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(54) Title: HUMAN TELOMERASE REVERSE TRANSCRIPTASE PEPTIDES

(57) Abstract: Tumor antigens can be categorized as tumor type specific or common. Telomerase reverse transcriptase (TRT) is the first bona fide common tumor antigen. While several 9mer peptides of the human TRT (hTRT) have been identified for HLA- A2, the most prevalent (~50%) HLA type in humans, little information exists on peptides for the remaining HLA types. As described herein, a multi-step approach was taken to select and characterize a panel of HLA-B79mer peptides as candidate immunogens. Specifically, several of algorithm based predictions, in vivo immunization of HLA-B7 transgenic mice, in vitro immunization of human blood lymphocytes, in vivo processing and supertype binding were employed to identify HLA-B7-restricted epitopes in hTRT. A correlation between in vivo immunogenicity and actual HLA-B7 binding avidity was found for the seven predicted peptides. Furthermore, endogenous processing was found to correlate with in vitro immunogenicity in human PBMC and HLA-B7 supertype binding.

HUMAN TELOMERASE REVERSE TRANSCRIPTASE PEPTIDES

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FIELD OF THE INVENTION

The present invention is directed to cancer immunotherapy and studies thereof. In particular, the present invention provides compositions and methods for inducing cytotoxic T lymphocyte responses to cells that present human telomerase reverse transcriptase peptides. In addition, the present invention provides tools for identifying immunogenic human telomerase reverse transcriptase peptides.

BACKGROUND OF THE INVENTION

Telomerase is a ribonucleoprotein that mediates RNA-dependent synthesis of telomeric DNA (1). Maintenance of a constant telomere length ensures chromosomal stability, prevents cells from aging, and confers immortality (2-4). *In vitro* studies show that the long-term ectopic expression of human telomerase reverse transcriptase (hTRT) in normal fibroblasts is sufficient for immortalization (5), and the expression of hTRT in combination with two oncogenes (SV40 T antigen and *Ras*) promotes tumor transformation in normal human epithelial and fibroblast cell lines (6). Thus, although telomerase *per se* is not tumorigenic, it plays a direct role in oncogenesis by allowing pre-cancerous cells to proliferate continuously and become immortal.

Studies of human cancer cells have shown a striking high expression (>85%) of telomerase activity in tumors of different histological origin and type (7, 8). In contrast, normal tissues display little or no telomerase activity (8, 9). For these reasons hTRT is considered the prototype common tumor antigen (10). To date numerous *in vitro* studies have been published demonstrating that hTRT peptides can be used to expand CD8 T cell precursors and generate cytotoxic T lymphocytes (CTL) in human peripheral blood mononuclear cells (PBMC) (11-15). Furthermore, several Phase 1 trials have also been completed proving that specific CD8 T cell responses can be induced *in vivo* (16-19) in cancer patients.

T lymphocytes recognize antigens through the intermediary of molecules of the major histocompatibility complex (MHC) or human leukocyte antigen (HLA), a polymorphic system composed of several hundred molecules ("MHC restriction"). CD8 T cells recognize antigen presented through MHC Class I molecules expressed at the surface of every cell after antigen peptides have been processed inside the cell and exported to the cell surface through the endogenous pathway (20). Under normal circumstances, MHC Class I molecules present a broad variety of peptides, mainly the product of processing of endogenous proteins. Upon infection by microbial pathogens or tumor transformation, peptides are generated that once complexed with the MHC molecules of an antigen presenting cell (APC) can activate CD8 T cells and induce CTL responses. However, since the MHC system is highly polymorphic among the human population, it requires that the immunogenicity of antigen peptides be studied in relation to each HLA molecule. An alternative and simpler approach is to test antigen peptides in relation to HLA alleles grouped into large supertype families (21). A HLA supertype is defined by the ability of a peptide to bind multiple HLA molecules (supermotif). The HLA alleleic variants that bind peptides possessing a particular HLA supertmotif are referred to as HLA supertype. The HLA-B7 supertype includes the B*0702, B*3501-03, B*51, B*5301, B*5401, B*0703-05, B*1508, B*5501-02, B*5601-02, B*6701 and B*7801 alleles. These HLA molecules share a peptide binding specificity for P in position 2 and a hydrophobic aliphatic (A, L, I, M, or V) or aromatic (F, W, or Y) residue at the C-terminal position (22).

To date specific information on the immunogenicity hTRT peptides is limited to one MHC allele (HLA-A*0201) with only initial reports on the HLA-A3 (13) and HLA-A24 (23) types, respectively. Although HLA-A*0201 is the most frequent in the human population (95% of HLA-A2 type which is itself expressed in ~50% of the Caucasian population (24-26)) immunogenic peptides for an equally large segment of the human population need to be identified. The goal of the work presented here was to identify immunogenic hTRT peptides restricted by HLA-B*0702 molecule, which is the most prevalent allele within the HLA-B7 type accounting for ~8.6% of the Caucasian population (27).

SUMMARY OF THE INVENTION

The present invention is directed to cancer immunotherapy and studies thereof. In particular, the present invention provides compositions and methods for inducing cytotoxic

T lymphocyte responses to cells that present human telomerase reverse transcriptase (hTRT) peptides. In addition, the present invention provides compositions and methods for identifying immunogenic hTRT peptides presented by the most frequently expressed major histocompatibility complex (MHC) class I types and supertypes. Specifically, in some embodiments the present invention provides compositions and methods comprising at least one human leukocyte antigen (HLA)-B7-restricted hTRT peptide. In further embodiments, compositions and methods comprising one or more of an HLA-A3-restricted hTRT peptide, an HLA-A2-restricted hTRT peptide, an HLA-A24-restricted hTRT peptide, an HLA-B44-restricted hTRT peptide, an HLA-A1-restricted hTRT peptide, and an HLA-B27-restricted hTRT peptide, are provided.

In still further embodiments, the present invention provides methods and compositions comprising an immunoglobulin molecule comprising an HLA class I restricted hTRT epitope inserted therein (e.g., recombinant antibody comprising an hTRT epitope expressed as part of a heavy or light chain variable region). The teaching of the production of antigenized antibodies can be found for instance in U.S. Patent Nos. 5,658,762, 5,583,202, and 5,508,386 to Zanetti et al. (herein incorporated by reference in their entirety).

In addition, in some embodiments the present invention provides methods and compositions for inducing a cytotoxic T lymphocyte response, comprising a first HLA Class I restricted hTRT peptide, wherein the first peptide is an HLA-A2-restricted hTRT peptide, and a second HLA Class I restricted hTRT peptide, wherein the second peptide comprises one or more of an HLA-B7-restricted hTRT peptide, an HLA-A3-restricted hTRT peptide, an HLA-A24-restricted hTRT peptide, an HLA-B44-restricted hTRT peptide, an HLA-A1-restricted hTRT peptide, and an HLA-B27-restricted hTRT. The teaching of HLA-A*0201-restricted hTRT peptides can be found for instance in U.S. Publication No. 20040086518 of Zanetti, and PCT Publication No. WO 00/25813 of Nadler et al. (both herein incorporated by reference in their entirety). In some embodiments, the HLA-A2-restricted hTRT peptide is selected from the group consisting of p540 (ILAKFLHWL, set forth as SEQ ID NO:10) and p865 (RLVDDFLLV, set forth as SEQ ID NO:11). In still further embodiments, the HLA-A2-restricted hTRT peptide comprises a modification which increases its binding affinity for HLA-A2 (e.g., p572Y, YLFFYRKSV, set forth as SEQ ID NO:12). Further teaching of HLA-A2-restricted peptides, with and without modifications for increasing their binding affinity for HLA-A2 can be found in

Minev et al., Proc Natl Acad Sci USA, 97:4796-4801, 2000; and Hernandez et al., Proc Natl Acad Sci USA, 99:12275-12280, 2002 (both herein incorporated by reference in their entirety).

Specifically, the present invention provides compositions for induction of a cytotoxic T lymphocyte response, comprising: at least one HLA-B7-restricted human telomerase reverse transcriptase (TRT) peptide from nine to twelve amino acid residues in length (e.g., 9, 10, 11 or 12 residues). In some embodiments, the HLA-B7 is selected from the group consisting of HLA-B*0702, HLA-B*3501, HLA-B*3502, HLA-B*3503, HLA-B*5101, HLA-B*5301, HLA-B*5401, HLA-B*0703, HLA-B*0704, HLA-B*0705, HLA-B*1508, HLA-B*5501, HLA-B*5502, HLA-B*5601, HLA-B*5602, HLA-B*6701, HLA-B*7801, and HLA-B*0801. In some preferred embodiments, the at least one TRT peptide consists of a sequence selected from the group consisting of SEQ ID NO:3 (p277), SEQ ID NO:4 (p342), SEQ ID NO:6 (p464), SEQ ID NO:8 (p1107), and SEQ ID NO:9 (p1123). In further embodiments, the composition also comprises a helper peptide, wherein the TRT peptide is not conjugated to the helper peptide. In an exemplary embodiments, the helper peptide corresponds to residues 128 to 140 of the hepatitis B core antigen (TPPAYRPPNAPIL, set forth as SEQ ID NO:13). In still further embodiments, the composition also comprises an adjuvant. In some embodiments, the compositions further comprise a physiologically acceptable carrier, which in preferred embodiments is a mammalian cell (e.g., antigen presenting cells such as a dendritic cell, a B lymphocyte or a macrophage having a TRT peptide bound to HLA class I molecules on the cell surface). Also provided are compositions in which the TRT peptide comprises a modification to enhance binding to HLA-B7. In some embodiments, the modification is a substitution of the first residue of a TRT nonamer with a tyrosine). In some preferred embodiments, the TRT peptide is a synthetic peptide.

Moreover, the present invention provides methods for inducing or enhancing a CTL response against target cells expressing human TRT and HLA-B7, comprising: harvesting leucocytes expressing HLA-B7; pulsing the leukocytes with a composition comprising an HLA-B7 restricted human TRT peptide from nine to twelve amino acid residues in length (e.g., 9, 10, 11 or 12 residues); and contacting target cells expressing human TRT and HLA-B7 with the pulsed leucocytes. In some embodiments, the contacting is accomplished *in vitro* or *ex vivo* while in alternative embodiments the contacting is accomplished *in vivo*. In some embodiments, the HLA-B7 is selected from the group consisting of HLA-B*0702,

HLA-B*3501, HLA-B*3502, HLA-B*3503, HLA-B*5101, HLA-B*5301, HLA-B*5401, HLA-B*0703, HLA-B*0704, HLA-B*0705, HLA-B*1508, HLA-B*5501, HLA-B*5502, HLA-B*5601, HLA-B*5602, HLA-B*6701, HLA-B*7801, and HLA-B*0801. In some preferred embodiments, the at least one TRT peptide consists of a sequence selected from the group consisting of SEQ ID NO:3 (p277), SEQ ID NO:4 (p342), SEQ ID NO:6 (p464), SEQ ID NO:8 (p1107), and SEQ ID NO:9 (p1123).

Additionally, the present invention provides methods for screening HLA class I-restricted human telomerase reverse transcriptase (TRT) peptides, comprising: a) using an algorithm to identify a human telomerase reverse transcriptase (TRT) peptide sequence in the full length TRT protein sequence that corresponds to a canonical HLA class I motif and comprises at least nine amino acid residues; b) testing HLA class I binding of the TRT peptide sequence by measuring HLA class I binding or stabilization in comparison to a reference peptide; and c) assessing immunogenicity of the TRT peptide sequence by measuring induction of TRT peptide-reactive cytotoxic T lymphocytes (CTL) of an HLA class I-positive subject. In some embodiments, the HLA class I-positive subject was immunized with a candidate human TRT vaccine (e.g., immunogenic composition) prior to the assessing of step c). In some preferred embodiments, the human TRT vaccine comprises human TRT DNA. In other preferred embodiments, the human TRT vaccine comprises a recombinant microorganism engineered to express human TRT. In further embodiments, the human TRT vaccine comprises a TRT peptide from nine to twelve amino acid residues in length (e.g., 9, 10, 11 or 12 residues), which in some embodiments is formulated with a liposome. In preferred methods, HLA class I is HLA-B7, while in particularly preferred embodiments the HLA-B7 binding comprises HLA-B*0702 binding, and one or more of HLA-B*3501, HLA-B*3502, HLA-B*3503, HLA-B*5101, HLA-B*5301, HLA-B*5401, HLA-B*0703, HLA-B*0704, HLA-B*0705, HLA-B*1508, HLA-B*5501, HLA-B*5502, HLA-B*5601, HLA-B*5602, HLA-B*6701, HLA-B*7801, and HLA-B*0801 binding. In alternative embodiments, the HLA class I is selected from the group consisting of HLA-A3, HLA-A24, HLA-B44, HLA-A1 and HLA-B27. In some embodiments, the HLA class I-positive subject is a transgenic mouse.

