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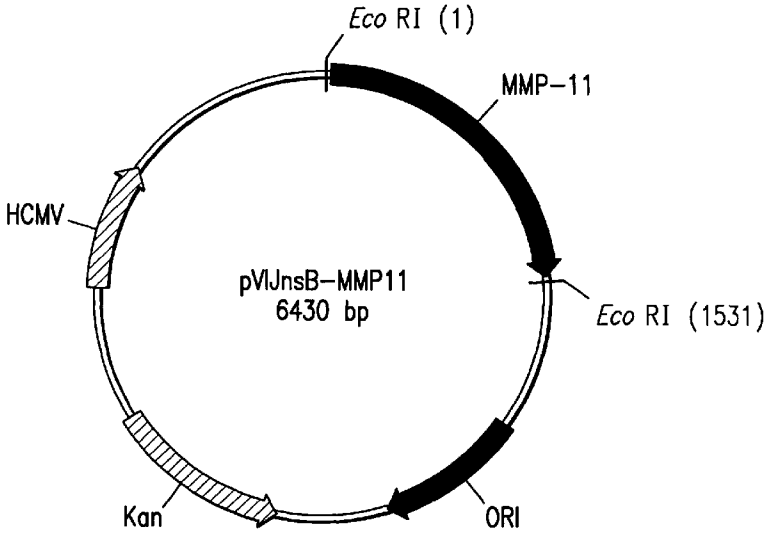
(54) Title: MATRIX METALLOPROTEINASE 11 VACCINE



(57) Abstract: Compositions comprising matrix metalloproteinase 11 (MMP-11) or stromelysin-3 (ST-3) or the nucleic acid encoding the MMP-11 for use in vaccines for treating tumors and cancers, which overexpress MMP-11, are described. In particular embodiments, the compositions comprise a nucleic acid encoding a fusion polypeptide that includes the catalytically inactivated MMP-11 linked at the C-terminus to an immunoenhancing element wherein the codons encoding the MMP-11 and the immunoenhancing element have been optimized for enhanced expression of the fusion polypeptide in human cells. In other embodiments, the compositions comprise the catalytically inactivated MMP-11 linked at the C-terminus to an immunoenhancing element. The compositions can be used alone or in synergy with vaccines against other tumor associated antigens as well as with conventional therapies such as radiation therapy and chemotherapy.

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MATRIX METALLOPROTEINASE 11 VACCINE

CROSS REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 60/724,498, filed October 7, 205, the contents of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

The present invention relates to compositions comprising matrix metalloproteinase 11 (MMP-11) or stromelysin-3 (ST-3) or the nucleic acid encoding the MMP-11 for use in vaccines for treating tumors and cancers, which overexpress MMP-11. In particular embodiments, the compositions comprise a nucleic acid encoding a fusion polypeptide that includes a catalytically inactivated MMP-11 linked at the C-terminus to an immunoenhancing element wherein the codons encoding the MMP-11 and the immunoenhancing element have been optimized for enhanced expression of the fusion polypeptide in human cells. In other embodiments, the compositions comprise the catalytically inactivated MMP-11 linked at the C-terminus to an immunoenhancing element. The compositions can be used alone or in synergy with vaccines against other tumor associated antigens as well as with conventional therapies such as radiation therapy and chemotherapy.

(2) Description of Related Art

Matrix Metalloproteinase-11 (MMP-11) or stromelysin 3 (ST3) is expressed in many, if not most, invasive primary carcinomas and in a number of their metastases and more rarely in sarcomas and other non-epithelial malignancies (*See* Basset *et al.*, Critical Reviews in Oncology/Hematology 26: 43-53, (1997)). Measuring levels of MMP-11 expression can be used to identify patients at greatest risk for cancer recurrence. It has been shown that recurrent breast carcinomas occurred more frequently in patients who had high levels of MMP-11 RNA or protein in their tumors than in patients who had low levels of MMP-11 RNA or protein in their tumors. Similarly, MMP-11 expression was found to be increased in pancreatic tumors as compared to normal tissue and the level of MMP-11 expression was strongly associated with lymph node involvement and overall survival (Jones *et al.*, Clin. Cancer Res. 10: 2832-2845, (2004)). MMP-11 mRNA expression is also significantly increased in colon carcinomas compared to MMP-11 mRNA expression in non-tumorous tissue (Thewes *et al.*, Diagn. Mol. Pathol. 5: 284-290, (1996)).

The role of MMP-11 in cancer progression has been demonstrated by several pre-clinical observations. For example, MMP-11 expression was shown to promote tumor take in mice (Noel et al., J Clin Invest 97: 1924-1930 (1996)). MMP-11 was also shown to promote homing of malignant epithelial cells in a paracrine manner and the homing appears to require extracellular matrix associated factors

(Masson *et al.*, J. Cell Biol. 140: 1535-1541 (1998)) such as basic fibroblast growth factor (bFGF) (Mari *et al.*, J. Biol. Chem. 273: 618-626 (1998)). MMP-11 protease activity can modulate cancer progression by remodeling extracellular matrix and inducing it to release microenvironmental factors (Noel *et al.*, Oncogene 19: 1605-1612 (2000)). MMP-11 has been shown to have an anti-apoptotic and anti-necrotic effect on tumorous cells (Boulay *et al.*, Cancer Res. 61: 2189-2193 (2001)), which appears to be mediated by its catalytic activity (Wu *et al.*, J. Cell Biochem. 82:549-555 (2001)). MMP-11 deficiency has been shown to increase tumor free survival and modulate local or distant invasion (Andarawewa *et al.*, Cancer Res. 63:5844-5849 (2003)). Knocking down MMP-11 mRNA in gastric cancer cells appears to dramatically suppresses tumor growth both in vitro and in vivo (Deng *et al.*, Biochem. Biophys. Res. Comm. 26: 274-281 (2005)). MMP-11 has also been shown to interfere with the immune system's response against tumors in that a cleavage product of a1-proteinase inhibitor, generated by MMP-11 cleavage, decreases the sensitivity of tumor cells to natural killer cells (NK) (Kataoka *et al.*, Am. J. Pathol. 154: 457-468, (1999)). In addition, an increased number of neutrophils and macrophages infiltrate tumors in MMP-11-null mice compared with wild-type mice, indicating that MMP-11 inhibits a chemoattractant for these cells (Boulay *et al.*, Cancer Res. 61: 2189-2193 (2001)). Thus, MMP-11 appears to play a crucial role in the initial stage of tumorigenesis.

Several agents have been developed that block the synthesis of MMPs, prevent them from interacting with the molecules that direct their activities to the cell surface, or inhibit their enzymatic activity (reviewed in Egeblad and Werb, Nature Reviews 2: 163-174 (2002)). Most of agents were not specifically directed against MMP-11 but interfered with functions of other members of MMP family. However, clinical trials with several of these MMP inhibitors have suggested the inhibitors have a limited antitumor effect. Therefore, in light of the above, there is a need for anti-cancer therapies and treatments that inhibit or interfere with MMP-11 activity.

BRIEF SUMMARY OF THE INVENTION

The present invention provides compositions comprising matrix metalloproteinase 11 (MMP-11) or stromelysin-3 (ST-3) or the nucleic acid encoding the MMP-11 for use in vaccines for treating tumors and cancers, which overexpress MMP-11. In particular embodiments, the compositions comprise a nucleic acid encoding a fusion polypeptide that includes a catalytically inactivated MMP-11 linked at the C-terminus to an immunoenhancing element wherein the codons encoding the MMP-11 and the immunoenhancing element have been optimized for enhanced expression of the fusion polypeptide in human cells. In other embodiments, the compositions comprise the catalytically inactivated MMP-11 linked at the C-terminus to an immunoenhancing element. The compositions can be used alone or in synergy with vaccines against other tumor associated antigens as well as with conventional therapies such as radiation therapy and chemotherapy.

Therefore, the present invention provides a nucleic acid comprising a nucleotide sequence encoding an MMP-11 polypeptide wherein one or more of the nucleotide codons encoding the

MMP-11 that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans (that is, the nucleotide sequence has been optimized for high expression of the nucleic acid in cells of human origin).

In a preferred embodiment of the nucleic acid, the MMP-11 encoded by the nucleotide sequence further includes a mutation that renders the MMP-11 catalytically inactive, in particular embodiments, the mutation is in the zinc binding domain of the MMP-11.

In further embodiments of the nucleic acid, the polynucleotide encodes an MMP-11, wherein the polynucleotide encodes a human MMP-11 or an MMP-11 of primate origin.

In further still embodiments of the nucleic acid, the nucleic acid includes a nucleotide sequence that has the nucleotide sequence of SEQ ID NO:4.

The present invention further provides a nucleic acid encoding a fusion polypeptide having an MMP-11 linked to an immunoenhancing element or substantial portion thereof. In preferred embodiments, one or more of the nucleotide codons encoding the fusion polypeptide that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans. In particular embodiments, the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and subunit B of the heat-labile toxin (LTB).

In a currently preferred embodiment of the nucleic acid, the immunoenhancing element is the *E. coli* LTB. In further embodiments, the LTB does not include a signal sequence. In further still embodiments of the nucleic acid, the LTB is encoded by the nucleotide sequence shown in SEQ ID NO:8.

In preferred embodiments of the above nucleic acid, the MMP-11 includes a mutation that renders the MMP-11 catalytically inactive.

In further embodiments of the nucleic acid, the MMP-11 is encoded by the nucleotide sequence shown in SEQ ID NO:4. In further still embodiments, the fusion polypeptide includes the nucleotide sequence shown in SEQ ID NO:11.

The present invention further provides an expression vector comprising the nucleic acid of any one of the aforementioned embodiments operably linked to a promoter. The present invention further provides a host cell containing any one of the embodiments of the above expression vector therein. The present invention further provides a process, comprising culturing the above host cell in a cell culture medium under conditions for producing the fusion polypeptide.

The present invention further provides a fusion polypeptide comprising an MMP-11 linked to an immunoenhancing element or substantial portion thereof.

In particular embodiments of the fusion polypeptide, the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and subunit B of the heat-labile toxin (LTB).

In a currently preferred embodiment of the fusion polypeptide, the immunoenhancing element polypeptide is the *E. coli* LTB. In further still embodiments, the LTB does not include a signal sequence. In further still embodiments, the LTB includes the amino acid sequence shown in SEQ ID NO:9.

In preferred embodiments of the fusion polypeptide, the MMP-11 includes a mutation that renders it catalytically inactive. Preferably, the mutation is in the zinc binding domain of the MMP-11. In further still embodiments, the MMP-11 polypeptide comprising the fusion polypeptide includes the amino acid sequence shown in SEQ ID NO:5 or the polypeptide includes the amino acid sequence shown in SEQ ID NO:10.

The present invention further provides a polynucleotide vaccine comprising a nucleotide sequence encoding an MMP-11 wherein one or more of the nucleotide codons encoding the MMP-11 that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

In a preferred embodiment of the polynucleotide vaccine, the MMP-11 encoded by the nucleotide sequence further includes a mutation that renders the MMP-11 catalytically inactive. In a currently preferred embodiment, the mutation is in the zinc binding domain of the MMP-11. In further still embodiments of the polynucleotide vaccine, the MMP-11 is an MMP-11 of human origin or of primate origin. In a further still embodiment of the polynucleotide vaccine, the nucleotide sequence includes the nucleotide sequence of SEQ ID NO:4.

The present invention further provides a polynucleotide vaccine encoding a fusion polypeptide having an MMP-11 linked to an immunoenhancing element or substantial portion thereof. In particular embodiments of the polynucleotide vaccine, the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and subunit B of the heat-labile toxin (LTB).

In a preferred embodiment of the polynucleotide vaccine, the MMP-11 encoded by the nucleotide sequence further includes a mutation that renders the MMP-11 catalytically inactive. In a currently preferred embodiment, the mutation is in the zinc binding domain of the MMP-11. In further still embodiments of the polynucleotide vaccine, the MMP-11 is an MMP-11 of human origin or of

primate origin. In a further still embodiment of the polynucleotide vaccine, the nucleotide sequence includes the nucleotide sequence of SEQ ID NO:4.

In a currently preferred embodiment of the polynucleotide vaccine, the immunoenhancing element polypeptide is the subunit B of heat labile toxin (LTB) of *E. coli*. In further still embodiments, the LTB does not include a signal sequence and in further still embodiments, the LTB is encoded by the nucleotide sequence shown in SEQ ID NO:8.

In a further preferred embodiment of the polynucleotide vaccine, one or more of the nucleotide codons encoding the fusion polypeptide that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

In a further embodiment, the MMP-11 is encoded by the nucleotide sequence shown in SEQ ID NO:4. In a further still embodiment, the fusion polypeptide includes the nucleotide sequence shown in SEQ ID NO:11.

In further still embodiments of the polynucleotide vaccine, the vaccine further includes one or more genetic adjuvants. Such genetic adjuvants include, but are not limited to, costimulatory molecules such as CD80 and CD86; proinflammatory cytokines such as interleukin-1α (IL-1α); tumor necrosis factor-α and β (TNF-α and TNF-β); Th1 cytokines such as IL-2, IL-12, IL-15, and IL-18; Th2 cytokines such as IL-4, IL-5, and IL-10; macrophage colony-stimulating factor (M-CSF); granulocyte colony-stimulating factor (G-CSF); granulocytes-monocyte colony-stimulating factor (GM-CSF); IL-8; interferon-γ-inducible protein-10 (γIP-10); macrophage inhibitory protein-1α (MIP-1α); and RANTES.

In further still embodiments of the polynucleotide vaccine, the vaccine further includes one or more conventional adjuvants. Conventional adjuvants include, but are not limited to, mineral salts such as aluminum phosphate or hydroxide, bacteria-derived adjuvants such as monophosphoryl lipid A, cholera toxin, muramyl peptides, lipid particles such as cationic liposomes and mannan-coated liposomes, emulsifier adjuvants such as QS-21, and synthetic adjuvants such as ubenimex.

The present invention further provides a polypeptide vaccine comprising a fusion polypeptide having an MMP-11 linked to an immunoenhancing element polypeptide or substantial portion thereof. In a preferred embodiment, the MMP-11 has a mutation that renders it catalytically inactive. In a currently preferred embodiment, the mutation is in the zinc binding domain of the MMP-11.

In particular embodiments of the polypeptide vaccine, the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from Vibrio cholerae, and subunit B of the heat-labile toxin (LTB).

In a currently preferred embodiment, the immunoenhancing element is the *E. coli* LTB. In further still embodiments, the LTB does not include a signal sequence. In further still embodiments, the LTB includes the amino acid sequence shown in SEQ ID NO:9.

In further still embodiments of the polypeptide vaccine, the MMP-11 includes the amino acid sequence shown in SEQ ID NO:5 or includes the amino acid sequence shown in SEQ ID NO:10.

In further still embodiments of the polypeptide vaccine, the vaccine includes one or more molecular adjuvants capable of modulating the immune response towards either a Th1 or Th2 response. Such molecular adjuvants include, but are not limited to, costimulatory molecules such as CD80 and CD86; proinflammatory cytokines such as interleukin-1α (IL-1α); tumor necrosis factor-α and β (TNF-α and TNF-β); Th1 cytokines such as IL-2, IL-12, IL-15, and IL-18; Th2 cytokines such as IL-4, IL-5, and IL-10; macrophage colony-stimulating factor (M-CSF); granulocyte colony-stimulating factor (G-CSF); granulocytes-monocyte colony-stimulating factor (GM-CSF); IL-8; interferon-γ-inducible protein-10 (γIP-10); macrophage inhibitory protein-1α (MIP-1α); and RANTES.

In further still embodiments of the polypeptide vaccine, the vaccine can include one or more conventional adjuvants. Conventional adjuvants include, but are not limited to, mineral salts such as aluminum phosphate or hydroxide, bacteria-derived adjuvants such as monophosphoryl lipid A, cholera toxin, muramyl peptides, lipid particles such as cationic liposomes and mannan-coated liposomes, emulsifier adjuvants such as QS-21, and synthetic adjuvants such as ubenimex.

The present invention further provides for the use of a nucleic acid a nucleotide sequence encoding an MMP-11 wherein one or more of the nucleotide codons encoding the fusion polypeptide that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans; use of a nucleic acid encoding a fusion polypeptide comprising an MMP-11 linked to an immunoenhancing element in a medicament for treating a carcinoma in an individual; and, use of a fusion polypeptide comprising an MMP-11 linked to an immunoenhancing element in a medicament for treating a carcinoma in an individual.

The present invention further provides a method for treating a carcinoma in an individual comprising providing a polynucleotide vaccine which includes a nucleic acid including a nucleotide sequence encoding an MMP-11 wherein one or more of the nucleotide codons encoding the MMP-11 that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans or a fusion polypeptide that includes an MMP-11 linked to an immunoenhancing element; and administering the vaccine to the individual to treat the cancer. In currently preferred embodiments of the nucleic acid encoding the fusion polypeptide, one or more of the nucleotide codons encoding the fusion polypeptide that are not present in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that are present in nucleic acids encoding the highly expressed proteins in humans.

In particular embodiments of the above method, the individual is undergoing one or more treatments selected from the group consisting of chemotherapy, radiation therapy, and vaccine against a tumor associated antigen. In further still embodiments, the individual has an invasive carcinoma selected from the group consisting of the breast, colon, head and neck, lung, ovary, pancreas, prostate, skin (basal cell carcinoma), uterus (cervix carcinoma and endometrial carcinoma) or the individual has a non-invasive carcinoma that has a risk of evolving towards invasion.

In a preferred embodiment of the method, the MMP-11 encoded by the nucleotide sequence further includes a mutation that renders the MMP-11 catalytically inactive. In a currently preferred embodiment, the mutation is in the zinc binding domain of the MMP-11. In further still embodiments of the polynucleotide vaccine, the MMP-11 is an MMP-11 of human origin or of primate origin. In a further still embodiment of the polynucleotide vaccine, the nucleotide sequence includes the nucleotide sequence of SEQ ID NO:4.

In particular embodiments of the method, the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and subunit B of the heat-labile toxin (LTB).

In a currently preferred embodiment of the method, the immunoenhancing element polypeptide is the subunit B of heat labile toxin of *E. coli* (LTB). In further still embodiments, the LTB does not include a signal sequence and in further still embodiments, the LTB is encoded by the nucleotide sequence shown in SEQ ID NO:8.

In a further embodiment, the MMP-11 is encoded by the nucleotide sequence shown in SEQ ID NO:4. In a further still embodiment, the fusion polypeptide includes the nucleotide sequence shown in SEQ ID NO:11.

In further still embodiments of the method, the vaccine further includes one or more genetic adjuvants. Such genetic adjuvants include, but are not limited to, costimulatory molecules such as CD80 and CD86; proinflammatory cytokines such as interleukin-1α (IL-1α); tumor necrosis factor-α and β (TNF-α and TNF-β); Th1 cytokines such as IL-2, IL-12, IL-15, and IL-18; Th2 cytokines such as IL-4, IL-5, and IL-10; macrophage colony-stimulating factor (M-CSF); granulocyte colony-stimulating factor (G-CSF); granulocytes-monocyte colony-stimulating factor (GM-CSF); IL-8; interferon-γ-inducible protein-10 (γIP-10); macrophage inhibitory protein-1α (MIP-1α); and RANTES.

In further still embodiments of the method, the vaccine further includes one or more conventional adjuvants. Conventional adjuvants include, but are not limited to, mineral salts such as aluminum phosphate or hydroxide, bacteria-derived adjuvants such as monophosphoryl lipid A, cholera toxin, muramyl peptides, lipid particles such as cationic liposomes and mannan-coated liposomes, emulsifier adjuvants such as QS-21, and synthetic adjuvants such as ubenimex.

The present invention further provides a method for treating a carcinoma in an individual comprising providing a vaccine that includes a fusion polypeptide having an MMP-11 linked to an immunoenhancing element; and administering the vaccine to the individual to treat the cancer.

In particular embodiments of the above method, the individual is undergoing one or more treatments selected from the group consisting of chemotherapy, radiation therapy, and vaccine against a tumor associated antigen. In further still embodiments, the individual has an invasive carcinoma selected from the group consisting of the breast, colon, head and neck, lung, ovary, pancreas, prostate, skin (basal cell carcinoma), uterus (cervix carcinoma and endometrial carcinoma) or the individual has a non-invasive carcinoma that has a risk of evolving towards invasion.

In particular embodiments of the method, the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from Vibrio cholerae, and subunit B of the heat-labile toxin (LTB).

In a currently preferred the method, the immunoenhancing element is the *E. coli* LTB. In further still embodiments, the LTB does not include a signal sequence. In further still embodiments, the LTB includes the amino acid sequence shown in SEQ ID NO:9.

In further still embodiments of the method, the MMP-11 includes the amino acid sequence shown in SEQ ID NO:5 or includes the amino acid sequence shown in SEQ ID NO:10.

In further still embodiments of the method, the vaccine includes one or more molecular adjuvants capable of modulating the immune response towards either a Th1 or Th2 response. Such molecular adjuvants include, but are not limited to, costimulatory molecules such as CD80 and CD86; proinflammatory cytokines such as interleukin-1α (IL-1α); tumor necrosis factor-α and β (TNF-α and TNF-β); Th1 cytokines such as IL-2, IL-12, IL-15, and IL-18; Th2 cytokines such as IL-4, IL-5, and IL-10; macrophage colony-stimulating factor (M-CSF); granulocyte colony-stimulating factor (G-CSF); granulocytes-monocyte colony-stimulating factor (GM-CSF); IL-8; interferon-γ-inducible protein-10 (γIP-10); macrophage inhibitory protein-1α (MIP-1α); and RANTES.

In further still embodiments of the method, the vaccine can include one or more conventional adjuvants. Conventional adjuvants include, but are not limited to, mineral salts such as aluminum phosphate or hydroxide, bacteria-derived adjuvants such as monophosphoryl lipid A, cholera toxin, muramyl peptides, lipid particles such as cationic liposomes and mannan-coated liposomes, emulsifier adjuvants such as QS-21, and synthetic adjuvants such as ubenimex.

The present invention further provides a method for identifying an analyte for inhibiting a cancer that overexpresses MMP-11, which comprises inducing the cancer in a mouse; administering the analyte to the mouse with the induced cancer; and determining whether the analyte inhibits the cancer in the mouse with the induced tumor, which identifies the analyte for inhibiting a cancer that overexpresses the MMP-11.

In particular embodiments, the analyte is determined to bind the MMP-11 before it is administered to the mouse.

In further still embodiments, the cancer that is induced in the mouse is a colon cancer, and in further still embodiments, the cancer is induced in the mouse by administering to the mouse 1-2dimethylhydrazine (DMH) in an amount sufficient to induce the cancer in the mouse.

Definitions

As used throughout the specification and in the appended claims, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise.

