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(54) Title: INDUCTION OF A STRONG IMMUNE RESPONSE TO A SELF-TUMOR ASSOCIATED ANTIGEN

(57) Abstract: The present invention relates to a method for enhancing the efficacy of an immune response against an antigen which is a self-antigen. The method of the invention comprises (i) immunization of a host with a mimic of the self-antigen to break tolerance to a self antigen, and (ii) increasing the response to the self-antigen by a booster with the original antigen. An immune response may be generated against any self antigen including, for example, tumor associated antigens, cell surface receptors, receptor ligands, cytokines, hormones, or a self antigen whose expression is associated with a disease or disorder. The method of the invention may be used to target destruction of targeted tumor cells bearing tumor associated antigens in cancer subjects through induction of an immune response.

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INDUCTION OF A STRONG IMMUNE RESPONSE TO A SELF-TUMOR ASSOCIATED ANTIGEN

The present invention relates to a method for enhancing the efficacy of an immune response against an antigen which is a self-antigen. The method of the invention comprises (i) immunization of a host with a mimic of the self-antigen to break tolerance to a self antigen, and (ii) increasing the response to the self-antigen by a booster with the original antigen. An immune response may be generated against any self antigen including, for example, tumor associated antigens, cell surface receptors, receptor ligands, cytokines, hormones, or a self antigen whose expression is associated with a disease or disorder. The method of the invention may be used to target destruction of targeted tumor cells bearing tumor associated antigens in cancer subjects through induction of an immune response.

BACKGROUND OF THE INVENTION

A number of immunotherapeutic approaches have been utilized for purposes of enhancing a host's ability to respond to certain antigens including self antigens (Bodey, 2000, *Anticancer Research* 20:2665-2676; Irvine et al., 1997, *J. Natl. Cancer Inst.* 89:1595-1601; Mamula et al., 1994, *J. Immunol.* 152:1453-1460; Cooney et al., 1993, *Proc. Natl. Acad. Sci. USA* 90:1882-1886; WO 97/39771 and U.S. Patent No. 5,798,100). Most tumor associated antigens expressed by a host are non-mutated self-antigens, thus it is difficult elicit an immune response against such antigens. However, immunogens which have been shown to be effective in inducing an immune response to a self-antigen include peptide mimics (WO 00/38515; US Patent No. 5,679,647) including, for example, xenogenic tumor associated antigens which display a high degree of homology, but not complete identity in their amino acid sequence with a self-antigen (Naftzger et al., 1996, *Proc. Natl. Acad. Sci. USA.*, 93:14809-14814; Overwijk et al., 1998, *J. Exp. Med.*, 188:277-286); (ii) peptides derived from the amino acid sequence of the tumor associated antigen which have been changed in the anchor residues to increase their affinity to MHC class I antigens

and/or in amino acids that contact T cell receptor (Bakker et al., 1997, *In tl. J. Cancer*, 70:302-309; Dyall et al., 1998, *J. Exp. Med.*, 188:1553-1561; Rivoltini et al., 1999, *Cancer Res.*, 59:301-306); and (iii) anti-idiotypic (anti-id) antibodies, which bear the internal image of tumor associated antigen (Shoenfeld et al., 1997, *Idiotypes in medicine: Autoimmunity, infection and cancer*, Elsevier Science, Amsterdam.; US
5 Patent No. 5,798,100; US Patent No. 5,780,029).

Anti-id antibodies which mimic various types of tumor associated antigens have been used as immunogens in clinical trials (for review, see Shoenfeld et al, *supra*). In at least three antigenic systems, anti-id antibodies have been found to be
10 more effective than the corresponding antigen in breaking tolerance to a self-antigen, since they have elicited antigen binding antibodies, while the corresponding nominal antigen has not (Von Kleist et al., 1966, *Immunology*, 10:507-515; Collatz et al., 1971, *Int. J. Cancer*, 8:298-303; Lo Gerfo et al., 1972, *Int. J. Cancer*, 9 : 344-348; Hamby et al., 1987, *Cancer Res.*, 47:5284-5289; Mittelman et al., 1992, *Proc. Natl. Acad. Sci. USA*, 89:466-470; Livingston et al., 1993, *Ann. NY. Acad. Sci.* 690:204-213; Chapman et al., 1994, *Vaccine Res.*, 3:59-69; Foon et al., 1995, *J. Clin. Invest.*, 96:334-342).
15

The lack of immunogenicity of a self antigen is likely to reflect the deletion during the establishment of self-identity, of B cell clones that recognize the
20 antigen with high affinity. In contrast, the immunogenicity of the corresponding anti-id antibody is likely to reflect its ability to stimulate B cell clones which have not been deleted during the establishment of self-identity, since they secrete antibodies reacting with the corresponding antigen with an affinity below the threshold required for deletion. Anti-id antibodies which are similar, but not identical to the nominal
25 antigen, stimulate B cell clones secreting antibodies which fit poorly antigenic determinants expressed on the self-antigen. As a result, the reactivity of the secreted anti anti-id antibodies with the nominal antigen is low, is spite of their high reactivity with the immunizing anti-id antibody. This model is supported by (i) the similarity, but not identity of the fine specificity of antigen binding anti-anti-id antibodies and
30 corresponding antibodies elicited by the nominal antigen (Viale et al., 1989, *J. Immunol.*, 143:4338-4344; Bhattacharya et al., 1990, *J. Immunol.*, 145:2758-2765;

Mariani et al., 1991, *J. Immunol.*, 147:1322-1330; Kageshita et al., 1995, *Int. J. Cancer*, 60:334-340) and (ii) the lower association constant of the antigen binding anti-anti-id mAb to the nominal antigen than the corresponding mAb elicited by the nominal antigen (Shinji et al., 1990, *J. Immunol.*, 144:4291-4297; Chen et al., 1993, *Ann. NY. Acad. Sci.* 690:398-401; Goldbaum et al., 1997, *Proc. Natl. Acad. Sci. USA* 94:8697-8701).

The structural basis of these immunological findings is the high degree of homology, but not identity in the amino acid sequence of the heavy and light chain variable regions of antigen binding anti-anti-id mAb and of the corresponding mAb elicited by the original antigen (Caton et al., 1990, *J. Immunol.*, 144:1965-1968; Garcia et al., 1992, *Science*, 257:528-531;24. Iwasaki et al., *Eur. J. Immunol.*, 24:2874-2881; Ferrone et al., 1997). Thus, there is an ongoing need in the field of active specific immunotherapy of malignant diseases to develop strategies to break tolerance to a self-antigen and to induce a strong immune response to it.

3. SUMMARY OF THE INVENTION

The present invention relates to a method for stimulating an immune response against an antigen which is a self-antigen. The method of the invention comprises (i) immunization of a host with a mimic of the self-antigen to break tolerance to the self antigen, and (ii) increasing the response to the self-antigen by a booster with the original antigen. The method may be used to generate an immune response against any self antigen. When the self antigen comprises a tumor associated antigen, the present invention provides a method for increasing the efficacy of active specific immunotherapy for malignant diseases.

4. BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Enhancement by booster with cultured human melanoma cells of the anti-HMW-MAA immune response triggered by mouse anti-id mAb MK2-23. Rabbits 97-6 and 97-7 were immunized on day 0, 14, 28 and 42 with KLH conjugated mouse anti-id mAb MK2k-23 mixed with Freund's adjuvant and on day 56 with irradiated, cultured human melanoma cells Colo 38 (2×10^6). Serum harvested

on day 49 showed a slightly higher reactivity with Colo 38 melanoma cells (Δ) in a binding assay (left panel) than the pre-immune serum (O). However, serum harvested on day 63 (right panel) showed a markedly higher reactivity with Colo 38 cells (Δ) in a binding assay (left panel) than the pre-immune serum (O). However, serum
5 harvested on day 63 (right panel) showed a markedly higher reactivity with Colo 38 cells (Δ) than the B lymphoid cells, L14 ($\blacktriangle$). Results are expressed as mean $\pm$ SD of bound cpm/well obtained with sera from the two rabbits.

