibodies, A Practical Approach, Vol. 1, IRL Press, Washington DC (1988); Klein, J., Immunology: The Science of Cell-Non-Cell Discrimination, John Wiley and Sons, New York (1982); Kennett, R., et al., Monoclonal Antibodies, Hybridoma, A New Dimension In Biological Analyses, Plenum Press, New York (1980); Campbell, A., Monoclonal Antibody Technology, in Laboratory Techniques and Biochemistry and Molecular Biology, Vol. 13 (Burdon, R. et al. EDS.), Elsevier Amsterdam (1984); and Eisen, H.N., Microbiology, third edition, Davis, B.D. et al. EDS. (Harper & Rowe, Philadelphia (1980).

The antibodies of the present invention thus are prepared by any of a variety of methods, including administering protein, fragments of protein, cells expressing the protein or fragments thereof and the like to an animal to induce polyclonal antibodies. The production of monoclonal antibodies is according to techniques well known in the art. As detailed herein, such antibodies may be used for example to identify tissues expressing protein or to purify protein. Antibodies also may be coupled to specific labeling agents for imaging or to antitumor agents, including, but not limited to, methotrexate, radioiodinated compounds, toxins such as ricin, other cytostatic or cytolytic drugs, and so forth. Antibodies prepared according to the invention also preferably are specific for the TRA/HLA complexes described herein.

When "disorder" is used herein, it refers to any pathological condition where the tumor rejection antigen precursor is expressed. An example of such a disorder is cancer, melanoma in particular.

Some therapeutic approaches based upon the disclosure are premised on a response by a subject's immune system, leading to lysis of TRA presenting cells, such as HLA-A2 cells. One such approach is the administration of autologous CTLs specific to the complex to a subject with abnormal cells of the phenotype at issue. It is within the skill of the artisan to develop such CTLs *in vitro*. Generally, a sample of cells taken from a subject, such as blood cells, are

contacted with a cell presenting the complex and capable of provoking CTLs to proliferate. The target cell can be a transfectant. such as a HeLa cell of the type described *supra*. These transfectants present the desired complex at their surface and, when combined with a CTL of interest, stimulate its proliferation. HeLa cells, such as those used herein, are widely available, as are other suitable host cells. Specific production of a CTL clone has been described above. The clonally expanded autologous CTLs then are administered to the subject. Other CTLs specific to brain glycogen phosphorylase may be isolated and administered by similar methods.

To detail a therapeutic methodology, referred to as adoptive transfer (Greenberg, J. Immunol. 136(5): 1917 (1986); Riddel et al., Science 257: 238 (7-10-92); Lynch et al. Eur. J. Immunol. 21: 1403-1410 (1991); Kast et al., Cell 59: 603-614 (11-17-89)), cells presenting the desired complex are combined with CTLs leading to proliferation of the CTLs specific thereto. The proliferated CTLs are then administered to a subject with a cellular abnormality which is characterized by certain of the abnormal cells presenting the particular complex. The CTLs then lyse the abnormal cells, thereby achieving the desired therapeutic goal.

The foregoing therapy assumes that at least some of the subject's abnormal cells present the relevant HLA/TRA complex. This can be determined very easily, as the art is very familiar with methods for identifying cells which present a particular HLA molecule, as well as how to identify cells expressing DNA of the pertinent sequences, in this case a brain glycogen phosphorylase sequence. Once cells presenting the relevant complex are identified via the foregoing screening methodology, they can be combined with a sample from a patient, where the sample contains CTLs. If the complex presenting cells are lysed by the mixed CTL sample, then it can be assumed that a brain glycogen phosphorylase derived TRA is being presented, and the subject is an appropriate candidate for the therapeutic approaches set forth *supra*.

Adoptive transfer is not the only form of therapy that is available in accordance with the invention. CTLs can also be provoked *in vivo*, using a number of approaches. One approach, i.e., the use of non-proliferative cells expressing the complex, has been elaborated upon *supra*. The cells used in this approach may be those that normally express the complex, such as irradiated tumor cells or cells transfected with one or both of the genes necessary for presentation of the complex. Chen et al., Proc. Natl. Acad. Sci. USA 88: 110-114 (January, 1991) exemplifies this approach, showing the use of transfected cells expressing HPVE7 peptides in a therapeutic regime. Various cell types may be used. Similarly, vectors carrying one or both of the genes of interest may be used. Viral or bacterial vectors are especially preferred. For

example, nucleic acids which encode a brain glycogen phosphorylase TRA may be operably linked to promoter and enhancer sequences which direct expression of the brain glycogen phosphorylase TRA in certain tissues or cell types. The nucleic acid may be incorporated into an expression vector. Expression vectors may be unmodified extrachromosomal nucleic acids, plasmids or viral genomes constructed or modified to enable insertion of exogenous nucleic acids, such as those encoding brain glycogen phosphorylase TRAs. Nucleic acids encoding a brain glycogen phosphorylase TRA also may be inserted into a retroviral genome, thereby facilitating integration of the nucleic acid into the genome of the target tissue or cell type. In these systems, the gene of interest is carried by a microorganism, e.g., a Vaccinia virus, retrovirus or the bacteria BCG, and the materials de facto "infect" host cells. The cells which result present the complex of interest, and are recognized by autologous CTLs, which then proliferate.

A similar effect can be achieved by combining the TRAP or a stimulatory fragment thereof with an adjuvant to facilitate incorporation into HLA-A2 presenting cells *in vivo*. The TRAP is processed to yield the peptide partner of the HLA molecule while the TRA is presented without the need for further processing. Generally, subjects can receive an intradermal injection of an effective amount of the brain glycogen phosphorylase TRAP, and/or TRAs derived therefrom. Initial doses can be followed by booster doses, following immunization protocols standard in the art.

As part of the immunization protocols, substances which potentiate the immune response may be administered with nucleic acid or peptide components of a cancer vaccine. Such immune response potentiating compound may be classified as either adjuvants or cytokines. Adjuvants may enhance the immunological response by providing a reservoir of antigen (extracellularly or within macrophages), activating macrophages and stimulating specific sets of lymphocytes. Adjuvants of many kinds are well known in the art; specific examples include MPL (SmithKline Beecham), a congener obtained after purification and acid hydrolysis of *Salmonella minnesota* Re 595 lipopolysaccharide, QS21 (SmithKline Beecham), a pure QA-21 saponin purified from *Quillja saponaria* extract, and various water-in-oil emulsions prepared from biodegradable oils such as squalene and/or tocopherol. Cytokines are also useful in vaccination protocols as a result of lymphocyte stimulatory properties. Many cytokines useful for such purposes will be known to one of ordinary skill in the art, including interleukin-12 (IL-12) which has been shown to enhance the protective effects of vaccines (Science 268: 1432-1434, 1995).

When administered, the therapeutic compositions of the present invention are administered in pharmaceutically acceptable preparations. Such preparations may routinely contain pharmaceutically acceptable concentrations of salt, buffering agents, preservatives, compatible carriers, supplementary immune potentiating agents such as adjuvants and cytokines and optionally other therapeutic agents.

The preparations of the invention are administered in effective amounts. An effective amount is that amount of a pharmaceutical preparation that alone, or together with further doses, stimulates the desired response. In the case of treating cancer, the desired response is inhibiting the progression of the cancer. This may involve only slowing the progression of the disease temporarily, although more preferably, it involves halting the progression of the disease permanently. This can be monitored by routine methods or can be monitored according to diagnostic methods of the invention discussed herein.

Where it is desired to stimulate an immune response using a therapeutic composition of the invention, this may involve the stimulation of a humoral antibody response resulting in an increase in antibody titer in serum, a clonal expansion of cytotoxic lymphocytes, or some other desirable immunologic response. It is believed that doses of immunogens ranging from one nanogram/kilogram to 100 milligrams/kilogram, depending upon the mode of administration, would be effective amounts for stimulating an immune response or inhibiting the progression of cancer. The preferred range is believed to be between 500 nanograms and 500 micrograms per kilogram. The absolute amount will depend upon a variety of factors, including the material selected for administration, whether the administration is in single or multiple doses, and individual patient parameters including age, physical condition, size, weight, and the stage of the disease. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

Other aspects of the invention will be clear to the skilled artisan and need not be repeated here.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

A Sequence Listing is followed by what is claimed.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: LUDWIG INSTITUTE FOR CANCER RESEARCH

(B) STREET:

(C) CITY: ZURICH

(E) COUNTRY: SWITZERLAND

(F) POSTAL CODE:

(i) APPLICANT:

(A) NAME: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

(B) STREET: 300 LAKESIDE DRIVE, 22ND FLOOR

(C) CITY: OAKLAND

(E) COUNTRY: CALIFORNIA

(F) POSTAL CODE: 94612-3550

(ii) TITLE OF INVENTION: BRAIN GLYCOGEN PHOSPHORYLASE CANCER ANTIGEN

(iii) NUMBER OF SEQUENCES: 25

(iv) CORRESPONDENCE ADDRESS:

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(E) COUNTRY: UNITED STATES OF AMERICA

(F) POSTAL CODE: 02110

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:

(B) FILING DATE:

(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: 08/672,351

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(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: VAN AMSTERDAM, JOHN R.