Also provided by the present invention are compositions for induction of a cytotoxic T lymphocyte response, comprising: at least one HLA class I-restricted human telomerase reverse transcriptase (hTRT) peptide from nine to twelve amino acid residues in length, wherein the hTRT peptide comprises one or more of an HLA-A3-restricted hTRT peptide,

an HLA-A24-restricted hTRT peptide, an HLA-B44-restricted hTRT peptide, an HLA-A1-restricted hTRT peptide, and an HLA-B27-restricted hTRT.

DESCRIPTION OF THE FIGURES

Figure 1. *In vivo* CTL responses against p277, p342, p444, p464, p966, p1107 and p1123 in HLA-B7 Tg mice. HLA-B7 Tg mice were vaccinated with 100 micrograms of individual hTRT peptide together with 120 micrograms of HBV helper peptide in IFA. Ten days after immunization, spleen lymphocytes were restimulated *in vitro* with peptide and fresh, irradiated syngeneic APC. Restimulations were performed on a weekly basis. A standard 4 hour ^{51}Cr-release assay was performed on day 5 after *in vitro* restimulation, using RMA-B7 cells pulsed with the homologous hTRT peptide as targets and an E:T ratio of 25:1. Results are expressed as the mean specific lysis plus or minus standard deviation of responder mice only, whose number is indicated in each panel. Tests were run in duplicate.

Figure 2. Examples of CTL responses induced *in vivo* by immunization with p277 and p1123. Spleen lymphocytes of HLA-B7 Tg immunized mice were restimulated *in vitro* with the homologous hTRT peptide on a weekly basis. A standard 4 hour ^{51}Cr-release assay was performed, using RMA-B7 cells pulsed or not pulsed with peptide as targets, at the indicated E:T ratios. CTL assay was performed after one (a and b), two (c and d) and three (e and f) rounds of *in vitro* restimulation.

Figure 3. Examples of CTL induction in a small scale *in vitro* immunization assay using normal donor PBMC. HLA-B7^{+} human PBMC were immunized *in vitro* in a 96 well plate assay, and tested for specific lysis of T2-B7 pulsed with peptide at day 10-11. The micro-CTL assay was performed as described in Material and Methods. All cultures but those with p444 were set with PBMC from the same donor.

Figure 4. Characterization of human CTL generated by *in vitro* immunization. An example of one of two HLA-B7^{+} normal donor PBMC from and a prostate cancer patient. Immunization *in vitro* was performed using a conventional method (12). **(A)** Specific lysis of T2-B7 cells pulsed with p1123 by CTL generated in normal donor PBMC. CTL were tested after 5 cycles of *in vitro* restimulation with homologous peptide. **(B)** Surface phenotype analysis using anti-CD3 and anti-CD8 monoclonal antibodies of the CTL shown in panel A. The percentage of double positive cells is indicated. **(C)** Specific lysis of T2-B7 cells pulsed with p1123 by CTL generated in prostate cancer patient PBMC. CTL were tested after 7 cycles of *in vitro* restimulation with homologous peptide. **(D)** Surface

phenotype analysis using anti-CD3 and anti-CD8 monoclonal antibodies of the CTL from the same patient shown on panel C after 5 cycles of *in vitro* restimulation with homologous peptide. Experiments shown in A and B are representative of set of similar data from two normal donors examined at different times.

Figure 5. p1123 is endogenously processed in JY lymphoblastoid cells. CTL from an HLA-B7^{+} human normal donor PBMC were tested in a 4 hour ^{51}Cr-release assay of T2-B7 cells pulsed with p1123 **(A)**, or JY cells **(B)**. Tests were run in duplicates at the indicated E:T ratios. CTL were used after 4 cycles of *in vitro* restimulation with homologous peptide. Tests were done in duplicate.

Figure 6. The nucleic acid sequence (SEQ ID NO:1) of hTRT is shown.

Figure 7. The amino acid sequence (SEQ ID NO:2) of hTRT is shown.

Figure 8. Murine CTL (mCTL) specific for p1123 recognizes hTRT+ human target cells (T1-B7 and BC1-B7). A mCTL line was expanded from p1123-immunized HLA-B7 Tg mice and re-stimulated five times in vitro. (A) Four-hour ^{51}Cr-release assay was performed with mCTL using human T2-B7 as target cells, with or without p1123 pulsing. (B) Intracellular IFN-gamma staining of mCTL upon overnight incubation with T1-B7, BC1-B7 lymphoblastoid cells and T2-B7 pulsed with p1123 (positive control) and p464 (negative control). Tests were repeated twice with similar results.

Definitions

To facilitate an understanding of the present invention, a number of terms and phrases are defined below:

As used herein, the terms "purified" and "isolated" refer to molecules (polynucleotides or polypeptides) or organisms that are removed or separated from their natural environment. "Substantially purified" molecules or organisms are at least 50% free, preferably at least 75% free, more preferably at least 90% and most preferably at least 95% free from other components with which they are naturally associated.

The term "wild-type" refers to a gene, gene product or organism that has the characteristics of that gene, gene product or organism when isolated from a naturally occurring source. A wild type gene or organism is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene or organism.

In contrast, the terms "modified," "mutant," and "variant" refer to a gene, gene product or organism that displays modifications in sequence and or functional properties (*i.e.*, altered characteristics) when compared to the wild-type gene, gene product or organism. It is noted that naturally occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene, gene product or organism.

As used herein, the term "immune response" refers to the reactivity of a subject's immune system in response to an antigen. In mammals, this may involve antibody production, induction of cell-mediated immunity, and/or complement activation. In preferred embodiments, the term immune response encompasses but is not limited to one or more of a "cytotoxic T lymphocyte response," a "lymphocyte proliferative response," a "cytokine response," and an "antibody response."

In particularly preferred embodiments, the immune response encompasses induction of CTL that are essentially specific for cells that present hTRT epitopes in the context of HLA class I molecules (e.g., HLA-A, HLA-B and/or HLA-C). In some embodiments, the cells that present hTRT epitopes are HLA class I positive cells that express hTRT or that have been pulsed with a peptide (e.g., nine to 29 amino acids in length, preferably 9, 10, 11, 12, 13, 14 or 15 amino acids, including but not limited to the peptides disclosed herein in Tables I and VI-XIX) of a hTRT protein consisting of the sequence set forth as SEQ ID NO: 2. In particularly preferred embodiments, the cells that present hTRT epitopes are hTRT-positive human tumor cell lines (e.g., melanoma, prostate, breast, colon, lung, etc.) obtained from the American Type Culture Collection (ATCC). Expression of hTRT by tumor cells is determined using art-recognized methods such as the PCR-based TRAPEZE assay of Intergen (Purchase, NY). Cellular cytotoxicity of hTRT-positive target cells is measured in a ^{51}Cr-labeled release assay at an E:T ration of 50:1. In some embodiments, tumor cell lines are incubated with 100 units/ml interferon-gamma before the assay.

The term "T cell epitope" as used herein refers to an antigenic determinant presented by a MHC class I or class II molecule for binding to a single T cell receptor. T cell epitopes are linear epitopes comprising at least seven amino acid residues. In some embodiments of the present invention, the term T cell epitope encompasses a CTL epitope, which is an antigen fragment presented by an MHC class I molecule for binding to T cell receptor on the surface of a cytotoxic T lymphocyte (*e.g.*, generally $CD8^+$), while in other embodiments the term T cell epitope encompasses a Th epitope, which is an antigen fragment presented

by an MHC class II molecule for binding to T cell receptor on the surface of a helper T cell (*e.g.*, generally $CD4^+$).

The term "specific for an epitope of interest" when made in reference to an immune response refers to an increased level of the immune response to cells presenting the epitope of interest (e.g., hTRT CTL epitope such as p277, p1123, p540, p865, etc.) as compared to the level of the immune response to cells presenting a control peptide (*e.g.*, irrelevant antigen).

The term "vaccine" as used herein refers to an immunogenic composition administered to a subject for the purpose of inducing an immune response. This term encompasses candidate prophylactic and therapeutic cancer vaccines that have not yet been demonstrated to protect a subject from developing cancer and/or to eradicate a tumor or malignant cells in a cancer patient.

The term "adjuvant" as used herein refers to any compound that when injected together with an antigen, non-specifically enhances the immune response to that antigen. Exemplary adjuvants include but are not limited to incomplete Freunds adjuvant (IFA), aluminum-based adjuvants (*e.g.*, AlOH, AlPO4, etc), and Montanide ISA 720.

The terms "excipient," "carrier" and "vehicle" as used herein refer to usually inactive accessory substances into which a pharmaceutical substance (*e.g.*, hTRT peptide) is suspended. Exemplary carriers include liquid carriers (such as water, saline, culture medium, aqueous dextrose, and glycols) and solid carriers (such as carbohydrates exemplified by starch, glucose, lactose, sucrose, and dextrans, anti-oxidants exemplified by ascorbic acid and glutathione, and hydrolyzed proteins).

The term "control" refers to subjects or samples that provide a basis for comparison for experimental subjects or samples. For instance, the use of control subjects or samples permits determinations to be made regarding the efficacy of experimental procedures. In some embodiments, the term "control subject" refers to animals or cells receiving a mock treatment (*e.g.*, adjuvant alone).

As used herein the terms "TRT," "TERT" and "telomerase reverse transcriptase" refer to the catalytic subunit of the telomerase enzyme of eukaryotic cells that adds telomeres to the ends of chromosomes after they divide. In particular, the terms "human TRT" and "hTRT" refer to the human protein set forth in SEQ ID NO:2 (FIG. 7) encoded by the nucleic acid sequence set forth in SEQ ID NO:1 (FIG. 6).

DESCRIPTION OF THE INVENTION

Defining the immunogenic components of hTRT for each HLA type is a formidable task but a necessary step to develop immunotherapies to target hTRT on tumor cells in the widest assortment of the human population. Previously, this (12, 14) and other (11) laboratories identified immunogenic peptides for the most frequent HLA type, HLA-A2. The outcome of these studies was that humans possess a residual CD8 T cell repertoire for both high and low affinity hTRT peptides that can be expanded by immunization *in vitro* (12, 14, 16). hTRT specific CD8 T cell precursors have been reported to persist in patients with advanced cancer (12, 14, 15). Here, we expanded our systematic effort to the identification and characterization of immunogenic hTRT peptides restricted to HLA-B7. The results of the present study lead to a series of general considerations.

The conventional algorithms used here proved to be overall poor predictors of immunogenic hTRT peptides for the HLA-B7 type. Previously, we successfully used BIMAS as a way to predict and select HLA-A2 restricted hTRT peptides that fulfill desired criteria for immunogenicity similar to those studied here. In contrast, BIMAS could not predict HLA-B7 immunogenic peptides overall. For instance, p444, the top peptide according to BIMAS, was not immunogenic *in vivo* in HLA-B7 Tg mice, was poorly immunogenic *in vitro* for human PBMC, and was apparently not processed in HLA-B7 Tg mice immunized with full length hTRT pDNA. Not surprisingly, p444 actual binding avidity for the HLA-B7 molecule was also poor, hence pointing to a discrepancy between predicted affinity, actual avidity and immunogenic function. SYFPEITHI did not predict two peptides (p966 and p464), which were poorly immunogenic, but at the same time did not distinguish between immunogenic and non-immunogenic peptides among the remaining five peptides studied. Finally, predictions based on proteasome cleavage were found not to be useful. For instance, the two peptides with the highest predicted probability for processing and immunogenicity turned out to be non-immunogenic in one case (p966) and poorly immunogenic in the other case (p342). This algorithm did, however, predict p277. Collectively, none of the three algorithms used to guide the initial selection of peptides was *per se* able to discriminate peptides that fulfill prerequisites for immunogenicity.