Figure 2. SDS-PAGE analysis of antigens immunoprecipitated from ¹²⁵I-labeled Colo 38 melanoma cells by sera from a rabbit sequentially immunized with the anti-id mAb MK2-23 and with melanoma cells Colo 38. Rabbits 97-6 was
10 immunized on day 0, 14, 28 and 42 with KLH conjugated mouse anti-id mAb MK2-23 mixed with Freund's adjuvant and on day 63 with cultured human melanoma cells Colo 38 (1×10^6). Sera harvested before immunization (NRS) and on day 40 (40) did not immunoprecipitate any component from a Colo 38 cell extract immunodepleted
15 with anti-96K MAA mAb 376.96. In contrast serum harvested on day 63 (63) immunoprecipitated HMW-MAA (left panel). Furthermore, serum harvested on day 63 (63) removed HMW-MAA recognized by mAb 763.74 from a Colo 38 cell extract (right panel). The anti-96K MAA mAb 376.96 and the anti-HMW-MAA mAb 763.74 were used as controls.

20 Figure 3. Strategy for Construction of the HMW-MAA minigene.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a method for enhancing the efficacy of an immune response against an antigen which is a self-antigen. The method of the
25 invention comprises (i) immunization of a host with a mimic of the self-antigen to break tolerance to the self antigen, and (ii) increasing the response to the self-antigen by a booster with the original antigen. The method may be used to generate an immune response against any self antigen.

In a non-limiting embodiment of the invention, a novel immunization
30 strategy is provided to elicit a strong immune response to a tumor associated antigen

which is a self-antigen. The method comprises immunization of hosts with antigens which are similar, but not identical to the original tumor associated antigens. The steps of the invention involve (i) first immunizing a host with a mimic of the tumor associated antigen to break unresponsiveness to a self-antigen, and (ii) increasing the response to the self-antigen by a booster with the original antigen. Although not intending to be bound by any theory, it is considered that the latter expands the population of B cells secreting antibodies that because of somatic hypermutations display a high, if not complete homology in heavy and/or light chain variable region amino acid sequences with the antibodies elicited by the original antigen.

The validity of this strategy is supported by the results obtained in rabbits utilizing the human high molecular weight-melanoma associated antigen (HMW-MAA) as a model system. HWM-MAA bearing human melanoma cells do not induce anti-HMW-MAA antibodies in rabbits which express HMW-MAA with an antigenic profile and a tissue distribution similar to those of the human counterpart (Schlingemann et al., 1990, *Am. J. Pathol.*, 136:1393-1405). However, the anti-id mAb MK2-23 which mimics a determinant of HMW-MAA (Mittelman et al., *Proc. Natl. Acad. Sci USA* 89:466-470, Kusama et al., 1989, *J. Immunol.*, 143:3844-3852; Chen et al., 1993, *Cancer Res.*, 53:112-119) elicited a low level of anti-HMW-MAA antibodies in rabbits. The level of these antibodies was markedly increased following a booster with HMW-MAA bearing human melanoma cells.

5.1. MIMICS OF SELF ANTIGENS

The present invention provides active immunotherapy of disorders, such as cancer, by utilizing as immunogens mimics of self-antigens. The method comprises immunization of hosts with a mimic comprising an antigen which is similar, but not identical to the original self antigen. Unlike the original self antigen, mimics are defined as those peptides capable of eliciting immunity to a self antigen.

Peptide mimics of self antigens may be identified by screening peptide libraries. For example, phage display peptide libraries may be panned with, for example, human or mouse antibodies immunoreactive with the targeted self antigen. In addition, mimics may comprise analogous self-antigens derived from other species,

peptides similar to self antigens but having amino acid alterations, or denatured self antigens, for example. Certain aspects of the invention are described below for tumor associated antigens, however, the principles of the invention may be applied to any self antigen.

5 Mimics of self antigens that can be used to break tolerance to a self-antigen include, but are not limited to, (i) peptide mimics of the self antigen; (ii) a nucleic acid molecule capable of encoding such a peptide mimic; (iii) or anti-idiotypic antibodies of a self antigen which bear the internal image of said self antigen. Nucleic acid molecules capable of encoding peptide mimics can be RNA or DNA or
10 derivatives or modified versions thereof, single-stranded or double-stranded. Mimics of a tumor associated antigen may relate to a fragment of an antigen, epitope, or antigenic determinant. In a specific non-limiting embodiment of the invention, the self-antigen is a tumor associated antigen.

Peptide mimics of self antigens may be identified by panning phage
15 display peptide libraries with, for example, human or mouse antibodies immunoreactive with the targeted self antigen. Such phage libraries are commercially available (Pharmacia), or alternatively, phage libraries may be constructed using routine techniques known in the art. Peptide mimics may include B cell defined epitopes of a tumor associated antigens identified through panning a phage display
20 library with human and/or mouse anti-tumor associated antigen antibodies. Once a phage expressing a peptide mimic has been identified the DNA sequence and deduced amino acid sequence of the peptide mimic may be determined. Such information may then be advantageously used to produce peptide mimics for use as immunogens. Additionally, peptide mimics that can be used to break tolerance
25 include, but are not limited to, those described in WO 00/38515 incorporated by reference herein.

Identified peptide mimics may be chemically synthesized using routine techniques known in the art. (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y.). In addition, the peptide
30 mimics may be modified to enhance their ability to stimulate an immune response in the immunized host. For example, the peptide mimic may be modified by

conjugation to an immunogenic reagent, such as a polysaccharide or peptidoglycan, or by glycosylation of the peptide mimic. Alternatively, the peptide mimics may be modified by haptenization of the peptide mimic.

Further, nucleic acid molecules capable of encoding peptide mimics
5 may also be prepared for use as immunogens by any method known in the art for the synthesis of DNA and RNA molecules. For example, the nucleic acids may be chemically synthesized using commercially available reagents and synthesizers by methods that are well known in the art (see, e.e., Gait, 1985, Oligonucleotide Synthesis: A Practical Approach, IRL Press, Oxford, England). The nucleic acids may
10 be purified by any suitable means, as are well known in the art. Alternatively, the peptide mimics may be advantageously produced by recombinant DNA technology using techniques well known in the art for expressing a nucleic acid containing gene sequences and/or coding sequences for peptide mimics. Such methods can be used to construct expression vectors containing nucleotide sequences encoding peptide
15 mimics and appropriate transcriptional and translational control signals. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

20 The nucleic acid encoding the peptide mimic of interest may be recombinantly engineered into a variety of host vector systems that also provide for replication of the nucleic acid in large scale and contain the necessary elements for directing the transcription of the nucleic acid. The use of such a construct to immunize a subject will result in the transcription of sufficient amounts of RNA
25 encoding the peptide mimic thereby facilitating the induction of an immune response directed against the peptide mimic. For example, a vector can be introduced *in vivo* such that it is taken up by a cell and directs the transcription of the nucleic acid molecule encoding the peptide mimic. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired
30 peptide, i.e., the peptide mimic. Such vectors can be constructed by recombinant DNA technology methods standard in the art.