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(C) REFERENCE/DOCKET NUMBER: L0461/7004WO

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 617-720-3500

(B) TELEFAX: 617-720-2441

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 110 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGGGCGAAC CGCTGACGGA CAGCGAGAAG CGGAAGCAGA TCAGCGTGCG CGGCCTGGCG 60

GGGCTAGGCG ACGTGGCCGA GGTGCGGAAG AGCTTCAACC GGCACTTGCA 110

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 102 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGGGCGAAC CGCTGACGGA CAGCGAGAAG CGGAAGCAGA TCAGCGTGCG CGGCCTGGCG 60

GGGCTAGGCG ACGTGGCCGA GGTGCGGAAG AGCTTCAACC GG 102

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 81 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGGCGAAC CGCTGACGGA CAGCGAGAAG CGGAAGCAGA TCAGCGTGCG CGGCCTGGCG 60

GGGCTAGGCG ACGTGGCCGA G 81

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gly Leu Ala Gly Leu Gly Asp Val Ala Glu Val Arg Lys Ser Phe Asn
1 5 10 15

Arg

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gly Leu Ala Gly Leu Gly Asp Val Ala Glu Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ala Gly Leu Gly Asp Val Ala Glu Val Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Gly Leu Gly Asp Val Ala Glu Val Arg Lys

1 5 10

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Gly Asp Val Ala Glu Val Arg Lys Ser

1 5 10

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly	Asp	Val	Ala	Glu	Val	Arg	Lys	Ser	Phe
1				5					10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Val Ala Glu Val Arg Lys Ser Phe Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Val Ala Glu Val Arg Lys Ser Phe Asn Arg

1 5 10

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Leu Ala Gly Leu Gly Asp Val Ala Glu Val

1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Gly Leu Gly Asp Val Ala Glu Val
1 5

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gly Leu Gly Asp Val Ala Glu Val
1 5

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Leu Gly Asp Val Ala Glu Val

1 5

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Gly Asp Val Ala Glu Val

1 5

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TGCCAGGCAC AGGTGGACCA

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CAGACCCCAG AATCCAGAGG C 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Gly Ile Val Gly Val Glu Asn Val Ala Glu Leu

1 5 10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Gly Leu Ala Gly Val Glu Asn Val Ile Glu Leu
1 5 10

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4066 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 35..2566

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

```
CCTCCATCTC TTTTCCTCCG CCTCCGCCGG CGCG ATG GGC GAA CCG CTG ACG          52
                                        Met Gly Glu Pro Leu Thr
                                          1               5

GAC AGC GAG AAG CGG AAG CAG ATC AGC GTG CGC GGC CTG GCG GGG CTA        100
Asp Ser Glu Lys Arg Lys Gln Ile Ser Val Arg Gly Leu Ala Gly Leu
            10                  15                  20

GGC GAC GTG GCC GAG GTG CGG AAG AGC TTC AAC CGG CAC TTG CAC TTC        148
Gly Asp Val Ala Glu Val Arg Lys Ser Phe Asn Arg His Leu His Phe
        25                  30                  35

ACG CTG GTC AAG GAC CGC AAT GTG GCC ACG CCC CGC GAC TAC TTC TTC        196
Thr Leu Val Lys Asp Arg Asn Val Ala Thr Pro Arg Asp Tyr Phe Phe
    40                  45                  50

GCG CTG GCG CAC ACG GTG CGC GAC CAC CTC GTG GGC CGC TGG ATC CGC        244
Ala Leu Ala His Thr Val Arg Asp His Leu Val Gly Arg Trp Ile Arg
55                  60                  65                  70

ACG CAG CAG CAC TAC TAC GAG CGC GAC CCC AAG CGC ATT TAT TAT CTT        292
```

Thr Gln Gln His Tyr Tyr Glu Arg Asp Pro Lys Arg Ile Tyr Tyr Leu
75 80 85

TCC CTG GAA TTC TAC ATG GGT CGC ACG CTG CAG AAC ACG ATG GTG AAC 340
Ser Leu Glu Phe Tyr Met Gly Arg Thr Leu Gln Asn Thr Met Val Asn
90 95 100

CTG GGC CTT CAG AAT GCC TGC GAT GAA GCC ATC TAT CAG TTG GGG TTA 388
Leu Gly Leu Gln Asn Ala Cys Asp Glu Ala Ile Tyr Gln Leu Gly Leu
105 110 115

GAC TTG GAG GAA CTC GAG GAG ATA GAA GAA GAT GCT GGC CTT GGG AAT 436
Asp Leu Glu Glu Leu Glu Glu Ile Glu Glu Asp Ala Gly Leu Gly Asn
120 125 130

GGA GGC CTG GGG AGG CTG GCA GCG TGT TTC CTT GAC TCA ATG GCT ACC 484
Gly Gly Leu Gly Arg Leu Ala Ala Cys Phe Leu Asp Ser Met Ala Thr
135 140 145 150

TTG GGC CTG GCA GCA TAC GGC TAT GGA ATC CGC TAT GAA TTT GGG ATT 532
Leu Gly Leu Ala Ala Tyr Gly Tyr Gly Ile Arg Tyr Glu Phe Gly Ile
155 160 165

TTT AAC CAG AAG ATT GTC AAT GGC TGG CAG GTA GAG GAG GCC GAT GAC 580
Phe Asn Gln Lys Ile Val Asn Gly Trp Gln Val Glu Glu Ala Asp Asp
170 175 180

TGG CTG CGC TAC GGC AAC CCC TGG GAG AAA GCG CGG CCT GAG TAT ATG 628
Trp Leu Arg Tyr Gly Asn Pro Trp Glu Lys Ala Arg Pro Glu Tyr Met
185 190 195

CTT CCC GTG CAC TTC TAC GGA CGC GTG GAG CAC ACC CCC GAC GGC GTG 676

Leu Pro Val His Phe Tyr Gly Arg Val Glu His Thr Pro Asp Gly Val
200 205 210

AAG TGG CTG GAC ACA CAG GTG GTG CTG GCC ATG CCC TAC GAC ACC CCA 724
Lys Trp Leu Asp Thr Gln Val Val Leu Ala Met Pro Tyr Asp Thr Pro
215 220 225 230

GTG CCC GGC TAC AAG AAC AAC ACC GTC AAC ACC ATG CGG CTG TGG TCC 772
Val Pro Gly Tyr Lys Asn Asn Thr Val Asn Thr Met Arg Leu Trp Ser
235 240 245

GCC AAG GCT CCC AAC GAC TTC AAG CTG CAG GAC TTC AAC GTG GGA GAC 820
Ala Lys Ala Pro Asn Asp Phe Lys Leu Gln Asp Phe Asn Val Gly Asp
250 255 260

TAC ATC GAG GCG GTC CTG GAC CGG AAC TTG GCT GAG AAC ATC TCC AGG 868
Tyr Ile Glu Ala Val Leu Asp Arg Asn Leu Ala Glu Asn Ile Ser Arg
265 270 275

GTC CTG TAT CCA AAT GAT AAC TTC TTT GAG GGG AAG GAG CTG CGG CTG 916
Val Leu Tyr Pro Asn Asp Asn Phe Phe Glu Gly Lys Glu Leu Arg Leu
280 285 290

AAG CAG GAG TAC TTC GTG GTG GCC GCC ACG CTC CAG GAC ATC ATC CGC 964
Lys Gln Glu Tyr Phe Val Val Ala Ala Thr Leu Gln Asp Ile Ile Arg
295 300 305 310

CGC TTC AAG TCG TCC AAG TTC GGC TGC CGG GAC CCT GTG AGA ACC TGT 1012
Arg Phe Lys Ser Ser Lys Phe Gly Cys Arg Asp Pro Val Arg Thr Cys
315 320 325

TTC GAG ACG TTC CCA GAC AAG GTG GCC ATC CAG CTG AAC GAC ACC CAC 1060

Phe Glu Thr Phe Pro Asp Lys Val Ala Ile Gln Leu Asn Asp Thr His
330 335 340

CCC GCC CTC TCC ATC CCT GAG CTC ATG CGG ATC CTG GTG GAC GTG GAG 1108
Pro Ala Leu Ser Ile Pro Glu Leu Met Arg Ile Leu Val Asp Val Glu
345 350 355

AAG GTG GAC TGG GAC AAG GCC TGG GAA ATC ACG AAG AAG ACC TGT GCA 1156
Lys Val Asp Trp Asp Lys Ala Trp Glu Ile Thr Lys Lys Thr Cys Ala
360 365 370

TAC ACC AAC CAC ACT GTG CTG CCT GAG GCC TTG GAG CGC TGG CCC GTG 1204
Tyr Thr Asn His Thr Val Leu Pro Glu Ala Leu Glu Arg Trp Pro Val
375 380 385 390

TCC ATG TTT GAG AAG CTG CTG CCG CGG CAC CTG GAG ATA ATC TAT GCC 1252
Ser Met Phe Glu Lys Leu Leu Pro Arg His Leu Glu Ile Ile Tyr Ala
395 400 405