As used throughout the specification and appended claims, the following definitions and abbreviations apply:

The term "promoter" refers to a recognition site on a DNA strand to which RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity of a nucleic acid sequence located downstream from the promoter. The promoter can be modified by including activating sequences termed "enhancers" or inhibiting sequences termed "silencers" within the promoter. The term further includes both promoters which are inducible and promoters which are constitutive.

The term "cassette" refers to a nucleotide or gene sequence that is to be expressed from a vector, for example, the nucleotide or gene sequence encoding the cDkk-4 protein. In general, a cassette comprises a gene sequence inserted into a vector which in some embodiments provides regulatory sequences for expressing the nucleotide or gene sequence. In other embodiments, the nucleotide or gene sequence provides the regulatory sequences for its expression. In further embodiments, the vector provides some regulatory sequences and the nucleotide or gene sequence provides other regulatory sequences. For example, the vector can provide a promoter for transcribing the nucleotide or gene sequence and the nucleotide or gene sequence provides a transcription termination sequence. The regulatory sequences which can be provided by the vector include, but are not limited to, enhancers, transcription termination sequences, splice acceptor and donor sequences, introns, ribosome binding sequences, and poly(A) addition sequences.

The term "vector" refers to some means by which DNA fragments can be introduced into a host organism or host tissue. There are various types of vectors including plasmid, viruses (including adenovirus), bacteriophages, and cosmids.

The term "MMP-11" refers to the MMP-11 protein or polypeptide.

The term "immunoenhancing element" refers to a polypeptide portion of the MMP-11 fusion polypeptides of the present invention that is capable of stimulating or enhancing the immune response to the associated MMP-11, relative to full-length wild-type MMP-11. Immunoenhancing elements of the present invention include, but are not limited to, polypeptides comprising all of or a substantial portion of the polypeptides selected from the group consisting of heat shock protein (HSP) 70,

lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin G1 (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and heat labile toxin B subunit (LTB) of *E. coli* or other bacterial species.

The term "fusion protein" or "fusion polypeptide" refers to a protein having at least two polypeptides covalently linked in which one polypeptide comes from one protein sequence or domain and the other polypeptide comes from a second protein sequence or domain. The fusion proteins of the present invention comprise an MMP-11, and a second polypeptide, which comprises an immunoenhancing element or substantial portion thereof, which, in some cases, is a bacterial toxin. The MMP-11 may be a human MMP-11 or MMP-11 from another species. The polypeptides that comprise the fusion protein are preferably linked N-terminus to C-terminus. The MMP-11 and the immunoenhancing element can be fused in any order. In some embodiments of this invention, the C-terminus of the MMP-11 is fused to the amino terminus of the immunoenhancing element or the immunoenhancing element is fused to the amino terminus of the MMP-11.

The term "MMP-11 fusion protein" is intended to be a general term which refers to a fusion protein as described above, which comprises an MMP-11 polypeptide or fragment or variant thereof fused to a polypeptide comprising an immunoenhancing element or portion thereof. The term "MMP-11 fusion protein" is interchangeable with the term "MMP-11 fusion polypeptide".

The term "recombinant MMP-11" refers to an MMP-11 that has been modified by genetic engineering. For example, the term includes the catalytically inactive MMP-11 and the MMP-11 fusion polypeptides disclosed herein.

The terms "polynucleotide", "nucleic acid" ,and "nucleic acid molecule" are intended to refer to any polymer of nucleotides bonded to one another by phosphodiester bonds, for example, ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) molecules of any length. Polynucleotides or nucleic acid can include genes and fragments or portions thereof, probes, oligonucleotides, and primers. DNA can be either complementary DNA (cDNA) or genomic DNA, e.g. a gene encoding an MMP-11 or variant thereof. The terms "nucleic acid" and "polynucleotide" are used interchangeably herein.

The term "recombinant polynucleotide" or "recombinant nucleic acid" refers to a polynucleotide which has been modified by genetic engineering. For example, the term includes a polynucleotide encoding an MMP-11 in which the polynucleotide includes a mutation which renders the MMP-11 catalytically inactive. The term further includes the polynucleotide encoding the MMP-11 or catalytically inactive MMP-11 wherein one or more of the nucleotide codons have been optimized for enhanced expression in humans. The term also includes the MMP-11 fusion polypeptides disclosed herein.

The term "variant thereof" refers to recombinant MMP-11 or polynucleotide. For example, the catalytically inactive MMP-11 or MMP-11 fusion polypeptide is a variant of the wild-type MMP-11. A polynucleotide encoding MMP-11 in which the codons have been optimized for enhanced

expression in humans, the catalytically inactive MMP-11, or the MMP-11 fusion polypeptide is a variant of the wide-type polynucleotide encoding the wild-type MMP-11.

The term "substantially similar" means that a given nucleic acid or amino acid sequence shares at least 75%, preferably 85%, more preferably 90%, and even more preferably 95% identity with a reference sequence. In the present invention, the reference sequence can be relevant portions of the wild-type MMP-11 nucleotide or amino acid sequence, or the wild-type nucleotide or amino acid sequence of an immunoenhancing element, as dictated by the context of the text. The reference sequence may be, for example, the wild-type human or non-human MMP-11 sequence. Thus, an MMP-11 sequence that is "substantially similar" to the wild-type MMP-11 or fragment thereof will share at least 75% identity with the relevant fragment of the wild-type MMP-11, along the length of the fragment, preferably 85% identity, more preferably 90% identity and even more preferably 95% identity. Whether a given MMP-11 or immunoenhancing element polypeptide or nucleotide sequence is "substantially similar" to a reference sequence can be determined for example, by comparing sequence information using sequence analysis software such as the GAP computer program, version 6.0, available from the University of Wisconsin Genetics Computer Group (UWGCG) . The GAP program utilizes the alignment method of Needleman and Wunsch (J. Mol. Biol. 48:443, 1970), as revised by Smith and Waterman (Ada. Appl. Math. 2:482, 1981).

The term "gene" refers both to the genomic nucleic acid encoding the gene product, which for many genes comprises a combination of exon and intron sequences, and the cDNA derived from the mRNA encoding the gene product, which does not include intron sequences.

The term "substantial portion" of a gene or polypeptide, variant, fragment, or subunit thereof, means a portion of at least 50%, preferably 75%, more preferably 90%, and even more preferably 95% of a reference sequence.

The phrases "codon-optimized", "nucleotide codons are optimized for enhanced expression in humans", "nucleotide sequence has been optimized for high expression", and the like for describing the polynucleotides of the present invention mean that one or more of the nucleotide codons of the MMP-11 and/or immunoenhancing element that occur at low frequency in nucleic acids encoding highly expressed proteins in an organism have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in the organism. The nucleotide codon for a particular amino acid with "low frequency" is that nucleotide codon with the lowest frequency of use in nucleic acids that encode highly expressed proteins in the organism. The nucleotide codon for a particular amino acid with "high frequency" is that nucleotide codon with the highest frequency of use or a frequency of use that is higher than the nucleotide codon with the lowest frequency in nucleic acids that encode highly expressed proteins in the organism.

The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Individuals in need of treatment include those already with the disorder as well as those prone to have the disorder or those in which the disorder is to be prevented.

The polynucleotides and polypeptides of the present invention are intended for use as treatments for disorders or conditions associated with overexpression of MMP-11 and which are characterized by aberrant cell proliferation, including, but not limited to, breast cancer, colorectal cancer, and lung cancer.

The term "effective amount" means sufficient vaccine composition is introduced to produce the adequate levels of the polypeptide, so that an immune response results. One skilled in the I art recognizes that this level may vary.

The term "analyte" includes molecule, compound, composition, drug, protein, peptide, nucleic acid, antibody and active fragment thereof, nucleic acid aptamer, peptide aptamer, and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows a map of the vector pV1JnsB-mMMP-11. The human CMV promoter and mouse MMP-11 cDNA are indicated.

Figure 2 shows the expression of mouse MMP-11. HeLa cells were transfected with pV1JnsB-MMP-11 and extracts analyzed by western blot. A band corresponding to the molecular weight of MMP-11 was detected.

Figure 3 shows the nucleotide sequence of codon-optimized nucleic acid encoding catalytically inactive mouse MMP-11 (mMMP-11opt) (SEQ ID NO:13). The nucleotides corresponding to the codon-optimized, catalytically inactive mMMP-11 are in black and the additional nucleotides for the polylinker comprising an *Xba*I site are underlined. Nucleotides for cloning sites, Kozak sequence, and stop codons are in shown in italics. The mMMP-11 start codon is in bold type..

Figure 4 shows the nucleotide sequence of codon-optimized nucleic acid encoding catalytically inactive mMMP-11 linked to *E. coli* LTB (mMMP-11-LTBopt) (SEQ ID NO:14). The nucleotides corresponding to the codon-optimized, catalytically inactive mMMP-11 are in black and the additional nucleotides for the polylinker comprising an *Xba*I site are underlined. The nucleotides encoding the *E. coli* LTB sequence are in lower-case letters. Nucleotides for cloning sites, Kozak sequence, and stop codons are in italics.

Figure 5 shows the amino acid sequence of the catalytically inactive mMMP-11-LTBopt fusion polypeptide (SEQ ID NO:15). The amino acids comprising the polypeptide corresponding to the catalytically inactive mMMP-11 are in black and the LTB sequence are in italics. The amino acids encoded by the polylinker are underlined.

Figure 6A shows a map of the vector pV1J-mMMP-11(cat-)opt comprising a codon-optimized polynucleotide encoding the catalytically inactive mMMP-11.

Figure 6B shows a map of the vector pV1J-mMMP-11(cat-)-LTBopt comprising a codon-optimized polynucleotide encoding the catalytically inactive mMMP-11 linked to the *E. coli* LTB.

Figure 7 shows a Western blot showing expression of pV1JnsA-MMP-11(cat-)-LTBopt in HeLa cells transfected with the pV1JnsA-MMP-11(cat-)-LTBopt using either anti-MMP-11 or anti-LTB antibodies.

Figure 8 shows the cell mediated immune response elicited by mMMP-11, mMMP-11opt and mMMP-11(cat-)-LTBopt. Six BALB/c mice were immunized with four weekly injections of DNA electroporation (DNA-EP). Immune response was measured by intracellular staining for IFNγ using peptides covering the C-term of mMMP-11 protein. Dots represent the % CD8+IFNγ+ for each single mouse. Horizontal bar represents the geometric mean of the group.

Figure 9 shows the humoral response elicited by catalytically inactive mMMP-11 and mMMP-11(cat-)-LTBopt. BALB/c mice were immunized with four weekly injections of DNA electroporation (DNA-EP). Presence of antibodies was measured by western blot. Detection of a 50 KDa band corresponding to mMMP-11 indicates a humoral response against mMMP-11.

Figure 10 shows mMMP-11 is overexpressed in Mouse Colon Adenomas induced by DMH. A/J mice were treated with six IP injections of DMH. Five weeks later, colon tissue is analyzed by IHC and western blotting. Veh means vehicle (PBS).

Figure 11A shows a schematic representation of the experiment performed to demonstrate therapeutic efficacy of the mMMP-11(cat-)-LTBopt genetic vaccine for preventing colon cancer. A/J mice were treated with six IP injections of DMH. A group of mice was left untreated (naive), a second group was vaccinated with the 50 μg of the plasmid pV1J-mMMP-11(cat-)-LTBopt. Seven to eight weeks after the last DMH injection, mice were sacrificed and colon analyzed at microscope for aberrant crypt formation (ACF) (Figures 11B and 11C), polyps (Figure 11D), and adenomas (Figure 11E).

Figure 11B shows the therapeutic efficacy of the mMMP-11(cat-)-LTBopt genetic vaccine in inhibiting ACF. Open dots indicate the number of formations per mouse; filled-in dot indicates the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 11C shows the therapeutic efficacy of the mMMP-11(cat-)-LTBopt genetic vaccine in inhibiting ACF. Open dots indicate the number of formations per mouse; filled-in dot indicates the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 11D shows the therapeutic efficacy of the mMMP-11(cat-)-LTBopt genetic vaccine in inhibiting polyps. Open dots indicate the number of formations per mouse; filled-in dot indicates the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 11E shows the therapeutic efficacy of the mMMP-11(cat-)-LTBopt genetic vaccine in inhibiting adenomas. Open dots indicate the number of formations per mouse; filled-in dot indicates the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 12A shows the immune response elicited by anti-mMMP-11 genetic vaccine. BALB/c mice were treated with six IP injections of DMH. A group of mice was left untreated (naive) and a second group was vaccinated with the 50 μg of the plasmid pV1J-mMMP-11(cat-)-LTBopt. The

immune response was measured by intracellular staining for IFNγ. Black dots represent the % CD8+IFNγ+ for each single mouse. Horizontal bar represents the geometric mean of the group.

Figure 12B shows the immune response elicited by anti-mMMP-11 genetic vaccine. BALB/c mice were treated with six IP injections of DMH. A group of mice was left untreated (naive) and a second group was vaccinated with the 50 μg of the plasmid pV1J-mMMP-11(cat-)-LTBopt. The immune response was measured using a CTL assay wherein effectors cells were stimulated for seven days with mMMP-11 peptides. p815 mastocytoma cells unloaded or loaded with mMMP-11 peptides were used as target.

Figure 13A shows the therapeutic efficacy of mMMP-11(cat-)-LTBopt genetic vaccine in BALB/c mice in inhibiting ACF. Seven to eight weeks after the last DMH injection, mice were sacrificed and colon analyzed at microscope for ACF. Open dots indicate number of formations per mouse; filled-in dots indicate the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 13B shows the therapeutic efficacy of mMMP-11(cat-)-LTBopt genetic vaccine in BALB/c mice in inhibiting ACF. Seven to eight weeks after the last DMH injection, mice were sacrificed and colon analyzed at microscope for ACF. Open dots indicate number of formations per mouse; filled-in dots indicate the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 13C shows the therapeutic efficacy of mMMP-11(cat-)-LTBopt genetic vaccine in BALB/c mice in inhibiting polyps. Seven to eight weeks after the last DMH injection, mice were sacrificed and colon analyzed at microscope for polyps. Open dots indicate number of formations per mouse; filled-in dots indicate the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 13D shows the therapeutic efficacy of mMMP-11(cat-)-LTBopt genetic vaccine in BALB/c mice in inhibiting adenomas. Seven to eight weeks after the last DMH injection, mice were sacrificed and colon analyzed at microscope for adenomas. Open dots indicate number of formations per mouse; filled-in dots indicate the geometric mean of the group. Statistic analysis (T student's test) is indicated.

Figure 14 shows the nucleotide sequence of a nucleic acid encoding catalytically inactive human MMP-11 (hMMP-11(cat-)opt) wherein the codons have been optimized for expression in humans (SEQ ID NO:4).

Figure 15 shows the amino acid sequence of catalytically inactive hMMP-11 (SEQ ID NO:5).

Figure 16 shows the nucleotide sequence of a nucleic acid encoding a fusion polypeptide comprising a catalytically-inactive hMMP-11 linked to the *E. coli* LTB wherein the codons encoding both the MMP-11 and the LTB have been optimized for expression in humans (SEQ ID NO:12) (hMMP-11(cat-)-LTBopt). The codons encoding amino acids 1 to 21 of the LTB are not included in the fusion

polypeptide. The nucleotides corresponding to the codon-optimized, catalytically inactive hMMP-11 are in black and the additional nucleotides for the polylinker comprising an *Xba*I site are underlined. The nucleotides encoding the *E. coli* LTB sequence are in lower-case letters.

Figure 17 shows the amino acid sequence for the catalytically-inactive hMMP-11-LTB fusion polypeptide (SEQ ID NO:10). The amino acids comprising the catalytically-inactive MMP-11 are in upper-case letters, the amino acids comprising the LTB are in italics, and the amino acids encoded by the polylinker are underlined. The start codon is in bold-faced type.

Figure 18 shows a map of the vector pV1J-hMMP-11(cat-)opt comprising a codon-optimized polynucleotide encoding the catalytically inactive hMMP-11.

Figure 19A shows that DMH does not interfere with the CD8+ immune response of BALB/c mice.

Figure 19B shows that DMH does not interfere with the CD8+ immune response of A/J mice.

Figure 19C shows that DMH does not interfere with the CD4+ immune response of A/J mice.

Figure 20 shows a map of the vector pV1J-hMMP-11(cat-)-LTBopt (*See* SEQ ID NO:18) comprising a codon-optimized polynucleotide encoding the catalytically inactive hMMP-11 linked to the *E. coli* LTB.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides compositions that can be used as anti-MMP-11 vaccines for inhibiting tumors and cancers that overexpress MMP-11 in an individual, in particular, for inhibiting invasive carcinomas that overexpress MMP-11 such as particular carcinomas of the breast, colon, head and neck, lung, ovary, pancreas, prostate, skin (basal cell carcinoma), uterus (cervix carcinoma and endometrial carcinoma) or non-invasive carcinomas that have a risk of evolving towards invasion. The anti-MMP-11 vaccine or anti-tumor associated antigen (anti-TAA) vaccine can be used, for example, in a mono-therapy regimen that targets tumor cells and stromal compartment; in a multi-therapy regimen with another anti-TAA vaccine, which targets tumor cells via a multi-specific cell mediated immune response and stromal compartment; in a multi-therapy regimen with other molecules or adjuvants; in a therapy that includes chemotherapy, the rationale being to get the stromal structure more permeable to cytotoxic agents; in a therapy that includes radiotherapy; and, in any one of the previous therapies wherein the MMP-11 is provided as part of a multi-epitope polypeptide or minigene. The anti-MMP-11 vaccines of the present invention can be a polypeptide vaccine, or preferably, a polynucleotide vaccine.

As shown in the Examples, it was found that administering an anti-MMP-11 vaccine to mice having colonic tumors, which had been induced with 1,2-dimethylhydrazine (DMH), caused a significant reduction of the DMH-induced carcinogenesis progression in the colon tissue of the mice. In susceptible mouse strains, such as A/J, but also to a lesser extent BALB/c, DMH induced carcinogenesis

progression in colon tissue goes through different stages: (1) aberrant crypt formation (ACF); (2) Adenoma; (3) Polyp; and (4) Adenocarcinoma (*See* Bird, Cancer Lett. 93(1): 55-71 (1995)). The inventors found that MMP-11 is overexpressed in the DMH-induced tumor tissue, which suggested the suitability of DMH-induced carcinogenesis in mice as a model for anti-MMP-11 therapy and vaccination. The inventors then found that a genetic vaccine comprising a nucleic acid encoding the mouse MMP-11 (mMMP-11), wherein the mMMP-11 had been inactivated catalytically by introducing a point mutation in the Zn binding domain in the catalytic site, induced an immune response in mice and that the immune response was further enhanced when the nucleic acid encoding the catalytically inactive mMMP-11 was linked to a nucleic acid encoding the subunit B heat-labile toxin (LTB) of *E. coli* without the signal peptide and the codons encoding the MMP-11 was optimized for enhanced expression in the mouse and the codons encoding the LTB were optimized for enhanced expression in humans. Finally, the inventors found that the genetic vaccine was efficacious in effecting a reduction in all phases of DMH-induced carcinogenesis in the mouse. Therefore, in light of the results of the mouse model, the present invention provides anti-MMP-11 vaccines comprising either a polynucleotide encoding the MMP-11 or the MMP-11 polypeptide, preferably an anti-MMP-11 vaccine having any one of the embodiments as set forth below.

In its most basic embodiment, the present invention provides a nucleic acid or polynucleotide, which comprises a nucleic acid or polynucleotide molecule encoding an MMP-11 or variant due to degeneracy of the genetic code, genetic engineering as disclosed below, or both, under the control of or operably linked to a suitable heterologous promoter and preferably wherein the nucleic acid encoding the MMP-11 has been modified to include a mutation that renders the encoded MMP-11 catalytically inactive. The mutation that renders the MMP-11 catalytically inactive can be introduced into the polynucleotide by genetic engineering. The recombinant polynucleotides encoding the catalytically inactive MMP-11 include polynucleotides derived from humans and non-human species wherein the polynucleotide is modified to include a mutation that renders the encoded MMP-11 catalytically inactive. Non-human species include primates, for example chimpanzee, rhesus monkey, cynomolgus monkey, and the like, and non-primate species, for example mouse, rat, dog, and the like. In a currently preferred embodiment, the recombinant polynucleotide encoding the MMP-11 is of human origin or encodes an MMP-11 having an amino acid sequence the same as or substantially similar to the amino acid sequence of human MMP-11. The nucleotide sequence of the cDNA encoding the human MMP-11 (hMMP-11) is set forth in GenBank Accession No. NM_005940 (SEQ ID NO:1) and encodes the MMP-11 having the amino acid sequence set forth in SEQ ID NO:2. The recombinant polynucleotide encoding the hMMP-11 is modified as shown below to encode variants which can be used in the anti-MMP-11 vaccines of the present invention. It is to be understood that while the currently preferred embodiments of the present invention comprise recombinant nucleic acids or polynucleotides encoding the hMMP-11, the present invention is not limited to recombinant polynucleotides encoding the hMMP-11. The present invention further include embodiments wherein the recombinant polynucleotides

encode MMP-11 of non-human origin and the recombinant nucleic acids or polynucleotides have been modified as shown below to encode MMP-11 variants that can be used in the anti-MMP-11 vaccines of the present invention.

In a preferred embodiment of the recombinant nucleic acid or polynucleotide, the MMP-11 encoded by the polynucleotide is a catalytically inactive variant of the MMP-11. A polynucleotide encoding a catalytically inactive MMP-11 can be produced by modifying by genetic engineering one or more of the nucleotide codons encoding the conserved amino acids comprising the zinc binding site H E X X H X X G X X H (SEQ ID NO:3) of the MMP-11 (for the hMMP-11, amino acids 215 to 225 of SEQ ID NO:2) to an alternative amino acid to produce a recombinant polynucleotide encoding a catalytically inactive MMP-11. For example, as shown by the nucleotide sequence in SEQ ID NO:4 encoding a catalytically inactive hMMP-11, the nucleotide codon GAA encoding the conserved glutamic acid at amino acid position 216 of the hMMP-11 was changed to the nucleotide codon GTG encoding the amino acid valine to produce a catalytically inactive hMMP-11 having the amino acid sequence shown in SEQ ID NO:5 wherein the amino acid at position 216 is valine. Noël *et al.*, Oncogene 19: 1605-1612 (2000)) have shown that changing the nucleotide codon at position 216 to a nucleotide codon encoding alanine rendered the hMMP-11 catalytically inactive and that changing the nucleotide codon encoding the glutamic acid at amino acid position 220 of the corresponding region of the mMMP-11 with a nucleotide codon that encodes alanine rendered the mMMP-11 catalytically inactive. While the nucleotide codon encoding the glutamic acid at amino acid position number two of SEQ ID NO:3 has been changed to a nucleotide codon encoding valine or alanine to produce a catalytically inactive MMP-11, the nucleotide codon can also be changed to other amino acids or the codons encoding one or more of the other conserved amino acids of the Zn binding domain can be changed to encode other amino acids without departing from the invention.