Vectors encoding the peptide mimic of interest can be plasmid, viral, or others known in the art, used for replication and expression in mammalian cells. Expression of the sequence encoding the peptide mimic can be regulated by any promoter known in the art to act in mammalian, preferably human cells. Such
5 promoters can be inducible or constitutive. Such promoters include but are not limited to: the SV40 early promoter region (Benoist, C. and Chambon, P. 1981, Nature 290:304-310), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto *et al.*, 1980, Cell 22:787-797), the herpes thymidine kinase promoter (Wagner *et al.*, 1981, Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445), the
10 regulatory sequences of the metallothionein gene (Brinster *et al.*, 1982, Nature 296:39-42), the viral CMV promoter, the human chorionic gonadotropin- β promoter (Hollenberg *et al.*, 1994, Mol. Cell. Endocrinology 106:111-119), etc. Any type of plasmid, cosmid, YAC or viral vector can be used to prepare the recombinant DNA construct which can be introduced directly into the tissue site. Alternatively, viral
15 vectors can be used which selectively infect the desired target cell.

In a preferred embodiment of the invention, a DNA minigene encoding a peptide mimic may be genetically engineered and used as an immunogen to break host tolerance to a self antigen. Such peptide mimics include but are not limited to peptide mimics of tumor associated antigens and/or peptide mimics of B cell epitopes
20 of tumor associated antigens.

The minigene for use in breaking tolerance to a self antigen comprises (i) nucleic acid molecules encoding peptide mimics, including for example, peptide mimics of B cell determinants of a targeted self antigen; (ii) a universal helper epitope, such as PADRE, which elicits helper function for differentiation of antigen
25 specific B cells into plasma cells; and (iii) a signal peptide that directs secretion of the expressed protein into the extra cellular space thereby facilitating antigen uptake by antigen specific cells. The order of the sequences in the minigene will consist of signal sequence, sequence of the helper peptide PADRE followed by sequences encoding the peptide mimics of B cell determinants of HMW-MAA. There will be no
30 intervening sequences between adjacent peptide sequences, so that all peptide sequences are part of a single open reading frame. In addition, the 5' end

oligonucleotide sequences of the minigene will contain sequences encoding the restriction enzyme site NheI and the Kozak consensus sequence to facilitate translation of the mRNA. The 3' end oligonucleotide of the minigene will contain an in frame termination codon and a restriction enzyme site KpnI. The different
5 restriction enzyme sites at the 5' and 3' ends of the minigene will allow its directional cloning into various expression vectors, such as those described above.

In addition to peptide mimics and nucleic acid molecules encoding such peptide mimics, anti-idiotypic antibodies may be utilized as mimics of self antigens. In an embodiment of the invention, anti-idiotypic antibodies and fragments
10 thereof, the antigen binding sites of which immuno-specifically bind to antibodies that recognize a self antigen, such as a tumor associated antigen, may be used to immunize a host. Methods for generating such anti-idiotypic antibodies are well known to those skilled in the art (Kennedy et al., 1983, *Science* 221:853-854; Kennedy et al., 1986, *Science* 232:220-223; Kieber-Emmons, TRE et al., 1986, *Int. Rev. Immunol.* 1:1-26).
15 Briefly, to generate anti-idiotypic antibodies, an antibody reactive with a self antigen, or hybridomas producing such antibodies, can be used as an immunogen. Immunizations are accomplished using standard procedures. The unit dose and immunization regimen will depend on the species of mammal immunized. Typically, the immunized mammals are bled and the serum from the blood sample is assayed for
20 the presence of anti-idiotypic antibodies. Anti-idiotypic polyclonal antibodies may be directly purified from the serum of the immunized animal. Alternatively, technology for producing monoclonal antibodies may be utilized to generate hybridoma cell lines that produce anti-idiotypic antibodies. The conditioned hybridoma culture supernatant may be collected and the antibodies purified by conventional methods.

25 5.2. IMMUNIZATION WITH MIMICS OF SELF ANTIGENS

The present invention relates to a method for enhancing the efficacy of an immune response against a self antigen, such as a tumor associated antigen. The method of the invention comprises two steps, first, immunization of a host with a mimic of the self antigen to break tolerance, and second, increasing the response to
30 self-antigen by a booster with the original antigen.

Mimics of tumor associated antigens that may be used to break tolerance in hosts suffering from diseases characterized by the production of the tumor associated antigens include, for example, peptide mimics, nucleic acid molecules capable of expressing such peptide mimics, and/or anti-idiotypic
5 antibodies. Stimulation of an immunological response to such antigens, is intended to elicit a more effective attack on tumor cells; such as inter alia inhibiting tumor cell growth or facilitating the killing of tumor cells.

In addition, mimics of self antigens may be used to break tolerance in hosts suffering from disorders or diseases associated with expression of specific self
10 antigens. Stimulation of an immunological response to such antigens is intended to remove the self antigen from the host's system, or alternatively, for targeted destruction of cells expressing such self antigens.

The mimics for use as immunogens can be prepared using the methods described in Section 5.1. When the mimic to be used for immunization comprises a
15 nucleic acid molecule formulation, various delivery systems are known and can be used to transfer the formulations of the invention into cells, *e.g.* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mimic, receptor-mediated endocytosis (see, *e.g.*, Wu and Wu, 1987, *J. Biol. Chem.* 262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector,
20 injection of DNA, electroporation, calcium phosphate mediated transfection, etc. Any of the methods for gene delivery into a host cell available in the art can be used according to the present invention. For general reviews of the methods of gene delivery see Strauss, M. and Barranger, J.A., 1997, *Concepts in Gene Therapy*, by Walter de Gruyter & Co., Berlin; Goldspiel *et al.*, 1993, *Clinical Pharmacy* 12:488-
25 505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 33:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; 1993, *TIBTECH* 11(5):155-215.

For administration, the mimics may be formulated with a suitable adjuvant in order to enhance the immunological response to the antigen. Suitable
30 adjuvants include, but are not limited to mineral gels, *e.g.* aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides,

oil emulsions, and potentially useful human adjuvants such as BCG (*Bacilli Calmett-Guerin*) and (*Corynebacterium parvum*).

Many methods may be used to introduce the formulations derived above; including but not limited to oral, intradermal, intramuscular, intraperitoneal, 5 intravenous, and subcutaneous. The methods and compositions for the introduction of an antigenic composition into an individual are well known to those skilled in the art.

The mimics will be administered in amounts which are effective to produce the desired effect, *i.e.*, the induction of an immune response to self antigens, such as tumor associated antigens, in the immunized host. Effective doses of the 10 mimics can be determined through procedures well known in the art. In addition, it may be necessary to immunized the host multiple times to stimulate an immune response. A variety of different methods may be utilized to determine whether an immune response to a self antigen has been stimulated in the immunized host. Such methods include western blots, immunoprecipitation followed by sodium dodecyl 15 sulfate polyacrylamide gel electrophoresis, immunocytochemistry, etc..

5.3. ENHANCEMENT BY BOOSTER IMMUNIZATION

The initial priming with the peptide mimics of self antigens, such as tumor associated self antigens, will induce low titer, low avidity anti- mimic 20 antibodies in the immunized host, since the encoded determinants are similar, but not identical to those of the self-antigen. This characteristic of the immunization which underlies its ability to break unresponsiveness to antigens, results in the stimulation of B clones secreting antibodies with low avidity for self antigens. In the course of the immune response antibodies will undergo somatic hypermutations and some of the 25 mutations will enhance the reactivity of the antibodies with self antigens. Thus, clones secreting the mutated antibodies may be expanded by booster(s) with the original self antigen. This expansion will result in a marked increase in the level of antibody populations reacting with high avidity with self antigens in the immunized host.

30 Thus, following immunization of the host with a mimic of a tumor associated antigen, the level of anti-self antigen antibodies, such as anti-tumor

associated antigen antibodies, in serum is measured. The efficacy of the immunization can be assessed by production of antibodies or immune cells that recognize the antigen, *i.e.*, the mimic. One skilled in the art recognizes the conventional methods used to assess the aforementioned parameters.