ATC AAC CAG CGG CAC CTG GAC CAC GTG GCC GCG CTG TTT CCC GGC GAT 1300
Ile Asn Gln Arg His Leu Asp His Val Ala Ala Leu Phe Pro Gly Asp
410 415 420

GTG GAC CGC CTG CGC AGG ATG TCT GTG ATC GAG GAG GGG GAC TGC AAG 1348
Val Asp Arg Leu Arg Arg Met Ser Val Ile Glu Glu Gly Asp Cys Lys
425 430 435

CGG ATC AAC ATG GCC CAC CTG TGT GTG ATT GGG TCC CAT GCT GTC AAT 1396
Arg Ile Asn Met Ala His Leu Cys Val Ile Gly Ser His Ala Val Asn
440 445 450

GGT GTG GCG AGG ATC CAC TCG GAG ATC GTG AAA CAG TCG GTC TTT AAG 1444

Gly Val Ala Arg Ile His Ser Glu Ile Val Lys Gln Ser Val Phe Lys
455 460 465 470

GAT TTT TAT GAA CTG GAG CCA GAG AAG TTC CAG AAT AAG ACC AAT GGC 1492
Asp Phe Tyr Glu Leu Glu Pro Glu Lys Phe Gln Asn Lys Thr Asn Gly
475 480 485

ATC ACC CCC CGC CGG TGG CTG CTG CTG TGC AAC CCG GGG CTG GCC GAT 1540
Ile Thr Pro Arg Arg Trp Leu Leu Leu Cys Asn Pro Gly Leu Ala Asp
490 495 500

ACC ATC GTG GAG AAA ATT GGG GAG GAG TTC CTG ACT GAC CTG AGC CAG 1588
Thr Ile Val Glu Lys Ile Gly Glu Glu Phe Leu Thr Asp Leu Ser Gln
505 510 515

CTG AAG AAG CTG CTG CCG CTG GTC AGT GAC GAG GTG TTC ATC AGG GAC 1636
Leu Lys Lys Leu Leu Pro Leu Val Ser Asp Glu Val Phe Ile Arg Asp
520 525 530

GTG GCC AAG GTC AAA CAG GAG AAC AAG CTC AAG TTC TCG GCC TTC CTG 1684
Val Ala Lys Val Lys Gln Glu Asn Lys Leu Lys Phe Ser Ala Phe Leu
535 540 545 550

GAG AAG GAG TAC AAG GTG AAG ATC AAC CCC TCC TCC ATG TTC GAT GTG 1732
Glu Lys Glu Tyr Lys Val Lys Ile Asn Pro Ser Ser Met Phe Asp Val
555 560 565

CAT GTG AAG AGG ATC CAC GAG TAC AAG CGG CAG CTG CTC AAC TGC CTG 1780
His Val Lys Arg Ile His Glu Tyr Lys Arg Gln Leu Leu Asn Cys Leu
570 575 580

CAC GTC GTC ACC CTG TAC AAT CGA ATC AAG AGA GAC CCG GCC AAG GCT 1828

His Val Val Thr Leu Tyr Asn Arg Ile Lys Arg Asp Pro Ala Lys Ala
585 590 595

TTT GTG CCC AGG ACT GTT ATG ATT GGG GGC AAG GCA GCG CCC GGT TAC 1876
Phe Val Pro Arg Thr Val Met Ile Gly Gly Lys Ala Ala Pro Gly Tyr
600 605 610

CAC ATG GCC AAG CTG ATC ATC AAG TTG GTC ACC TCC ATC GGC GAC GTC 1924
His Met Ala Lys Leu Ile Ile Lys Leu Val Thr Ser Ile Gly Asp Val
615 620 625 630

GTC AAT CAT GAC CCA GTT GTG GGT GAC AGG TTG AAA GTG ATC TTC CTG 1972
Val Asn His Asp Pro Val Val Gly Asp Arg Leu Lys Val Ile Phe Leu
635 640 645

GAG AAC TAC CGT GTG TCC TTG GCT GAG AAA GTG ATC CCG GCC GCT GAT 2020
Glu Asn Tyr Arg Val Ser Leu Ala Glu Lys Val Ile Pro Ala Ala Asp
650 655 660

CTG TCG CAG CAG ATC TCC ACT GCA GGC ACC GAG GCC TCA GGC ACA GGC 2068
Leu Ser Gln Gln Ile Ser Thr Ala Gly Thr Glu Ala Ser Gly Thr Gly
665 670 675

AAC ATG AAG TTC ATG CTC AAC GGG GCC CTC ACC ATC GGC ACC ATG GAC 2116
Asn Met Lys Phe Met Leu Asn Gly Ala Leu Thr Ile Gly Thr Met Asp
680 685 690

GGC GCC AAC GTG GAG ATG GCC GAG GAG GCC GGG GCC GAG AAC CTC TTC 2164
Gly Ala Asn Val Glu Met Ala Glu Glu Ala Gly Ala Glu Asn Leu Phe
695 700 705 710

ATC TTC GGC CTG CGG GTG GAG GAT GTC GAG GCC TTG GAC CGG AAA GGG 2212

Ile Phe Gly Leu Arg Val Glu Asp Val Glu Ala Leu Asp Arg Lys Gly
715 720 725

TAC AAT GCC AGG GAG TAC TAC GAC CAC CTG CCC GAG CTG AAG CAG GCC 2260
Tyr Asn Ala Arg Glu Tyr Tyr Asp His Leu Pro Glu Leu Lys Gln Ala
730 735 740

GTG GAC CAG ATC AGC AGT GGC TTT TTT TCT CCC AAG GAG CCA GAC TGC 2308
Val Asp Gln Ile Ser Ser Gly Phe Phe Ser Pro Lys Glu Pro Asp Cys
745 750 755

TTC AAG GAC ATC GTG AAC ATG CTG ATG CAC CAT GAC AGG TTC AAG GTG 2356
Phe Lys Asp Ile Val Asn Met Leu Met His His Asp Arg Phe Lys Val
760 765 770

TTT GCA GAC TAT GAA GCC TAC ATG CAG TGC CAG GCA CAG GTG GAC CAG 2404
Phe Ala Asp Tyr Glu Ala Tyr Met Gln Cys Gln Ala Gln Val Asp Gln
775 780 785 790

CTG TAC CGG AAC CCC AAG GAG TGG ACC AAG AAG GTC ATC AGG AAC ATC 2452
Leu Tyr Arg Asn Pro Lys Glu Trp Thr Lys Lys Val Ile Arg Asn Ile
795 800 805

GCC TGC TCG GGC AAG TTC TCC AGT GAC CGG ACC ATC ACG GAG TAT GCA 2500
Ala Cys Ser Gly Lys Phe Ser Ser Asp Arg Thr Ile Thr Glu Tyr Ala
810 815 820

CGG GAG ATC TGG GGT GTG GAG CCC TCC GAC CTG CAG ATC CCG CCC CCC 2548
Arg Glu Ile Trp Gly Val Glu Pro Ser Asp Leu Gln Ile Pro Pro Pro
825 830 835