In vitro immunization studies support the conclusion that there exists a residual CD8 T cell repertoire for the majority (5 out of 7) peptide specificities investigated. Since these peptides also possess good binding avidity for the HLA-B7 molecule, the present findings indicate that thymic negative selection (central tolerance) of hTRT CD8 T cell clonotypes

restricted to HLA-B7 did not occur or occurred to a only limited extent. The response of HLA-B7 Tg mice to *in vivo* immunization with peptide in immunological adjuvant was immediate and stronger than that of HLA-A2 Tg mice similarly immunized (12, 14, 39). It appears as if, at least with respect of hTRT, HLA-B7/peptide complexes are highly immunogenic. Similarly, high immunogenicity was documented in studies where HLA-B7 Tg mice were immunized with influenza virus peptides (28).

The supertype binding studies proved to be an excellent final checkpoint in the selection of immunogenic peptides. For instance, p1123 and to a lesser degree p277 and p1107, bound to various alleles of the HLA-B7 supertype. Taken together the results of our study indicate that candidate immunogenic peptides need to satisfy at least two general criteria; good avidity interaction with the HLA-B7 molecule and good supertype binding. One may also need to consider the quality of the interaction between the MHC/peptide complex with the TCR as an additional factor in immunogenicity. As to the second characteristic, our data indicate that supertype binding peptides are preferentially processed and possess a selective advantage for interaction with molecules of the transporter associated with antigen processing (TAP) complex (14, 44, 45). Nonetheless, an understanding of the mechanism(s) is not necessary in order to make and use the present invention, and it is not intended that the present invention be limited to any particular mechanism.

In conclusion, we presented the successful identification of several immunogenic hTRT peptides restricted to HLA-B7. We show that this identification required a multi-step approach and involved an ensemble of *in vitro* and *in vivo* steps using both mice and human PBMC. This implies that the selection of immunogenic peptides for potential clinical use rests on a series of checkpoints and an element of empiricism overall. To date, such systematic approach has enabled the identification of HLA-A2 (10), and now HLA-B7 peptides with characteristics of immunogenicity that could justify their use in immunotherapy of cancer patients. Together, HLA-A2 and HLA-B7 account for ~60% of the Caucasian population. If one takes into account supertype binding of some of the peptides identified in this study one may achieve greater than 70% coverage irrespective of ethnicity. Thus, for a complete coverage of the human population, immunogenic peptides for the alleles accounting for the remaining 30-40% of the population still need to be identified systematically using a strategy similar to the one followed herein.

EXPERIMENTAL

The following example is provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and is not to be construed as limiting the scope thereof. In the experimental disclosure which follows, the following abbreviations apply: eq (equivalents); M (Molar); μM (micromolar); N (Normal); mol (moles); mmol (millimoles); μmol (micromoles); nmol (nanomoles); g (grams); mg (milligrams); μg (micrograms); ng (nanograms); l or L (liters); ml (milliliters); μl (microliters); cm (centimeters); mm (millimeters); μm (micrometers); nm (nanometers); °C (degrees Centigrade); U (units), mU (milliunits); min. (minutes); sec. (seconds); % (percent); kb (kilobase); bp (base pair); PCR (polymerase chain reaction); TRT (telomerase reverse transcriptase); WT (wild type); Tg (transgenic); TCR (T cell receptor); Th (helper T cell); MHC (major histocompatibility complex); mAb (monoclonal antibody), APC (antigen presenting cell); and CTL (cytotoxic T lymphocyte).

Materials and Methods

Mice. HLA-B7 transgenic mice express a chimeric HLA-B7/H2-D^b MHC Class I molecule, are on a C57BL/6 background and have been previously described (28). Mice were originally produced at the Institut Pasteur (Paris, France). A colony was bred and maintained under specific pathogen-free conditions in the vivarium of the University of California, San Diego (La Jolla, CA). All experimental procedures were performed according to an approved protocol and the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Cell lines. The human T2-B7 transfectants and murine RMA-B7 transfectants lines have been transfected with the HLA-B*0702 allele as described previously in (28, 29). The Epstein Barr Virus transformed B lymphoblastoid (HLA-A2/B7) JY cells were obtained from Dr. Antonella Vitiello (PRI Johnson & Johnson, La Jolla, CA).

Human blood cells. Buffy coats from HLA-$B7^+$ normal donors were purchased from the San Diego Blood Bank (San Diego, CA). Prostate cancer patients were recruited through the Division of Hematology Oncology and blood was obtained by venipuncture. HLA-B7 positivity was assessed by flow cytometry. Experiments were performed in accordance with approved Institutional Review Board (IRB) protocols.

Peptides and monoclonal antibodies. All synthetic peptides were purchased from the Peptide Synthesis Core Facility of Ohio State University (Columbus, OH). The

monoclonal antibody against HLA-B7, BB7.1, was purchased from American Tissue Type Collection (Manassas,VA). Other antibodies used were fluorescein isothiocyanate (FITC)-conjugated mouse IgG anti-human CD8 Beta (mAb 53-6.7) and phycoerythrin (PE)-conjugated mouse IgG anti-human CD3 (BD PharMingen, San Diego, CA), and FITC-conjugated goat anti-mouse IgG antibody (Jackson Immunoresearch, West Grove, PA).

Predictive algorithms. The following predictive algorithms were used: (A) BIMAS algorithm, which is based on highly favorable and unfavorable dominant anchor residues, as well as auxiliary anchor residues, and sores peptides according to a coefficient (30) (access via: thr.cit.nih.gov/molbio/hla_bind/). (B) SYFPEITHI algorithm, which is based known T cell epitopes and MHC ligands (31, 32) (access via: www.uni-tuebingen.de/uni/kxi/) and takes into consideration the amino acids in the anchor and auxiliary anchor positions, and scores peptides according to the cumulative (positive or negative) effects of contributing amino acids with ideal anchor residues accounting for 10 points and amino acids regarded as having a negative effect on binding accounting for −1 and 3 points. (C) PAProC (Prediction Database for Proteasomal Cleavages) algorithm, which is a computer-based theoretical model for the cleavage of substrate proteins by yeast and human 20S proteasomes. PAProC predicts cleavability of amino acids sequence (cuts per amino acids) and individual cleavages (positions and estimated strength). Specifically, we used the Type III model, based on human erythrocyte proteasome cleavage of enolase and ovalbumin (33, 34) (access via: www.paproc.de/).

MHC binding assays. Relative avidity measurements. The relative avidity of hTRT peptides for HLA B7 was measured using a MHC stabilization assay on T2-B7 cells in comparison with a reference peptide as described previously (14). Results are expressed as values of relative avidity, which is the ratio of the concentration of test peptide necessary to reach 20% of the maximal binding by the reference peptide, so that the lower the value the stronger the binding.

Supertype analysis. Quantitative assays to measure the binding affinity of peptides to purified HLA B7- supertype molecules (B*0702, B*3501, B*5101, B*5301, B*5401) and B*0801 were based on the inhibition of binding of a radiolabeled standard peptide, and were performed as previously described (22, 35). Briefly, 1-10 nM of radiolabeled peptide was co-incubated at room temperature with 1 microM to 1 nM of purified MHC in the presence of 1-3 μM human beta $_2$-microglobulin (Scripps Laboratories, San Diego, CA) and a cocktail of protease inhibitors. After a two-day incubation, binding of the radiolabeled

peptide to the corresponding MHC class I molecule was determined by capturing MHC/peptide complexes on Greiner Lumitrac 600 microplates (Greiner Bio-one, Longwood, FL) coated with the W6/32 antibody, and measuring bound counts per minute (cpm) using the TopCount microscintillation counter (Packard Instrument Co.). Results are expressed as the concentration of peptide yielding 50% inhibition of the binding of the radiolabeled reference peptide. Peptides were typically tested at 6 different concentrations covering a 100,000-fold dose range, and in 3 or more independent assays. Under the conditions utilized, where [label]<[MHC] and $IC_{50} \geq$ [MHC], the measured IC_{50} values are reasonable approximations of the true K_d values.

In vitro immunization procedures. In experiments shown in Table III and Figure 3, immunizations were performed in 96 well plates. Briefly, $2x10^5$ irradiated (6000 rads) human PBMC were plated in 96 (flat) well-plate in 100 micro-liters of complete human medium (RPMI 1640 medium containing 10% heat inactivated human AB serum, 2 mM glutamine, 50 micro-grams/ml streptomycin and 50 micro-grams/ml penicillin) with 100 micro-grams/ml of peptide. 12 wells per peptide were plated per patient. Then $2x10^5$ PBMC in 100 micro-liters of complete human medium were added into each well. Four days later 100 micro-liters of medium were replaced with 100 micro-liters of fresh complete human medium containing 80 IU/ml of IL-2. At day 6-7, 100 IU/ml of IL-2 were added and wells were split into two. On day 10-11 micro-cytotoxicity assay was performed. In experiments shown in Figure 4 and 5 human PBMC were stimulated *in vitro* in 24 well plate with autologous, irradiated, peptide-pulsed adherent cells in the presence of IL-2 and IL-7 as previously described (12). On day 4 to 5 after restimulation, effector CTL were tested in a standard ^{51}Cr-release assay.

In vivo immunization procedures. Peptide immunization. HLA B7 transgenic mice (28) were injected s.c. at the base of the tail with 100 micro-grams of hTRT peptides along with 120 micro-grams of I-A^b MHC Class II helper peptide 128–140 of the hepatitis B virus core protein in incomplete Freunds' adjuvant as described previously (12). Long-term CTL lines were maintained in culture by weekly restimulation with irradiated, peptide-pulsed syngeneic spleen cells in RPMI-1640 medium containing 10% heat inactivated fetal bovine serum, 2 mM glutamine, 5×10^{-5} M 2-Mercaptoethanol, 50 micro-grams/ml streptomycin, and 50 micro-grams/ml penicillin (complete medium) and supplemented with 40 IU/ml of recombinant human IL-2.

DNA immunization. A DNA vector coding for the hTRT expressed under the control of CMV promoter was purified on plasmid Giga Kit columns under endotoxin-free conditions (Qiagen, Hilden, Germany). Anesthetized HLA-B*0702 transgenic mice were injected with 50 micro-liters of cardiotoxin into each tibialis anterior muscle 5-6 days prior DNA injection. For vaccination, 50 micro-liters of DNA (1 micro-gram/micro-liter in PBS) was injected into each pretreated muscle at day 0 and day 14. Ten days later, spleen cells of individual mice were separately restimulated *in vitro* with each relevant peptide (10 micro-grams/ml) for 6 days. Effector CTL cells were tested in a standard 4 hr ^{51}Cr-release assay, using RMA-B7 cells (HLA-B*0702 transfected RMA cells) pulsed with test peptide or control peptide (CMV p65-derived R10TV restricted to HLA-B7). Specific % lysis as indicated below. *In vivo* immunization procedures were preformed in accordance with approved animal protocols at the University of California, San Diego or the Pasteur Institute, respectively.