5 Once an immune response has been detected, the host is then boosted with the original tumor associated antigen using routine immunization techniques known to those of skill in the art. The antigens that may be used to enhance the immune response include tumor cells expressing the tumor associated antigen, purified tumor associated antigen, peptides derived from the sequence of the tumor
10 associated antigen and nucleic acid molecules, such as minigene constructs, capable of expressing the tumor associated antigen. The level of anti-tumor associated antigen antibodies is monitored in the host's serum.

6. EXAMPLE: ENHANCEMENT BY A BOOSTER WITH HMW-MAA
15 BEARING CULTURED HUMAN MELANOMA CELLS

Rabbits 97-6 and 97-7 were immunized on day 0, 14, 28 and 42 with the mouse anti-id mAb MK-23 conjugated to KLH (300 µg/injection) and mixed with Freund's adjuvant. Rabbits express the HMW-MAA with a tissue distribution and an antigenic profile similar to those of the human counterpart (Schlingemann, R.O, et al.,
20 1990, *Am. J. Pathol.* 136:1393-1405).

The anti-id mAb MK2-23 bears the internal image of HMW-MAA (Mittelman, A. et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:466-470; Kasuama M., et al., 1989, *J. Immunol.* 143:3844-3852; Chen, Z.J. et al., 1993, *Cancer Res.*, 53:112-119). Sera harvest on day 49 displayed a low reactivity with HMW-MAA bearing
25 melanoma cells Colo 38 (Fig. 1), but did not immunoprecipitate HMW-MAA from radiolabelled melanoma cells (Fig. 2). Rabbits were boosted with Colo 38 cells on day 56. Sera harvested on day 63 displayed an increased reactivity with Colo 38 cells (Figure 1) and immunoprecipitated the HMW-MAA from radiolabelled melanoma cells (Figure 2). The data indicate that a mimic of an antigen can break
30 unresponsiveness to a self-antigen. The low immune response triggered by the mimic can be enhanced by a booster with the original antigen.

7. EXAMPLE: IDENTIFICATION OF PEPTIDE MIMICS OF
DISTINCT ANTIGENIC DETERMINANTS OF HMW-MAA BY
PANNING PHAGE DISPLAY PEPTIDE LIBRARIES

The human scFv C21 and the mouse mAb 149.53, 225.28, 763.79,
5 GH704 and TP61.5 recognize distinct antigenic determinants of HMW-MAA.
Panning phage display peptide libraries LX-8 and X15 (Morgan, D.J. et al., 1998, *J. Immunol.*, 160:643-651) with HMW-MAA binding human scFv C21 and mouse mAb 149.53, 225.28, 763.79, GH704 and TP61.5 following the methodology described by Bonnycastle *et al.* (Bonnycastle, L.L. et al., 1996, *J. Mol. Biol.*, 258:747-762), led to
10 the isolation of clones which reacted with the corresponding antibody in the immunoscreening assay (Valadon, P. et al., 1996, *J. Immunol. Methods*, 197:171-179). Nucleotide sequence analysis of randomly selected positive clones by the dideoxy nucleotide chain termination method (Sanger, F. et al., 1977, *Proc. Natl. Acad. Sci. USA*, 74:5463-5467) identified inserts with partial homology to the HMW-
15 MAA. Representative examples are shown in Table 1.

Table 1 Amino acid sequence of peptides identified by panning phage display peptide libraries with anti-HMW-MAA mAb 149.53, 225.28 and GH704

mAb	Peptide	Homology with HMW-MAA149.53
149.53	EELHPPGSRAPSIRK	1846 GSRAPISR 1853
225.28	TQYTRTDPWGLEPPK	1398 LEPP 1401
GH704	GCIKSHPFVRCP	1457 SHPV 1460

Synthetic peptides corresponding to peptide sequences identified by panning phage display peptide libraries with antibodies have been shown to react *in vitro* with the corresponding antibodies. Furthermore synthetic peptides have been
25 shown to induce antibodies reacting with melanoma cells in BALB/c mice.

8. EXAMPLE: IMMUNIZATION WITH A DNA MINIGENE

ENCODING PEPTIDE MIMICS OF β CELL EPITOPES

The following section describes experiments involving i) immunizations with a DNA minigene encoding peptide mimics of B cell epitopes of the human HMW-MAA in hosts with a constitutive expression of HMW-MAA and ii) sequential boosting with a DNA minigene encoding native B cell epitopes of HMW-MAA corresponding to peptide mimics.

A DNA minigene encoding peptide mimics of B cell epitopes of human HMW-MAA is tested for its ability to trigger the immune response to HMW-MAA in hosts with a constitutive expression of this antigen. The minigene has been selected as the immunogen to break tolerance to HMW-MAA, since minigenes have been shown to be highly immunogenic.

The hosts utilized include mice transgenic for human HMW-MAA and rabbits. Mice transgenic for human HMW-MAA will be utilized because they are suitable for testing a large number of variables which may influence the immunogenicity of DNA minigene; they provide a source of hosts with a defined and standardized genetic background which minimizes the variability in the host's immune response; and they allow the testing of variables, *i.e.* cytokines, dendritic cells, which cannot be tested in other animal species due to the unavailability of reagents and/or methodology.

Rabbits are also used in the experiments, since they express the HMW-MAA with a tissue distribution and an antigenic profile similar to those observed in humans. Rabbits are tested to determine the effect of similarity, but not identity between rabbit and human HMW-MAA on the host's immune response using the methods described herein.

8.1. MATERIALS AND METHODS8.1.1 PREPARATION OF A MINIGENE ENCODING PEPTIDE MIMICS OF CELL DETERMINANTS AND OF NATIVE B CELL DETERMINANTS OF HMW-MAA

Oligonucleotide sequences encoding peptides that express cytotoxic T cell, B cell and helper T cell epitopes can be assembled together in the form of "minigenes". Following transfer into an appropriate expression vector, such as viral or plasmid based mammalian expression vectors, the minigenes are known to be highly immunogenic (Whitton, J.L. et al., 1993, *J. Virol*, 67:348-352; An, L.L. et al., 1997, *J. Virol*, 71:2292-2302; Ishioka, G.Y. et al., 1999, *J. Immunol.*, 162:3915-3925).

The minigene used in this study consists of (i) oligonucleotide sequences encoding peptide mimics of B cell determinants of HMW-MAA, (ii) a universal helper epitope PADRE that elicits help for differentiation of antigen specific B cells into plasma cells, and (iii) a signal peptide. The signal peptide will direct secretion of the expressed protein into the extracellular space facilitating antigen uptake by antigen specific B cells.

The minigene is constructed following published procedures (Ishioka, G.Y. et al., 1999, *J. Immunol.*, 162:3915-3925). The order of sequences in the minigene consist of signal sequence, sequence of the helper peptide PADRE followed by sequences encoding the peptide mimics of B cell determinants of HMW-MAA. As in the published procedures, there are no intervening sequences between neighboring peptide sequences, so that all peptide sequences are part of a single open reading frame. In addition, the 5' end oligonucleotide sequences of the minigene contains sequences encoding the restriction enzyme site NheI and the Kozak consensus sequences to facilitate translation of the mRNA. The 3' end oligonucleotide of the minigene will contain an in frame termination codon and a restriction enzyme site KpnI. The different restriction enzyme sites at the 5' and 3' ends of the minigene will permit directional cloning into the pcDNA3.1 expression vector.