It has been shown that codon optimization of genes or transcription units coding for particular polypeptides leads to increased expression of the encoded polypeptide, that is increased translation of the mRNA encoding the polypeptide. In the case of a polynucleotide vaccine, the increased expression of the encoded polypeptide produces more of the encoded polypeptide which can lead to increased immunogenecity of the vaccine *in vivo*, which in turn, can enhance the efficacy of the vaccine. In the context of codon optimization, the term "expression" and its variants refer to translation of the mRNA encoding the polypeptide and not to transcription of the polynucleotide encoding the polypeptide. The term "gene" as used herein refers to both the genomic DNA or RNA encoding a polypeptide and to the cDNA encoding the polypeptide.

Codon optimization is a process that seeks to improve heterologous expression of a gene when that gene is moved into a foreign genetic environment that exhibits a different nucleotide codon usage from the gene's native genetic environment or improve ectopic expression of a gene in its native genetic environment when the gene naturally includes one or more nucleotide codons that are not usually used in genes native to the genetic environment that encode highly expressed genes. In other words,

codon optimization involves replacing those nucleotide codons of a gene that are used at a relatively low frequency in a particular genetic environment or organism with nucleotide codons that are used in genes that are expressed at a higher frequency in the genetic environment or organism. In that way, the expression (translation) of the gene product (polypeptide) is increased. The assumption is that the nucleotide codons that appear with high frequency in highly expressed genes are more efficiently translated than nucleotide codons that appear at low frequency.

In general, methods for optimizing nucleotide codons for a particular gene depend on identifying the frequency of the nucleotide codons for each of the amino acids used in genes that are highly expressed in an organism and then replacing those nucleotide codons in a gene of interest that are used with low frequency in the highly expressed genes with nucleotide codons that are identified as being used in the highly expressed genes (*See* for example Lathe, *Synthetic Oligonucleotide Probes Deduced from Amino Acid Sequence Data: Theoretical and Practical Considerations*, J. Molec. Biol.: 183: 1-12 (1985); Nakamura *et al.*, Nuc. Acid Res. 28: 292 (2000); Fuglsang, Protein Expression & Purification 31: 247-249 (2003). There are numerous computer programs that will automatically analyze the nucleotide codons of a nucleic acid of an organism encoding a gene and suggest nucleotide codons to replace nucleotide codons, which occur with low frequency in the organism, with nucleotide codons that are found in genes that are highly expressed in the organism. For convenience, a table of nucleotide codon usage for humans derived from Nakamura (*ibid.*) is shown below in Table 1 and identifies which nucleotide codons occur at low frequency in nucleic acids encoding highly expressed proteins in humans and which nucleotide codons occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

Table 1

Codon	Amino Acid	Frequency	Codon	Amino Acid	Frequency
UUU	F	17.4	UCU	S	15.1
UUC	F	20.4	UCC	S	17.7
UUA	L	7.5	UCA	S	12.1
UUG	L	12.8	UCG	S	4.5
CUU	L	13.1	CCU	P	17.5
CUC	L	19.7	CCC	P	20.0
CUA	L	7.1	CCA	P	16.9
CUG	L	39.9	CCG	P	7.0
AUU	I	15.8	ACU	T	13.0
AUC	I	20.9	ACC	T	19.0
AUA	I	7.4	ACA	T	15.0
AUG	M	22.0	ACG	T	5.1
GUU	V	11.0	GCU	A	18.5
GUC	V	14.6	GCC	A	28.1
GUA	V	7.1	GCA	A	15.9
GUC	V	28.4	GCG	A	7.5
UAU	Y	12.1	UGU	C	10.4
UAC	Y	15.3	UGC	C	12.6
UAA	*	1.0	UGA	*	1.6
UAG	*	0.8	UGG	W	13.2
CAU	H	10.8	CGU	R	4.6
CAC	H	15.1	CGC	R	10.6
CAA	Q	12.1	CGA	R	6.2
CAG	Q	34.2	CGG	R	11.6
AAU	N	16.7	AGU	S	12.1
AAC	N	19.1	AGC	S	19.4
AAA	K	24.1	AGA	R	11.9
AAG	K	32.0	AGG	R	11.9
GAU	D	21.7	GGU	G	10.8
GAC	D	25.2	GGC	G	22.5
GAA	E	28.6	GGA	G	16.4
GAG	E	39.7	GGG	G	16.5

*Stop codon.

Therefore, in further embodiments, a recombinant polynucleotide is provided wherein one or more of the nucleotide codons encoding the amino acids comprising the MMP-11 are optimized to enhance the expression of the encoded MMP-11 and thereby in the case of the anti-MMP-11 vaccine, enhance the efficacy of the anti-MMP-11 vaccine. That is, one or more of the nucleotide codons encoding the MMP-11 and/or immunoenhancing element that occur at low frequency in nucleic acids encoding highly expressed proteins in an organism have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in the organism. Preferably, the nucleotide codons are optimized for enhanced expression of the MMP-11 in humans. However, recombinant polynucleotides codon-optimized for enhanced expression in another organism, for example, primates, are equivalents of recombinant polynucleotides codon-optimized for enhanced expression in humans. Currently, it is preferable that the MMP-11 be catalytically inactive and it is currently preferred that the catalytically inactive MMP-11 be the hMMP-11. Where there are multiple nucleotide codons for a particular amino acid of MMP-11 and two or more of the nucleotide codons have the same relative frequency of use in highly expressed human genes or a frequency of use greater in highly expressed human genes than the nucleotide codon having the lowest frequency of use in highly expressed human genes, each of the nucleotide codons for the amino acid in the MMP-11 can independently be any one of the nucleotide codons of the same frequency of use or frequency of use greater than the nucleotide codon having the lowest frequency of use. Not all nucleotide codons in the codon optimized polynucleotide encoding the MMP-11 need be the nucleotide codon having the highest frequency of use in highly expressed human genes. An example of a nucleotide sequence of hMMP-11 that is catalytically inactive and in which the codons encoding the catalytically inactive hMMP-11 have been optimized for expression in humans is shown in SEQ ID NO:6.

The mouse model showed that the efficacy of an anti-MMP-11 vaccine comprising a recombinant polynucleotide encoding a catalytically inactive mMMP-11 in which the nucleotide codons encoding the mMMP-11 were optimized for enhanced expression in the mouse (mMMP-11-opt) was further enhanced when the nucleotide codon encoding the carboxy terminal amino acid of the mMMP-11-opt was linked or fused to nucleotide codons encoding a substantial portion of the immunoenhancing heat-labile toxin B (LTB) of *E. coli* to produce a fusion polypeptide comprising the mMMP-11 and the *E. coli* LTB. Therefore, in a preferred embodiment, the recombinant polynucleotide comprises a nucleic acid encoding a the catalytically inactive MMP-11 linked to a nucleic acid encoding an immunoenhancing element polypeptide or substantial portion thereof (MMP-11 fusion polypeptide). In currently preferred embodiments, the recombinant polynucleotide comprises a nucleic acid encoding the catalytically inactive hMMP-11 linked to a nucleic acid encoding an immunoenhancing element which is the LTB of *E. coli* such that the polynucleotide encodes an hMMP-11-LTB fusion protein. In further embodiments, the nucleic acid encoding the LTB does not include the codons encoding the LTB signal peptide. The nucleic acid sequence encoding the *E. coli* LTB is available in GenBank Accession No. AB011677 and the amino acid sequence for the *E. coli* LTB is shown in GenBank Accession No.

BAA25726. The signal peptide includes amino acid residues 1 to 21 of the amino acid sequence shown in BAA25726. The nucleotide sequence of the *E. coli* LTB without the signal peptide is shown in SEQ ID NO:7 and its amino acid sequence is shown in SEQ ID NO:9. The polynucleotide sequence encoding the *E. coli* LTB without the signal peptide and in which the nucleotide codons encoding the LTB have been optimized for enhanced expression in humans sis shown in SEQ ID NO:8. In a particularly preferred embodiment, the nucleotide codons of the polynucleotide encoding the catalytically inactive hMMP-11 and the LTB are optimized for expression in humans.

While the *E. coli* LTB was the source for the immunoenhancing element polypeptide that was used in the fusion polypeptide embodiments disclosed herein, the present invention further contemplates embodiments comprising recombinant polynucleotides encoding fusion polypeptides comprising MMP-11 fused to other immunoenhancing element polypeptides or substantial portions thereof. Examples of immunoenhancing element polypeptides include, but are not limited to, heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and LTB from other bacterial species.

Therefore, in light of the above, the present invention further provides a recombinant nucleic acid or polynucleotide which encodes a single fusion polypeptide comprising a catalytically inactive MMP-11 linked to an LTB or substantial portion thereof or another immunoenhancing element polypeptide or substantial portion thereof. An example of such a polynucleotide comprises a polynucleotide encoding a polypeptide comprising the amino acid of SEQ ID NO:5 (catalytically inactive hMMP-11) and the amino acid sequence of SEQ ID NO:8 (*E. coli* LTB without signal peptide). The polynucleotide can comprise the nucleotide sequence of SEQ ID NO:4 encoding the catalytically inactive hMMP-11 and SEQ ID NO:7 encoding the *E. coli* LTB without signal peptide or SEQ ID NO:8 (polynucleotide encoding the *E. coli* LTB without signal peptide codon-optimized for enhanced expression in humans). As an example, the polynucleotide can encode a catalytically inactive hMMP-11-LTB fusion polypeptide having the amino acid sequence shown in SEQ ID NO:10. Such a polypeptide can be encoded by the nucleotide sequence shown in SEQ ID NO:11.

In a preferred embodiment, the nucleotide codons of any one of the recombinant nucleic acids and polynucleotides disclosed herein are optimized for enhanced expression of the recombinant polypeptide encoded thereon in humans. That is, one or more of the nucleotide codons of the recombinant nucleic acids and polynucleotides that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans. It is further preferable in the case of recombinant polynucleotides encoding any one of the fusion polypeptides disclosed herein that the nucleotide codons of the recombinant polynucleotides encoding the immunoenhancing element polypeptide or LTB comprising the recombinant polypeptide are also optimized for enhanced expression

of the fusion polypeptide in humans. An example of such a recombinant polynucleotide would comprise the codon-optimized nucleotide sequence of SEQ ID NO:6 encoding the catalytically inactive hMMP-11 and the codon-optimized nucleotide sequence of SEQ ID NO:8 encoding the *E. coli* LTB without signal peptide. As an example, the recombinant polynucleotide encoding the codon-optimized, catalytically inactive hMMP-11-LTB fusion polypeptide has the nucleotide sequence shown in SEQ ID NO:12.

The present invention further provides recombinant polypeptides comprising a catalytically inactive MMP-11 wherein the one or more of the conserved amino acids comprising the zinc binding site H E X X H X X G X X H (SEQ ID NO:3) of MMP-11 are changed to an alternative amino acid. For example, as shown in SEQ ID NO:5 for hMMP-11 wherein the conserved glutamic acid at position 216 of the hMMP-11 was changed to the amino acid valine to produce a catalytically inactive MMP-11. Preferably, the catalytically inactive MMP-11 polypeptide comprises a fusion polypeptide wherein the MMP-11 is linked at its carboxy terminus to an immunoenhancing element polypeptide or substantial portion of (MMP-11 fusion polypeptide). In currently preferred embodiments, the immunoenhancing element polypeptide is the *E. coli* LTB. In further embodiments, the LTB does not include its signal peptide. An example of an MMP-11 fusion polypeptide comprises the catalytically inactive hMMP-11 having the amino acid sequence shown in SEQ ID NO:5 linked at its carboxy terminus to the amino terminus of the *E. coli* LTB polypeptide without signal peptide having the amino acid sequence shown in SEQ ID NO:7. The MMP-11 fusion polypeptide can be encoded by, for example, the polynucleotide shown in SEQ ID NO:11 or the codon-optimized polynucleotide shown in SEQ ID NO:12.

While the *E. coli* LTB is the source for the immunoenhancing element that was used in the MMP-11 fusion polypeptide embodiments exemplified herein, the present invention further contemplates embodiments comprising MMP-11 fusion polypeptides comprising MMP-11 fused to other immunoenhancing element polypeptides or substantial portions thereof. Examples of immunoenhancing element polypeptides include, but are not limited to, heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and LTB from other bacterial species.

The present invention further provides vectors which comprise at least one of the nucleic acid molecules disclosed throughout this specification (herein after "recombinant polynucleotides"), preferably wherein the nucleic acid molecule is operably linked to a heterologous promoter. These vectors can comprise DNA or RNA. For most purposes, DNA plasmid or viral expression vectors are preferred. Typical expression vectors include plasmids, modified viruses, bacteriophage, cosmids, yeast artificial chromosomes, and other forms of episomal or integrated DNA, any of which expresses any one of the recombinant polynucleotides disclosed herein. Preferably, the nucleotide codons encoding the any one of the aforementioned embodiments is optimized for enhanced expression in humans.

An expression vector comprising a polynucleotide encoding any one of the recombinant polynucleotides disclosed herein wherein the DNA is preferably codon-optimized for enhanced expression in humans and operably linked to a heterologous promoter can be used for expression of the any one of the recombinant polynucleotides disclosed herein in a recombinant host cell. Such recombinant host cells can be cultured under suitable conditions to produce any one of the recombinant polynucleotides disclosed herein. Expression vectors include, but are not limited to, cloning vectors, modified cloning vectors, specifically designed plasmids, or specifically designed viruses. The expression vectors described in the examples are acceptable expression vectors.

The nucleic acids of the present invention are preferably assembled into an expression cassette that comprises sequences which provide for efficient expression of any one of the recombinant polynucleotides disclosed herein encoded thereon in a human cell. The cassette preferably contains homologous or heterologous transcriptional and translational control sequences operably linked to the nucleic acid. Such control sequences include at least a transcription promoter (constitutive or inducible) and transcription termination sequences and can further include other regulatory elements such as transcription enhancers, ribosome binding sequences, splice junction sequences, and the like. In most embodiments, the promoter is a heterologous promoter; however, in particular embodiments, the promoter can the native promoter for the MMP-11. In a particularly useful embodiment, the promoter is the constitutive cytomegalovirus immediate early promoter with or without the intron A sequence (CMV) although those skilled in the art will recognize that any of a number of other known promoters such as the strong immunoglobulin promoter, Rous sarcoma virus long terminal repeat promoter, SV40 small or large T antigen promoter, or the like. Transcriptional terminators include the bovine growth hormone terminator although other known transcriptional terminators such as SV40 termination sequences can also be used. The plasmids pV1JnsB and pV1JnsA, each of which contain the cytomegalovirus (CMV) immediate/early region promoter and enhancer with intron A followed by a cloning site and the BGH polyadenylation signal, are examples of a useful an expression vector. The MMP-11 in any one of the aforementioned embodiments can be cloned into the cloning site to complete the expression cassette.

Commercially available mammalian expression vectors which are suitable for expression of any one of the recombinant polynucleotides disclosed herein include, but are not limited to, pV1JnsA, pV1JnsB, pVAX1 (Invitrogen, Carlsbad, AC), pcDNA3.neo (Invitrogen), pcDNA3.1 (Invitrogen), pcDNA3.1/Myc-His (Invitrogen), pCI-neo (Promega, Madison, WI), pLITMUS28, pLITMUS29, pLITMUS38 and pLITMUS39 (New England Bioloabs, Beverly, MA), pcDNAI, pcDNAIamp (Invitrogen), pcDNA3 (Invitrogen), pMC1neo (Stratagene, La Jolla, CA), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593) pBPV-1(8-2) (ATCC 37110), pdBPV-MMTneo (342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), and lZD35 (ATCC 37565).

Also, a variety of bacterial expression vectors can be used to express any one of the recombinant polynucleotides disclosed herein in bacterial cells. Commercially available bacterial

expression vectors which may be suitable for expression include, but are not limited to, pCR2.1 (Invitrogen), pET11a (Novagen, Madison, WI), lambda gt11 (Invitrogen), and pKK223-3 (Pharmacia).

In addition, a variety of fungal cell expression vectors may be used to express any one of the recombinant polynucleotides disclosed herein in fungal cells. Commercially available fungal cell expression vectors that are suitable for expression include, but are not limited to, pYES2 (Invitrogen) and Pichia expression vector (Invitrogen).

Also, a variety of insect cell expression vectors can be used to express any one of the recombinant polynucleotides disclosed herein in insect cells. Commercially available insect cell expression vectors which can be suitable for expression include, but are not limited to, pBlueBacIII and pBlueBacHis2 (Invitrogen), and pAcG2T (Pharmingen).

Viral vectors that can be used for expression of any one of the recombinant polynucleotides disclosed herein in mammalian cells include, but are not limited to, adenovirus vectors, adeno-associated virus vectors, herpesvirus vectors, Sindbis virus vectors, Simliki forest virus vectors, parvovirus vectors, pox virus vectors (such as vaccinia virus, fowl pox, canary pox, and the like), retrovirus vectors, bacteriophage vectors, and baculovirus vectors. Many of the viral vectors for making recombinant viruses encoding any one of the recombinant polynucleotides herein are commercially available.

In currently preferred embodiments, the viral vector used for making recombinant viruses is an adenoviral or plasmid vector, although linear DNA linked to a promoter, or other vectors, such as adeno-associated virus or a modified vaccinia virus, retroviral or lentiviral vector may also be used. If the vector chosen is an adenovirus, it is currently preferred that the vector be a so-called first-generation adenoviral vector. These adenoviral vectors are characterized by having a non functional E1 gene region, and preferably a deleted adenoviral E1 gene region. In some embodiments, the expression cassette is inserted in the position where the adenoviral E1 gene is normally located. In addition, these vectors optionally have a non-functional or deleted E3 region. It is also preferred that the adenovirus genome used be deleted of both the E1 and E3 regions (AE1AE3).

The adenovirus vectors can be multiplied in known cell lines which express the viral E1 gene, such as 293 cells, or PERC.6 cells, or in cell lines derived from 293 or PERC.6 cell which are transiently or stably transformed to express an extra protein. For examples, when using constructs that have a controlled gene expression, such as a tetracycline regulatable promoter system, the cell line may express components involved in the regulatory system. One example of such a cell line is TRex-293, others are known in the art.

For convenience in manipulating the adenoviral vector, the adenovirus may be in a shuttle plasmid form. This invention is also directed to a shuttle plasmid vector, which comprises a plasmid portion and an adenovirus portion, the adenovirus portion comprising an adenoviral genome which has a deleted E1 and optional E3 deletion, and has an inserted expression cassette comprising a nucleic acid encoding a catalytically inactive MMP-11 polypeptide or catalytically inactive MMP-11

fusion polypeptide. In preferred embodiments, there is a restriction site flanking the adenoviral portion of the plasmid so that the adenoviral vector can easily be removed. The shuffle plasmid may be replicated in prokaryotic cells or eukaryotic cells.

In a currently preferred embodiment of the invention, an expression cassette comprising a nucleic acid encoding any one of the recombinant MMP-11 disclosed herein is inserted into the pMRKAdS-HV0 adenovirus plasmid (*See* Emini *et al.*, WO0222080). This plasmid comprises an Ads adenoviral genome deleted of the E1 and E3 regions. The design of the pMRKAd5-HV0 plasmid was improved over prior adenovectors by extending the 5' *cis*-acting packaging region further into the E1 gene to incorporate elements found to be important in optimizing viral packaging, resulting in enhanced virus amplification. Advantageously, this enhanced adenoviral vector is capable of maintaining genetic stability following high passage propagation.

The present invention further provides recombinant host cells transformed or transfected with a vector comprising any one of the recombinant polynucleotides disclosed herein, particularly host cells transformed or transfected with a vector comprising any one of the aforementioned nucleic acid molecules wherein the nucleic acid molecule is operably linked to a promoter. Recombinant host cells include bacteria such as *E. coli*, fungal cells such as yeast, plant cells, mammalian cells including, but not limited to, cell lines of bovine, porcine, monkey, human, or rodent origin; and insect cells including, but not limited to, Drosophila and silkworm-derived cell lines. For instance, one insect expression system utilizes Spodoptera frugiperda (Sf21) insect cells (Invitrogen) in tandem with a baculovirus expression vector (pAcG2T, Pharmingen, San Diego, CA). Also, mammalian species which may be suitable and which are commercially available, include but are not limited to, L cells L-M(TK-) (ATCC CCL-1.3), L cells L-M (ATCC CCL-1.2), Saos-2 cells (ATCC HTB-85), 293 cells (ATCC CRL-1573), Raji cells (ATCC CCL-86), CV-1 cells (ATCC CCL-70), COS-1 cells (ATCC CRL-1650), COS-7 cells (ATCC CRL-1651), CHO-K1 cells (ATCC CCL-61), 3T3 cells (ATCC CCL-92), NIH/3T3 cells (ATCC CRL-1658), HeLa cells (ATCC CCL-2), C127I cells (ATCC CRL-1616), BS-C-1 cells (ATCC CCL-26), MRC-5 cells (ATCC CCL-171), HEK293T cells (ATCC CRL-1573), ST2 cells (Riken Cell bank, Tokyo, Japan RCB0224), C3H10T1/2 cells (JCRB0602, JCRB9080, JCRB0003, or IFO50415), and CPAE cells (ATCC CCL-209).

As noted above, an expression vector containing any one of the recombinant polynucleotides disclosed herein can be used to express the recombinant MMP-11 encoded therein in a recombinant host cell. Therefore, the present invention provides a process for expressing any one of the recombinant polynucleotides disclosed herein in a recombinant host cell comprising introducing the vector comprising a nucleic acid which encodes the recombinant MMP-11 into a suitable host cell and culturing the host cell under conditions which allow expression any one of the recombinant polynucleotides disclosed herein. The polynucleotide encoding the recombinant MMP-11 is operably linked to a heterologous promoter which can be constitutive or inducible.

Following expression of any one of the recombinant nucleic acids or polynucleotides disclosed herein in a host cell, the recombinant MMP-11 polypeptide can be recovered for use in a polypeptide-based vaccine. Methods for purifying polypeptides are well known in the art and include purification from cell lysates and extracts by various combinations of, or individual application of salt fractionation, ion exchange chromatography, size exclusion chromatography, hydroxylapatite adsorption chromatography, or hydrophobic interaction chromatography. In addition, recombinant MMP-11 can be separated from other cellular polypeptides by use of an immunoaffinity column made with monoclonal or polyclonal antibodies specific for MMP-11 or the immunoenhancing element polypeptide in the case of the MMP-11 fusion polypeptide.

Cloning, expression vectors, transfections and transformations, and protein isolation of expressed proteins are well known in the art and have been described, for example, in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual 2nd Edition; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, (1989) or Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd Edition. Cold Spring Harbor Laboratory Press, Plainview, NY (2001).