CTL assays. Both murine and human CTL were detected by the ^{51}Cr release assay performed as previously described (14). Briefly, HLA-B7^{+} antigen presenting cells (RMA-B7 or T2-B7 cells) were labeled for 1 hr with 100 micro-Ci of $Na_2{}^{51}CrO_4$ (Perkin Elmer). Washed cells ($5x10^3$ per well) were mixed in 96-well plates in 100 micro-liters/well with each peptide (at 10 micro-grams/ml or lower concentration) and 100 micro-liters of the CTL effector cells (at various E:T ratio) in RPMI medium. The plates were incubated for 4-5 hrs at 37 °C (5 % CO_2). The supernatants were harvested and counted on a Wallac 1470 Wizard Gamma counter. The percent lysis was calculated as 100 ($cpm_{exp} - cpm_{spont}$) / ($cpm_{max} - cpm_{spont}$).

FACS analysis. The phenotypic characteristics of *in vitro* expanded CTL were determined by FACS analysis. Briefly, on day 6 or 7 after stimulation, cells (0.5×10^6) were incubated with FITC-conjugated mouse anti-human CD8 mAb and PE-conjugated mouse anti-human CD3 mAb (2 micro-grams/ml) in Hank's Balanced Solution containing 0.1% BSA and 0.05% sodium azide for 30 min at 4°C. For human PBMC typing, cells were incubated with 10 micro-l of BB7.1 mouse B cell hybridoma supernatant for 20 min at 4°C, followed by 30 min incubation with FITC-conjugated rabbit anti-mouse IgG antibody. Samples were analyzed on a FACSCalibur (Becton Dickinson, San Jose, CA). One hundred thousand events were collected and analyzed using the CellQuest software (Becton Dickinson).

Results

Selection of peptides on predicted algorithms.

To limit the number of candidate peptides to a manageable panel we used two predictive algorithms BIMAS and SYFPEITHI. These were used independently to predict nine aminoacid peptides for the HLA-B*0702 allele which accounts for the majority of the members of the HLA-B7 type (27). While BIMAS predicts HLA binding based on overall binding characteristics and the presence of canonic anchor residues, SYFPEITHI predicts peptides whose binding characteristics are extrapolated from naturally occurring MHC ligands as a matrix database. PAProC (Prediction Database for Proteasomal Cleavages), which predicts the proteasomal cleavage of full-length proteins, was used to define cleavage accessibility.

We initially selected ten 9mer peptides with high predicted scores in either of the two algorithms or both, and synthesized seven peptides (Table I). These peptides were selected based on a consensus prediction by both BIMAS and SYFPEITHI. Among the seven peptides only three had a score greater than 180 using BIMAS, and all but two had a score of 23 using SYFPEITHI. Interestingly, the two peptides that could not be predicted using SYPEITHI scored among the best using BIMAS.

Table I. Prediction of HLA-B7 binding for hTRT peptides

hTRT peptides			Predictive Algorythm		
a.a.	**Sequence**	**SEQ ID NO**	**BIMAS**	**SYFPEITHI**	PAProC
p277	RPAEEATSL	3	80	23	XX
p342	RPSFLLSSL	4	80	23	XXX
p444	DPRRLVQLL	5	800	23	X
p464	FVRACLRRL	6	200	NP	0
p966	AGRNMRRKL	7	180	NP	XXX
p1107	LPGTTLTAL	8	80	23	0
p1123	LPSDFKTIL	9	80	23	0

HLA-B*0702 binding affinity was predicted by BIMAS and SYFPEITHI, where for the former the minimum numerical value for 9mer peptides possessing canonical anchor

residues is 180, and for the latter is 20. C-terminus proteasomal cleavage of the predicted 9mers out of the full-length (1132 amino acids) hTRT by proteasomal cleavage (PAProC). The predicted proteasomal cleavage strength is arbitrarily scored as 0 (for no cleavage), X, XX and XXX (for cleavage strength).
NP = not predicted

Next, we assessed the actual binding avidity for HLA-B7 (HLA-B*0702). Two independent assays were used: binding stabilization assay on T2-B7 cells by flow cytometry (12) and a competitive solid-phase radioimmunoassay on immobilized purified HLA-B7 molecule (35). As shown in Table II five out of seven peptides (p277, p342, p464, p1107 and p1123) displayed high avidity binding. The two peptides with weak binding (p444 and p966) were among the top three peptides predicted by BIMAS. There was excellent concordance between the two types of binding assays utilized.

TABLE II. Relative avidity of predicted hTRT peptides for HLA-B7

hTRT peptide	RA[a]	IC50[b] (nM)
p277	4.7	6.3
p342	2.5	0.56
p444	>20	239
p464	3.2	4.1
p966	>20	-
p1107	3.8	0.96
p1123	1.8	11

[a] Relative avidity was tested by MHC stabilization assay on T2-B7.
[b] IC50 was calculated by competition solid-phase radioimmunoassay.
Dash indicates an IC50 > 50,000 nM

In vivo immunization of HLA-B7 Tg mice

In order to assign immunogenicity to each of the peptides and correlate this property with the binding characteristics and the scores of the predictive algorithms, we immunized HLA-B7 Tg mice (28). Ten to eleven days after immunization mice were sacrificed, the spleen harvested and splenocytes put in culture with LPS/Dextran activated APC, and tested in a 4 hour ^{51}Cr-release assay. As shown, only five out of seven peptides yielded a meaningful, specific CTL response even after a third cycle of *in vitro* restimulation (Figure 1). All immunogenic peptides induced a response from the first *in vitro* restimulation and this response increased upon subsequent rounds of antigen restimulation. An example of CTL for two of the immunogenic peptides is shown in Figure 2. As noted the lysis of peptide-pulsed RMA-B7 target cells increased at each round of *in vitro* restimulation. No lysis occurred on RMA-B7 cells not pulsed with peptide. Thus, the *in vivo* results together with the actual measure of the avidity of HLA-B7 binding avidity distinguished two groups of 9mer hTRT peptides. One group (p277, p342, p464, p1107 and p1123), displayed both high binding *in vitro* and good immunogenicity *in vivo*. The other group (p444 and p966), showed poor binding and poor immunogenicity.

In vitro immunization of human PBMC from normal donors

To further assess the immunogenicity of the selected peptide candidates as well as their ability to expand precursor CD8 T cell in human PBMC, the following experiment was performed. PBMC from eight HLA-B7^{+} normal donors were screened in a small scale *in vitro* immunization assay (96 well plate assay) to determine the level of responses against each individual peptide. The cumulative data of this screening step are shown in Table III. As indicated the response to these peptides varied among the eight donors. Overall, two peptides (p277 and p1123) yielded strong responses in the majority of the subjects. Three peptides (p342, p464 and p1107) induced strong responses but in fewer instances only. Notably, p444 that was poorly immunogenic *in vivo* in HLA-B7 Tg mice also displayed poor immunogenicity in this micro-CTL assay. The response against p966 was not tested because of repeated negative results in HLA-B7 Tg mice. Thus, the results of this *in vitro* assay narrowed the spectrum of immunogenic peptides beyond those identified *in vivo* in HLA-B7 Tg mice. A typical result of this type of analysis is shown in Figure 3, which depicts the induction of CTL and their specificity in each of the twelve wells. As shown, there is considerable variability in the number of positive wells per peptide as well as in the

percentage lysis which itself varied from peptide to peptide. This variation in the response to each peptide may be related to either an intrinsic characteristic of the peptide (e.g., its avidity) or a variation in the frequency of CD8 T cell precursors for that peptide among donors particularly in view of the format of the assay used.

TABLE III. CTL response *in vitro* following immunization of normal donors PBMC with HLA-B7 restricted hTRT peptides

hTRT peptide	Donor 1	Donor 2	Donor 3	Donor 4	Donor 5	Donor 6	Donor 7	Donor 8	High R /Total	Low R /Total
p277	>50%	>50%	>50%	>50%	>50%	<25%	<25%	<25%	5/8	0/8
p342	>25%	>25%	>25%	>50%	0	<25%	>25%	0	1/8	4/8
p444	ND	ND	<25%	>25%	0	0	0	<25%	0/8	1/8
p464	>50%	>50%	0	>25%	<25%	<25%	<25%	0	2/8	1/8
p966	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
p1107	>50%	>50%	0	>50%	>25%	<25%	>25%	0	3/8	2/8
p1123	>50%	>50%	>50%	<25%	>50%	>50%	>50%	>50%	7/8	0/8

PBMC from HLA-B7^{+} normal blood donors were pulsed with the candidate peptide in 96 well plate assay (described in Material and Methods), and tested for lysis of T2-B7 pulsed with peptide on day 10-11. A micro ^{51}Cr-release assay was performed as described in Material and Methods. Responders were considered at >50% specific CTL lysis. CTL assays were performed at an approximate E:T ratio of 10:1. ND = not done

In vivo processing

Next, we established which among the various candidate peptides was processed and presented from full-length hTRT. To this end, we immunized HLA-B7 Tg mice with hTRT plasmid DNA. Mice were sacrificed on day 24, and splenocytes were restimulated *in vitro* with each of the following peptides: p277, p342, p444, p464, p1107 and p1123. As shown in Table IV some but not all the peptides were processed and presented *in vivo*. Three peptides (p277, p1107 and p1123) yielded greater CTL responses than the other peptides, implying either preferential processing and/or better immunogenicity once displayed at the surface of the APC. The remaining three peptides (p342, p444, p464) were marginally immunogenic if any. Based on this analysis, it appears that only three of the original seven

peptides were processed and presented efficiently *in vivo*. Interestingly, we found that among the three most immunogenic peptides only one (p277) was predicted by PAProC, whereas the other two (p1107 and p1123) were not (Table I). Thus, selection using PAProC algorithm was per se unable to predict hTRT peptides that would be cleaved and become immunogenic *in vivo*.

TABLE IV. *In vivo* processing and immunogenicity of hTRT peptides in HLA-B7 Tg mice

hTRT peptide	Responders/Total	%Responders	Specific Lysis (%)
p277	4/7	57	6, 3, **20, 16, 34**, 6, **31**
p342	2/7	29	3,4,7,**13**,6,2,**20**
p444	0/4	0	5,8,6,4
p464	2/6	17	9,**16**,**12**,9,7,2
p1107	3/6	50	**19**,8,**30**,**20**,9,4
p1123	3/6	50	**20**,9,**14**,**31**,**11**,9

HLA-B7 Tg mice were immunized with a pDNA coding for full-length hTRT under the CMV promoter. ^{51}Cr-release assay was performed after 6 days of *in vitro* restimulation with respective peptide. Mice were considered responders when >10% specific lysis was observed. Tests were run in duplicate at an E:T ratio of 60:1, using RMA-B7 target cells.

Supertype analysis

HLA molecules are highly polymorphic posing problems to the identification of peptides, which could be used to cover the totality of the human population. However, HLA alleles can be clustered into a relatively small number of groups termed supertypes (21). The HLA-B7 supertype includes ten alleles (22). Here, we decided to test the selected hTRT peptides for their ability to bind five out of ten members of the HLA-B7 supertype (B*3501, B*5101, B*5301, B*5401) and B*0801. The B*0801 allele shares binding features with B*0702, although is not officially part of the HLA-B7 supertype. This analysis had the purpose to further narrow the selection of putative HLA-B7 immunogens based on supertype binding (Table V). Only one peptide (p1123) had measurable avidity for all alleles examined. Another peptide (p1107) bound with high avidity three out of five alleles. Two additional peptides (p277 and p342) bound four alleles with intermediate

avidity. The remaining peptides (p444, p464 and p966) displayed little HLA-B7 supertype binding. Thus, it appears as if the peptides retained through the *in vitro* and *in vivo* screening processes described above for immunogenicity and processing *in vivo,* ranked best as HLA-B7 supertype binders. This demonstrates that the supertype analysis is a pivotal step in refining the selection process.