The total length of the minigene is 255 nucleotides. This includes a 120-nucleotide long 5' region containing the 5' NheI restriction site, the Kozak sequence, the signal peptide and the PADRE sequence. The 3' part of the minigene is 135 nucleotides long and contains sequences encoding the three peptide mimics, the termination codon and the 3' end KpnI restriction site. These two parts are assembled separately. The 5' part is assembled as a 135 nucleotide long cDNA since it also

includes a 15 overlap with the 3' part. Two overlapping oligonucleotide primers including a 75 mer sense primer and a 75 mer antisense primer with a central overlapping segment of 15 nucleotides is annealed and extended in a PCR to yield a 135 nucleotide long cDNA product. The cDNA is purified by electrophoresis on an agarose gel. The 3' part is similarly assembled separately. It is assembled as a 135 nucleotide long cDNA since the central overlap segment between the 5' and 3' parts is provided by the 5' part. Thus, the 3' part of the minigene is assembled with two overlapping oligonucleotide primers including a 75 mer sense primer and a 75 mer antisense primer with a 15 nucleotide overlap at their 3' ends. The two primers are annealed and extended in a PCR for 10 cycles and the resulting 135 nucleotide long cDNA purified by electrophoresis on an agarose gel. The gel purified 5' and 3' parts of the minigene are mixed together and carried through 10 cycles of PCR to yield a 255 nucleotides long minigene. This full-length minigene is selectively amplified using two 25 mer primers from the 5' and 3' ends of the minigene. The cycling conditions of the PCR amplifications are 95°C, 15 sec. for denaturation; 5°C below the calculate melting temperature of the overlapping segments of the oligonucleotide for 30 sec. for annealing and 72°C for 1 min. for DNA synthesis. The 255 nucleotide long minigene is then purified by agarose gel electrophoresis and cloned in the vector pCR2.1 (Invitrogen). Individual clones are isolated and sequenced to confirm their sequence identity. The minigene is excised from the pCR2.1 vector by digestion with NheI and KpnI enzymes and re-cloned into the same restriction sites of the expression vector pcDNA 3.1.

8.1.2 SYNTHESIS OF FULL LENGTH HMW-MAA cDNA

A cDNA library is constructed from two micrograms of poly(A)+ RNA isolated from A385 met human melanoma cells. An oligo(dT)primer adapter containing a NotI site is used for priming first-strand cDNA with SuperScript II RNase H- reverse transcriptase (Gibco BRL). Double-stranded cDNAs is generated using the rest of the SuperScript cDNA synthesis system followed by a Sal I adapter ligation and Not I digestion. All of the recovered double-stranded cDNAs are ligated into Not I/Sal I sites of a lambda vector. The ligated cDNA/phage DNA mixtures are

packaged using Gigapack Gold packaging extracts (Stratagene) and titered according to the manufacturer's specifications.

To identify cDNAs corresponding to the HMW-MAA, the above cDNA library members are screened using ^{32}P labeled PCR product as a probe at 1×10^6 cpm/ml of hybridization solution (50% formamide/5X Denhardt's solution/1 % SDS/6X SSPE/0.2 mg/ml salmon sperm DNA) at 42°C for 16 hrs. Membranes will be rinsed in 1X SSC/0.5% SDS at room temperature for 30 min. and then in 0.1X SSC/0.5% SDS at 65°C for 30 min. prior to autoradiography. Isolated clones with expected sizes are sequenced in their entirety at least two times per nucleotide using the Prism Ready Reaction Cycle Sequencing system (Perkin Elmer).

Because of the size of the HMW-MAA message, 6.966 bases long for the coding sequence (Pluschke, G. et al., 1996, *Proc. Natl. Acad. Sci. USA*, 93: 9710-9715), it may be difficult to obtain single full length cDNA clones with conventional cloning strategy. Alternatively, a RT-PCR based SMART RACE cDNA amplification protocol may be used in combination with a Marathon cDNA amplification kit (Clontech). In order to avoid difficulties associated with the long RNA template the cDNA construction will be performed in three parts (Figure 3).

The synthetic oligonucleotide shown in Table II are designed according to the sequence of chosen unique restriction sites for convenient cloning.

Table II. Synthetic oligonucleotides used for preparing the HMW-MAA cDNA fragments by reverse transcription and PCR amplification

5' Primers	3' Primers
1. CTA <u>GCGG CCGC GCT AGC</u> GCC ACC ATG CAG TCC GGC Not I Nhe I	GCA GCT <u>GGA</u> TCC AGC CAC GGT Bamh I
2. ACC GTG GCT <u>GGA TCC</u> AGC TGC Bamh I	CG <u>GAA TTC AAT ATT</u> CCC AGC GTA GAC CTC Ecor I SspI
3. GCT GGG <u>AAT ATT</u> CTG TAT GAG Ssp I	GC <u>ATC GAT</u> TTT TTT TTT TTT TTT TTT TTT TCC AGA Bsp106 I

The recognition sites for restriction enzymes are underlined.

In order to obtain a full length cDNA clone of the HMW-MAA gene, the termini of every cDNA fragment have overlapped sequences shared by the cDNA

fragments of the neighboring regions (Figure 3). PCR products will be sequentially cloned into vector pBluescript II (Stratagene) and the nucleotide sequence will be determined using an automatic DNA sequencer (ABI Prism Model 388 PE-Biosystem, Foster City; CA).

5 To complete the HMW-MAA minigene, a 280 bp NotI/NotI fragment, 5' promoter region of the mouse tyrosinase for human and mouse melanocyte-specific expression, (Kluppel, M. et al., 1991, *Proc. Natl. Acad. Sci. USA*, 88:3777-3781) is ligated into pBluescript II containing HMW-MAA cDNA, and the simian virus 40 splice and polyadenylation sites (Figure 3). The direction of cloned NotI/NotI
10 fragment is determined by sequencing.

8.1.3. GENERATION AND ESTABLISHMENT OF MICE TRANSGENIC FOR HUMAN HMW-MAA

After removal of vector sequences by cleavage with BssHII, the minigene is isolated from a low-gelling temperature agarose gel and purified with
15 glass powder (GeneClean; Bio 101) followed by dialysis against injection buffer (10 mM Tris.HCL, pH 7.5/0.1 mM EDTA) on dialysis filters (Millipore).

Approximately 500-1,000 copies (1-2 pl of 2-5 ng/μl DNA soln.) of the transgene is injected into the male pronucleus which allows easier introduction of the injection needle as it is larger in size than female's. Approximately 200 eggs are
20 injected during each day of microinjection. After microinjection, eggs are cultured in 5% CO₂ incubator, and in the following day, transferred into the oviduct of pseudopregnant female at the 2-cell stage. Offspring are usually born 19 days after the transfer.

Transgene integration is screened initially by polymerase chain
25 reaction (PCR) analysis and subsequently confirmed by Southern analysis of tail DNA purified from each offspring. Only transgene positive animals are backcrossed to original strain of mice to establish transgenic mouse strains. As founder transgenic animals (G0: generation 0) may be chimeric in copy number and integration site of the transgene in germ cells, the next generation of animals (G1) are thoroughly
30 investigated for transgene segregation. After establishment of transgenic mouse lines

a subset of the mice are sacrificed and tested for RNA and protein expression in various tissue utilizing Northern blot and immunohistochemistry. One or two representative transgenic lines in which the transgene is highly expressed are selected for further experimentation. It is expected that expression of the HMW-MAA genes is induced in melanocytes. All techniques described here are routine. (Reilly, M.P. et al., 1994, *Academic Press, New York*, pp. 403-434; Katsumata, M. et al., 1995 *Nature Medicine*, 1:644-648).