AAC ATC CCC CGG GAC TAGGCACACC CTGCCTTGGC GGGACCAGCG GGCATTTGTT 2603

Asn Ile Pro Arg Asp
840

TTCTTGCTGA CTTTGCACCT CCTTTTTTCC CCAAACACTT TGCCAGCCAC TGGTGGTCCC 2663

TGCTTTTCTG AGTACCATGT TTCCAGGAGG GGCCATGGGG GTCAGGGTGG TTTTGAGAGA 2723

GCAGGGTAAG GAAGGAATGT GCTAGAAGTG CTCCTAGTTT CTTGTAAAGG AAGCCAGAGT 2783

TGACAGTACA AAGGGTCGTG GCCAGCCCTG CAGCTTCAGC ACCTGCCCCA CCCAGAGTGG 2843

GAGTCAGGTG GAGCCACCTG CTGGGCTCCC CCAGAACTTT GCACACATCT TGCTATGTAT 2903

TAGCCGATGT CTTTAGTGTT GAGCCTCTGG ATTCTGGGGT CTGGGCCAGT GGCCATAGTG 2963

AAGCCTGGGA ATGAGTGTTA CTGCAGCATC TGGGCTGCCA GCCACAGGGA AGGGCCAAGC 3023

CCCATGTAGC CCCAGTCATC CTGCCCAGCC CTGCCTCCTG GCCATGCCGG GAGGGGTCGG 3083

ATCCTCTAGG CATCGCCTTC ACAGCCCCCT GCCCCCTGCC CTCTGTCCTG GCTCTGCACC 3143

TGGTATATGG GTCATGGACC AGATGGGGCT TTCCCTTTGT AGCCATCCAA TGGGCATTGT 3203

GTGGGTGCTT GGAACCCGGG ATGACTGAGG GGGACACTGG AGTGGGTGCT TGTGTCTGCT 3263

GTCTCAGAGG CCTTGGTCAG GATGAAGTTG GCTGACACAG CTTAGCTTGG TTTTGCTTAT 3323

TCAAAAGAGA AAATAACTAC ACATGGAAAT GAAACTAGCT GAAGCCTTTT CTTGTTTTAG 3383

CAACTGAAAA TTGTACTTGG TCACTTTTGT GCTTGAGGAG GCCCATTTTC TGCCTGGCAG 3443

GGGCAGGTCT GTGCCCTCCC GCTTGACTCC TGCTGTGTCC TGAGGTGCAT TTCCTGTTTG 3503

TTACACACAA GGGCCAGGCT CCATTCTCCC TCCCTTTCCA CCAGTGCCAC AGCCTCGTCT 3563

GGAAAAAGGA CCAGGGGTCC CGGAGGAACC CATTTGTGCT CTGCTTGGAC AGCAGGCCTG 3623

GCACTGGGAG GTGGGGGTGA GCCCCTCACA GCCTTGCCCC TCCCCAAGGC TCGAACCTGC 3683

CTCCCATTGC CCAAGAGAGA GGGCAGGGAA CAGGCTACTG TCCTTCCCTG TGGAATTGCC 3743

GAGAAATCTA GCACCTTGCA TGCTGGATCT GGGCTGCGGG GAGGCTCTTT TTCTCCCTGG 3803

CCTCCAGTGC CCACCAGGAG GATCTGCGCA CGGTGCACAG CCCACCAGAG CACTACAGCC 3863

TTTTATTGAG TGGGGCAAGT GCTGGGCTGT GGTCGTGCCC TGACAGCATC TTCCCCAGGC 3923

AGCGGCTCTG TGGAGGAGGC CATACTCCCC TAGTTGGCCA CTGGGGCCAC CACCCTGACC 3983

ACCACTGTGC CCCTCATTGT TACTGCCTTG TGAGATAAAA ACTGATTAAA CCTTTGTGGC 4043

TGTGGTTGGC TGAAAAAAAA AAA 4066

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 843 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met Gly Glu Pro Leu Thr Asp Ser Glu Lys Arg Lys Gln Ile Ser Val

1 5 10 15

Arg Gly Leu Ala Gly Leu Gly Asp Val Ala Glu Val Arg Lys Ser Phe
20 25 30

Asn Arg His Leu His Phe Thr Leu Val Lys Asp Arg Asn Val Ala Thr
35 40 45

Pro Arg Asp Tyr Phe Phe Ala Leu Ala His Thr Val Arg Asp His Leu
50 55 60

Val Gly Arg Trp Ile Arg Thr Gln Gln His Tyr Tyr Glu Arg Asp Pro
65 70 75 80

Lys Arg Ile Tyr Tyr Leu Ser Leu Glu Phe Tyr Met Gly Arg Thr Leu
85 90 95

Gln Asn Thr Met Val Asn Leu Gly Leu Gln Asn Ala Cys Asp Glu Ala
100 105 110

Ile Tyr Gln Leu Gly Leu Asp Leu Glu Glu Leu Glu Glu Ile Glu Glu
115 120 125

Asp Ala Gly Leu Gly Asn Gly Gly Leu Gly Arg Leu Ala Ala Cys Phe
130 135 140

Leu Asp Ser Met Ala Thr Leu Gly Leu Ala Ala Tyr Gly Tyr Gly Ile
145 150 155 160

Arg Tyr Glu Phe Gly Ile Phe Asn Gln Lys Ile Val Asn Gly Trp Gln
165 170 175

Val Glu Glu Ala Asp Asp Trp Leu Arg Tyr Gly Asn Pro Trp Glu Lys
180 185 190

Ala Arg Pro Glu Tyr Met Leu Pro Val His Phe Tyr Gly Arg Val Glu
195 200 205

His Thr Pro Asp Gly Val Lys Trp Leu Asp Thr Gln Val Val Leu Ala
210 215 220

Met Pro Tyr Asp Thr Pro Val Pro Gly Tyr Lys Asn Asn Thr Val Asn
225 230 235 240

Thr Met Arg Leu Trp Ser Ala Lys Ala Pro Asn Asp Phe Lys Leu Gln
245 250 255

Asp Phe Asn Val Gly Asp Tyr Ile Glu Ala Val Leu Asp Arg Asn Leu
260 265 270

Ala Glu Asn Ile Ser Arg Val Leu Tyr Pro Asn Asp Asn Phe Phe Glu
275 280 285

Gly Lys Glu Leu Arg Leu Lys Gln Glu Tyr Phe Val Val Ala Ala Thr
290 295 300

Leu Gln Asp Ile Ile Arg Arg Phe Lys Ser Ser Lys Phe Gly Cys Arg
305 310 315 320

Asp Pro Val Arg Thr Cys Phe Glu Thr Phe Pro Asp Lys Val Ala Ile
325 330 335

Gln Leu Asn Asp Thr His Pro Ala Leu Ser Ile Pro Glu Leu Met Arg
340 345 350

Ile Leu Val Asp Val Glu Lys Val Asp Trp Asp Lys Ala Trp Glu Ile
355 360 365

Thr Lys Lys Thr Cys Ala Tyr Thr Asn His Thr Val Leu Pro Glu Ala
370 375 380

Leu Glu Arg Trp Pro Val Ser Met Phe Glu Lys Leu Leu Pro Arg His
385 390 395 400

Leu Glu Ile Ile Tyr Ala Ile Asn Gln Arg His Leu Asp His Val Ala
405 410 415

Ala Leu Phe Pro Gly Asp Val Asp Arg Leu Arg Arg Met Ser Val Ile
420 425 430

Glu Glu Gly Asp Cys Lys Arg Ile Asn Met Ala His Leu Cys Val Ile
435 440 445

Gly Ser His Ala Val Asn Gly Val Ala Arg Ile His Ser Glu Ile Val
450 455 460

Lys Gln Ser Val Phe Lys Asp Phe Tyr Glu Leu Glu Pro Glu Lys Phe
465 470 475 480

Gln Asn Lys Thr Asn Gly Ile Thr Pro Arg Arg Trp Leu Leu Leu Cys
485 490 495

Asn Pro Gly Leu Ala Asp Thr Ile Val Glu Lys Ile Gly Glu Glu Phe
500 505 510

Leu Thr Asp Leu Ser Gln Leu Lys Lys Leu Leu Pro Leu Val Ser Asp
515 520 525

Glu Val Phe Ile Arg Asp Val Ala Lys Val Lys Gln Glu Asn Lys Leu
530 535 540

Lys Phe Ser Ala Phe Leu Glu Lys Glu Tyr Lys Val Lys Ile Asn Pro
545 550 555 560

Ser Ser Met Phe Asp Val His Val Lys Arg Ile His Glu Tyr Lys Arg
565 570 575

Gln Leu Leu Asn Cys Leu His Val Val Thr Leu Tyr Asn Arg Ile Lys
580 585 590

Arg Asp Pro Ala Lys Ala Phe Val Pro Arg Thr Val Met Ile Gly Gly
595 600 605

Lys Ala Ala Pro Gly Tyr His Met Ala Lys Leu Ile Ile Lys Leu Val
610 615 620

Thr Ser Ile Gly Asp Val Val Asn His Asp Pro Val Val Gly Asp Arg
625 630 635 640

Leu Lys Val Ile Phe Leu Glu Asn Tyr Arg Val Ser Leu Ala Glu Lys
645 650 655

Val Ile Pro Ala Ala Asp Leu Ser Gln Gln Ile Ser Thr Ala Gly Thr
660 665 670

Glu Ala Ser Gly Thr Gly Asn Met Lys Phe Met Leu Asn Gly Ala Leu
675 680 685

Thr Ile Gly Thr Met Asp Gly Ala Asn Val Glu Met Ala Glu Glu Ala
690 695 700

Gly Ala Glu Asn Leu Phe Ile Phe Gly Leu Arg Val Glu Asp Val Glu
705 710 715 720

Ala Leu Asp Arg Lys Gly Tyr Asn Ala Arg Glu Tyr Tyr Asp His Leu
725 730 735

Pro Glu Leu Lys Gln Ala Val Asp Gln Ile Ser Ser Gly Phe Phe Ser
740 745 750

Pro Lys Glu Pro Asp Cys Phe Lys Asp Ile Val Asn Met Leu Met His
755 760 765

His Asp Arg Phe Lys Val Phe Ala Asp Tyr Glu Ala Tyr Met Gln Cys
770 775 780

Gln Ala Gln Val Asp Gln Leu Tyr Arg Asn Pro Lys Glu Trp Thr Lys
785 790 795 800

Lys Val Ile Arg Asn Ile Ala Cys Ser Gly Lys Phe Ser Ser Asp Arg
805 810 815

Thr Ile Thr Glu Tyr Ala Arg Glu Ile Trp Gly Val Glu Pro Ser Asp
820 825 830

Leu Gln Ile Pro Pro Pro Asn Ile Pro Arg Asp
835 840

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GGGCTAGGCG ACGTGGCCGA GGTG 24

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCGGGGCTAG GCGACGTGGC CGAGGTG 27

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CTGGCGGGGC TAGGCGACGT GGCCGAGGTG 30

CLAIMS

1. An isolated fragment of brain glycogen phosphorylase, the fragment comprising not more than 80 consecutive amino acids of the amino acid sequence set forth in SEQ ID NO:22 and containing the amino acid sequence of SEQ ID NO:15.