TABLE V. HLA-B7 supertype binding assay

hTRT peptide	Sequence	SEQ ID NO	HLA class I binding capacity (IC50 nM)					
			B*0702	B*3501	B*5101	B*5301	B*54011	B*0801
p277	RPAEEATSL	3	**6.3**	510	-	10618	45158	**207**
p342	RPSFLLSSL	4	**0.56**	1019	-	2199	12648	**37**
p444	DPRRLVQLL	5	**239**	-	7069	-	21933	**217**
p464	FVRACLRRL	6	**4.1**	-	-	-	18843	**123**
p966	AGRNMRRKL	7	-	-	-	-	34065	-
p1107	LPGTTLTAL	8	**0.96**	**132**	-	**120**	-	**192**
p1123	LPSDFKTIL	9	**11**	**5**	1625	**2.4**	19877	**74**

Dash indicates an IC50>50000 nM

Characterization of human CTL against p1123

To better characterize the response against the peptide with the best characteristics for immunogenicity overall (p1123), new *in vitro* immunization experiments were performed, using PBMC from two HLA-B7^{+} normal blood donors and one cancer patient. These experiments were performed using a conventional *in vitro* immunization assay (12). After repeated rounds of *in vitro* restimulation high efficiency CTL were induced that specifically killed T2-B7 target cells pulsed with p1123 (Figure 4A). These CTL showed to CD3/CD8 double positive T cells (80%) (Figure 4B). Thus, p1123 expanded CD8 T cell precursors, which developed into CTL. A similar approach was used with PBMC from a prostate cancer patient. Again, after repeated restimulations we were able to expand CTL that killed T2-B7 target cells pulsed with p1123 (Figure 4C). Compared with the efficiency of induction observed in both normal blood donors, the CTL induced in the cancer patient

were less efficient. The activity was seemingly mediated by CD3/CD8$^+$ lymphocytes double positive T cells (75%) as indicated by FACS analysis (Figure 4D). Collectively, these data confirm that CD8 T cell precursors for p1123 exist in the normal CD8 T cell repertoire, and persist after cancer development.

Finally, it was important to demonstrate that CTL against p1123 were able to lyse transporter associated with antigen processing protein (TAP) competent/hTRT positive target cells. To this end, we used the JY (a HLA-A2$^+$/B7$^+$ EBV transformed B lymphoblastoid human cell line), which is highly positive for hTRT (our unpublished data). CTL from normal donors that efficiently killed T2-B7 target cells pulsed with p1123, also killed JY cells in the absence of any peptide pulsing (Figure 5), suggesting that p1123 is naturally processed from endogenous hTRT, and that HLA-B7/p1123 complexes are presented at the cell surface in a way that is recognized by CTL induced by peptide immunization.

A mCTL line recognizes human cells

To further characterize the endogenous processing and presentation of p1123 in human cells, we used a mCTL line specific for p1123 with high lytic activity for human target cells (T2-B7) pulsed with peptide (p1123) (Fig. 8A). Two HLA-B7+ human lymphoblastoid cells were used, T1-B7 and BC1-B7. Although TAP-deficient T2-B7 cells pulsed with p1123 are highly susceptible to lysis by mCTL, non-pulsed TAP competent hTRT+ HLA-B7+ EBV-transformed B lymphoblastoid human cell lines, T1-B7 and BC1-B7, were not. This indicates that the low-affinity interaction between the murine CD8 co-receptor molecule and the human MHC may be compensated by the abundance of MHC–peptide complexes on T2-B7 cells pulsed with peptide.

As an alternative approach, we tested intracellular synthesis IFN-gamma in a mCTL line specific for p1123 in the presence of T1-B7 and BC1-B7 cells, reasoning that specific recognition of p1123 would engender IFN-gamma synthesis. This was assessed by measuring intracellular staining. As shown in Fig. 8B, overnight contact with T1-B7 and BC1-B7 lymphoblastoid cells produced an increase in CD8/IFN-gamma double-positive cells. This was only slightly at variance with the percentage CD8/IFN-gamma double positive CTL incubated with control T2-B7 cells pulsed with p1123 (positive control). This confirms, therefore, endogenous processing and presentation of hTRT p1123 in human cells.

All publications and patents mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and compositions of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the art, are intended to be within the scope of the present invention.

HLA-A3 SUPERTYPE HTRT PEPTIDES

Table VI. HLA-A3 hTRT peptides

1	535	RLREEILAK	SEQ ID NO: 14
2	1081	KLTRHRVTY	SEQ ID NO: 15
3	817	AVRIRGKSY	SEQ ID NO: 16
4	740	CVRRYAVVQ	SEQ ID NO: 17
5	143	RVGDDVLVH	SEQ ID NO: 18
6	973	KLFGVLRLK	SEQ ID NO: 19
7	130	ALRGSGAWG	SEQ ID NO: 20
8	79	ELVARVLQR	SEQ ID NO: 21
9	378	RLPRLPQRY	SEQ ID NO: 22
10	418	AVTPAAGVC	SEQ ID NO: 23

Table VII. HLA-A*1101 hTRT peptides

1	535	RLREEILAK	SEQ ID NO: 24
2	562	YVTETTFQK	SEQ ID NO: 25
3	973	KLFGVLRLK	SEQ ID NO: 26
4	881	KTFLRTLVR	SEQ ID NO: 27
5	550	SVYVVELLR	SEQ ID NO: 28
6	83	RVLQRLCER	SEQ ID NO: 29
7	995	QTVCTNIYK	SEQ ID NO: 30

Table VIII. HLA-A*3101 hTRT peptides

1	83	RVLQRLCER	SEQ ID NO: 31
2	881	KTFLRTLVR	SEQ ID NO: 32
3	1003	KILLLQAYR	SEQ ID NO: 33
4	550	SVYVVELLR	SEQ ID NO: 34
5	513	SVRDCAWLR	SEQ ID NO: 35

Table IX. HLA-A*6801 hTRT peptides

1	147	DVLVHLLAR	SEQ ID NO: 36
2	605	EVRQHREAR	SEQ ID NO: 37
3	663	SVLNYERAR	SEQ ID NO: 38
4	639	VVGARTFRR	SEQ ID NO: 39
5	638	YVVGARTFR	SEQ ID NO: 40
6	83	RVLQRLCER	SEQ ID NO: 41
7	550	SVYVVELLR	SEQ ID NO: 42
8	55	LVCVPWDAR	SEQ ID NO: 43
9	513	SVRDCAWLR	SEQ ID NO: 44
10	1089	YVPLLGSLR	SEQ ID NO: 45
11	79	ELVARVLQR	SEQ ID NO: 46
12	727	EVIASIIKP	SEQ ID NO: 47
13	135	GAWGLLLRR	SEQ ID NO: 48
14	503	LSLQELTWK	SEQ ID NO: 49
15	995	QTVCTNIYK	SEQ ID NO: 50

HLA-B44 SUPERTYPE HTRT PEPTIDES

Table X. HLA-B*4403 hTRT peptides

1	911	DEALGGTAF	SEQ ID NO: 51
2	554	VELLRSFFY	SEQ ID NO: 52
3	19	REVLPLATF	SEQ ID NO: 53
4	317	WDTPCPPVY	SEQ ID NO: 54

Table XI. HLA-B*4402 hTRT peptides

1	440	EEDTDPRRL	SEQ ID NO: 55
2	338	KEQLRPSFL	SEQ ID NO: 56
3	19	REVLPLATF	SEQ ID NO: 57
4	89	CERGAKNVL	SEQ ID NO: 58
5	208	REAGVPLGL	SEQ ID NO: 59
6	532	AEHRLREEI	SEQ ID NO: 60
7	537	REEILAKFL	SEQ ID NO: 61
8	554	VELLRSFFY	SEQ ID NO: 62
9	892	PEYGCVVNL	SEQ ID NO: 63
10	911	DEALGGTAF	SEQ ID NO: 64
11	667	YERARRPGL	SEQ ID NO: 65
12	1115	LEAAANPAL	SEQ ID NO: 66

Table XII. HLA-B*60 hTRT peptides

1	208	REAGVPLGL	SEQ ID NO: 67
2	1115	LEAAANPAL	SEQ ID NO: 68
3	537	REEILAKFL	SEQ ID NO: 69
4	440	EEDTDPRRL	SEQ ID NO: 70
5	667	YERARRPGL	SEQ ID NO: 71
6	338	KEQLRPSFL	SEQ ID NO: 72
7	89	CERGAKNVL	SEQ ID NO: 73

Table XIII. HLA-B*61 hTRT peptides

1	506	QELTWKMSV	SEQ ID NO: 74
2	604	AEVRQHREA	SEQ ID NO: 75
3	280	EEATSLEGA	SEQ ID NO: 76
4	781	QETSPLRDA	SEQ ID NO: 77
5	199	CERAWNHSV	SEQ ID NO: 78
6	428	REKPQGSVA	SEQ ID NO: 79
7	208	REAGVPLGL	SEQ ID NO: 80
8	1115	LEAAANPAL	SEQ ID NO: 81

HLA-A1 SUPERTYPE HTRT PEPTIDES

Table XIV. HLA-A*01 hTRT peptides

1	325	YAETKHFLY	SEQ ID NO: 82
2	1036	ISDTASLCY	SEQ ID NO: 83
3	442	DTDPRRLVQ	SEQ ID NO: 84
4	699	AQDPPPELYDF	SEQ ID NO: 85
5	766	LTDLQPYMR	SEQ ID NO: 86
6	943	QSDYSSYAR	SEQ ID NO: 87
7	838	STLLCSLCY	SEQ ID NO: 88
8	764	STLTDLQPY	SEQ ID NO: 89
9	938	RTLEVQSDY	SEQ ID NO: 90
10	563	VTETTFQKN	SEQ ID NO: 91
11	659	KALFSVLNY	SEQ ID NO: 92
12	1081	KLTRHRVTY	SEQ ID NO: 93
13	941	EVQSDYSSY	SEQ ID NO: 94

Table XV. HLA-A*26 hTRT peptides

1	941	EVQSDYSSY	SEQ ID NO: 95
2	552	YVVELLRSF	SEQ ID NO: 96
3	727	EVIASIIKP	SEQ ID NO: 97
4	565	ETTFQKNRL	SEQ ID NO: 98
5	790	VVIEQSSSL	SEQ ID NO: 99
6	362	ETIFLGSRP	SEQ ID NO: 100
7	147	DVLVHLLAR	SEQ ID NO: 101
8	1034	RVISDTASL	SEQ ID NO: 102
9	281	EATSLEGAL	SEQ ID NO: 103
10	327	ETKHFLYSS	SEQ ID NO: 104

HLA-A24 SUPERTYPE HTRT PEPTIDES

Table XVI. HLA-A*24 hTRT peptides

1	1088	TYVPLLGSL	SEQ ID NO: 105
2	845	CYGDMENKL	SEQ ID NO: 106
3	167	AYQVCGPPL	SEQ ID NO: 107
4	461	VYGFVRACL	SEQ ID NO: 108
5	324	VYAETKHFL	SEQ ID NO: 109
6	1009	AYRFHACVL	SEQ ID NO: 110
7	385	RYWQMRPLF	SEQ ID NO: 111
8	637	DYVVGARTF	SEQ ID NO: 112
9	622	RFIPKPDGL	SEQ ID NO: 113
10	869	DFLLVTPHL	SEQ ID NO: 114
11	1011	RFHACVLQL	SEQ ID NO: 115
12	486	RFLRNTKKF	SEQ ID NO: 116

HLA-B27 SUPERTYPE HTRT PEPTIDES

Table XVII. HLA-B*2705 hTRT peptides

1	485	RRFLRNTKK	SEQ ID NO: 117
2	358	RRLVETIFL	SEQ ID NO: 118
3	858	RRDGLLLRL	SEQ ID NO: 119
4	646	RREKRAERL	SEQ ID NO: 120
5	649	KRAERLTSR	SEQ ID NO: 121
6	222	RRRGGSASR	SEQ ID NO: 122
7	377	RRLPRLPQR	SEQ ID NO: 123
8	742	RRYAVVQKA	SEQ ID NO: 124
9	810	LRFMCHHAV	SEQ ID NO: 125
10	29	RRLGPQGWR	SEQ ID NO: 126
11	971	RRKLFGVLR	SEQ ID NO: 127
12	384	QRYWQMRPL	SEQ ID NO: 128
13	229	SRSLPLPKR	SEQ ID NO: 129
14	260	GRTRGPSDR	SEQ ID NO: 130

Table XVIII. HLA-B*2702 hTRT peptides

1	470	RRLVPPGLW	SEQ ID NO: 131
2	742	RRYAVVQKA	SEQ ID NO: 132
3	978	LRLKCHSLF	SEQ ID NO: 133
4	107	ARGGPPEAF	SEQ ID NO: 134
5	536	LREEILAKF	SEQ ID NO: 135
6	10	VRSLLRSHY	SEQ ID NO: 136
7	357	ARRLVETIF	SEQ ID NO: 137
8	630	LRPIVNMDY	SEQ ID NO: 138
9	646	RREKRAERL	SEQ ID NO: 139
10	858	RRDGLLLRL	SEQ ID NO: 140
11	358	RRLVETIFL	SEQ ID NO: 141

Table XIX. HLA-B*1510 hTRT peptides

1	608	QHREARPAL	SEQ ID NO: 142
2	1084	RHRVTYVPL	SEQ ID NO: 143
3	150	VHLLARCAL	SEQ ID NO: 144
4	16	SHYREVLPL	SEQ ID NO: 145
5	533	EHRLREEIL	SEQ ID NO: 146
6	778	AHLQETSPL	SEQ ID NO: 147
7	761	SHVSTLTDL	SEQ ID NO: 148
8	1074	CHQAFLLKL	SEQ ID NO: 149
9	189	HASGPRRRL	SEQ ID NO: 150
10	751	AHGHVRKAF	SEQ ID NO: 151

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CLAIMS

We claim:

1. A composition for induction of a cytotoxic T lymphocyte response, comprising: at least one HLA-B7-restricted human telomerase reverse transcriptase (TRT) peptide from nine to twelve amino acid residues in length.