The anti-MMP-11 vaccines of the present invention include both polynucleotide vaccines encoding any one of the embodiments of recombinant MMP-11 or MMP-11 fusion polypeptides disclosed herein and polypeptide vaccines comprising any one of the embodiments of recombinant MMP-11 or MMP-11 fusion polypeptides disclosed herein. Individuals suffering from invasive carcinomas that overexpress MMP-11 such as those of the breast, colon, head and neck, lung, ovary, pancreas, prostate, skin (basal cell carcinoma), uterus (cervix carcinoma and endometrial carcinoma) or non-invasive carcinomas that have a risk of evolving towards invasion can benefit from immunization by the vaccines of the present invention. The anti-MMP-11 vaccines further include adenovirus anti-MMP-11 vaccines which comprise a recombinant adenovirus having any one of the recombinant polynucleotides disclosed herein.

In its most basic embodiment, the nucleic acid or polynucleotide anti-MMP-11 vaccine comprises any one of the recombinant polynucleotides disclosed herein, for example, a recombinant nucleic acid molecule or polynucleotide encoding anyone of the aforementioned embodiments of the recombinant MMP-11 polypeptides or MMP-11 fusion polypeptides under the control of or operably linked to a suitable heterologous promoter. The encoded recombinant MMP-11 polypeptide can have the amino acid sequence of the MMP-11 polypeptide of any species, including, but not limited to, the MMP-11 from humans; primates such as chimpanzees, Rhesus monkey, Cynomolgus monkey; non-primates such as mouse, rat, dog, and the like. Preferably, the encoded recombinant MMP-11 has the amino acid sequence of the human MMP-11. The following illustrates the currently preferred embodiments of the nucleic acids encoding the MMP-11 for inclusion in the aforementioned gene cassettes and expression vectors for use as a polynucleotide anti-MMP-11 vaccine.

In a preferred embodiment, the MMP-11 encoded by the polynucleotide of the anti-MMP-11 vaccine is catalytically inactive. For example, the catalytically inactive hMMP-11 is shown by

the nucleotide sequence in SEQ ID NO:4 wherein the nucleotide codon GAA encoding the conserved glutamic acid at position 216 of the hMMP-11 had been changed to the nucleotide codon GTG encoding the amino acid valine to produce a catalytically inactive hMMP-11 having the amino acid sequence shown in SEQ ID NO:5. While the nucleotide codon encoding the glutamic acid residue was changed to a nucleotide codon encoding valine, the nucleotide codon can also be changed to other amino acids or the histidine residues changed to other amino acids without departing from the invention.

As discussed previously, it has been shown that codon optimization of genes or transcription units coding for particular polypeptides leads to increased expression of the encoded polypeptide, that is increased translation of the mRNA encoding the polypeptide. In the case of a polynucleotide vaccine, the increased expression of the encoded polypeptide produces more of the encoded polypeptide which can lead to increased immunogenecity of the vaccine *in vivo*, which in turn, can enhance the efficacy of the vaccine. Therefore, in further embodiments, the nucleotide codons encoding the amino acids comprising the MMP-11 are optimized to enhance expression of the MMP-11 and thus, the efficacy of the anti-MMP-11 vaccine. That is, one or more of the nucleotide codons encoding the MMP-11 that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans. The nucleotide sequence of hMMP-11 that is catalytically inactive and in which the codons encoding the catalytically inactive hMMP-11 have undergone codon optimization is shown in SEQ ID NO:6.

The mouse model of the examples show that the efficacy of an anti-MMP-11 vaccine comprising a polynucleotide encoding a catalytically inactive, codon-optimized mouse MMP-11 was enhanced when the carboxy terminal codon of the codons encoding the mMMP-11-opt was linked to codons encoding the immunoenhancing element polypeptide: the *E. coli* LTB. Therefore, in a preferred embodiment of the anti-MMP-11 vaccine, the polynucleotide comprises a nucleic acid encoding the catalytically inactive MMP-11 linked to a polynucleotide encoding an immunoenhancing element polypeptide or substantial portion (MMP-11 fusion polypeptide). In currently preferred embodiments, the polynucleotide comprises a nucleic acid encoding the catalytically inactive MMP-11 linked to a polynucleotide encoding the *E. coli* LTB or substantial portion of such that the polynucleotide encodes an MMP-11-LTB fusion protein. In further embodiments, the polynucleotide encoding the LTB does not include the codons encoding the LTB signal peptide. Currently, it is preferable that the MMP-11 be the hMMP-11. The nucleotide sequence of the *E. coli* LTB without the signal peptide is shown in SEQ ID NO:7 and its amino acid sequence is shown in SEQ ID NO:9. The polynucleotide sequence encoding the *E. coli* LTB without the signal peptide in which the nucleotide codons have been optimized for enhanced expression in humans is shown in SEQ ID NO:8. In a particularly preferred embodiment, the nucleotide codons comprising the polynucleotide encoding the catalytically inactive hMMP-11 and the LTB are optimized for enhanced expression in humans.

While the *E. coli* LTB was the source of the immunoenhancing element polypeptide that was used in the fusion polypeptide embodiments of the anti-MMP-11 vaccines disclosed herein, the present invention further contemplates embodiments comprising polynucleotides encoding fusion polypeptides comprising MMP-11 fused to other immunoenhancing element polypeptides or substantial portions thereof. Examples of immunoenhancing element polypeptides include, but are not limited to, heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and LTB from other bacterial species.

Therefore, in light of the above, the present invention provides a nucleic acid or polynucleotide anti-MMP-11 vaccine which encodes a single fusion polypeptide comprising a catalytically inactive MMP-11 linked to an immunoenhancing element or substantial portion thereof, for example, the *E. coli* LTB. An example of such a vaccine comprises a polynucleotide encoding a polypeptide comprising the amino acid of SEQ ID NO:5 (catalytically inactive hMMP-11) and the amino acid of SEQ ID NO:8 (LTB) or the nucleotide sequence of SEQ ID NO:4 (catalytically inactive hMMP-11) and SEQ ID NO:7 (encoding LTB without signal peptide) or SEQ ID NO:8 (wherein nucleotide codons encoding the LTB without signal peptide have been optimized for expression in humans), respectively. As an example, the vaccine comprises a polynucleotide that encodes a catalytically inactive hMMP-11-LTB fusion polypeptide having the amino acid sequence shown in SEQ ID NO:10. Such a polypeptide can be encoded by the nucleotide sequence shown in SEQ ID NO:11.

In a preferred embodiment of the polynucleotide anti-MMP-11 vaccine, the nucleotide codons of the polynucleotide encoding the catalytically inactive MMP-11 are optimized for enhanced expression in humans as described above. It is further preferable that the nucleotide codons of the polynucleotide encoding the immunoenhancing element are also enhanced for enhanced expression in humans. An example of such a polynucleotide would comprise the codon-optimized nucleotide sequence of SEQ ID NO:6 encoding the catalytically inactive hMMP-11 and the codon-optimized nucleotide sequence of SEQ ID NO:8 encoding the LTB. As an example, the polynucleotide encoding the codon-optimized, catalytically inactive hMMP-11-LTB fusion polypeptide has the nucleotide sequence shown in SEQ ID NO:12.

The polynucleotide anti-MMP-11 vaccines can be administered by a variety of delivery mechanisms such as direct injection, electroporation, mucosal delivery, and the like. In some preferred embodiments, the vaccine is administered intramuscularly, intranasally, intraperitoneally, subcutaneously, intradermally, bombardment by gene gun, topically, or orally. For example, the vaccine can be administered intramuscularly into the deltoid muscle and can be administered using a 0.5 mL syringe followed by an electrical stimulus within two minutes of the injection. The electrical stimulus can be provided using the MEDPULSER DNA delivery system (Inovio Biomedical Corporation, San Diego, CA). Preferably, the polynucleotide anti-MMP-11 vaccines comprise any one of the above

polynucleotides in a pharmaceutically acceptable carriers and excipients such as water, saline, dextrose, glycerol, ethanol, and the like, and combinations thereof. In a currently preferred embodiment, the vaccine is formulated in a saline solution. In some cases it is anticipated that the polynucleotide vaccines can comprise the expression vector within a bacterium such as an attenuated strains of *Shigella flexneri*, *Salmonella* spp., *Yersinia enterocolitica*, or *Listeria monocytogenes*. The polynucleotide anti-MMP-11 vaccines can also contain auxiliary substances such as wetting agents, emulsifying agents, buffers, and the like.

The polynucleotide anti-MMP-11 vaccine can include one or more genetic adjuvants (nucleic acids encoding one or more molecular adjuvants) capable of modulating the immune response towards either a Th1 or Th2 response. Such genetic adjuvants include, but are not limited to, costimulatory molecules such as CD80 and CD86; proinflammatory cytokines such as interleukin-1α (IL-1α); tumor necrosis factor-α and β (TNF-α and TNF-β); Th1 cytokines such as IL-2, IL-12, IL-15, and IL-18; Th2 cytokines such as IL-4, IL-5, and IL-10; macrophage colony-stimulating factor (M-CSF); αgranulocyte colony-stimulating factor (G-CSF); granulocytes-monocyte colony-stimulating factor (GM-CSF); IL-8; interferon-γ-inducible protein-10 (γIP-10); macrophage inhibitory protein-1α (MIP-1α); and RANTES. Sasaki *et al.*, Methods 31: 243-254 (2003), provides a good discussion on adjuvant formulations and delivery systems for DNA vaccines (*See* also Kim *et al.*, J. Interferon Cytokine Res. 20: 487-498 (2000) and Kim *et al.*, Human Gene Therapy 11: 305-321 (2000)). The genetic adjuvants can be provided in an expression cassette on an expression vector separate from the expression vector encoding the hMMP-11 in any one of the aforementioned embodiments or on the same expression vector encoding the hMMP-11 in any one of the aforementioned embodiments.

The polynucleotide anti-MMP-11 vaccine can include one or more conventional adjuvants. Conventional adjuvants include, but are not limited to, mineral salts such as aluminum phosphate or hydroxide, bacteria-derived adjuvants such as monophosphoryl lipid A, cholera toxin, muramyl peptides, lipid particles such as cationic liposomes and mannan-coated liposomes, emulsifier adjuvants such as QS-21, and synthetic adjuvants such as ubenimex. Additional adjuvants and excipients can be found in "A Compendium of Vaccine Adjuvants and Excipients (2nd Edition)" by Vogel *et al.*, Vaccine and Prevention Research Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892.

The polynucleotide anti-MMP-11 vaccines of the present invention are preferably administered as a solution or suspension in a pharmaceutically acceptable carrier, at a DNA concentration in the range of about 10 μg/mL to about 5 mg/mL. In general, an immunologically or prophylactically effective dose of about 5 mg, and preferably about 0.05, of a plasmid vaccine vector is administered directly into muscle tissue. The appropriate dosage will depend upon the individual to be vaccinated, and can depend upon the capacity of the individual's ability to express the nucleic acids encoding the hMMP-11 contained in the vaccine and the individual's immune system to react to the

expressed hMMP-11. T he exact dosage chosen may also depend, in part, upon the judgment of the medical practitioner administering or requesting administration of the vaccine.

The anti-MMP-11 vaccines further include recombinant adenoviruses comprising any one of the aforementioned recombinant polynucleotides. Adenovirus vaccine is currently preferably administered intramuscularly in deltoid diluted in a diluent such as phosphate buffered saline in a final volume of less than about 1 mL. A effective dose of the recombinant adenovirus is generally about 10^6 to 10^{12} viral particles, preferably, about 10^7 to 10^{11} viral particles.

In some embodiments of this invention, the adenovirus and polynucleotide anti-MMP-11 vaccines disclosed herein are used in various prime/boost combinations in order to induce an enhanced immune response. In this case, the two vectors are administered in a "prime and boost" regimen. For example the first type of vector is administered one or more times, then after a predetermined amount of time, for example, 2 weeks, 1 month, 2 months, six months, or other appropriate interval, a second type of vector is administered one or more times. Preferably the vectors carry expression cassettes encoding the same polynucleotide or combination of polynucleotides. In the embodiment where a plasmid vector is also used, it is preferred that the vector contain one or more promoters recognized by mammalian or insect cells. In a preferred embodiment, the plasmid vector would contain a strong promoter such as, but not limited to, the CMV promoter.

As stated above, an adenoviral vector anti-MMP-11 vaccine and a polynucleotide anti-MMP-11 vaccine may be administered to a vertebrate as part of a single therapeutic regime to induce an immune response. In one embodiment, the first vector is a plasmid and the second vector is an adenovirus vector. In an alternative embodiment, the first vector is an adenovirus vector and the second vector is a plasmid. In the method described above, the first type of vector may be administered more than once, with each administration of the vector separated by a predetermined amount of time. Such a series of administration of the first type of vector may be followed by administration of a second type of vector one or more times, after a predetermined amount of time has passed. Similar to treatment with the first type of vector, the second type of vector may also be given one time or more than once, following predetermined intervals of time.

Another embodiment of the present invention is a kit comprising the adenovirus vector or polynucleotide anti-MMP-11 vaccines of the present invention packaged in suitably sterilized containers such as ampules, bottles, vials, and the like, either in multi-dose or in unit-dosage forms. The containers are preferably hermetically sealed after being filled with a vaccine preparation. Preferably, the polynucleotide anti-MMP-11 vaccines are packaged in a container having a label affixed thereto, which label identifies the vaccine, and bears a notice in a form prescribed by a government regulatory agency such as the United States Food and Drug Administration reflecting approval of the vaccine under appropriate laws, dosage information, and the like. The label preferably contains information about the vaccine that is useful to a health care professional administering the vaccine to a patient. The kit also

preferably contains printed informational materials relating to the administration of the vaccine, instructions, indications, and any necessary required warnings.

In its most basic embodiment, the anti-MMP-11 polypeptide vaccines of the present invention comprises a catalytically inactive MMP-11 wherein the one or more of the conserved amino acids comprising the zinc binding site H E X X H X X G X X H (SEQ ID NO:3) of the MMP-11 are changed to an alternative amino acid. For example, as shown in SEQ ID NO:5 wherein the conserved glutamic acid at position 216 of the hMMP-11 was changed to the amino acid valine to produce a catalytically inactive MMP-11. Preferably, the catalytically inactive MMP-11 is an MMP-11 fusion polypeptide wherein the MMP-11 is fused or linked at its carboxy terminus to a an immunoenhancing element polypeptide or substantial portion thereof. In currently preferred embodiments, the immunoenhancing element polypeptide is the LTB polypeptide, preferably an LTB in which the signal peptide had been removed, for example, the catalytically inactive hMMP-11 shown in SEQ ID NO:5 linked to the LTB polypeptide comprising the amino acid sequence shown in SEQ ID NO:7.

The polypeptide anti-MMP-11 vaccines can be administered by a variety of delivery mechanisms such as direct injection, mucosal delivery, oral delivery, and the like. In some preferred embodiments, the vaccine is administered intramuscularly, intranasally, intraperitoneally, subcutaneously, intradermally, topically, or orally. Preferably, the polynucleotide anti-MMP-11 vaccines are formulated with pharmaceutically acceptable carriers and excipients such as water, saline, dextrose, glycerol, ethanol, and the like, and combinations thereof. The polynucleotide anti-MMP-11 vaccines can also contain auxiliary substances such as wetting agents, emulsifying agents, buffers, and the like. The polypeptide anti-MMP-11 vaccine can include one or more molecular adjuvants capable of modulating the immune response towards either a Th1 or Th2 response. Such molecular adjuvants include, but are not limited to, costimulatory molecules such as CD80 and CD86, proinflammatory cytokines such as interleukin-1α (IL-1α), tumor necrosis factor-α and β (TNF-α and TNF-β), Th1 cytokines such as IL-2, IL-12, IL-15, and IL-18), Th2 cytokines such as IL-4, IL-5, and IL-10, macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (G-CSF), granulocytes-monocyte colony-stimulating factor (GM-CSF), IL-8, interferon-γ-inducible protein-10 (γIP-10), macrophage inhibitory protein-1α (MIP-1α), and RANTES. The polypeptide anti-MMP-11 vaccine can include one or more conventional adjuvants. Conventional adjuvants include, but are not limited to, mineral salts such as aluminum phosphate or hydroxide, bacteria-derived adjuvants such as monophosphoryl lipid A, cholera toxin, muramyl peptides, lipid particles such as cationic liposomes and mannan-coated liposomes, emulsifier adjuvants such as QS-21, and synthetic adjuvants such as ubenimex. Additional adjuvants and excipients can be found in aforementioned "A Compendium of Vaccine Adjuvants and Excipients (2nd Edition)" by Vogel *et al.*

The present invention further provides a method for identifying an analyte for inhibiting a cancer that overexpresses MMP-11, which comprises inducing the cancer in a mouse; administering the analyte to the mouse with the induced cancer; and determining whether the analyte inhibits the cancer in

the mouse with the induced tumor, which identifies the analyte for inhibiting a cancer that overexpresses the MMP-11. In particular embodiments, the analyte is determined to bind the MMP-11 before it is administered to the mouse. In further still embodiments, the cancer that is induced in the mouse is a colon cancer, and in further still embodiments, the cancer is induced in the mouse by administering to the mouse 1-2dimethylhydrazine (DMH) in an amount sufficient to induce the cancer in the mouse.

The following examples are provided to further illustrate the features and embodiments of the present invention, and are not meant to be limiting.

EXAMPLE 1

To construct a vector expressing the mouse MMP-11, cDNA was cloned from mouse fibroblast cells, which were part of stromal compartment. Total RNA was extracted from NIH-3T3 cells and oligonucleotides specific for the mouse MMP-11 were used to amplify the cDNA using Polymerase chain reaction (PCR). The PCR primers used were forward 5'-MMP-11, having the nucleotide sequence 5'-CCCGGGGCGG ATGGCACGGG CCGCCTGTC-3' (SEQ ID NO:16) and the degenerated oligonucleotide reverse 3'-MMP-11-1473 having the nucleotide sequence 5'-GTCAGMGGAA AGTRTTGGCA GGCTCAGCAC AG-3' (SEQ ID NO:17) wherein M is A or C and R is A or G . The RT-PCR reaction was performed as follows: 45°C for 30 minutes; 94°C for 2 minutes, and then 40 cycles at 94°C for 15 seconds, 58°C for 30 seconds, and 68°C for 2 minutes.

An amplification product of about 1630 bp was obtained and cloned into the TA cloning vector pCR2.1 (Invitrogen, Carlsbad, CA) to produce plasmid pCR2.1-MMP-11. DNA sequence analysis the cloned amplification product showed that the DNA sequence of the cloned amplification product was a complete match with the nucleotide sequence for the mouse MMP-11 cDNA (Accession number: NM_008606). The cDNA encoding the mouse MMP-11 was removed from the pCR2.1-MMP-11 by digesting with *Eco*RI and cloning into the *Eco*RI site of plasmid vector pV1JnsB to produce expression vector pV1JnsB-MMP-11 (Figure 1). As shown in Figure 1, the cDNA encoding MMP-11 is downstream of a human CMV promoter. The PV1j vectors have been described by Montgomery *et al.* in DNA Cell Biol. 12: 777-783 (1993).

To verify the expression of MMP-11, HeLa cells were transfected with pV1JnsB-MMP-11. Cell extracts were analyzed by western blot using an antibody for human MMP-11 cross reacting with mouse MMP-11. As shown in Figure 2, a band of about 50KDa was detected, indicating that MMP-11 was expressed by the vector.

EXAMPLE 2

It has been shown that codon optimization of genes coding for various types of antigens can lead to increased expression and enhanced immunogenicity in vivo. Therefore, to increase expression and enhance immunogenicity of the mMMP-11, the mMMP-11 coding sequence was codon optimized.

The mMMP-11 cDNA sequence was converted to a polynucleotide sequence encoding the same amino acid sequence but with codon usage optimized for expression in mouse cells (For a general discussion on codon optimization, *see* Lathe, J. Molec. Biol.: 183: 1-12 (1985)). The methodology generally consisted of identifying codons in the wild-type mMMP-11 polynucleotide sequence that are not commonly associated with highly expressed genes in mice and replacing them with codons commonly associated with highly expressed genes in mice to produce a polynucleotide having only codons commonly associated with highly expressed genes for high expression of the polynucleotide in cells of mouse origin. The new gene sequence was then inspected for undesired sequences generated by these codon replacements (e.g., "ATTTA" sequences, inadvertent creation of intron splice recognition sites, unwanted restriction enzyme sites, high GC content, etc.). Undesirable sequences were eliminated by substitution of the codons comprising the undesirable sequences with other codons, preferably, if practical, with another codon associated with highly expressed genes that codes for the same amino acid. The synthetic gene segments are then tested for improved expression. The codon optimized gene for mouse expression was designed using the Vector NTI program algorithm (InforMax, Rockville, MD). To increase the level of transcription, an optimized Kozak sequence was inserted 5' to the ATG start codon. Moreover, two consecutive stop codons were inserted downstream of the coding sequence to enhance translation termination.

The codon-optimized cDNA encoding mMMP-11 was synthesized by oligonucleotide assembly performed at GENEART GmbH, Germany and then cloned into the *BglII/SalI* site of the pV1JnsA vector, thus generating pV1JnsA-mMMP-11opt. In order to abrogate enzymatic activity of the MMP-11 while not modifying its immunogenic properties, a point mutation was introduced in the catalytic site, which changed the glutamic acid (E) in position 220 to an alanine (A) (Noel *et al.*, Oncogene. 19: 1605-12 (2000)). This produced vector pV1JnsA-mMMP-11(cat-)opt. The nucleotide sequence of the codon-optimized, catalytically inactive variant of mMMP-11 (mMMP-11(cat-)opt) is shown in Figure 3 (SEQ ID NO:13) and the map for the vector pV1JnsA-mMMP-11(cat-)opt is shown in Figure 6A.

WO2005077977 showed that a genetic fusion of the carcinoembryonic antigen (CEA) to immunoenhancing elements such as the heat labile toxin B (LTB) of *E. Coli* further increased the efficacy of vaccination against CEA. Therefore, to enhance the efficacy of the MMP-11, the codon-optimized polynucleotide encoding the catalytically inactive mMMP-11 fused to the *E. coli* LTB with the signal sequence removed was synthesized and cloned into the *BglII/SalI* site of the pV1JnsA vector, thus generating pV1JnsA-mMMP-11(cat-)-LTBopt (Figure 6B). The catalytically inactive mMMP-11-LTB fusion was synthesized by oligonucleotide assembly performed at GENEART GmbH, Germany. The codons encoding the LTB were optimized for expression in cells of human origin. Figure 4 shows the codon-optimized nucleotide sequence encoding the catalytically inactive mMMP-11-LTB fusion polypeptide and Figure 5 shows the amino acid sequence of the catalytically inactive mMMP-11-LTB fusion polypeptide, respectively.