2. The isolated fragment of claim 1, wherein the fragment comprises not more that 19 consecutive amino acids of SEQ ID NO:22.

3. The isolated fragment of claim 1 consisting essentially of a molecule between 7 and 50 consecutive amino acids of SEQ ID NO:22, comprising the sequence of SEQ ID NO:15.

4. The isolated fragment of claim 3, wherein the fragment consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:5.

5. The isolated fragment of claim 4, wherein the fragment consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

6. An isolated nucleic acid encoding a polypeptide, wherein the polypeptide is selected from the group consisting of the fragment of any of claims 1-5.

7. The isolated nucleic acid of claim 6, wherein the nucleic acid encodes a polypeptide selected from the group consisting of the fragment of any of claims 4 or 5.

8. The isolated nucleic acid of claim 6, wherein the nucleic acid comprises a molecule encoding a polypeptide having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

9. An expression vector comprising the isolated nucleic acid of claim 6 operably linked to a promoter.

10. The expression vector of claim 9 wherein the isolated nucleic acid comprises SEQ ID NO:23, SEQ ID NO:24 or SEQ ID NO:25.

11. The isolated nucleic acid or expression vector of claims 8, 9 or 10 further comprising a nucleic acid which codes for HLA-A2.

12. A host cell transfected or transformed with an expression vector selected from the group consisting of the expression vector of claim 9, the expression vector of claim 10, and the expression vector of claim 11.

13. A method for enriching selectively a population of T cells with cytolytic T cells specific for a brain glycogen phosphorylase tumor rejection antigen comprising:

contacting an isolated population of T cells with an agent presenting a complex of a brain glycogen phosphorylase tumor rejection antigen with a HLA presenting molecule in an amount sufficient to selectively enrich said isolated population of T cells with said cytolytic T cells.

14. The method of claim 13 wherein the HLA presenting molecule is HLA-A2 and wherein the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15.

15. The method of claim 14 wherein the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:5.

16. The method of claim 15 wherein the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

17. A method for diagnosing a disorder characterized by expression of a brain glycogen phosphorylase tumor rejection antigen, comprising:

contacting a biological sample isolated from a subject with an agent that is specific for the brain glycogen phosphorylase tumor rejection antigen, wherein the biological sample is isolated from non-brain, and non-retinal pigment epithelium tissue, and

determining the interaction between the agent and the brain glycogen phosphorylase tumor rejection antigen as a determination of the disorder.

18. The method of claim 17 wherein the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15.

19. The method of claim 18 wherein the peptide is between 7 and 100 consecutive amino acids of SEQ ID NO:21.

20. The method of claim 19 wherein the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:5.

21. The method of claim 20 wherein the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

22. A method for diagnosing a disorder characterized by expression of a brain glycogen phosphorylase tumor rejection antigen which forms a complex with HLA-A2 molecules, comprising:

contacting a biological sample isolated from a subject with an agent that binds the complex; and

determining binding between the complex and the agent as a determination of the disorder.

23. The method of claim 22 wherein the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15.

24. The method of claim 23 wherein the peptide is between 7 and 100 consecutive amino acids of SEQ ID NO:21.

25. The method of claim 24 wherein the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13,

SEQ ID NO:12, and SEQ ID NO:5.

26. The method of claim 25 wherein the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

27. The use of an agent comprising an isolated nucleic acid molecule which encodes a brain glycogen phosphorylase tumor rejection antigen or a polypeptide which includes a brain glycogen phosphorylase tumor rejection antigen, which enriches selectively in the subject the presence of complexes of HLA with brain glycogen phosphorylase tumor rejection antigen in the preparation of a medicament.

28. The use of claim 27 wherein the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising the amino acids of SEQ ID NO:15.

29. The use of claim 28 wherein the peptide consists essentially of a molecule having a sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:5.

30. The use of claim 29 wherein the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

31. The use of autologous cytolytic T cells specific for complexes of an HLA molecule with a brain glycogen phosphorylase tumor rejection antigen in the preparation of a medicament.

32. The use of claim 31 wherein the HLA presenting molecule is HLA-A2 and wherein the brain glycogen phosphorylase tumor rejection antigen is a peptide comprising a molecule having the amino acids of SEQ ID NO:15.

33. The use of claim 32 wherein the peptide is between 7 and 100 consecutive amino acids of SEQ ID NO:21.

34. The use of claim 33 wherein the peptide consists essentially of a molecule having a

sequence selected from the group consisting of SEQ ID NO:15, SEQ ID NO:14, SEQ ID NO:13, SEQ ID NO:12, and SEQ ID NO:5.

35. The use of claim 34 wherein the peptide consists of a molecule having a sequence selected from the group consisting of SEQ ID NO:14, SEQ ID NO:13, and SEQ ID NO:12.

36. A method for diagnosing a disorder characterized by expression of brain glycogen phosphorylase, comprising:

contacting a biological sample isolated from cells of a subject with an agent that binds the brain glycogen phosphorylase, wherein the biological sample is isolated from non-brain, non-retinal pigment epithelium, non-heart, non-renal cell carcinoma, non-hepatoma, or non-stomach adenocarcinoma tissue, and

determining binding between the brain glycogen phosphorylase and the agent as a determinant of the disorder.

37. A method for diagnosing a disorder characterized by expression of a nucleic acid which encodes brain glycogen phosphorylase, comprising:

contacting a biological sample isolated from a subject with an agent that is specific for said nucleic acid or an expression product thereof, wherein the biological sample is isolated from non-brain, non-retinal pigment epithelium, non-renal cell carcinoma, non-hepatoma, or non-stomach adenocarcinoma tissue, and wherein the nucleic acid hybridizes under stringent conditions to a molecule consisting of the nucleic acid sequence of claim 8, and

determining the interaction between said agent and said nucleic acid or said expression product as a determination of said disorder.

38. A pharmaceutical preparation comprising a pharmaceutically effective amount of brain glycogen phosphorylase or a fragment thereof that binds an HLA molecule, and a pharmaceutically acceptable carrier.

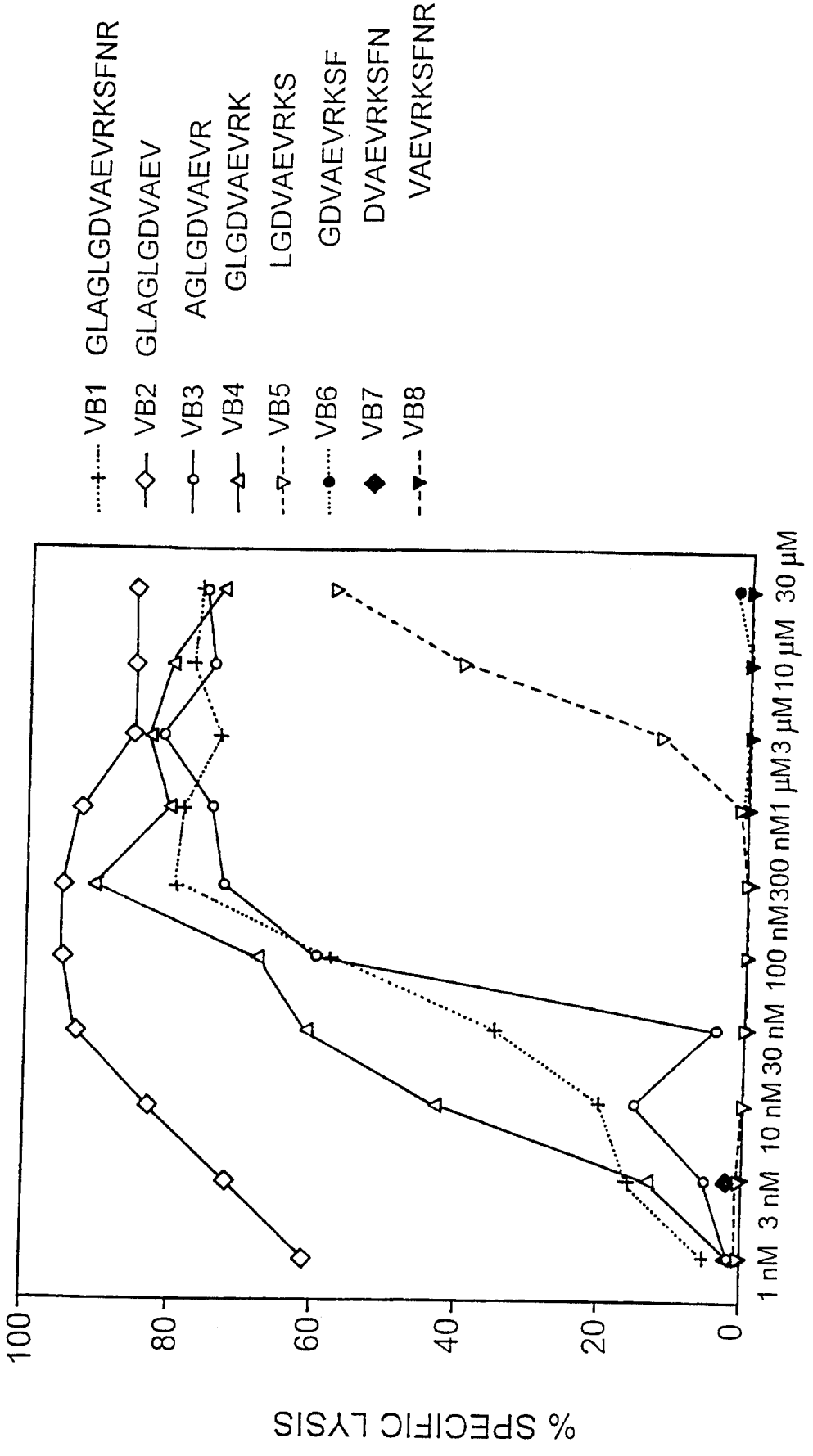
39. The pharmaceutical preparation of claim 38 wherein the brain glycogen phosphorylase or a fragment thereof that binds an HLA molecule comprises a peptide consisting of the amino acids of SEQ ID NO:15.