2. The composition of Claim 1, wherein said HLA-B7 is selected from the group consisting of HLA-B*0702, HLA-B*3501, HLA-B*3502, HLA-B*3503, HLA-B*5101, HLA-B*5301, HLA-B*5401, HLA-B*0703, HLA-B*0704, HLA-B*0705, HLA-B*1508, HLA-B*5501, HLA-B*5502, HLA-B*5601, HLA-B*5602, HLA-B*6701, HLA-B*7801, and HLA-B*0801.

3. The composition of Claim 1, wherein said HLA-B7 is HLA-B*0702.

4. The composition of Claim 1, wherein said at least one TRT peptide consists of a sequence selected from the group consisting of SEQ ID NO:3 (p277), SEQ ID NO:4 (p342), SEQ ID NO:6 (p464), SEQ ID NO:8 (p1107), and SEQ ID NO:9 (p1123).

5. The composition of Claim 1, wherein said at least one TRT peptide consists of a sequence set forth as SEQ ID NO:9 (p1123).

6. The composition of Claim 1, further comprising a helper peptide, wherein said TRT peptide is not conjugated to said helper peptide.

7. The composition of Claim 1, further comprising an adjuvant.

8. The composition of Claim 1, further comprising a physiologically acceptable carrier.

9. The composition of Claim 8, wherein said carrier is a mammalian cell.

10. The composition of Claim 1, wherein said TRT peptide comprises a modification to enhance binding to HLA-B7.

11. The composition of Claim 1, wherein said TRT peptide is a synthetic peptide.

12. A method for inducing or enhancing a CTL response against target cells expressing human TRT and HLA-B7, comprising: harvesting leucocytes expressing HLA-B7; pulsing said leucocytes with the composition of Claim 1 comprising an HLA-B7 restricted human TRT peptide; and contacting target cells expressing human TRT and HLA-B7 with said pulsed leucocytes.

13. The method of Claim 12, wherein said contacting is accomplished *in vitro*.

14. The method of Claim 12, wherein said contacting is accomplished *in vivo*.

15. A method for screening HLA class I-restricted human telomerase reverse transcriptase (TRT) peptides, comprising:

a) using an algorithm to identify a human telomerase reverse transcriptase (TRT) peptide sequence in the full length TRT protein sequence that corresponds to a canonical HLA class I motif and comprises at least nine amino acid residues;

b) testing HLA class I binding of said TRT peptide sequence by measuring HLA class I binding or stabilization in comparison to a reference peptide; and

c) assessing immunogenicity of said TRT peptide sequence by measuring induction of TRT peptide-reactive cytotoxic T lymphocytes (CTL) of an HLA class I-positive subject.

16. The method of Claim 15, wherein said HLA class I-positive subject was immunized with a human TRT vaccine prior to said assessing of step c).

17. The method of Claim 16, wherein said human TRT vaccine comprises human TRT DNA.

18. The method of Claim 16, wherein said human TRT vaccine comprises a recombinant microorganism engineered to express human TRT.

19. The method of Claim 15, wherein said HLA class I is HLA-B7.

20. The method of Claim 15, wherein said HLA class I binding comprises HLA-B*0702 binding, and one or more of HLA-B*3501, HLA-B*3502, HLA-B*3503, HLA-B*5101, HLA-B*5301, HLA-B*5401, HLA-B*0703, HLA-B*0704, HLA-B*0705, HLA-B*1508, HLA-B*5501, HLA-B*5502, HLA-B*5601, HLA-B*5602, HLA-B*6701, HLA-B*7801, and HLA-B*0801 binding.

21. The method of Claim 15, wherein said HLA class I is selected from the group consisting of HLA-A3, HLA-A24, HLA-B44, HLA-A1, and HLA-B27.

22. The method of Claim 15, wherein said HLA class I-positive subject is a transgenic mouse.

23. A composition for induction of a cytotoxic T lymphocyte response, comprising: at least one HLA class I-restricted human telomerase reverse transcriptase (hTRT) peptide from nine to twelve amino acid residues in length, wherein said hTRT peptide comprises one or more of an HLA-A3-restricted hTRT peptide, an HLA-A24-restricted hTRT peptide, an HLA-B44-restricted hTRT peptide, an HLA-A1-restricted hTRT peptide, and an HLA-B27-restricted hTRT.

Figure 1

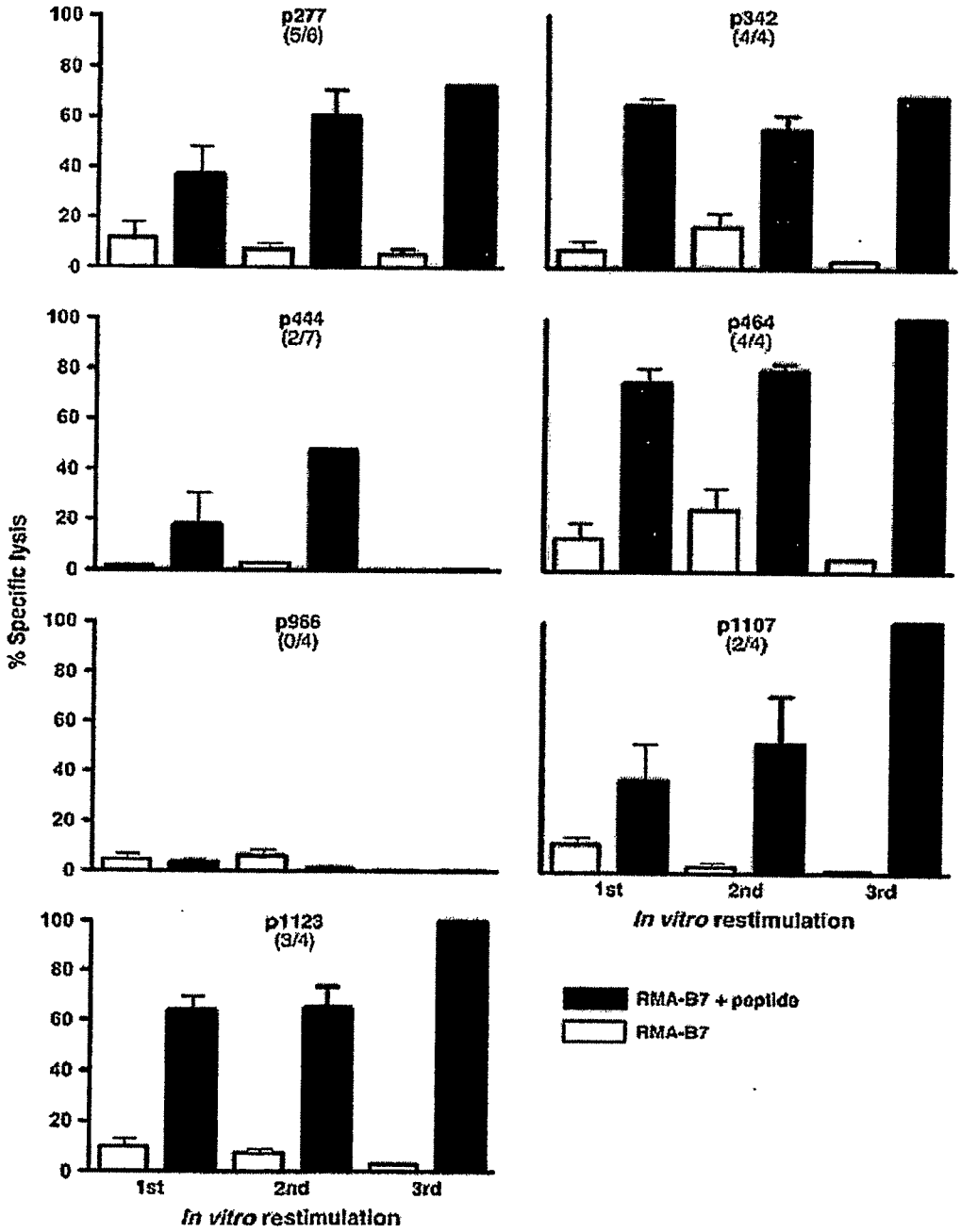

p277
(5/6)
p342
(4/4)
p444
(2/7)
p464
(4/4)
p966
(0/4)
p1107
(2/4)
p1123
(3/4)
% Specific lysis
100
80
60
40
20
0
1st
2nd
3rd
In vitro restimulation
RMA-B7 + peptide
RMA-B7