To test the expression of pV1JnsA-mMMP-11(cat-)-LTBopt, HeLa cells were transfected with pV1jnsA-mMMP-11(cat-)-LTBopt by Lipofectamine2000 (Invitrogen). Whole Cell extracts were prepared using Lysis buffer (2%SDS, 5mM EGTA, 5mM EDTA, 20mM Tris-HCl, pH7.4) and analyzed by Western blot for expression of the catalytically inactive mMMP-11-LTB fusion protein. The catalytically inactive mMMP-11-LTB fusion protein was detected using anti-MMP-11 and anti-LTB antibodies following standard Western blot protocols. Anti-MMP-11 and -LTB antibodies were from BIOMOL (Exter, UK and Plymouth Meeting, PA, Anti-MMP-11 cat. # SA-371) and Abcam (Cambridge, UK and MA, Anti-*E. Coli* heat labile toxin, cat# ab9199). As shown in Figure 7, both antibodies bound to a band of about 60 KDa, which corresponded to the molecular weight of the catalytically inactive mMMP-11-LTB fusion protein.

EXAMPLE 3

To test the immunogenic potential of mMMP-11(cat-)opt and mMMP-11(cat-)-LTBopt as compared to the wild-type mMMP-11, BALB/c mice were each immunized intramuscularly with four DNA injections of 50 μg of plasmid DNA in saline followed by electroporation (EP) 1 week apart, according to Zucchelli *et al.* (*Enhancing B and T cell Immune response to an HCV E2 DNA vaccine by muscle electro gene transfer.* J. Virol. 74: 11598-11607, (2000)). Two weeks after last injection, mice were sacrificed and the immune response against mMMP-11 peptides was measured by intracellular staining for interferon gamma (IFNγ). As shown in Figure 8, both wild type and catalytically inactive mMMP-11opt were efficient to break tolerance in mice (%CD8+IFNγ+>0.1%). There did not appear to be any significant difference between mMMP-11 and catalytically inactive mMMP-11opt. However, as also shown in Figure 8, fusion of catalytically inactive mMMP-11opt to LTB significantly increased the immune response ($p<0.05$).

The humoral response was measured in a Western blot. To measure the humoral immune response, whole cell extracts from HeLa cells transfected with pV1JnsB-MMP-11 were separated on a polyacrylamide gel and transferred onto nitrocellulose membranes. Sera from the above immunized mice were incubated with the membranes. Afterwards, detection of mouse antibodies against mMMP-11 was by using an anti-mouse IgG conjugated with alkaline phosphatases (Sigma Chemicals, St. Louis, MO). Detection of a band corresponding to the molecular weight of mMMP-11 indicates the presence of antibodies against mMMP-11. As shown in Figure 9, no apparent significant difference was observed in the humoral response between mice immunized with mMMP-11 and mice immunized with catalytically inactive mMMP-11(cat-)-LTBopt.

Based on the results shown in Figure 8, mMMP-11(cat-)-LTBopt was selected as the best immunogen to be used for the vaccination studies.

EXAMPLE 4

A tumor model overexpressing MMP-11 was produced as follows. 1,2-dimethylhydrazine (DMH) or its metabolite azoxymethane are often used as the initiating carcinogen in tumor-induction studies. DMH has been found to induce colonic tumors in numerous species of animals (Choudhary and H. Hansen, Chemosphere 37: 801–843 (1998)), even after a single oral exposure in some cases, but typically 6 to 10 weekly treatments are used. DMH is an alkylating agent and treatment with this chemical has been shown to induce methyl adducts to DNA bases, point mutations, micronuclei, and sister chromatid exchanges. Treatment with DMH induces apoptosis in the colon (Blakey *et al.*, Cancer Res. 45: 242–249 (1985)) as well as an increase in cellular proliferation of colonic epithelial cells (Ma *et al.*, World J. Gastroenterol. 8: 847–852 (2002)), which is a characteristic of human colon cancer.

In susceptible mouse strains, such as A/J, but also to a lesser extent BALB/c, DMH induced carcinogenesis progression in colon tissue goes through different stages: (1) aberrant crypt formation (ACF); (2) Adenoma; (3) Polyp; and (4) Adenocarcinoma. In order to verify the expression of mMMP-11 in this process of tumorigenesis, A/J mice received six weekly injections of DMH and were sacrificed five weeks after last DMH injection: at this stage both aberrant crypts and some adenomas were present in mouse colon tissue. Gut tissue was frozen and analyzed by western blot and immunohistochemistry (IHC) using an antibody against mMMP-11. In untreated mice (vehicle), IHC analysis showed expression of mMMP-11 at the basis of normal crypt: it appears that expression of mMMP-11 was limited to colonic stem cells. Strong and diffused expression was detected in aberrant crypt and adenoma formations (Figure 10). This observation was confirmed by western blot analysis of colon tissue extracts from mice treated with DMH or left untreated: the activated form of mMMP-11 was present in DMH treated colon (Figure 10), thus indicating overexpression of the proteinase by the tumorous tissue. These data indicate the suitability of DMH induced carcinogenesis as model for anti-MMP-11 therapy and vaccination.

EXAMPLE 5

This example shows the therapeutic efficacy of anti-MMP-11 vaccine. As shown in the previous example, MMP-11 is overexpressed in aberrant crypt formations (ACF) and adenomas induced by the administration of 1-2dimethylhydrazine (DMH) in A/J mice. Other studies have demonstrated that DMH does not interfere with the immune system and efficacy of genetic vaccination. The following experiment was performed to determine whether DMH would interfere with the functionality of the immune system and efficacy of genetic vaccination.

Groups of 10 BALB/c or A/J mice were treated with six intraperitoneal (IP) injections of DMH starting from the fifth week of age or left untreated (Mock). At weeks eight and 11, all of the mice received injections of 50 μg of the plasmid pV1J-CEAopt (*See* WO2005077977 for pV1J-CEAopt). Two weeks later, the mice were bled and their immune responses to the CEA encoded by pV1J-CEAopt analyzed by intracellular staining upon stimulation with 15^{mer} peptides covering CEA protein. For the

BALB/c mice, the CD8+ immune response was measured. Figure 19A shows that there was no significant difference in CD8+ response between DMH-treated and mock-treated BALB/c mice. For the A/J mice, the CD8+ and CD4+ immune responses were measured. Figures 19B and 19C show that there was no significant difference in CD8+ and CD4+ responses between DMH-treated and mock-treated A/J mice. These results demonstrated that DMH did not appear to influence the immune system activity. Taken together, these data suggested that MMP-11 is a tumor associated antigen and that a vaccine against MMP-11 would be feasible means for treating cancers that overexpress MMP-11.

To test efficacy of an anti-MMP-11 vaccine, groups of 60 A/J mice were treated with DMH with six weekly injections: one group is left untreated (Naïve), a second group is immunized with pV1jnsA-mMMP-11(cat-)-LTBopt followed by electroporation, as indicated in the scheme shown in Figure 11A. Two weeks after the last immunization, cell-mediated immunity (CMI) against mMMP-11 was analyzed with pool of 15mer peptides encompassing the entire protein; however, poor immune-response was detected in the analyzed groups (data not shown). Seven to eight weeks after the last injection of DMH, 20 mice per group were sacrificed and the colon was analyzed for the presence of ACF, Adenomas, polyps, and adenocarcinomas. Vaccinated mice show significant reduction of DMH-induced presence of ACF, Adenomas, polyps, and adenocarcinomas (Figures 11B to 11E).

EXAMPLE 6

To confirm the efficacy of tumor protection in DMH carcinogenesis model in another mouse strain, the same treatment and immunization scheme as above was followed using BALB/c mice. Two weeks after the last immunization, CMI against mMMP-11 was analyzed with a pool of 15mer peptides encompassing the entire protein.

For preparation of splenocytes from immunized mice, spleens were removed from sacrificed mice in a sterile manner and disrupted by scratching through a grid. Erythrocyte lysis was obtained by incubation for 10 minutes with ACK lysing buffer (Life Technologies, Bethesda, MD). After centrifugation at 1200 rpm for 10 minutes, white cells were resuspended in R10 medium. About 1 to 2 x 10^6 splenocytes or PBMC (peripheral blood mononuclear cells) were resuspended in 1 mL R10 medium. Antigen peptides were added to a final concentration of 1 µg/mL with Brefeldin A. The mMMP-11 antigen peptides comprised a pool of 15mer peptides, which together encompassed the entire MMP-11 peptide. The total number of peptides was 121 and was divided in 4 pools (A, from 1 to 30; B, from 31 to 60; C, from 61 to 90; D, from 91 to 121).

After 12 hours incubation at 37°C, the cells were washed with 3 mL FACS buffer (PBS supplemented with either 1% FBS and containing 0.05% NaN_3) and centrifuged for 10 minutes at room temperature. The cells were incubated with anti-mouse CD16/CD32 in 100 µL FACS buffer for 15 minutes at 4°C. Then, after washing the cells with FACS buffer, the cells were analyzed for the secretion of IFNγ upon incubation with 15mer mMMP-11 antigen peptides.

For surface antigen staining, allophycocyanin (APC) conjugated anti-mouse CD3□ phycoerythrin (PE) conjugated anti-mouse CD4, and peridininchlorophyll protein (PerCP) conjugated anti-mouse CD8α, all diluted 1:50 in FACS buffer, were added to the cells in 100 µL final volume and the cells incubated for 30 minutes at room temperature in the dark. After washing with PERMWASH (Pharmingen), cells were resuspended in 100 µL of CYTOFIX-CYTOPERM solution (Pharmingen), vortexed, and incubated for 20 minutes at 4°C in the dark.

For intracellular staining, the cells were incubated with fluorescein (FITC) conjugated anti-mouse interferon-γ diluted 1:50 in PermWash (100 µL final volume) for 30 minutes at room temperature in the dark. After washing, the cells were resuspended in 250 to 300 µL 1% formaldehyde in PBS and analyzed with a FACS CALIBER (Becton Dickinson, San Jose, CA).

A significant immune-response was detected in the immunized groups mainly directed against C-term of the protein and was CD8+ specific (Figure 12A). Elicited CD8+ effectors were functional since they were able to lyse tumorous target cells loaded with mMMP-11 antigen peptides (Figure 12B). Most importantly, highly significant protection in vaccinated mice was observed at all stages from ACF to adenomas (Figures 13A to 13D). These data indicate that MMP-11 is an optimal target for active specific immunotherapy and genetic vaccination is extremely efficient in tumor protection.

EXAMPLE 7

Cloning and optimization of nucleotide sequence encoding catalytically inactive human MMP-11.

Similar to the mouse MMP-11, the human MMP-11 was codon optimized according to human cell most frequent codon usage and rendered catalytically inactive by changing the codon for the glutamic acid (E) in the catalytic site in position 220 with an alanine (A). The polynucleotide comprising the codon-optimized, catalytically inactive hMMP-11 was synthesized by oligonucleotide assembly (GENEART, GmbH) and cloned into the *BglII*/*Eco*RI site of the vector pV1JnsA, generating pV1JnsA-hMMP-11(cat-)opt (Figure 18). The nucleotide sequence of the codon-optimized polynucleotide encoding the catalytically inactive hMMP-11 is shown in Figure 14. The amino acid sequence of the catalytically inactive hMMP-11 is shown in Figure 15. Vector pV1JnsA- hMMP-11(cat-)opt was designed for use in humans and may be used in preclinical models such as mice transgenic for human MHC class I, such as HLA-A2.1 to identify immunogenic epitopes.

To improve efficacy of the anti-MMP-11 vaccine comprising the hMMP-11, the codon-optimized polynucleotide encoding the catalytically inactive hMMP-11 fused to the *E. coli* LTB with the signal sequence removed is synthesized and cloned into the *BglII*/*SalI* site of the pV1JnsA vector, thus generating pV1JnsA-MMP-11(cat-)-LTBopt (Figure 20). The nucleotide sequence of pV1JnsA-MMP-11(cat-)-LTBopt is shown in SEQ ID NO:18. The nucleotide sequence starts at the second nucleotide of the *Xba*I polylinker separating the nucleotide codons encoding the catalytically inactive hMMP-11 from

the nucleotide codons encoding the LTB. In the nucleotide sequence of SEQ ID NO:18, the CMV promoter includes nucleotides 3647 to 4261, intron A includes nucleotides 4396 to 5221, catalytically inactive hMMP-11 includes nucleotides 5253 to 6715, the *Xba*I polylinker includes nucleotides 6715 to 5, the LTB includes nucleotides 6 to 315, and the BGH polyA includes nucleotides 382 to 599. The polynucleotide encoding catalytically inactive human MMP-11-LTB fusion can be synthesized by oligonucleotide assembly, which can be performed at GENEART GmbH, Germany.

While the present invention is described herein with reference to illustrated embodiments, it should be understood that the invention is not limited hereto. Those having ordinary skill in the art and access to the teachings herein will recognize additional modifications and embodiments within the scope thereof. Therefore, the present invention is limited only by the claims attached herein.

WHAT IS CLAIMED:

1. A nucleic acid comprising a nucleotide sequence encoding a matrix metalloproteinase 11 (MMP-11) wherein one or more of the nucleotide codons encoding the MMP-11 that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

2. The nucleic acid of Claim 1 wherein the MMP-11 encoded by the nucleotide sequence further includes a mutation that renders the MMP-11 catalytically inactive.

3. The nucleic acid of Claim 2 wherein the mutation is in the zinc binding domain of the MMP-11.

4. The nucleic acid of Claim 1 wherein the MMP-11 is a human MMP-11.

5. The nucleic acid of Claim 1 wherein the MMP-11 is an MMP-11 of primate origin.

6. The nucleic acid of Claim 2 wherein the nucleotide sequence comprises the nucleotide sequence of SEQ ID NO:4.

7. A nucleic acid encoding a fusion polypeptide having a matrix metalloproteinase 11 (MMP-11) linked to an immunoenhancing element polypeptide or substantial portion thereof.

8. The nucleic acid of Claim 7 wherein the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from Vibrio cholerae, and subunit B of the heat-labile toxin (LTB).

9. The nucleic acid of Claim 7 wherein the immunoenhancing element is the subunit B of heat labile toxin (LTB) of *E. coli*.

10. The nucleic acid of Claim 7 wherein the LTB does not include a signal sequence.

11. The nucleic acid of Claim 10 wherein the LTB is encoded by the nucleotide sequence shown in SEQ ID NO:8.

12. The nucleic acid of Claim 7 wherein the MMP-11 includes a mutation that renders it catalytically inactive.

13. The nucleic acid of Claim 7 wherein one or more of the nucleotide codons encoding the fusion polypeptide that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

14. The nucleic acid of Claim 7 wherein the MMP-11 is encoded by the nucleotide sequence shown in SEQ ID NO:4

15. The nucleic acid of Claim 7 wherein the fusion polypeptide includes the nucleotide sequence shown in SEQ ID NO:11.

16. An expression vector comprising the nucleic acid of Claim 1 operably linked to a promoter.

17. A host cell containing the expression vector of Claim 16 therein.

18. A process, comprising culturing the host cell of Claim 17 in a cell culture medium under conditions for producing the fusion polypeptide.

19. An expression vector comprising the nucleic acid of Claim 7 operably linked to a promoter.

20. A host cell containing the expression vector of Claim 19 therein.

21. A process, comprising culturing the host cell of Claim 20 in a cell culture medium under conditions for producing the fusion polypeptide.

22. A fusion polypeptide comprising a matrix metalloproteinase 11 (MMP-11) linked to an immunoenhancing element or substantial portion thereof.

23. The fusion polypeptide of Claim 22 wherein the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from Vibrio cholerae, and subunit B of the heat-labile toxin (LTB).

24. The fusion polypeptide of Claim 22 wherein the immunoenhancing element is the subunit B of heat labile toxin (LTB) of *E. coli*.

25. The fusion polypeptide of Claim 24 wherein the LTB does not include a signal sequence.

26. The fusion polypeptide of Claim 25 wherein the LTB includes the amino acid sequence shown in SEQ ID NO:9.

27. The fusion polypeptide of Claim 22 wherein the MMP-11 includes a mutation that renders it catalytically inactive.

28. The fusion polypeptide of Claim 27 wherein the mutation is in the zinc binding domain of the MMP-11.

29. The fusion polypeptide of Claim 22 wherein the MMP-11 includes the amino acid sequence shown in SEQ ID NO:5.

30. The fusion polypeptide of Claim 22 wherein the polypeptide includes the amino acid sequence shown in SEQ ID NO:10.

31. A polynucleotide vaccine comprising a nucleotide sequence encoding a matrix metalloproteinase 11 (MMP-11) wherein one or more of the nucleotide codons encoding the MMP-11 that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

32. The polynucleotide vaccine of Claim 32 wherein the MMP-11 encoded by the nucleotide sequence further includes a mutation that renders the MMP-11 catalytically inactive.

33. The polynucleotide vaccine of Claim 33 wherein the mutation is in the zinc binding domain of the MMP-11.

34. The polynucleotide vaccine of Claim 32 wherein the MMP-11 is a human MMP-11.

35. The polynucleotide vaccine of Claim 32 wherein the MMP-11 is an MMP-11 of primate origin.

36. The polynucleotide vaccine of Claim 33 wherein the nucleotide sequence includes the nucleotide sequence of SEQ ID NO:4.

37. A polynucleotide vaccine encoding a fusion polypeptide having a matrix metalloproteinase 11 (MMP-11) linked to an immunoenhancing element or substantial portion thereof.

38. The polynucleotide vaccine of Claim 38 wherein the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from Vibrio cholerae, and subunit B of the heat-labile toxin (LTB).

39. The polynucleotide vaccine of Claim 38 wherein the immunoenhancing element is the subunit B of heat labile toxin (LTB) of *E. coli*.

40. The polynucleotide vaccine of Claim 38 wherein the LTB does not include a signal sequence.

41. The polynucleotide vaccine of Claim 41 wherein the LTB is encoded by the nucleotide sequence shown in SEQ ID NO:8.

42. The polynucleotide vaccine of Claim 38 wherein the MMP-11 includes a mutation that renders it catalytically inactive.

43. The polynucleotide vaccine of Claim 38 wherein one or more of the nucleotide codons encoding the fusion polypeptide that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

44. The polynucleotide vaccine of Claim 38 wherein the MMP-11 is encoded by the nucleotide sequence shown in SEQ ID NO:4.

45. The polynucleotide vaccine of Claim 38 wherein the fusion polypeptide includes the nucleotide sequence shown in SEQ ID NO:11.

46. The polynucleotide vaccine of Claim 38 further including a genetic adjuvant.

47. A polypeptide vaccine comprising fusion polypeptide having a matrix metalloproteinase 11 (MMP-11) linked to an immunoenhancing element or substantial portion thereof.

48. The polypeptide vaccine of Claim 50 wherein the immunoenhancing element is selected from the group consisting of heat shock protein (HSP) 70, lysosome-associated membrane protein (LAMP), fragment C of tetanus toxoid (FrC), the N-terminal domain of FrC (DOM), the heavy fragment of constant chain of immune globulin Gl (FcIgG), the vesicular stomatitis virus glycoprotein (VSV-G), cholera toxin (CT) from *Vibrio cholerae*, and subunit B of the heat-labile toxin (LTB).

49. The polypeptide vaccine of Claim 50 wherein the immunoenhancing element is the subunit B of heat labile toxin (LTB) of *E. coli*.

50. The polypeptide vaccine of Claim 52 wherein the LTB does not include a signal sequence.

51. The polypeptide of vaccine Claim 53 wherein the LTB includes the amino acid sequence shown in SEQ ID NO:9.

52. The polypeptide of vaccine Claim 50 wherein the MMP-11 includes a mutation that renders it catalytically inactive.

53. The polypeptide of vaccine Claim 55 wherein the mutation is in the zinc binding domain of the MMP-11.

54. The polypeptide of vaccine Claim 50 wherein the MMP-11 includes the amino acid sequence shown in SEQ ID NO:5

55. The polypeptide of vaccine Claim 50 wherein the fusion polypeptide includes the amino acid sequence shown in SEQ ID NO:10.

56. Use of a nucleic acid a nucleotide sequence encoding a matrix metalloproteinase 11 (MMP-11) wherein one or more of the nucleotide codons encoding the fusion polypeptide that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans.

57. Use of a nucleic acid encoding a fusion polypeptide comprising a matrix metalloproteinase 11 (MMP-11) linked to an immunoenhancing element in a medicament for treating a carcinoma in an individual.

58. Use of a fusion polypeptide comprising a matrix metalloproteinase 11 (MMP-11) linked to an immunoenhancing element in a medicament for treating a carcinoma in an individual.

59. A method for treating a carcinoma in an individual comprising:

(a) providing a vaccine which includes a nucleic acid including a nucleotide sequence encoding a matrix metalloproteinase 11 (MMP-11) wherein one or more of the nucleotide codons encoding the MMP-11 that occur at low frequency in nucleic acids encoding highly expressed proteins in humans have been replaced with nucleotide codons that occur at a higher frequency in the nucleic acids encoding the highly expressed proteins in humans or a fusion polypeptide that includes an MMP-11 linked to an immunoenhancing element; and

(b) inoculating the individual with the vaccine to treat the cancer.

60. The method of Claim 62 wherein the individual is undergoing one or more treatments selected from the group consisting of chemotherapy, radiation therapy, and vaccine against a tumor associated antigen.

61. The method of Claim 62 wherein the individual has an invasive carcinoma selected from the group consisting of the breast, colon, head and neck, lung, ovary, pancreas, prostate, skin (basal cell carcinoma), uterus (cervix carcinoma and endometrial carcinoma).

62. The method of Claim 62 wherein the individual has a non-invasive carcinoma that has a risk of evolving towards invasion.

63. A method for treating a cancer in an individual comprising:

(a) providing a vaccine which includes a fusion polypeptide that has a matrix metalloproteinase 11 (MMP-11) linked to an immunoenhancing element; and

(b) inoculating the individual with the vaccine to treat the cancer.

64. The method of Claim 66 wherein the individual has an invasive carcinoma selected from the group consisting of the breast, colon, head and neck, lung, ovary, pancreas, prostate, skin (basal cell carcinoma), uterus (cervix carcinoma and endometrial carcinoma).

65. The method of Claim 66 wherein the individual has a non-invasive carcinoma that has a risk of evolving towards invasion.

66. A method for identifying an analyte for inhibiting a cancer that overexpresses matrix metalloproteinase 11 (MMP-11), which comprises:

(a) inducing the cancer in a mouse;

(b) administering the analyte to the mouse with the induced cancer; and

(c) determining whether the analyte inhibits the cancer in the mouse with the induced tumor, which identifies the analyte for inhibiting a cancer that overexpresses the MMP-11.

67. The method of Claim 69 wherein the analyte is determined to bind the MMP-11 before it is administered to the mouse.

68. The method of Claim 69 wherein the cancer that is induced in the mouse is a colon cancer.

69. The method of Claim 69 wherein the cancer is induced in the mouse by administering to the mouse 1-2dimethylhydrazine (DMH) in an amount sufficient to induce the cancer in the mouse.