40. A pharmaceutical preparation comprising a pharmaceutically effective amount of isolated autologous T cells specific for complexes of an HLA molecule and a brain glycogen phosphorylase tumor rejection antigen, and a pharmaceutically acceptable carrier.

41. A functional variant of the isolated fragment of any of claims 1-5, comprising a polypeptide which is presented by HLA-A2 and is recognized by CTL264/76.

42. An isolated nucleic acid comprising a nucleotide sequence which encodes the functional variant of claim 41.

AMENDED SHEET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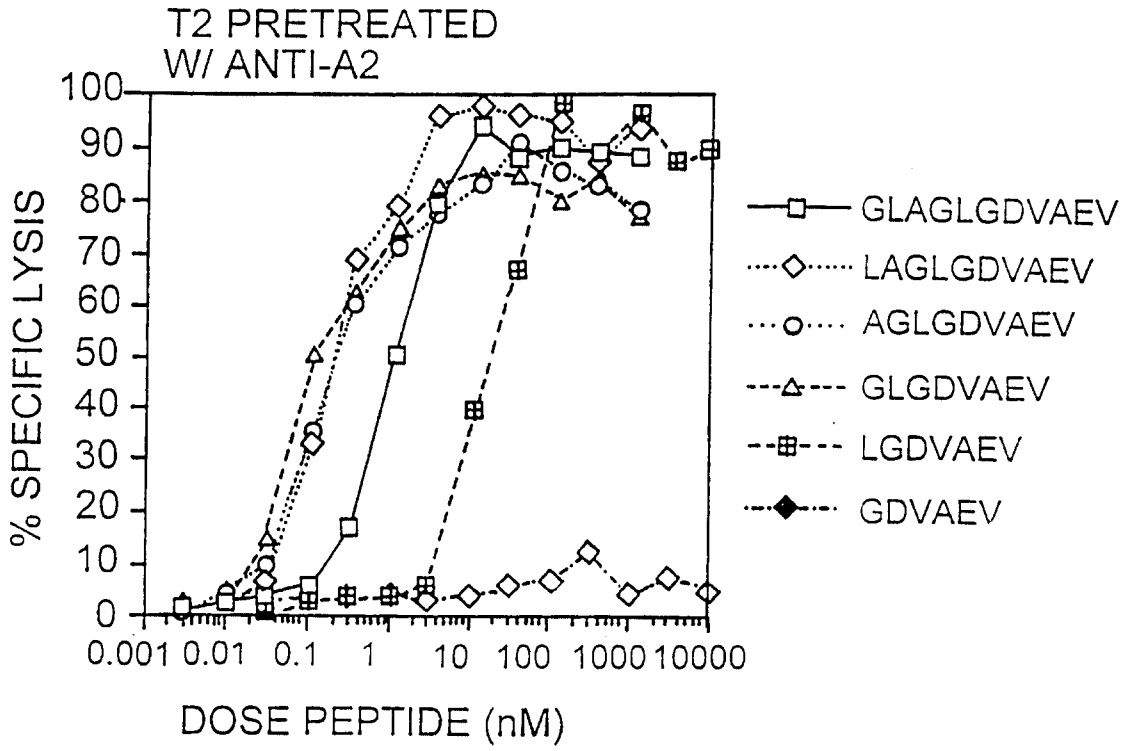
VB3 AGLGDVAEVR
VB4 GLGDVAEVRK
VB5 LGDVAEVRKS
VB6 GDVAEVRKSF
VB7 DVAEVRKSFN
VB8 VAEVRKSFNR
100
80
60
40
20
0
% SPECIFIC LYSIS
1 nM 3 nM 10 nM 30 nM 100 nM 300 nM 1 µM 3 µM 10 µM 30 µM
[PEPTIDES]

Fig. 1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W/ ANTI-A2
% SPECIFIC LYSIS
100
90
80
70
60
50
40
30
20
10
0
0.001 0.01 0.1 1 10 100 1000 10000
DOSE PEPTIDE (nM)
GLAGLGDVAEV
LAGLGDVAEV
AGLGDVAEV
GLGDVAEV
LGDVAEV
GDVAEV

Fig. 2A

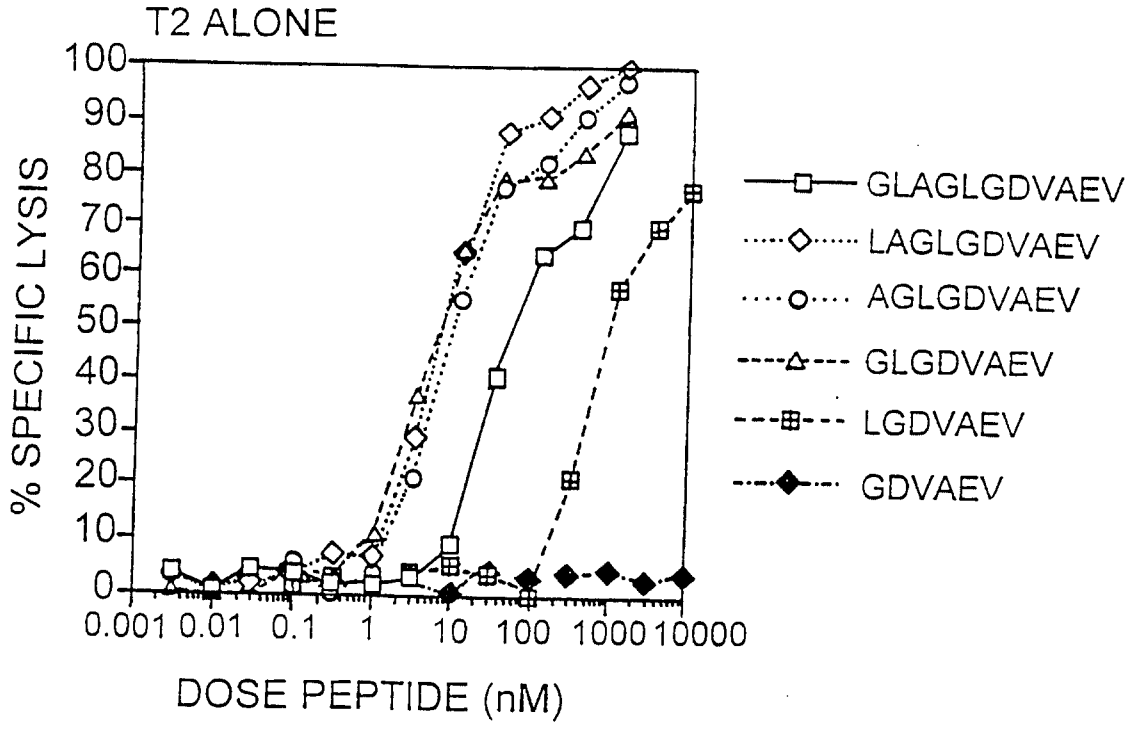

T2 ALONE
% SPECIFIC LYSIS
100
90
80
70
60
50
40
30
20
10
0
0.001 0.01 0.1 1 10 100 1000 10000
DOSE PEPTIDE (nM)
GLAGLGDVAEV
LAGLGDVAEV
AGLGDVAEV
GLGDVAEV
LGDVAEV
GDVAEV