The C57BL6/J inbred strain is chosen to produce transgenic animals since its genetic and physiological background are well characterized, although, the use of inbred mice is sometimes not suitable for obtaining sufficient numbers of properly fertilized eggs, and highly viable embryos for microinjection. In addition to this, reproduction of inbred mice is relatively poor. This low reproductivity is mostly due to insufficient mating ability of male mice. To overcome this problem several (Waldman, T.A., 1991, *Science*, 252:1657-1662; von-Mehren, et al., 1996, *Curr. Opin. Oncol.*, 8:493-498) male mice are raised in the same cage to induce competition among the animals so that only one dominant male can mate. T-cell receptor gene transgenic mice have been efficiently produced using this approach. To obtain sufficient numbers of fertilized eggs, larger numbers (up to fifteen) of females are used for the mating. Each female produces at least 10-20 properly fertilized eggs although the proportion of unhealthy eggs is high. If *in vitro* survival of those embryos appears poor, the embryos will be transferred the same day of microinjection.

8.1.4. IMMUNIZATION SCHEDULE

Ten HMW-MAA transgenic mice are immunized with the DNA minigene following published procedures which rely on gene gun-mediated delivery of cDNA under the skin (Sundaram, P. et al., 1996, *Nucl. Acids Res.*, 24:1375-1377). Briefly, DNA coated gold particles are prepared by adding supercoiled plasmid DNA to 25 mg of gold particles (1-3 mm average diameter) in 100 µl of 0.1M spermidine, followed by 10 sec. sonication. Two hundred µl of a 2.5M CaCl₂ are then added with continuous vortexing and the mixture is incubated at 20°C for 10 min. with

intermittent vortexing. DNA coated particles are then pelleted at 12,000 for 30 sec., washed three times with 100% ethanol and resuspended in 3 ml of ethanol. A 21 inch long Tefzel tubing (1/8th inch outside diameter, 3/32 inch internal diameter) is filled with the suspension and laid flat for 10 min. to allow the particles to settle. The ethanol is then decanted, and the tubing is manually rotated to coat the inner walls with the particles. The tubing is then dried in a stream of nitrogen gas for 3-5 min. Individual shots are generated by cutting the tubing into ½ inch length, each containing 1 µg/0.5 mg gold and loaded into 12-shot barrels of a helium-driven gene gun. Mice are anesthetized with ketamine (30 mg/kg) and xylazine (3mg/kg). Hair is clipped from a 100 cm² area on mice backs, and residual hair and superficial keratin are treated with a commercial depilatory. The gene gun is held against the skin and each shot is inoculated by instantaneous release of 350 psi of helium pressure through the barrel. For each primary and booster immunization, mice are inoculated at 10 sites. The primary immunization is given on day 0, and booster immunizations on days 21 and 42. Sera are obtained prior to primary immunization and 10 days after each booster immunization.

Ten rabbits are immunized with the DNA minigene encoding peptide mimics of B cell epitopes of HMW-MAA utilizing the methodology described for mice. The detection and characterization of anti-HMW-MAA antibodies will be performed as described above. Rabbits (10/group) immunized with a DNA minigene encoding peptide mimics of B cell determinants of HMW-MAA are boosted with a DNA minigene encoding a fragment of HMW-MAA expressing the corresponding native B cell determinants, utilizing the methodology described above.

8.1.5. DETECTION OF ANTI-HMW-MAA ANTIBODIES

The methodology utilized to analyze the humoral immune response has been extensively utilized (Mittelman, A. et al., 1992, *Proc. Natl. Acad. Sci. USA* 89:466-470; Kusama, M. et al., 1989, *J. Immunol.*, 143:3844-3852; Chen, Z.J. et al., 1993, *Cancer Res.*, 53:112-119). Briefly, the level of HMW-MAA binding antibodies is measured by testing serial two fold dilutions of sera from immunized mice with HMW-MAA bearing cultured human melanoma cells in a binding assay.

The binding of antibodies is detected using biotinylated anti-mice IgG and anti-mice IgM xenoantibodies. The specificity of the binding is assessed by testing immune sera for binding to lymphoid cells, which do not express HMW-MAA and by testing melanoma cells with preimmune sera and sera from mice immunized with a minigene encoding an unrelated peptide.

The specificity of immune sera for HMW-MAA is assessed by SDS-PAGE analysis of components immunoprecipitated from ¹²⁵I- or ³⁵S-labelled melanoma cells and by sequential immunoprecipitation utilizing anti-HMW-MAA mAb as a reference. Radio-labeling of cells, solubilization of cells, immunoprecipitation, SDS-PAGE and autoradiography or fluorography is performed utilizing procedures that have been extensively utilized (Chen, Z.J. et al., 1993, *Cancer Res.*, 53:112-119). If sera from immunized mice do not immunoprecipitate any components from radiolabeled melanoma cells because of the low titer and/or avidity of the antibodies, sera will be tested with HMW-MAA purified from a melanoma cell extract by binding to a microtiter plate coated with an anti-HMW-MAA mAb. The latter recognizes a determinant distinct and spatially distant from those encoded by DNA minigene. The binding of antibodies to HMW-MAA is detected with biotinylated anti-mouse IgG Fc xenoantibodies. The specificity of the binding is monitored utilizing plates coated with an unrelated antigen.

Sera containing HMW-MAA binding antibodies is tested for staining of melanoma lesions in the immunoperoxidase reaction (Schlingemann, R.O., et al., 1990 *Am. J. Pathol.*, 136:1393-1405), to prove that the antibodies react with the antigen *in vivo*. If the results are not reliable because of high background, antibodies will be purified by affinity chromatography on synthetic peptides and used to stain melanoma lesions and control tissues in the immunoperoxidase reaction.

Cross blocking experiments is used to map the antigenic determinant recognized by antibodies present in immune sera. To this end, melanoma cells are incubated with saturating amounts (20 µg/well) of F(ab')₂ fragments of mouse monoclonal antibodies or of human scFv fragments recognizing the determinants encoded by the DNA minigene. Following washing, immune sera are added to cells and the binding of antibodies is detected using biotinylated anti-mouse IgG Fc

xenoantibodies. Immune sera is tested for its ability to inhibit the binding to melanoma cells of monoclonal antibodies and scFv fragments recognizing determinants encoded by the DNA minigene. To this end, melanoma cells are coated with immune sera and then tested for their ability to biotinylated monoclonal
5 antibodies and seFv fragments recognizing the determinants encoded by the DNA minigene. The specificity of these assays is assessed using mAb and antisera recognizing unrelated antigens.

8.1.6. ENHANCEMENT BY BOOSTS WITH A DNA MINIGENE

Mice (10/group) immunized with a DNA minigene encoding peptide
10 mimics of β cell determinants of HMW-MAA are boosted with a DNA minigene encoding a fragment of HMW-MAA expressing the corresponding native B cell determinants. In preliminary experiments mice are immunized at biweekly intervals with four injections of the DNA minigene encoding peptide mimics of B cell determinants of HMW-MAA and one with a DNA minigene encoding a fragment of
15 HMW-MAA expressing the corresponding native B cell determinants. On the basis of the results obtained the number of immunizations with the two DNA minigenes may be changed. Controls include immunizations with an unrelated DNA minigene.

The level of anti-HMW-MAA antibodies is monitored in sequential bleedings from mice following the methodology described above. The increase in the
20 reactivity with melanoma cells of sera from mice following a booster encoding a fragment of HMW-MAA may reflect an increase of the antibody population elicited by the minigene encoding peptide mimics of HMW-MAA or determinant spreading (Secarz, E., 1998, *Immunol. Rev.*, 164:5-264). To distinguish between these two possibilities, sera from rabbits is tested for their susceptibility to inhibition in their
25 binding to melanoma cells by the mAb used to identify the peptide. The specificity of the inhibition is assessed using unrelated mAb and antisera. If the mAb used does not inhibit the binding of antiserum to melanoma cells, it is likely that the increase in reactivity of antisera with melanoma cells reflects determinant spreading.