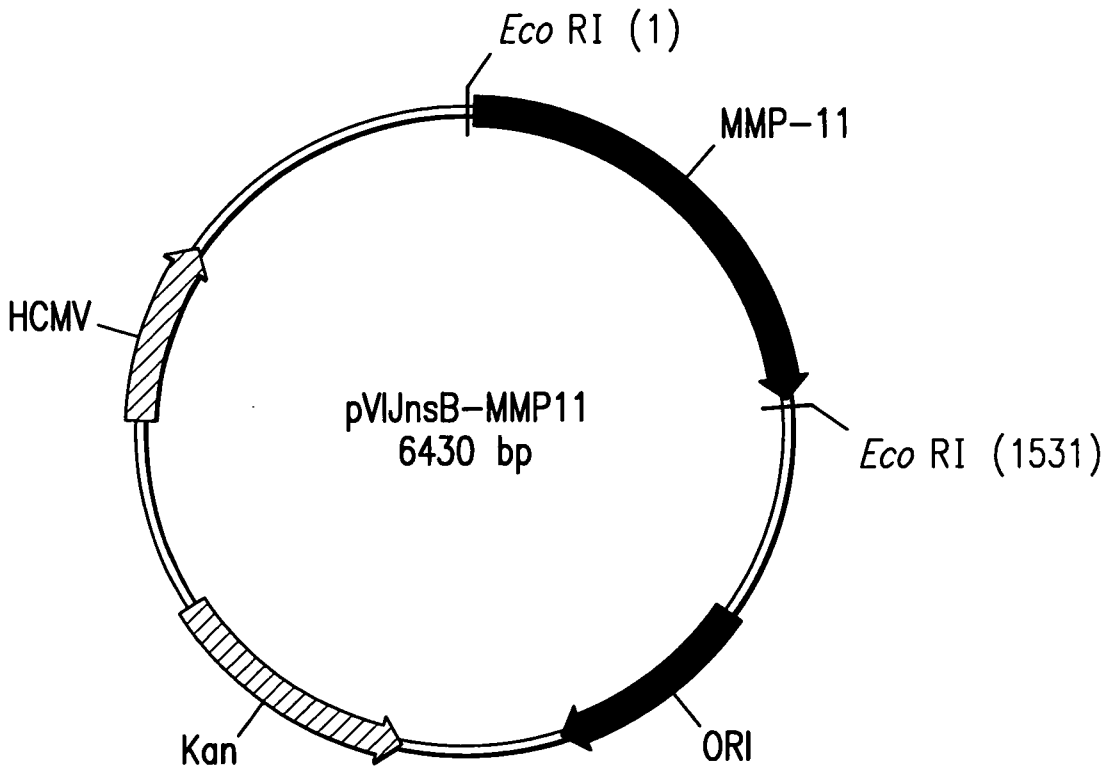
Eco RI (1)
MMP-11
HCMV
pVIJnsB-MMP11
6430 bp
Eco RI (1531)
Kan
ORI

FIG.1

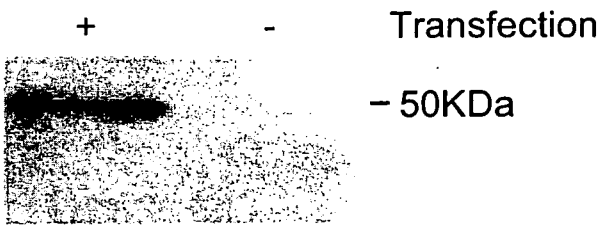
+
-
Transfection
- 50KDa

FIG.2

1 *CTGCAGAGAT CTGGTACCGA TATCGCCACC* **ATG**GCCAGAG CCGCCTGCCT
GCTGAGAGCC ATCAGCAGAG TGCTGCTGCT GCCTCTGCCA CTGCTGCTCC
101 TGTTGCTGCT CCTGCTGCCT AGCCCTCTGA TGGCCAGAGC TAGGCCTCCT
GAGAGCCACA GACACCACCC TGTGAAGAAG GGCCCTAGAC TGCTGCACGC
201 CGCCCTGCCT AACACCCTGA CCAGCGTGCC TGCCAGCCAC TGGGTGCCAA
GCCCTGCCGG CAGCAGCAGA CCTCTGAGAT GTGGCGTGCC TGACCTGCCT
301 GACGTGCTGA ACGCCAGGAA CAGGCAGAAG CGGTTCGTGC TGAGCGGCGG
CAGATGGGAA AAGACCGACC TGACCTACAG GATCCTGAGA TTCCCCTGGC
401 AGCTGGTGCG TGAGCAAGTG CGTCAGACCG TGGCCGAGGC CCTCCAGGTG
TGGAGCGAGG TGACCCCTCT GACCTTCACC GAGGTGCACG AGGGCAGAGC
501 CGACATCATG ATCGACTTCG CCAGATACTG GCACGGCGAC AACCTGCCTT
TCGACGGCCC TGGCGGCATC CTGGCCCACG CCTTTTTCCC CAAGACCCAC
601 AGAGAGGGCG ACGTGCACTT CGACTACGAC GAGACCTGGA CCATCGGCGA
TAACCAGGGC ACCGACCTGC TCCAGGTGGC CGCCCACGCT TTCGGCCACG
701 TGCTGGGCCT CCAGCACACC ACCGCCGCCA AGGCCCTGAT GAGCCCCTTC
TACACCTTCA GATACCCCCT GAGCCTGAGC CCTGACGACA GAAGAGGCAT
801 CCAGCACCTG TACGGCAGAC CTCAGATGGC CCCTACCAGC CCTGCCCCTA
CCCTGAGCAG CCAGGCCGGC ACCGACACCA ACGAGATCGC CCTGCTGGAG
901 CCTGAGACCC CTCCTGATGT GTGCGAGACC AGCTTCGACG CCGTGTCTAC
CATCAGAGGC GAGCTGTTCT TCTTCAAGGC CGGCTTTGTG TGGAGACTGA
1001 GGAGCGGCAG ACTCCAGCCT GGCTACCCTG CCCTGGCCAG CAGACACTGG
CAGGGCCTGC CTTCCCCTGT GGACGCCGCC TTCGAGGACG CCCAGGGCCA
1101 GATTTGGTTC TTCCAGGGCG CCCAGTACTG GGTGTACGAC GGCGAGAAGC
CTGTGCTGGG CCCTGCCCCA CTGAGCAAGC TGGGACTCCA GGGCAGCCCT
1201 GTGCACGCTG CCCTGGTGTG GGGACCTGAA AAGAACAAAA TCTATTTCTT
CAGAGGCGGC GACTACTGGA GATTCCACCC CAGGACCCAG AGAGTGGACA
1301 ACCCCGTGCC CAGAAGAAGC ACCGACTGGA GAGGCGTGCC TAGCGAGATC
GACGCCGCTT TCCAGGATGC TGAGGGCTAC GCCTACTTCC TGAGGGGCCA
1401 CCTGTACTGG AAGTTCGACC CCGTGAAGGT GAAGGTGCTG GAGGGCTTCC
CTAGACCTGT GGGCCCTGAC TTCTTCGACT GCGCCGAGCC TGCCAACACC
1501 TTCCGG**TCTA** **GA***TGATAAGT GACTAAATGA GAATTCGTCG ACGCGGCCGC*
CGGCGGTAGT CGTACCTCTT AACTATTAGA TCTACTATTC ACTGATTTAC
1601 *TCTTAAGCAG CTGCGCCGGC G*

FIG.3

1 *CTGCAGAGAT CTGGTACCGA TATCGCCACC* **ATG**GCCAGAG CCGCCTGCCT
GCTGAGAGCC ATCAGCAGAG TGCTGCTGCT GCCTCTGCCA CTGCTGCTCC
101 TGTTGCTGCT CCTGCTGCCT AGCCCTCTGA TGGCCAGAGC TAGGCCTCCT
GAGAGCCACA GACACCACCC TGTGAAGAAG GGCCCTAGAC TGCTGCACGC
201 CGCCCTGCCT AACACCCTGA CCAGCGTGCC TGCCAGCCAC TGGGTGCCAA
GCCCTGCCGG CAGCAGCAGA CCTCTGAGAT GTGGCGTGCC TGACCTGCCT
301 GACGTGCTGA ACGCCAGGAA CAGGCAGAAG CGGTTCGTGC TGAGCGGCGG
CAGATGGGAA AAGACCGACC TGACCTACAG GATCCTGAGA TTCCCCTGGC
401 AGCTGGTGCG TGAGCAAGTG CGTCAGACCG TGGCCGAGGC CCTCCAGGTG
TGGAGCGAGG TGACCCCTCT GACCTTCACC GAGGTGCACG AGGGCAGAGC
501 CGACATCATG ATCGACTTCG CCAGATACTG GCACGGCGAC AACCTGCCTT
TCGACGGCCC TGGCGGCATC CTGGCCCACG CCTTTTTCCC CAAGACCCAC
601 AGAGAGGGCG ACGTGCACTT CGACTACGAC GAGACCTGGA CCATCGGCGA
TAACCAGGGC ACCGACCTGC TCCAGGTGGC CGCCCACGCT TTCGGCCACG
701 TGCTGGGCCT CCAGCACACC ACCGCCGCCA AGGCCCTGAT GAGCCCCTTC
TACACCTTCA GATACCCCCT GAGCCTGAGC CCTGACGACA GAAGAGGCAT
801 CCAGCACCTG TACGGCAGAC CTCAGATGGC CCCTACCAGC CCTGCCCCTA
CCCTGAGCAG CCAGGCCGGC ACCGACACCA ACGAGATCGC CCTGCTGGAG
901 CCTGAGACCC CTCCTGATGT GTGCGAGACC AGCTTCGACG CCGTGTCTAC
CATCAGAGGC GAGCTGTTCT TCTTCAAGGC CGGCTTTGTG TGGAGACTGA
1001 GGAGCGGCAG ACTCCAGCCT GGCTACCCTG CCCTGGCCAG CAGACACTGG
CAGGGCCTGC CTTCCCCTGT GGACGCCGCC TTCGAGGACG CCCAGGGCCA
1101 GATTTGGTTC TTCCAGGGCG CCCAGTACTG GGTGTACGAC GGCGAGAAGC
CTGTGCTGGG CCCTGCCCCA CTGAGCAAGC TGGGACTCCA GGGCAGCCCT
1201 GTGCACGCTG CCCTGGTGTG GGGACCTGAA AAGAACAAAA TCTATTTCTT
CAGAGGCGGC GACTACTGGA GATTCCACCC CAGGACCCAG AGAGTGGACA
1301 ACCCCGTGCC CAGAAGAAGC ACCGACTGGA GAGGCGTGCC TAGCGAGATC
GACGCCGCTT TCCAGGATGC TGAGGGCTAC GCCTACTTCC TGAGGGGCCA
1401 CCTGTACTGG AAGTTCGACC CCGTGAAGGT GAAGGTGCTG GAGGGCTTCC
CTAGACCTGT GGGCCCTGAC TTCTTCGACT GCGCCGAGCC TGCCAACACC
1501 TTCCGG**TCTA** **GA**gccccca gagcatcacc gagctgtgca gcgagtaccg
gaacacccag atttacacca tcaacgacaa gatcctgagc tacaccgaga
1601 gcatggccgg caagagggag atggtgatca tcaccttcaa gagcggcgcc
accttccagg tggaggtgcc cggcagccag cacatcgaca gccagaagaa
1701 ggccatcgag cggatgaagg acaccctgcg gatcacctac ctcaccgaga
ccaagatcga caagctgtgc gtgtggaaca acaagacccc caacagcatc
1801 gccgccatca gcatggagaa t*TGATAATCT AGATGATAAG TGACTAAATG*
AGAATTCGTC GACGCGGCCG CCGGCGGTAG TCGTACCTCT TAACTATTAGA
1901 *TCTACTATTC ACTGATTTAC TCTTAAGCAG CTGCGCCGGC G*

FIG.4

```
  1 MARAACLLRA ISRVLLLPLP LLLLLLLLLP SPLMARARPP ESHRHHPVKK
 51 GPRLLHAALP NTLTSVPASH WVPSPAGSSR PLRCGVPDLP DVLNARNRQK
101 RFVLSGGRWE KTDLTYRILR FPWQLVREQV RQTVAEALQV WSEVTPLTFT
151 EVHEGRADIM IDFARYWHGD NLPFDGPGGI LAHAFFPKTH REGDVHFDYD
201 ETWTIGDNQG TDLLQVAAHA FGHVLGLQHT TAAKALMSPF YTFRYPLSLS
251 PDDRRGIQHL YGRPQMAPTS PAPTLSSQAG TDTNEIALLE PETPPDVCET
301 SFDAVSTIRG ELFFFKAGFV WRLRSGRLQP GYPALASRHW QGLPSPVDAA
351 FEDAQGQIWF FQGAQYWVYD GEKPVLGPAP LSKLGLQGSP VHAALVWGPE
401 KNKIYFFRGG DYWRFHPRTQ RVDNPVPRRS TDWRGVPSEI DAAFQDAEGY
451 AYFLRGHLYW KFDPVKVKVL EGFPRPVGPD FFDCAEPANT FRSRAPQSIT
501 ELCSEYRNTQ IYTINDKILS YTESMAGKRE MVIITFKSGA TFQVEVPGSQ
551 HIDSQKKAIE RMKDTLRITY LTETKIDKLC VWNNKTPNSI AAISMEN
```

FIG.5

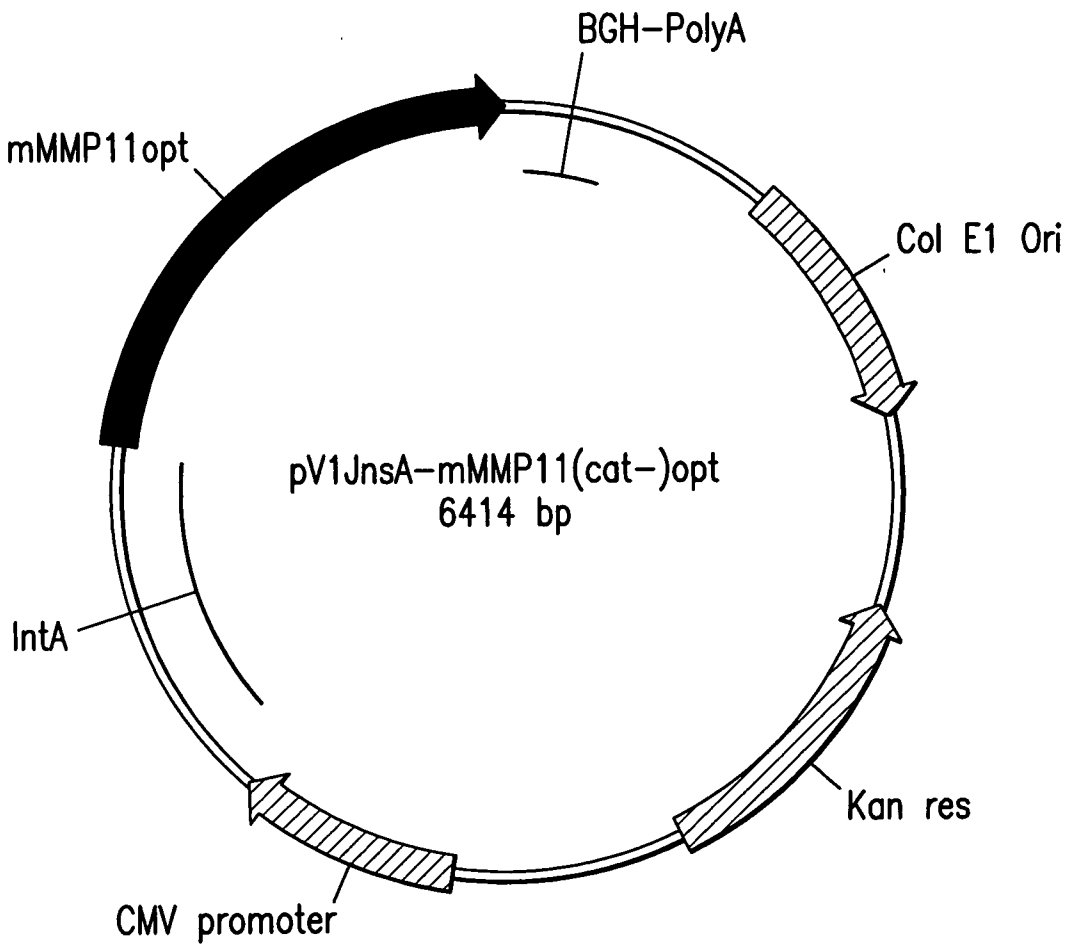
BGH-PolyA
mMMP11opt
Col E1 Ori
pV1JnsA-mMMP11(cat-)opt
6414 bp
IntA
Kan res
CMV promoter

FIG.6A

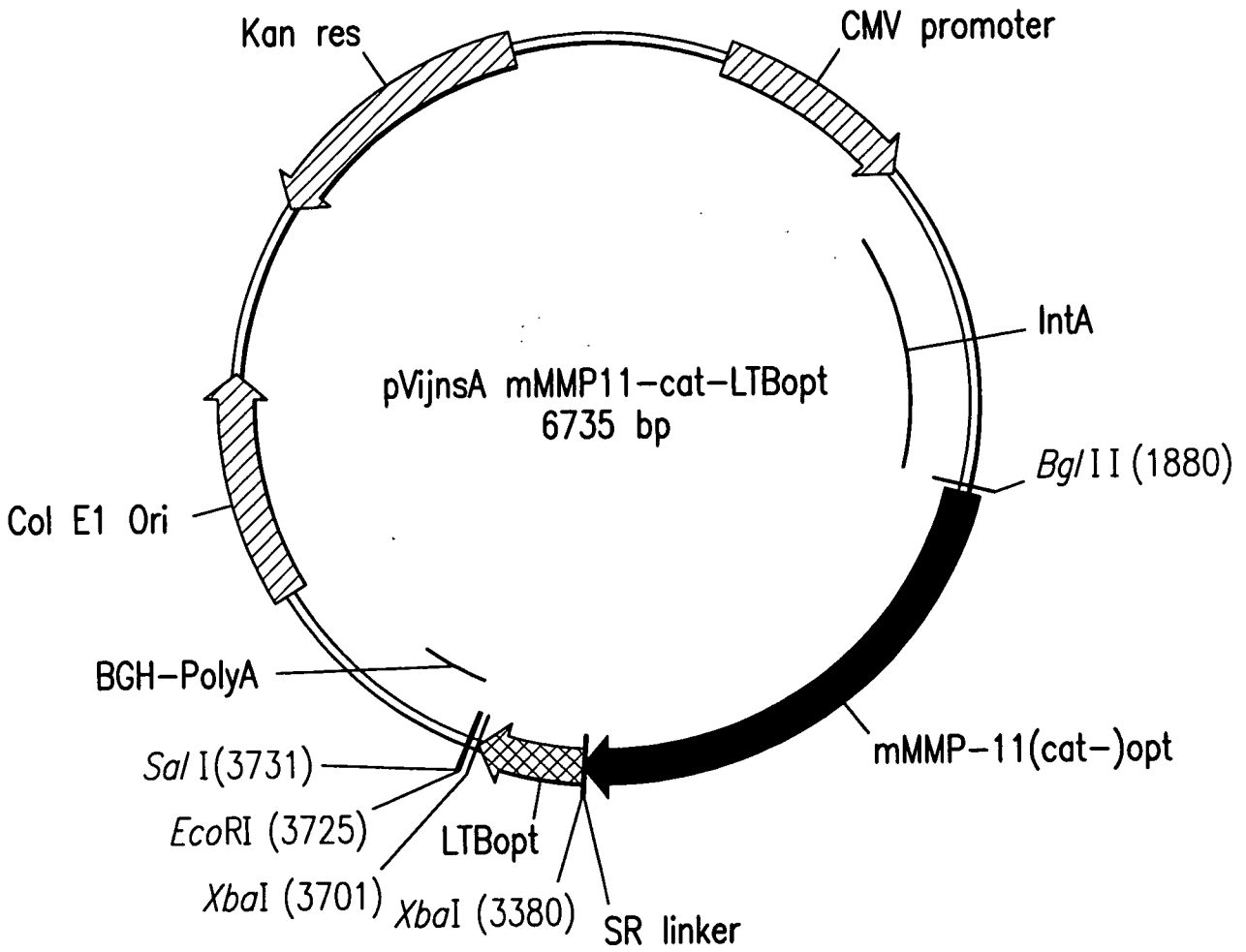
Kan res
CMV promoter
IntA
pVijnsA mMMP11-cat-LTBopt
6735 bp
Bgl II (1880)
Col E1 Ori
BGH-PolyA
mMMP-11(cat-)opt
Sal I(3731)
EcoRI (3725)
LTBopt
XbaI (3701)
XbaI (3380)
SR linker

FIG.6B

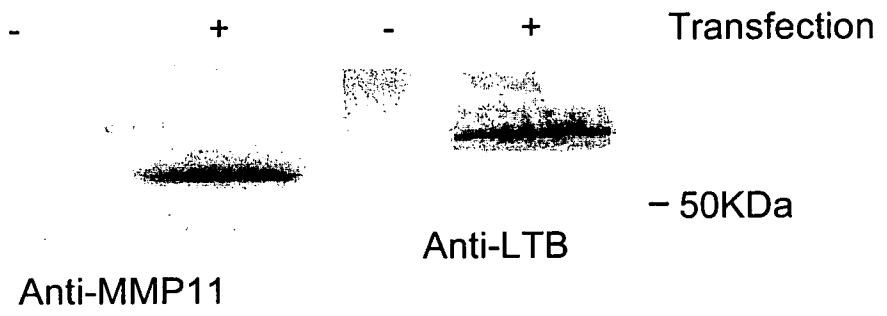

FIG.7

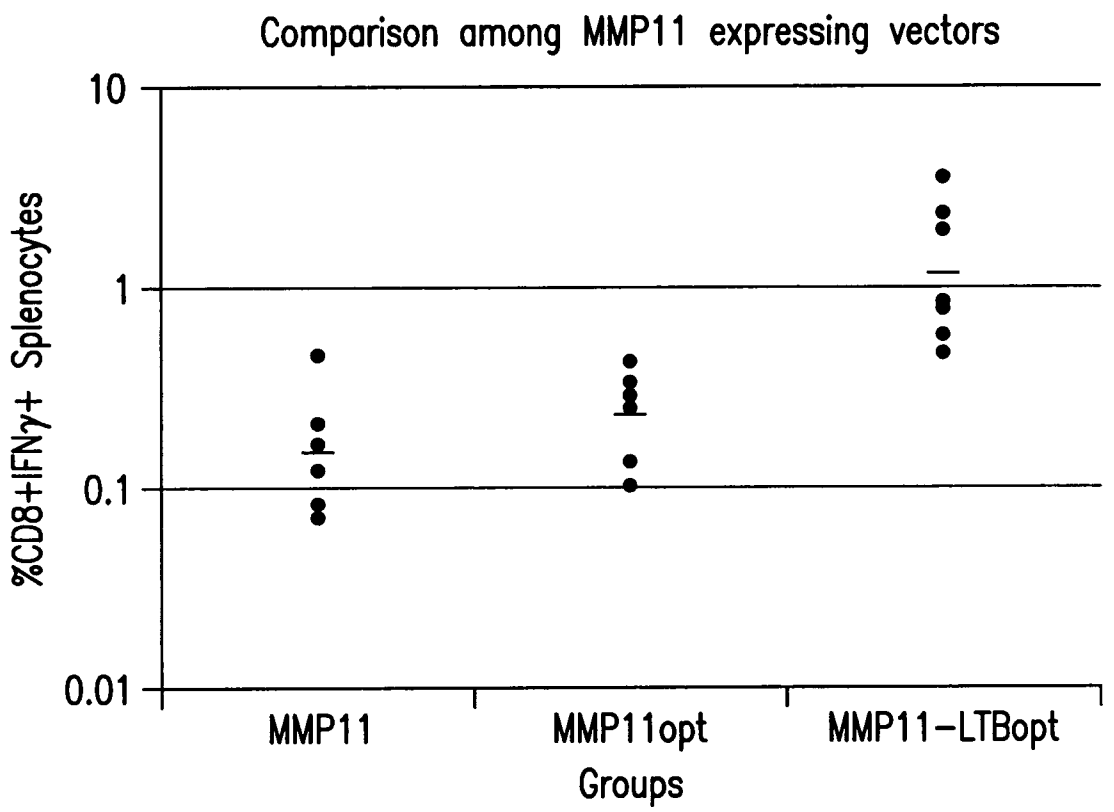
Comparison among MMP11 expressing vectors
10
1
0.1
0.01
%CD8+IFNγ+ Splenocytes
MMP11
MMP11opt
MMP11-LTBopt
Groups