Fig. 2B

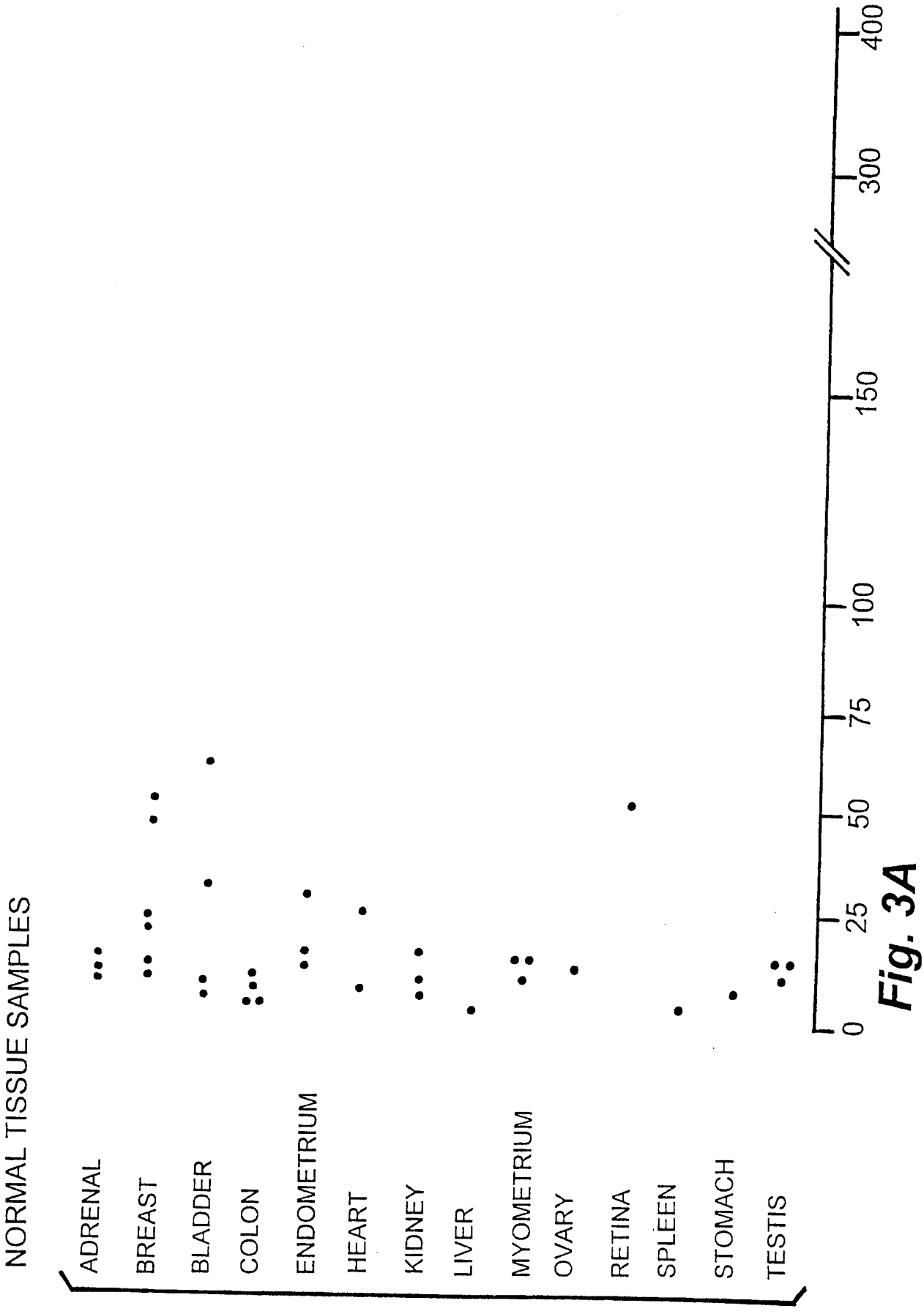

NORMAL TISSUE SAMPLES
ADRENAL
BREAST
BLADDER
COLON
ENDOMETRIUM
HEART
KIDNEY
LIVER
MYOMETRIUM
OVARY
RETINA
SPLEEN
STOMACH
TESTIS
0
25
50
75
100
150
300
400

Fig. 3A

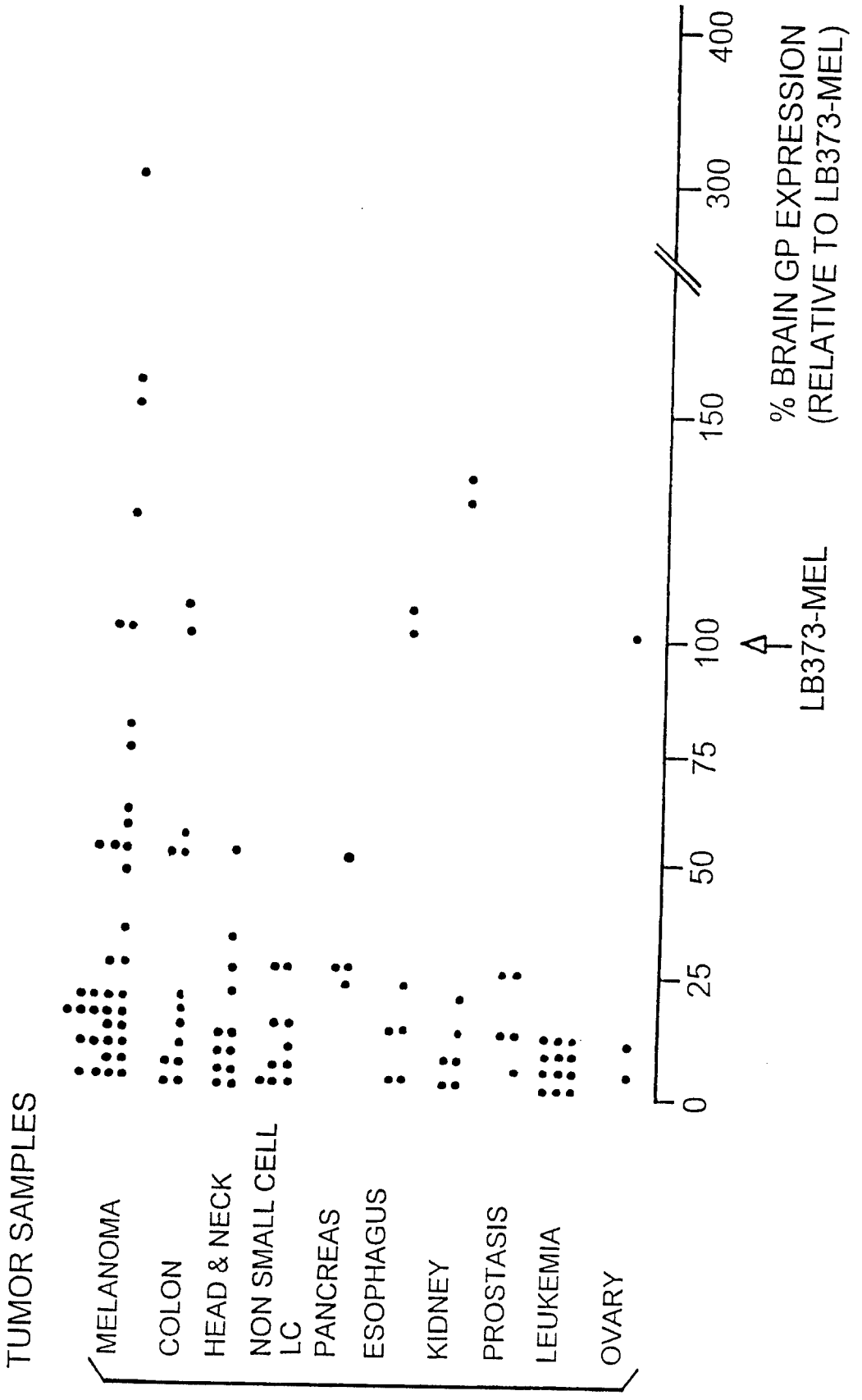
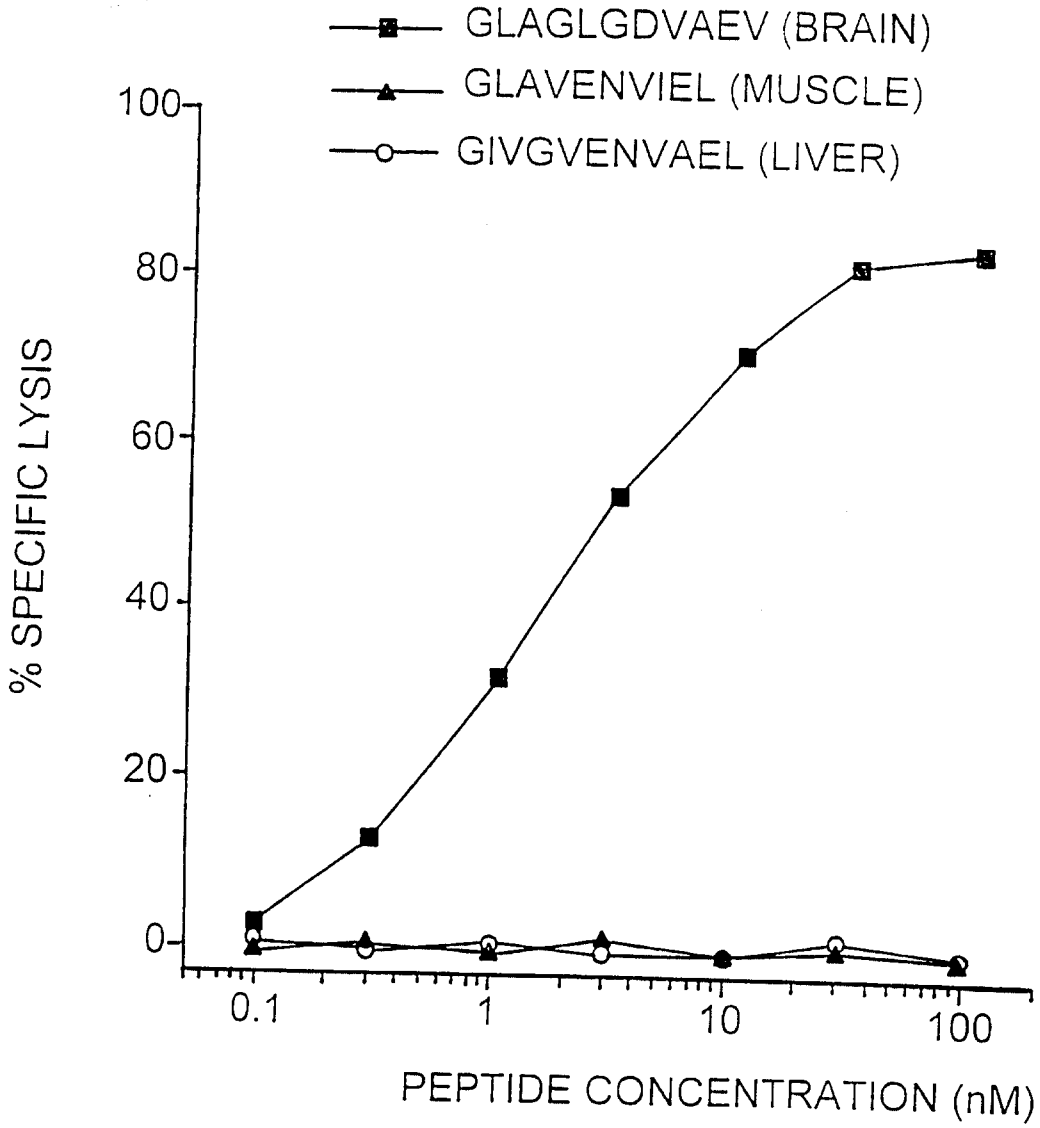