Figure 2

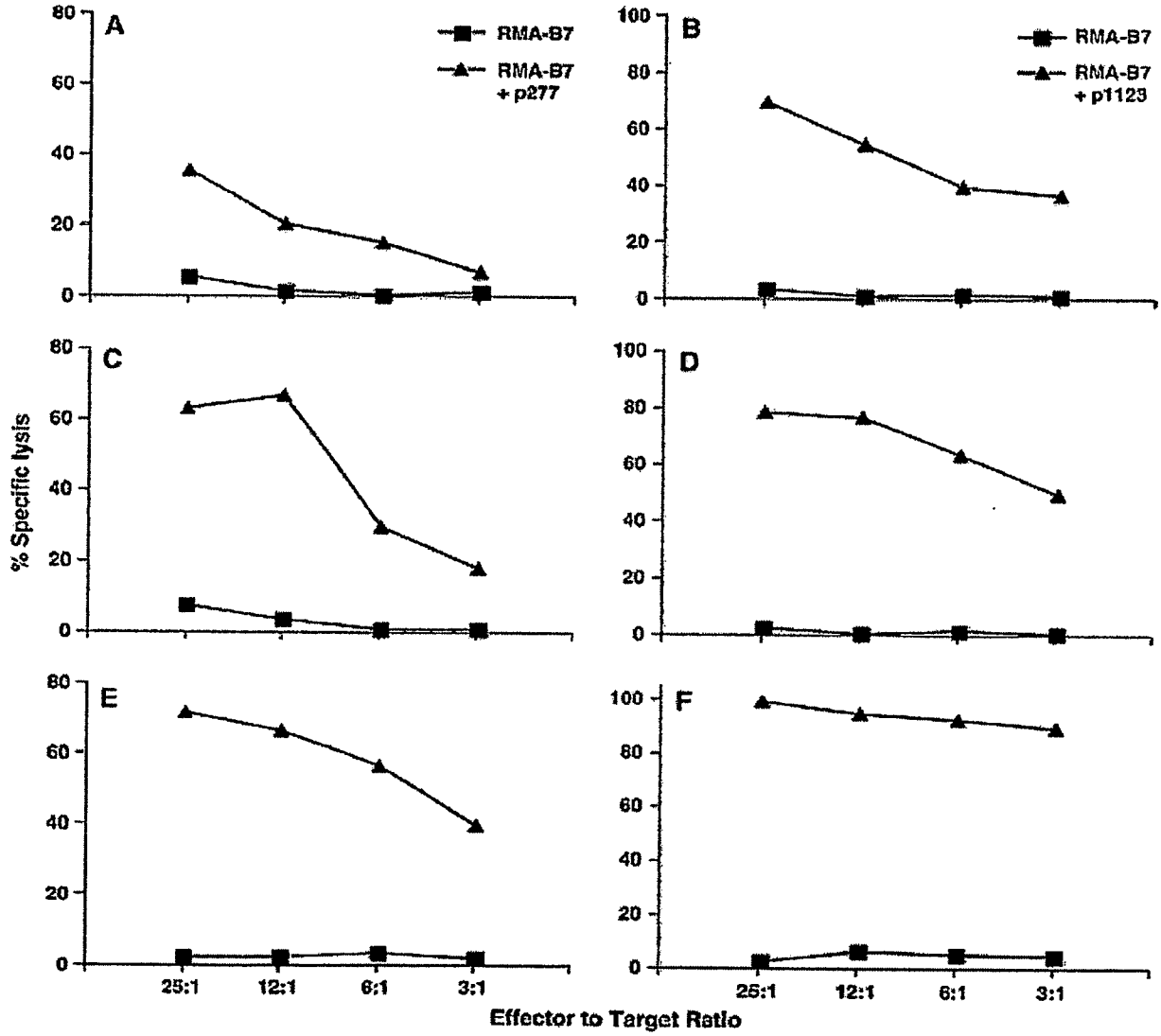

A
RMA-B7
RMA-B7 + p277
B
RMA-B7
RMA-B7 + p1123
C
D
E
F
% Specific lysis
0
20
40
60
80
100
25:1
12:1
6:1
3:1
Effector to Target Ratio

Figure 3

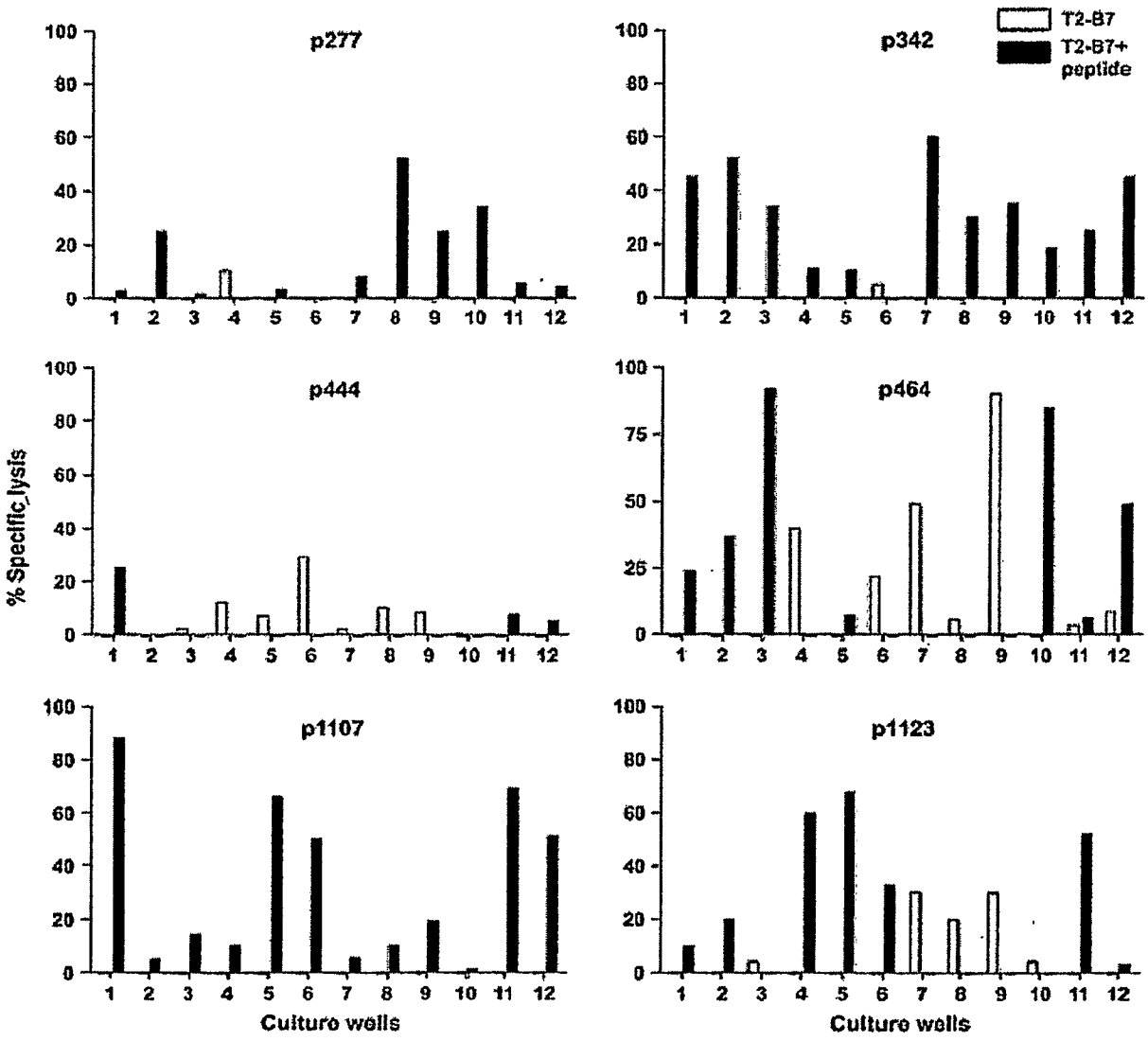

p277
p342
p444
p464
p1107
p1123
T2-B7
T2-B7+ peptide
% Specific lysis
Culture wells
Culture wells

Figure 4

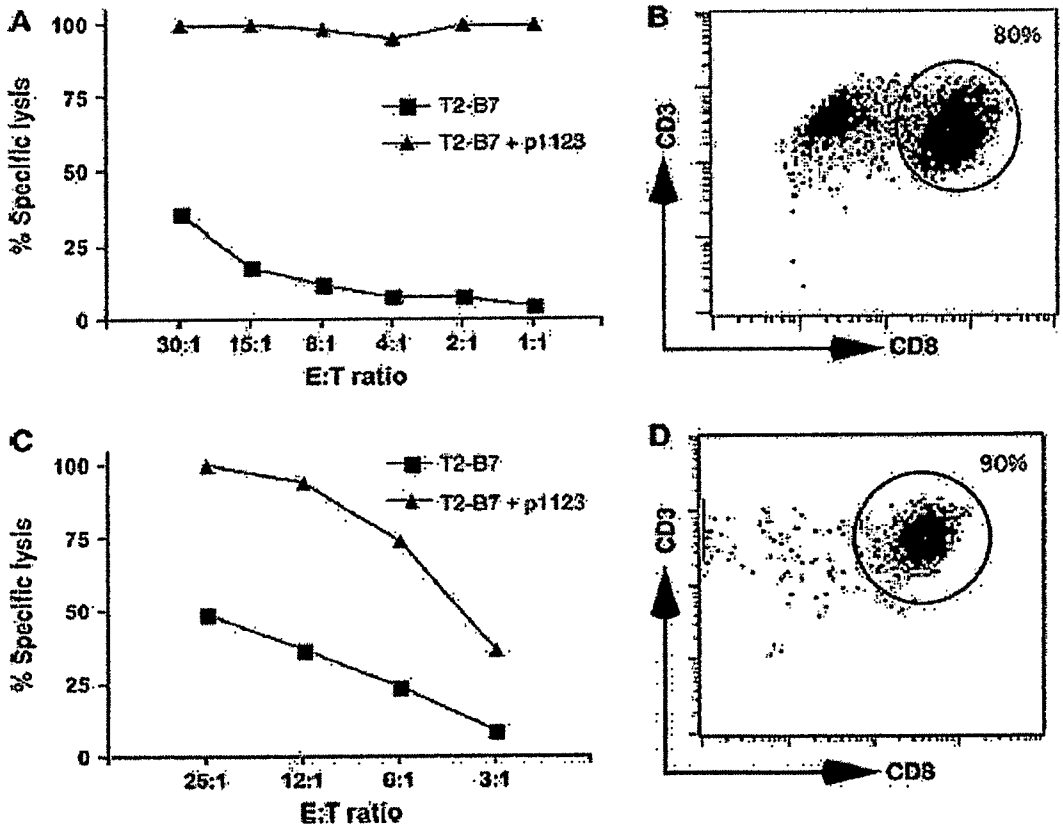

A
100
75
50
25
0
% Specific lysis
T2-B7
T2-B7 + p1123
30:1
15:1
8:1
4:1
2:1
1:1
E:T ratio
B
80%
CD3
CD8
C
100
75
50
25
0
% Specific lysis
T2-B7
T2-B7 + p1123
25:1
12:1
6:1
3:1
E:T ratio
D
90%
CD3
CD8

Figure 5

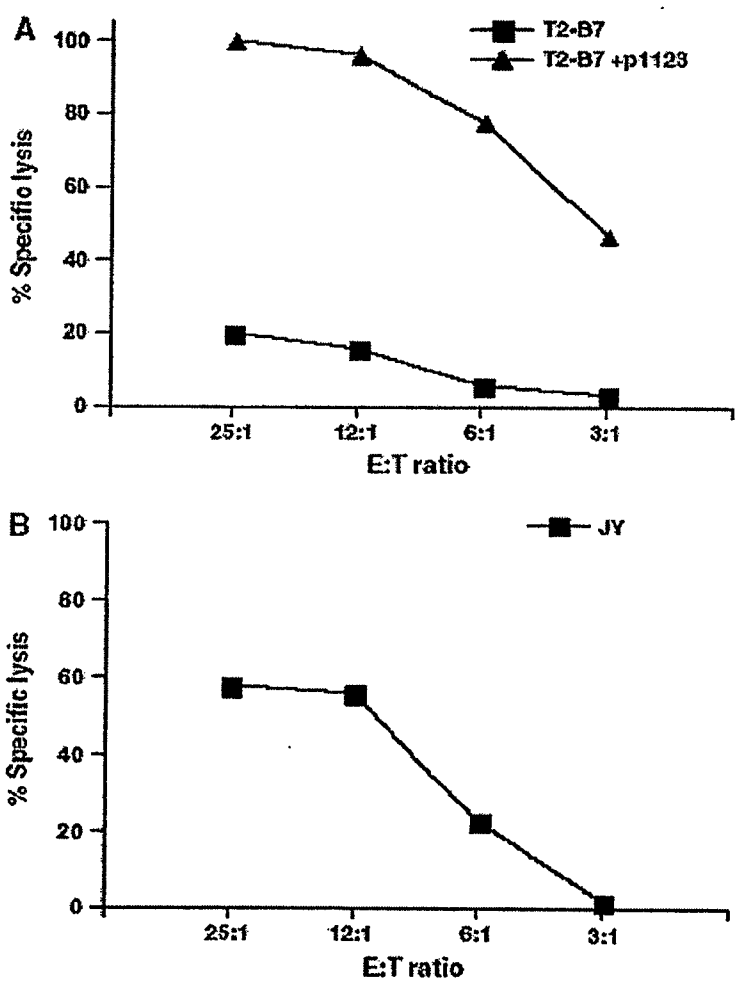

A
100
80
60
40
20
0
% Specific lysis
T2-B7
T2-B7 +p1128
25:1
12:1
6:1
3:1
E:T ratio
B
100
80
60
40
20
0
% Specific lysis
JY
25:1
12:1
6:1
3:1
E:T ratio