FIG.8

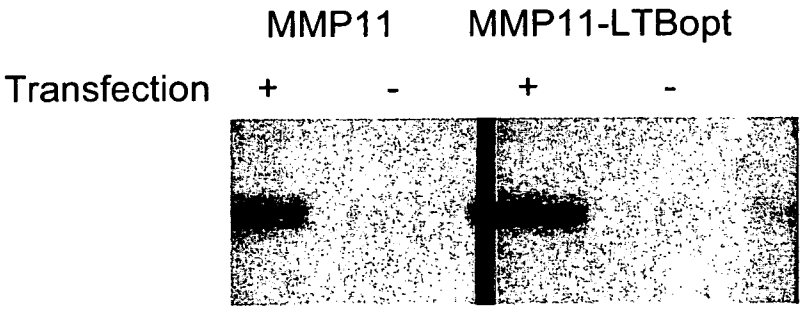
MMP11
MMP11-LTBopt
Transfection
+
-
+
-

FIG.9

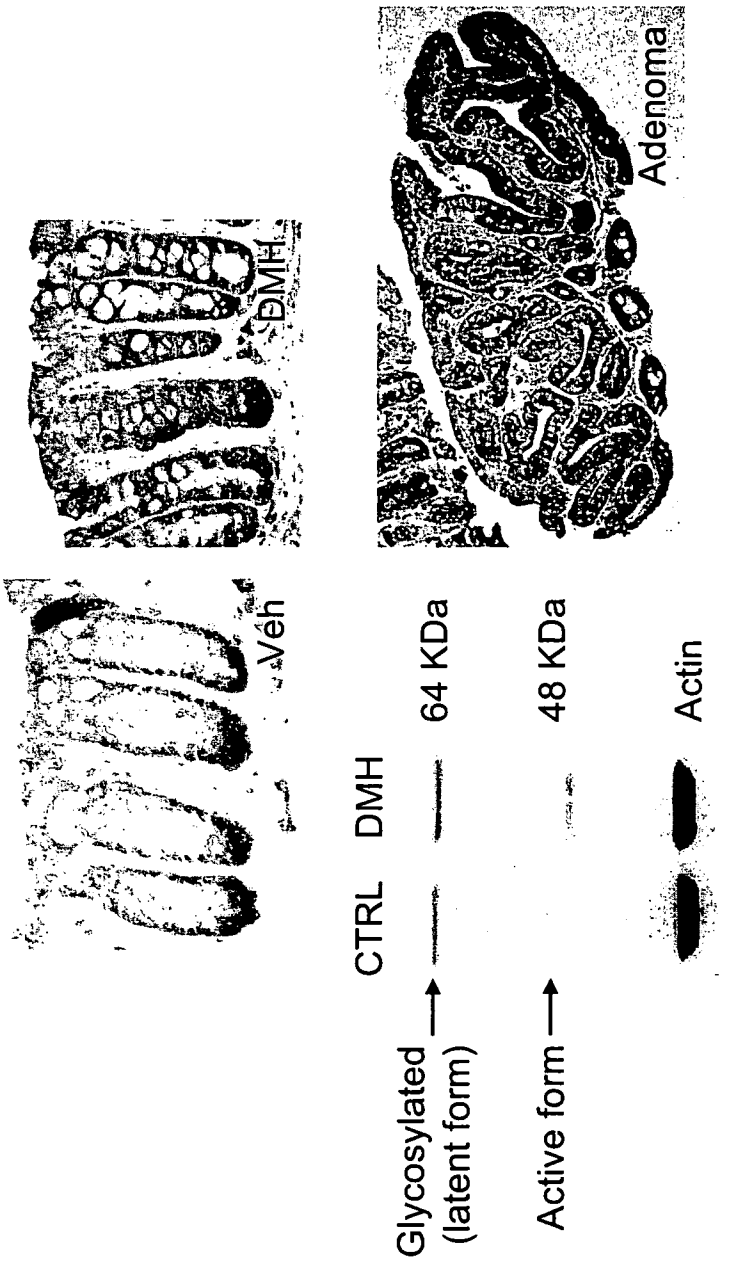
Veh
DMH
Adenoma
CTRL
DMH
64 KDa
48 KDa
Actin
Glycosylated (latent form)
Active form