8.1.7. STATISTICAL ANALYSIS

Depending on whether the values have or do not have a normal distribution, a two sample statistical procedure such as the two sample test or the two sample Mann-Whitney test will be used to analyze the statistical difference in the level of antibodies elicited with the various immunization protocols.

9. EXAMPLE: IDENTIFICATION OF HLA-A2 RESTRICTED CTL DEFINED EPITOPES

The minigene evaluated for its ability to induce HMW-MAA specific CTL is constructed with HLA-A2 (A*0201) restricted epitopes, since the HLA-A2 allospecificity is the most frequent in all ethnic populations. HLA-A2/Kb transgenic mice are used to identify immunogenic HLA-A2 restricted, T cell epitopes of HMW-MAA, because their CTL repertoire has been shown to be similar, if not identical, to that in humans (Wentworth PA, 1996, Eur. J. Immunol. 26:67-101). The ability of the HMW-MAA DNA minigene to induce HLA-A2 restricted, HMW-MAA specific CTL will be tested utilizing mice transgenic for human HMW-MAA and for HLA-A2 antigen. The latter are generated by crossing HLA-A2 transgenic mice with HMW-MAA transgenic mice. HMA-MAA specific CTL epitopes defined in the animal model will be tested for their ability to generate in vitro HLA-A2-restricted, HMW-MAA specific CTL utilizing PBMC from patients with malignant melanoma.

Two approaches will be used for identification of HLA Class I antigen restricted CTL epitopes. First, CTL are induced by immunization with peptides derived from the sequence of target antigen, expanded by in vitro stimulation with antigen presenting cells (APC) pulsed with the immunizing peptides followed by evaluation for their capability to recognize target cells expressing the antigen. Second, CTL are generated by immunization with cDNA followed by in vitro stimulation with cDNA transfected APC and then testing on target cells pulsed with peptides. cDNAs are likely to be better immunogens than peptides when engineered like minigenes to contain sequences encoding an helper epitope and the signal peptide. The peptides to be used in both approaches are selected on the basis of HLA-A2 binding motifs identified with a computer program (Parker et al., 1994, J.

Immunol. 152:163-175).

From the foregoing, it will be obvious to those skilled in the art the various modifications in the above-described method, and compositions can be made without departing from the spirit and scope of the invention. Accordingly, the

5 invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. Throughout the application, various publications are referred to, the citations for which appear at the end of the specification. All of these references are incorporated herein by reference.

CLAIMS

1. A method for stimulating an immune response against a self antigen in a host comprising:
 - (i) immunization of a host with a mimic of the self-antigen; and
 - 5 (ii) increasing the response to the self-antigen by a booster with the self antigen.
2. The method of claim 1 wherein the self antigen is a tumor associated antigen.
3. The method of claim 2 wherein the tumor associated antigen is
10 expressed on the surface of a melanoma cell.
4. The method of claim 1 wherein the mimic is a peptide mimic.
5. The method of claim 1 wherein the mimic is a nucleic acid molecule encoding a peptide mimic.
6. The method of 5 wherein the nucleic acid molecule is a minigene
15 construct.
7. The method of claim 5 wherein the nucleic acid molecule encodes a peptide mimic of a tumor associated antigen.
8. The method of claim 7 wherein the tumor associated antigen is expressed on the surface of a melanoma cell.
- 20 9. The method of claim 1 wherein the mimic is an anti-idiotypic antibody.

10. The method of claim 9 wherein the anti-idiotypic antibody is a self antigen anti-idiotypic antibody.
12. The method of claim 10 wherein the self antigen is a tumor associated antigen.
- 5 13. The method of claim 12 wherein the tumor associated antigen is expressed on the surface of a melanoma cell.
14. The method of claim 1 wherein the host is a mammal.
15. The method of claim 1 wherein the host is a human.
16. The method of claim 14 or 15 wherein the host expresses tumor
10 associated antigens.
17. The method of claim 16 wherein the tumor associated antigen is expressed on a melanoma cell.