FIG. 6

Human Telomerase Reverse Transcriptase cDNA sequence (SEQ ID NO:1)

```
   1 atgccgcgcg ctccccgctg ccgagccgtg cgctccctgc tgcgcagcca ctaccgcgag
  61 gtgctgccgc tggccacgtt cgtgcggcgc ctggggcccc agggctggcg gctggtgcag
 121 cgcggggacc cggcggcttt ccgcgcgctg gtggcccagt gcctggtgtg cgtgccctgg
 181 gacgcacggc cgccccccgc cgcccctcc ttccgccagg tgtcctgcct gaaggagctg
 241 gtggcccgag tgctgcagag gctgtgcgag cgcggcgcga agaacgtgct ggccttcggc
 301 ttcgcgctgc tggacggggc ccgcgggggc ccccccgagg ccttcaccac cagcgtgcgc
 361 agctacctgc ccaacacggt gaccgacgca ctgcgggga gcggggcgtg ggggctgctg
 421 ctgcgccgcg tgggcgacga cgtgctggtt cacctgctgg cacgctgcgc gctctttgtg
 481 ctggtggctc ccagctgcgc ctaccaggtg tgcgggccgc cgctgtacca gctcggcgct
 541 gccactcagg cccggccccc gccacacgct agtggacccc gaaggcgtct gggatgcgaa
 601 cgggcctgga accatagcgt cagggaggcc ggggtccccc tgggcctgcc agccccgggt
 661 gcgaggaggc gcgggggcag tgccagccga agtctgccgt tgcccaagag gcccaggcgt
 721 ggcgctgccc ctgagccgga gcggacgccc gttgggcagg ggtcctgggc ccacccgggc
 781 aggacgcgtg gaccgagtga ccgtggtttc tgtgtggtgt cacctgccag acccgccgaa
 841 gaagccacct ctttggaggg tgcgctctct ggcacgcgcc actcccaccc atccgtgggc
 901 cgccagcacc acgcgggccc cccatccaca tcgcggccac cacgtccctg ggacacgcct
 961 tgtcccccgg tgtacgccga gaccaagcac ttcctctact cctcaggcga caaggagcag
1021 ctgcggccct ccttcctact cagctctctg aggcccagcc tgactggcgc tcggaggctc
1081 gtggagacca tctttctggg ttccaggccc tggatgccag ggactccccg caggttgccc
1141 cgcctgcccc agcgctactg gcaaatgcgg cccctgtttc tggagctgct tgggaaccac
1201 gcgcagtgcc cctacggggt gctcctcaag acgcactgcc cgctgcgagc tgcggtcacc
1261 ccagcagccg gtgtctgtgc ccgggagaag ccccagggct ctgtggcggc ccccgaggag
1321 gaggacacag acccccgtcg cctggtgcag ctgctccgcc agcacagcag cccctggcag
1381 gtgtacggct tcgtgcgggc ctgcctgcgc cggctggtgc ccccaggcct ctggggctcc
1441 aggcacaacg aacgccgctt cctcaggaac accaagaagt tcatctccct ggggaagcat
1501 gccaagctct cgctgcagga gctgacgtgg aagatgagcg tgcgggactg cgcttggctg
1561 cgcaggagcc caggggttgg ctgtgttccg gccgcagagc accgtctgcg tgaggagatc
1621 ctggccaagt tcctgcactg gctgatgagt gtgtacgtcg tcgagctgct caggtctttc
1681 ttttatgtca cggagaccac gtttcaaaag aacaggctct ttttctaccg gaagagtgtc
1741 tggagcaagt tgcaaagcat tggaatcaga cagcacttga agagggtgca gctgcgggag
1801 ctgtcggaag cagaggtcag gcagcatcgg gaagccaggc ccgccctgct gacgtccaga
1861 ctccgcttca tccccaagcc tgacgggctg cggccgattg tgaacatgga ctacgtcgtg
1921 ggagccagaa cgttccgcag agaaaagagg gccgagcgtc tcacctcgag ggtgaaggca
1981 ctgttcagcg tgctcaacta cgagcgggcg cggcgccccg gcctcctggg cgcctctgtg
2041 ctgggcctgg acgatatcca cagggcctgg cgcaccttcg tgctgcgtgt gcgggcccag
2101 gacccgccgc ctgagctgta ctttgtcaag gtggatgtga cgggcgcgta cgacaccatc
2161 ccccaggaca ggctcacgga ggtcatcgcc agcatcatca aaccccagaa cacgtactgc
2221 gtgcgtcggt atgccgtggt ccagaaggcc gcccatgggc acgtccgcaa ggccttcaag
2281 agccacgtct ctaccttgac agacctccag ccgtacatgc gacagttcgt ggctcacctg
2341 caggagacca gcccgctgag ggatgccgtc gtcatcgagc agagctcctc cctgaatgag
2401 gccagcagtg gcctcttcga cgtcttccta cgcttcatgt gccaccacgc cgtgcgcatc
2461 aggggcaagt cctacgtcca gtgccagggg atcccgcagg gctccatcct ctccacgctg
2521 ctctgcagcc tgtgctacgg cgacatggag aacaagctgt ttgcggggat tcggcgggac
2581 gggctgctcc tgcgtttggt ggatgatttc ttgttggtga cacctcacct cacccacgcg
2641 aaaaccttcc tcaggaccct ggtccgaggt gtccctgagt atggctgcgt ggtgaacttg
2701 cggaagacag tggtgaactt ccctgtagaa gacgaggccc tgggtggcac ggcttttgtt
2761 cagatgccgg cccacggcct attcccctgg tgcggcctgc tgctggatac ccggaccctg
2821 gaggtgcaga gcgactactc cagctatgcc cggacctcca tcagagccag tctcaccttc
2881 aaccgcggct tcaaggctgg gaggaacatg cgtcgcaaac tctttggggt cttgcggctg
2941 aagtgtcaca gcctgtttct ggatttgcag gtgaacagcc tccagacggt gtgcaccaac
```

FIG. 6 continued

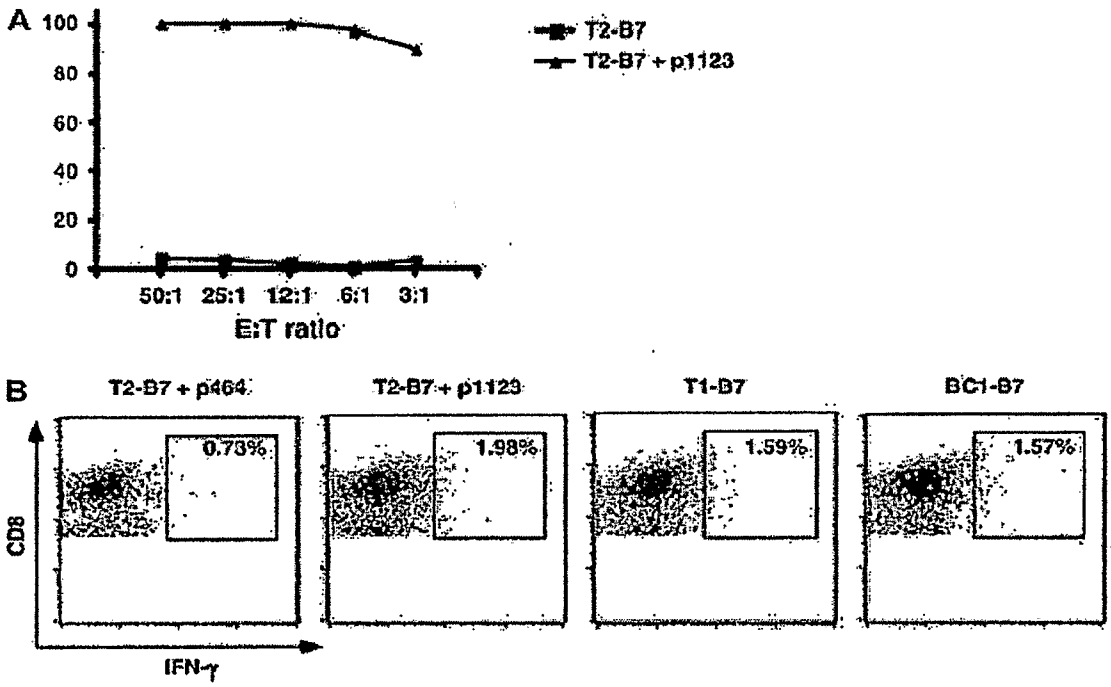
```
3001 atctacaaga tcctcctgct gcaggcgtac aggtttcacg catgtgtgct gcagctccca
3061 tttcatcagc aagtttggaa gaaccccaca tttttcctgc gcgtcatctc tgacacggcc
3121 tccctctgct actccatcct gaaagccaag aacgcaggga tgtcgctggg ggccaagggc
3181 gccgccggcc ctctgccctc cgaggccgtg cagtggctgt gccaccaagc attcctgctc
3241 aagctgactc gacaccgtgt cacctacgtg ccactcctgg ggtcactcag gacagcccag
3301 acgcagctga gtcggaagct cccggggacg acgctgactg ccctggaggc cgcagccaac
3361 ccggcactgc cctcagactt caagaccatc ctggactga
```

FIG. 7

Human Telomerase Reverse Transcriptase protein sequence (SEQ ID NO:2)

```
   1 mpraprcrav rsllrshyre vlplatfvrr lgpqgwrlvq rgdpaafral vaqclvcvpw
  61 darpppaaps frqvsclkel varvlqrlce rgaknvlafg falldgargg ppeafttsvr
 121 sylpntvtda lrgsgawgll lrrvgddvlv hllarcalfv lvapscayqv cgpplyqlga
 181 atqarpppha sgprrrlgce rawnhsvrea gvplglpapg arrrggsasr slplpkrprr
 241 gaapepertp vgqgswahpg rtrgpsdrgf cvvsparpae eatslegals gtrhshpsvg
 301 rqhhagppst srpprpwdtp cppvyaetkh flyssgdkeq lrpsfllssl rpsltgarrl
 361 vetiflgsrp wmpgtprrlp rlpqrywqmr plflellgnh aqcpygvllk thcplraavt
 421 paagvcarek pqgsvaapee edtdprrlvq llrqhsspwq vygfvraclr rlvppglwgs
 481 rhnerrflrn tkkfislgkh aklslqeltw kmsvrdcawl rrspgvgcvp aaehrlreei
 541 lakflhwlms vyvvellrsf fyvtettfqk nrlffyrksv wsklqsigir qhlkrvqlre
 601 lseaevrqhr earpalltsr lrfipkpdgl rpivnmdyvv gartfrrekr aerltsrvka
 661 lfsvlnyera rrpgllgasv lglddihraw rtfvlrvraq dpppelyfvk vdvtgaydti
 721 pqdrltevia siikpqntyc vrryavvqka ahghvrkafk shvstltdlq pymrqfvahl
 781 qetsplrdav vieqsssine assglfdvfl rfmchhavri rgksyvqcqg ipqgsilstl
 841 lcslcygdme nklfagirrd glllrlvddf llvtphltha ktflrtlvrg vpeygcvvnl
 901 rktvvnfpve dealggtafv qmpahglfpw cgllldtrtl evqsdyssya rtsirasltf
 961 nrgfkagrnm rrklfgvlrl kchslfldlq vnslqtvctn iykilllqay rfhacvlqlp
1021 fhqqvwknpt fflrvisdta slcysilkak nagmslgakg aagplpseav qwlchqafll
1081 kltrhrvtyv pllgslrtaq tqlsrklpgt tltaleaaan palpsdfkti ld
```

Figure 8


A
100
80
60
40
20
0
50:1
25:1
12:1
6:1
3:1
E:T ratio
T2-B7
T2-B7 + p1123
B
T2-B7 + p464
T2-B7 + p1123
T1-B7
BC1-B7
0.73%
1.98%
1.59%
1.57%
CD8
IFN-γ