TUMOR SAMPLES
MELANOMA
COLON
HEAD & NECK
NON SMALL CELL LC
PANCREAS
ESOPHAGUS
KIDNEY
PROSTASIS
LEUKEMIA
OVARY
0
25
50
75
100
150
300
400
LB373-MEL
% BRAIN GP EXPRESSION (RELATIVE TO LB373-MEL)

Fig. 3B


GLAGLGDVAEV (BRAIN)
GLAVENVIEL (MUSCLE)
GIVGVENVAEL (LIVER)
% SPECIFIC LYSIS
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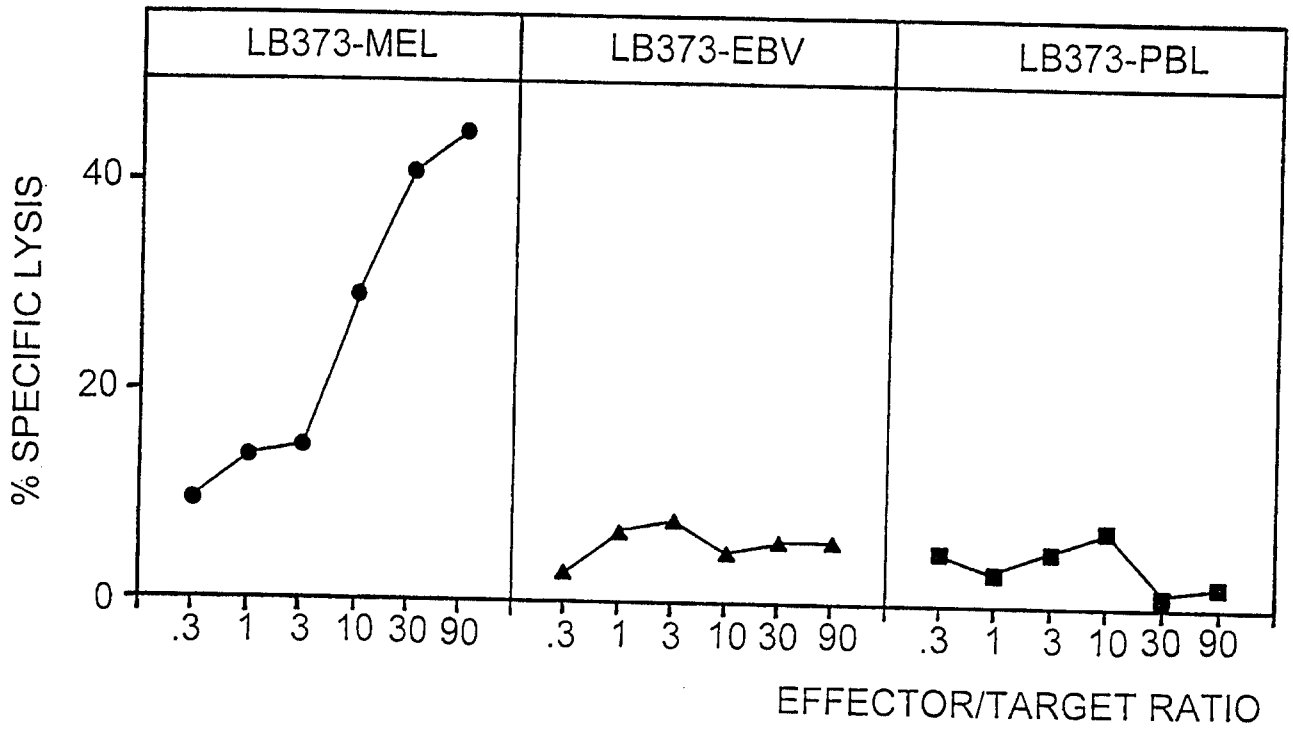
80
60
40
20
0
0.1
1
10
100
PEPTIDE CONCENTRATION (nM)

Fig. 4


LB373-MEL
LB373-EBV
LB373-PBL
% SPECIFIC LYSIS
40
20
0
.3 1 3 10 30 90
EFFECTOR/TARGET RATIO

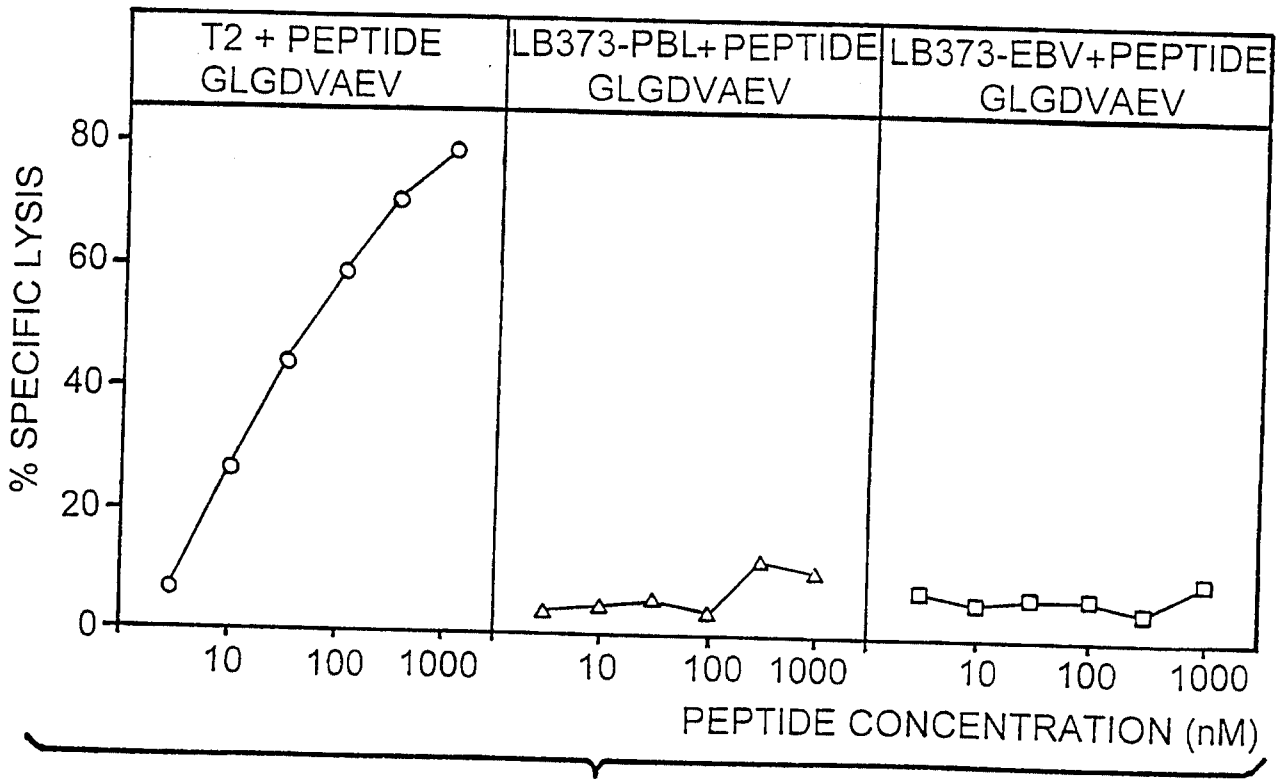

T2 + PEPTIDE GLGDVAEV
LB373-PBL+ PEPTIDE GLGDVAEV
LB373-EBV+PEPTIDE GLGDVAEV
% SPECIFIC LYSIS
80
60
40
20
0
10 100 1000
PEPTIDE CONCENTRATION (nM)

Fig. 5