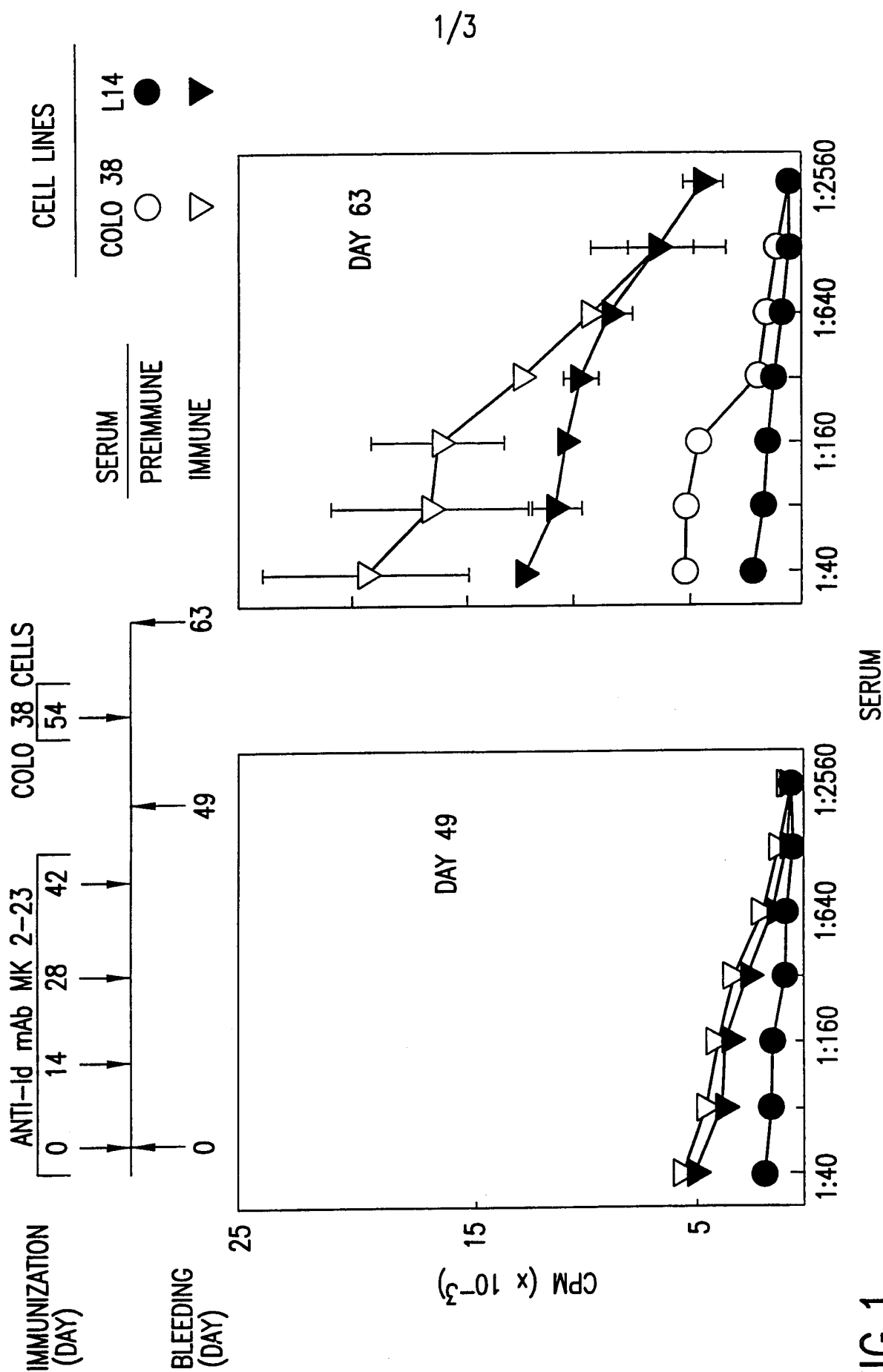


FIG.1

2/3

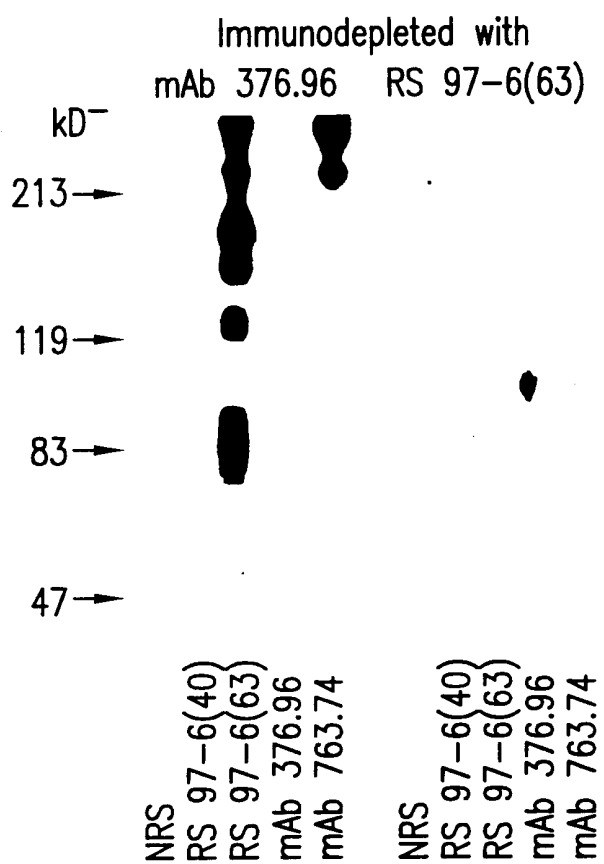


FIG.2

3/3

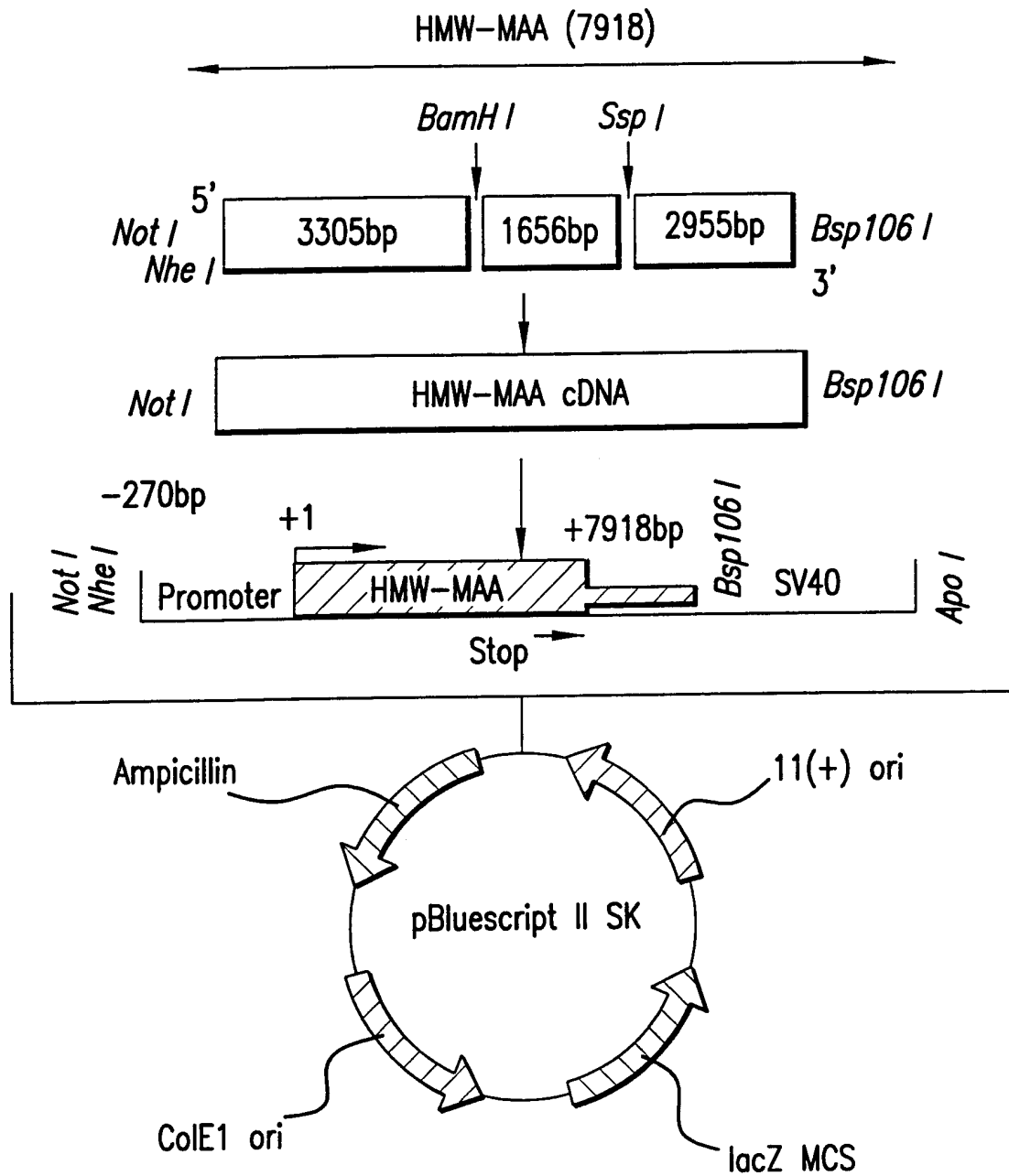


FIG.3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/28517

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/00, 31/70, 39/395, 39/40, 39/42, 39/44; A01N 43/04

US CL : 514/12,13,14,15,16,17,18,44; 424/130.1,184.1

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/12,13,14,15,16,17,18,44; 424/130.1,184.1

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

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

BIOSIS, CAPLUS, WPIDS, MEDLINE, CANCERLIT

search terms: anti-idiotype, self-antigen, tumor-associated antigen, melanoma, nucleic acid, minigene, peptide

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DREXLER, I. et al. Modified vaccinia virus ankara for delivery of human tyrosinase as melanoma-associated antigen: Induction of tyrosinase- and melanoma-specific human leukocyte antigen A*0201-restricted cytotoxic T cells in vitro and in vivo. Can. Res. 01 October 1999, Vol. 59, pages 4955-4963, especially page 4957, last paragraph of column 1 through first paragraph of column 2.	1-2, 4

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

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

Date of the actual completion of the international search

10 JANUARY 2001

Date of mailing of the international search report

27 MAR 2001

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/28517

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OVERWIJK et al, Gp100/pmel 17 is a murine tumor rejection antigen: Induction of "self"-reactive, tumoricidal T cells using high-affinity, altered peptide ligand, J. Exp. Med. July 1998, Vol. 188, No. 2, pages 277-286, see entire document.	1-4, 14, 16-17
X	ZAJAC et al, Generation of tumoricidal cytotoxic T lymphocytes from healthy donors after in vitro stimulation with a replication-incompetent vaccinia virus encoding MART-1/MELAN-A 27-35 epitope, Int. J. Canc. 1997, Vol. 71, pages 491-496, especially Page 491, column 2 and Page 492.	5-8
X	MITTELMAN et al, Human high molecular weight melanoma-associated antigen (HMW-MAA) mimicry by mouse anti-idiotypic monoclonal antibody MK2-23: Induction of humoral anti-HMW-MAA immunity and prolongation of survival in patients with stage IV melanoma, Proc. Natl. Acad. Sci. January 1992, Vol. 89, pages 466-470, especially Page 467, column 2, second paragraph.	1-3, 9-17