FIG.10

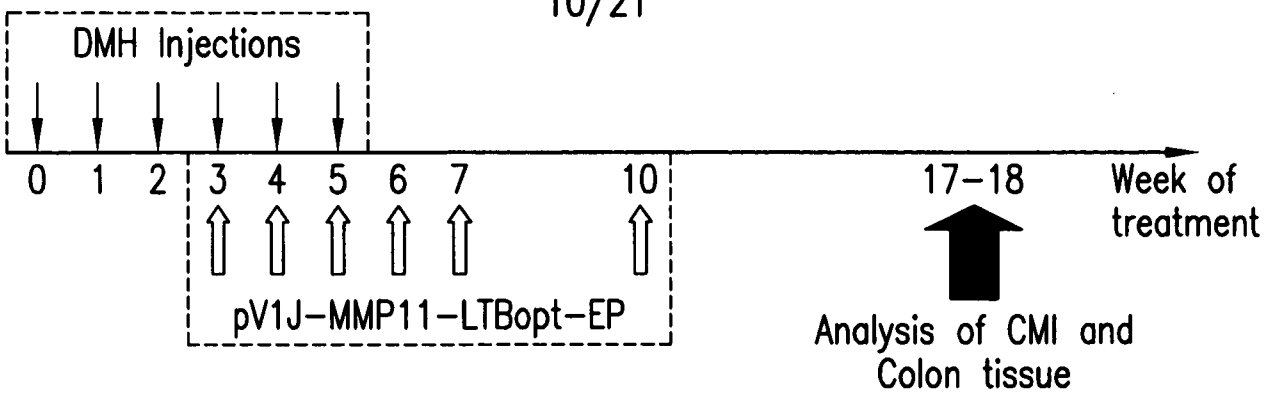

FIG.11A

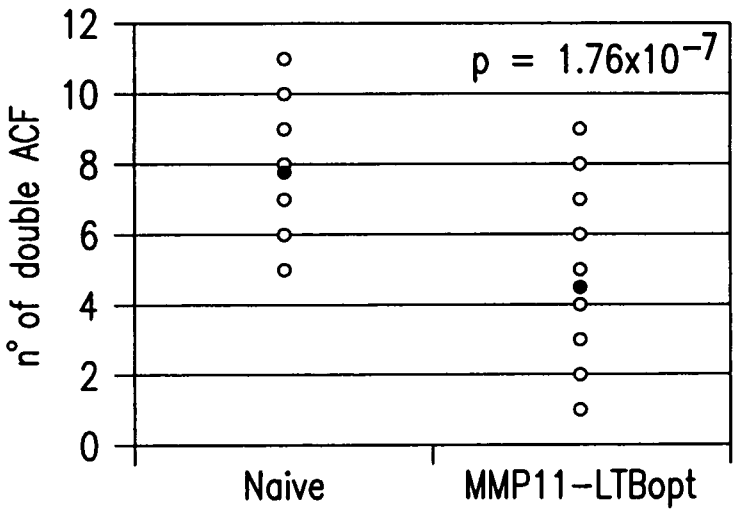

FIG.11B

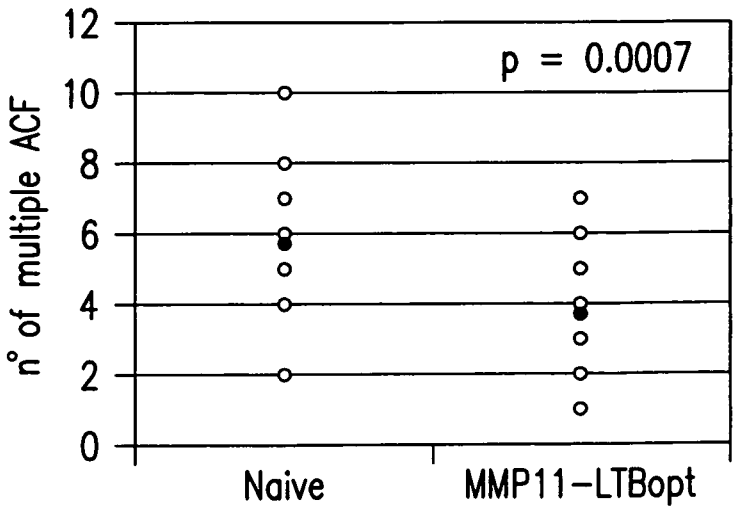

FIG.11C

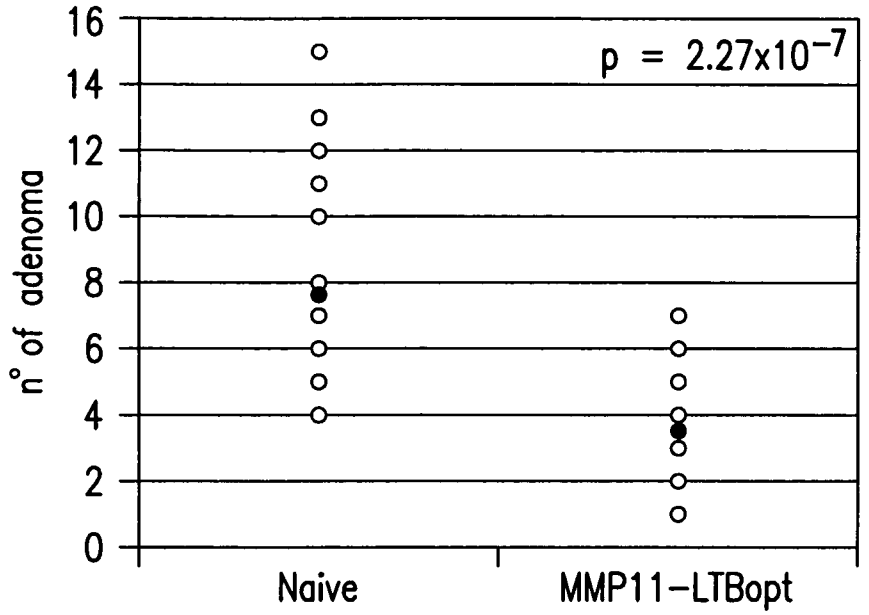

FIG.11D

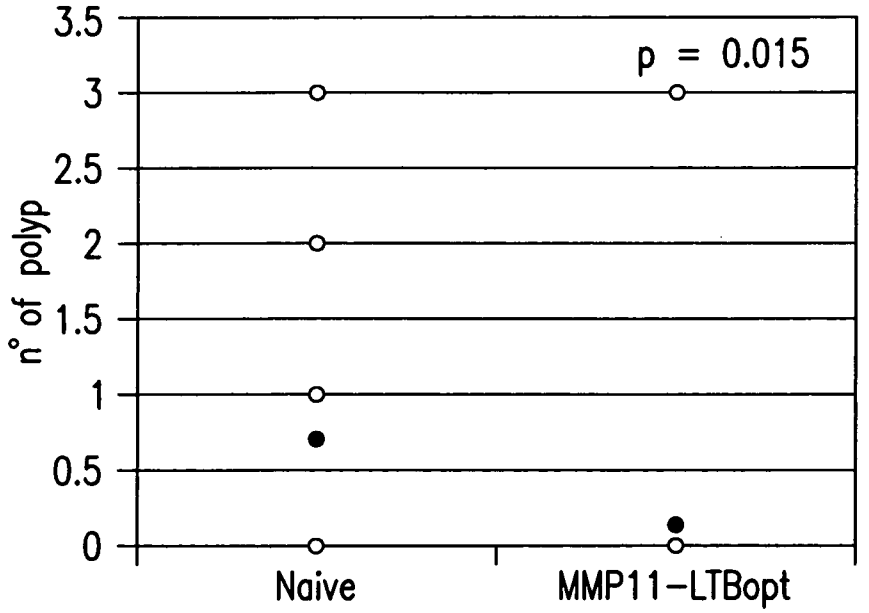

FIG.11E

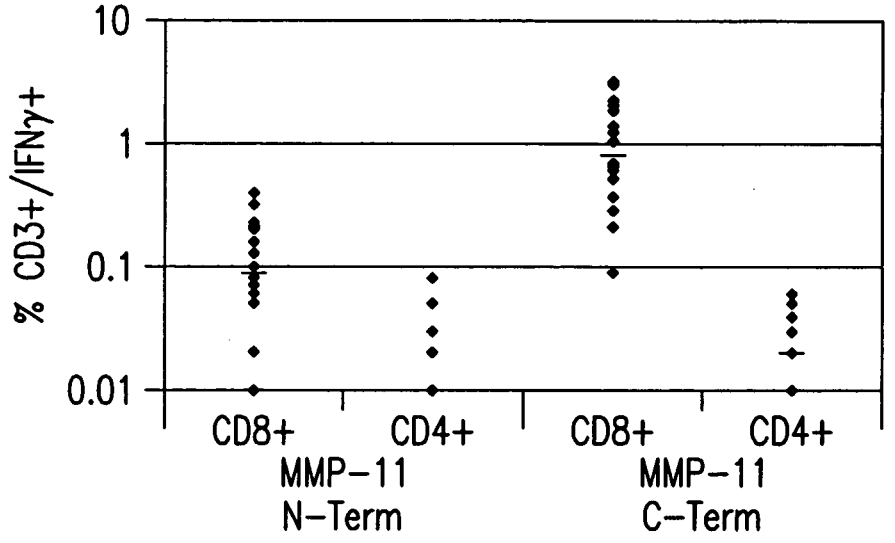

FIG.12A

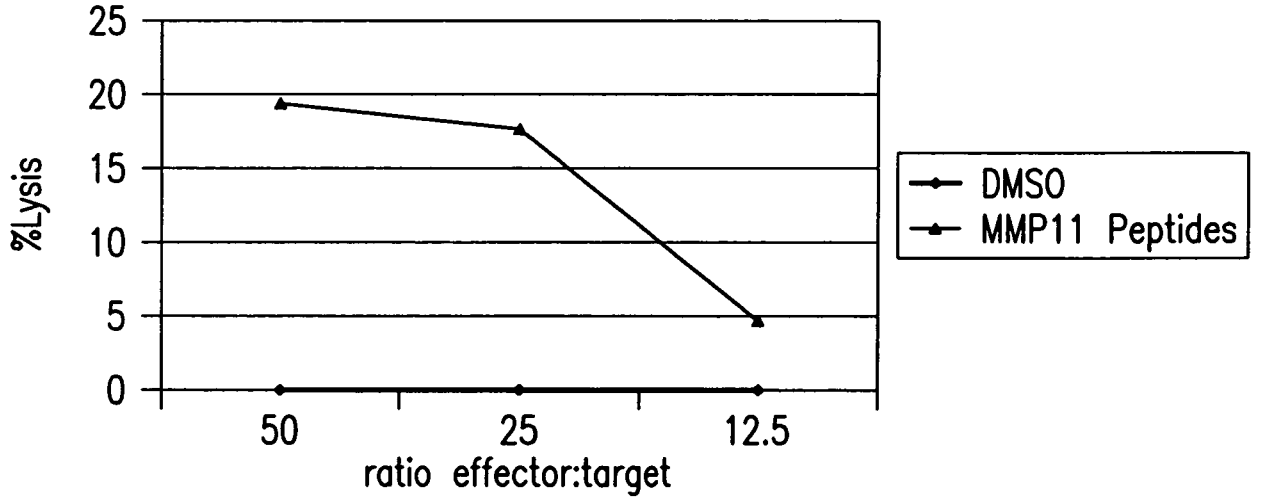

FIG.12B

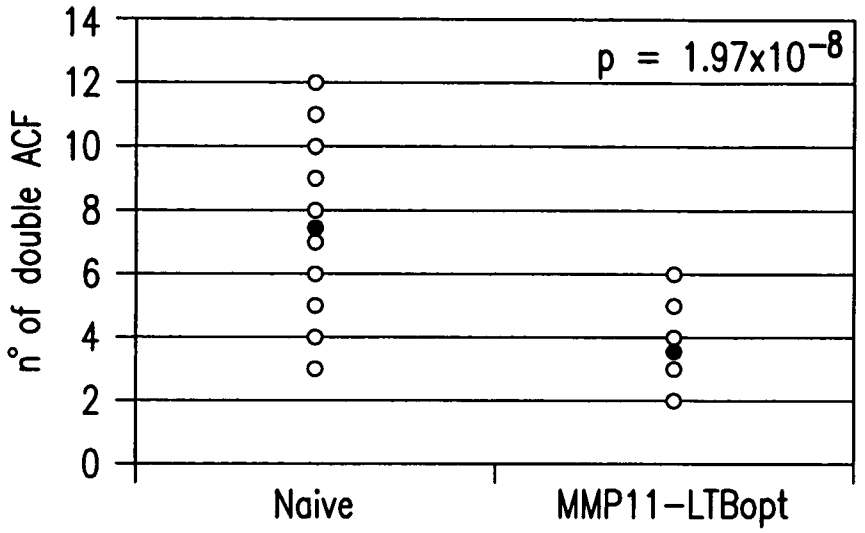

FIG.13A

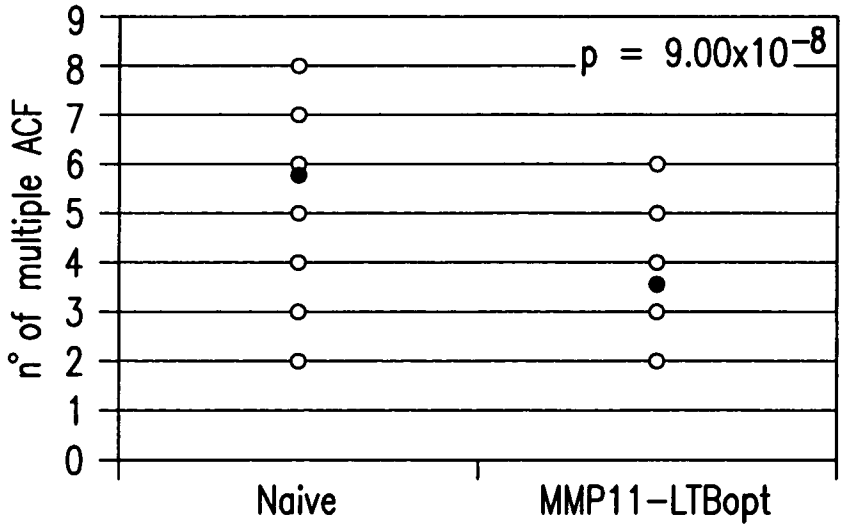

FIG.13B

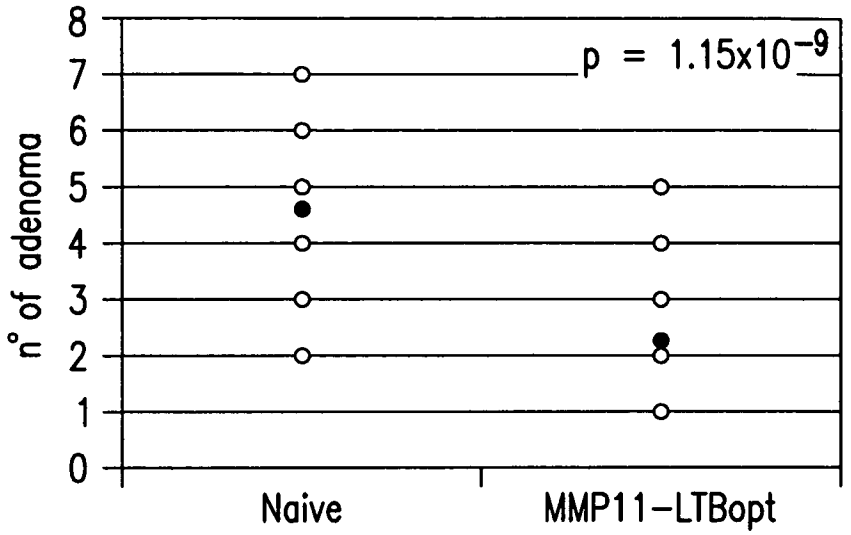

FIG.13C

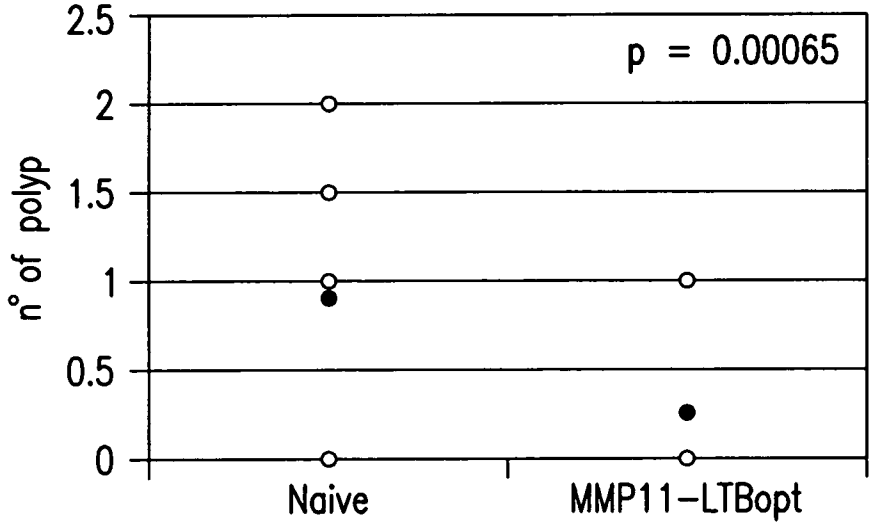

FIG.13D

```
   1  ATGGCTCCTG CCGCCTGGCT GAGAAGCGCT GCCGCTAGAG CCCTGCTGCC
      CCCTATGCTG CTGCTCCTGC TGCAGCCTCC TCCTCTGCTG GCTCGGGCTC
 101  TGCCTCCTGA CGTGCACCAC CTGCATGCCG AGAGGAGGGG GCCACAGCCC
      TGGCATGCTG CCCTGCCCAG TAGCCCTGCT CCTGCCCCTG CCACACAGGA
 201  AGCCCCCAGA CCTGCCAGCA GCCTGAGGCC TCCCAGATGT GGCGTGCCCG
      ACCCATCTGA TGGGCTGAGT GCCCGCAACC GGCAGAAGAG ATTCGTGCTG
 301  TCTGGCGGAC GCTGGGAGAA AACCGACCTG ACCTACAGGA TCCTGCGGTT
      CCCATGGCAG CTGGTGCAGG AACAGGTGCG GCAGACAATG GCTGAGGCCC
 401  TGAAAGTGTG GAGCGATGTG ACCCCACTGA CCTTTACTGA AGTGCACGAG
      GGCAGGGCTG ACATCATGAT CGACTTCGCC CGGTACTGGC ATGGGGACGA
 501  CCTGCCTTTT GATGGGCCTG GGGGCATCCT GGCCCATGCC TTCTTCCCCA
      AAACTCACCG GGAAGGGGAT GTGCACTTCG ACTATGATGA GACCTGGACT
 601  ATCGGGGATG ACCAGGGCAC AGACCTGCTG CAGGTGGCCG CCCATGTGTT
      TGGCCACGTG CTGGGGCTGC AGCACACAAC AGCTGCCAAG GCCCTGATGT
 701  CCGCCTTCTA CACCTTTCGC TACCCACTGA GTCTGAGCCC AGATGACTGC
      AGGGGCGTGC AGCACCTGTA TGGCCAGCCC TGGCCCACTG TGACCTCCAG
 801  GACCCCAGCC CTGGGCCCCC AGGCTGGGAT TGACACCAAT GAGATTGCCC
      CCCTGGAGCC AGACGCCCCT CCAGATGCCT GTGAGGCCTC CTTTGACGCC
 901  GTGTCCACCA TCAGAGGCGA GCTGTTTTTC TTCAAGGCCG GCTTTGTGTG
      GAGACTGAGA GGGGGCCAGC TGCAGCCCGG CTACCCAGCT CTGGCCTCTC
1001  GCCACTGGCA GGGACTGCCC AGCCCTGTGG ACGCTGCCTT CGAGGATGCC
      CAGGGCCACA TTTGGTTCTT CCAGGGCGCT CAGTACTGGG TGTACGACGG
1101  CGAAAAGCCA GTGCTGGGCC CTGCTCCCCT GACCGAGCTG GGCCTGGTGA
      GATTCCCAGT GCATGCCGCC CTGGTGTGGG GACCCGAGAA GAACAAAATC
1201  TACTTCTTCC GGGGCAGGGA CTACTGGAGA TTCCACCCCA GCACCCGGAG
      AGTGGACAGT CCCGTGCCCA GAAGGGCCAC TGACTGGAGA GGAGTGCCCT
1301  CTGAGATCGA CGCCGCCTTC CAGGACGCTG ATGGCTATGC CTACTTCCTG
      CGCGGCAGGC TGTACTGGAA GTTTGACCCT GTGAAAGTGA AGGCTCTGGA
1401  AGGCTTCCCC AGACTGGTGG GCCCTGACTT CTTTGGCTGT GCCGAGCCTG
      CCAACACTTT CCTGTGATAA
```

FIG.14

```
  1 MAPAAWLRSA AARALLPPML LLLLQPPPLL ARALPPDVHH LHAERRGPQP
 51 WHAALPSSPA PAPATQEAPR PASSLRPPRC GVPDPSDGLS ARNRQKRFVL
101 SGGRWEKTDL TYRILRFPWQ LVQEQVRQTM AEALKVWSDV TPLTFTEVHE
151 GRADIMIDFA RYWHGDDLPF DGPGGILAHA FFPKTHREGD VHFDYDETWT
201 IGDDQGTDLL QVAAHVFGHV LGLQHTTAAK ALMSAFYTFR YPLSLSPDDC
251 RGVQHLYGQP WPTVTSRTPA LGPQAGIDTN EIAPLEPDAP PDACEASFDA
301 VSTIRGELFF FKAGFVWRLR GGQLQPGYPA LASRHWQGLP SPVDAAFEDA
351 QGHIWFFQGA QYWVYDGEKP VLGPAPLTEL GLVRFPVHAA LVWGPEKNKI
401 YFFRGRDYWR FHPSTRRVDS PVPRRATDWR GVPSEIDAAF QDADGYAYFL
451 RGRLYWKFDP VKVKALEGFP RLVGPDFFGC AEPANTFL
```

FIG.15

ATGGCTCCTGCCGCCTGGCTGAGAAGCGCTGCCGCTAGAGCCCTGCTGCCCCCTAT
GCTGCTGCTCCTGCTGCAGCCTCCTCCTCTGCTGGCTCGGGCTCTGCCTCCTGACG
TGCACCACCTGCATGCCGAGAGGAGGGGGCCACAGCCCTGGCATGCTGCCCTGCCC
AGTAGCCCTGCTCCTGCCCCTGCCACACAGGAAGCCCCCAGACCTGCCAGCAGCCT
GAGGCCTCCCAGATGTGGCGTGCCCGACCCATCTGATGGGCTGAGTGCCCGCAACC
GGCAGAAGAGATTCGTGCTGTCTGGCGGACGCTGGGAGAAAACCGACCTGACCTAC
AGGATCCTGCGGTTCCCATGGCAGCTGGTGCAGGAACAGGTGCGGCAGACAATGGC
TGAGGCCCTGAAAGTGTGGAGCGATGTGACCCCACTGACCTTTACTGAAGTGCACG
AGGGCAGGGCTGACATCATGATCGACTTCGCCCGGTACTGGCATGGGGACGACCTG
CCTTTTGATGGGCCTGGGGGCATCCTGGCCCATGCCTTCTTCCCCAAAACTCACCG
GGAAGGGGATGTGCACTTCGACTATGATGAGACCTGGACTATCGGGGATGACCAGG
GCACAGACCTGCTGCAGGTGGCCGCCCATGTGTTTGGCCACGTGCTGGGGCTGCAG
CACACAACAGCTGCCAAGGCCCTGATGTCCGCCTTCTACACCTTTCGCTACCCACT
GAGTCTGAGCCCAGATGACTGCAGGGGCGTGCAGCACCTGTATGGCCAGCCCTGGC
CCACTGTGACCTCCAGGACCCCAGCCCTGGGCCCCCAGGCTGGGATTGACACCAAT
GAGATTGCCCCCCTGGAGCCAGACGCCCCTCCAGATGCCTGTGAGGCCTCCTTTGA
CGCCGTGTCCACCATCAGAGGCGAGCTGTTTTTCTTCAAGGCCGGCTTTGTGTGGA
GACTGAGAGGGGGCCAGCTGCAGCCCGGCTACCCAGCTCTGGCCTCTCGCCACTGG
CAGGGACTGCCCAGCCCTGTGGACGCTGCCTTCGAGGATGCCCAGGGCCACATTTG
GTTCTTCCAGGGCGCTCAGTACTGGGTGTACGACGGCGAAAAGCCAGTGCTGGGCC
CTGCTCCCCTGACCGAGCTGGGCCTGGTGAGATTCCCAGTGCATGCCGCCCTGGTG
TGGGGACCCGAGAAGAACAAAATCTACTTCTTCCGGGGCAGGGACTACTGGAGATT
CCACCCCAGCACCCGGAGAGTGGACAGTCCCGTGCCCAGAAGGGCCACTGACTGGA
GAGGAGTGCCCTCTGAGATCGACGCCGCCTTCCAGGACGCTGATGGCTATGCCTAC
TTCCTGCGCGGCAGGCTGTACTGGAAGTTTGACCCTGTGAAAGTGAAGGCTCTGGA
AGGCTTCCCCAGACTGGTGGGCCCTGACTTCTTTGGCTGTGCCGAGCCTGCCAACA
CTTTCCTGTCTAGAgccccccagagcatcaccgagctgtgcagcgagtaccggaac
Acccagatttacaccatcaacgacaagatcctgagctacaccgagagcatggccgg
Caagagggagatggtgatcatcaccttcaagagcggcgccaccttccaggtggagg
Tgcccggcagccagcacatcgacagccagaagaaggccatcgagcggatgaaggac
Accctgcggatcacctacctcaccgagaccaagatcgacaagctgtgcgtgtggaa
caacaagacccccaacagcatcgccgccatcagcatggagaattgataa

FIG.16

MAPAAWLRSA AARALLPPML LLLLQPPPLL ARALPPDVHH LHAERRGPQP
WHAALPSSPA PAPATQEAPR PASSLRPPRC GVPDPSDGLS ARNRQKRFVL
SGGRWEKTDL TYRILRFPWQ LVQEQVRQTM AEALKVWSDV TPLTFTEVHE
GRADIMIDFA RYWHGDDLPF DGPGGILAHA FFPKTHREGD VHFDYDETWT
IGDDQGTDLL QVAAHVFGHV LGLQHTTAAK ALMSAFYTFR YPLSLSPDDC
RGVQHLYGQP WPTVTSRTPA LGPQAGIDTN EIAPLEPDAP PDACEASFDA
VSTIRGELFF FKAGFVWRLR GGQLQPGYPA LASRHWQGLP SPVDAAFEDA
QGHIWFFQGA QYWVYDGEKP VLGPAPLTEL GLVRFPVHAA LVWGPEKNKI
YFFRGRDYWR FHPSTRRVDS PVPRRATDWR GVPSEIDAAF QDADGYAYFL
RGRLYWKFDP VKVKALEGFP RLVGPDFFGC AEPANTFLSR *APQSITELCS*
EYRNTQIYTI NDKILSYTES MAGKREMVII TFKSGATFQV EVPGSQHIDS
QKKAIERMKD TLRITYLTET KIDKLCVWNN KTPNSIAAIS MEN

FIG.17

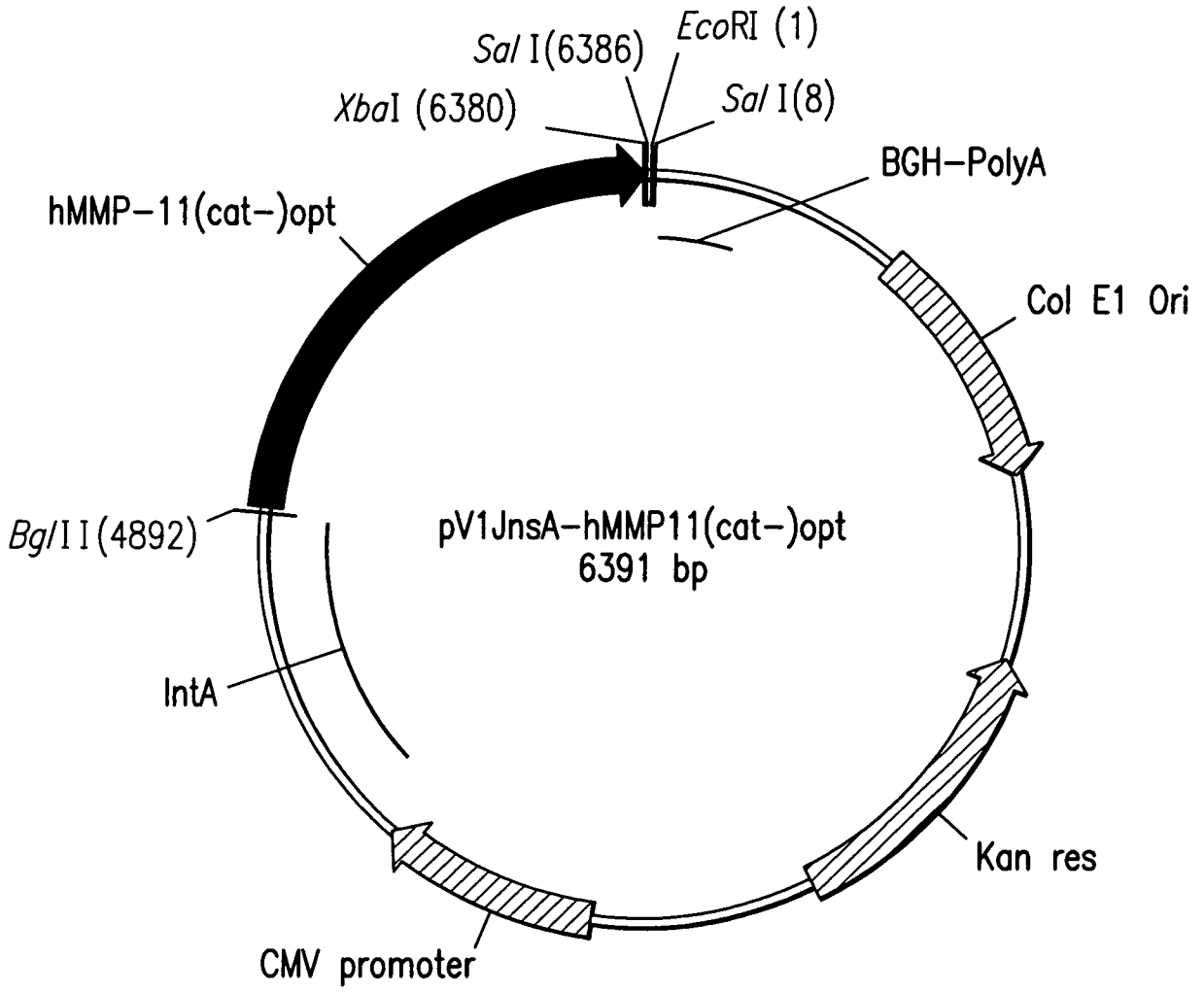

FIG.18

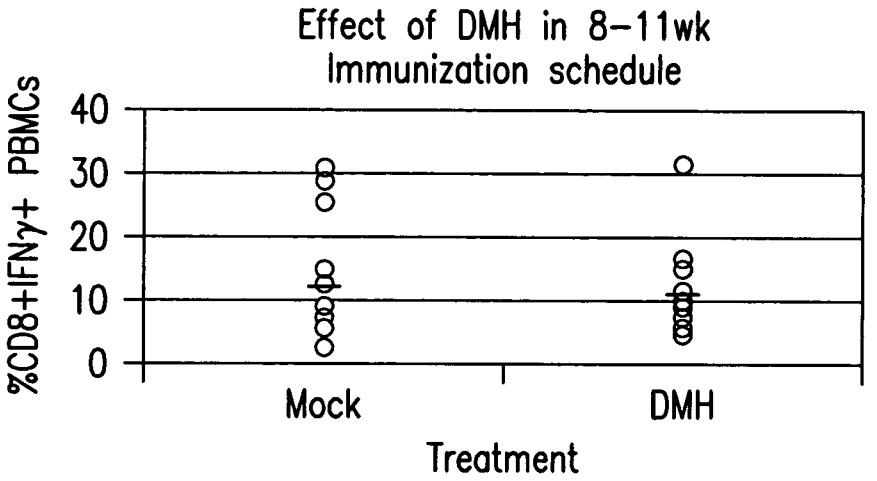

FIG.19A

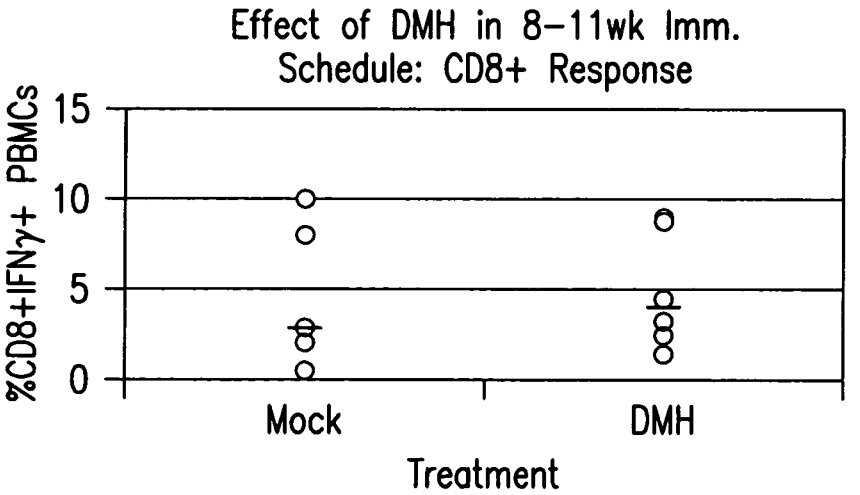

FIG.19B

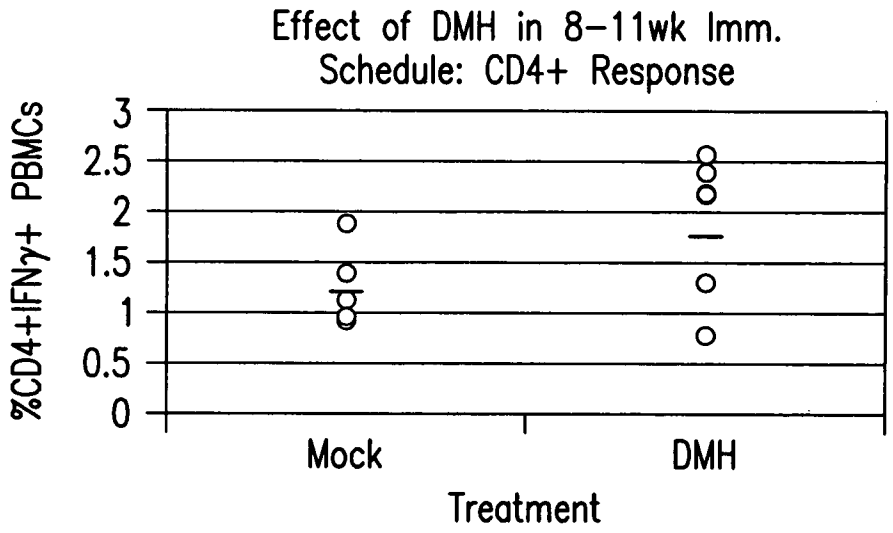
Effect of DMH in 8–11wk Imm.
Schedule: CD4+ Response
%CD4+IFNγ+ PBMCs
3
2.5
2
1.5
1
0.5
0
Mock
DMH
Treatment

FIG.19C

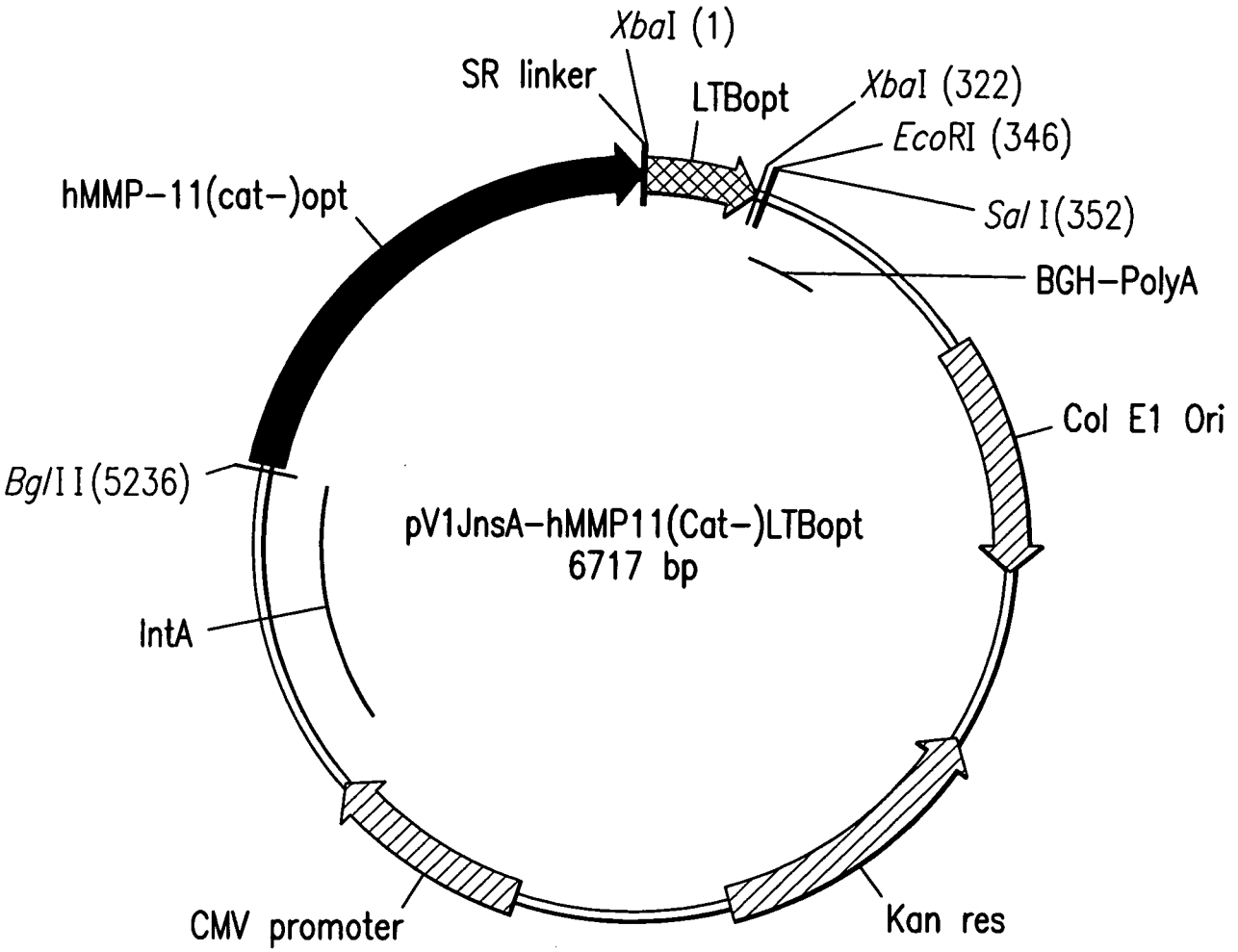
XbaI (1)
SR linker
LTBopt
XbaI (322)
EcoRI (346)
SalI (352)
hMMP-11(cat-)opt
BGH-PolyA
Col E1 Ori
BglII (5236)
pV1JnsA-hMMP11(Cat-)LTBopt
6717 bp
IntA
CMV promoter
Kan res

FIG.20