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(54) Title: DNA MOLECULES AND RECOMBINANT DNA MOLECULES FOR PRODUCING HUMANIZED MONOCLONAL ANTIBODIES TO *S. MUTANS*

(57) Abstract: Dental caries in man may be prevented or treated by oral ingestion of human or humanized murine monoclonal IgG and IgM antibodies that bind to surface antigens of cariogenic organisms, such as *S. mutans*. The genetically engineered monoclonal antibodies engage the effector apparatus of the human immune system when they bind to cariogenic organisms, resulting in their destruction. In a preferred embodiment, monoclonal antibodies to cariogenic organisms are produced by edible plants, including fruits and vegetables, transformed by DNA sequences that code on expression for the desired antibodies. The antibodies are applied by eating the plants.

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PATENT

PATENT APPLICATION IN THE U.S. PATENT AND TRADEMARK  
OFFICE

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for

DNA MOLECULES AND RECOMBINANT DNA MOLECULES  
FOR PRODUCING HUMANIZED MONOCLONAL ANTIBODIES  
TO *S. MUTANS*

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by

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Background of the Invention

This application is a continuation-in-part of United States patent application serial number 09/378,577 filed August 20, 1999.

This application relates to an immunologic methodology for the treatment and prevention of dental caries. This invention has special application to patients who are without the ability or motivation to apply established principles of self care, such as very young children, the infirm and poorly educated populations.

Dental caries (tooth decay) and periodontal disease are probably the most common chronic diseases in the world. The occurrence of cavities in teeth is the result of bacterial infection. Hence the occurrence of dental caries is properly viewed as an infectious microbiological disease that results in localized destruction of the calcified tissues of the teeth.

*Streptococcus mutans* is believed to be the principal cause of tooth decay in man. When *S. mutans* occurs in large numbers in dental plaque, and metabolizes complex sugars, the resulting organic acids cause demineralization of the tooth surface. The result is carious lesions, commonly known as cavities. Other organisms, such as *Lactobaccilli* and *Actinomyces* are also believed to be involved in the progression and formation of carious lesions. Those organisms that cause tooth decay are referred to herein as "cariogenic organisms."

Removal of the damaged portion of a tooth and restoration by filling can, at least temporarily, halt the damage caused by oral infection with cariogenic organisms. However, the "drill and fill" approach does not eliminate the causative bacterial agent. Proper oral hygiene can control the accumulation of dental plaque, where cariogenic organisms grow and attack the tooth

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surfaces. However, dental self-care has its limits, particularly in populations that are unable to care for themselves, or where there is a lack of knowledge of proper methods of self care. Administration of fluoride ion has been shown to decrease, but not eliminate the incidence of dental caries.

5 In view of the overwhelming evidence of the involvement of cariogenic organisms in the pathogenesis of dental caries, it is not surprising that there have been a number of different attempts to ameliorate the condition using traditional methods of anti-microbial therapy. The disadvantage of 10 antimicrobial agents is that they are not selective for cariogenic organisms. Administration of non-specific bacteriocidal agents disturbs the balance of organisms that normally inhabit the oral cavity, with consequences that cannot be predicted, but may include creation of an environment that provides 15 opportunities for pathogenic organisms. In addition, long term use of antimicrobial agents is known to select for organisms that are resistant to them. Hence long term and population-wide use of antimicrobial agents to prevent tooth decay is not practical.

Vaccination of humans to elicit an active immune response to *S. mutans*, or other cariogenic organisms, is also not a practical solution at this time. One drawback of this approach is that vaccination elicits production of predominantly IgG and IgM antibodies, but they are not secreted into saliva. The majority of antibodies present in saliva are of the IgA isotype, which can 20 bind to, but cannot activate lymphocytes or complement components to kill bacteria. Accordingly, vaccination is not believed likely to be capable of producing antibodies that can trigger the immune system to kill cariogenic organisms in the mouth. There is no known method for selectively increasing 25 the titer of vaccination induced antibodies of the IgG or IgM isotypes in the oral cavity.

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There have been a number of reported attempts to passively immunize patients to *S. mutans* using monoclonal IgA antibodies raised in mice to prevent tooth decay in animals and in man. Because IgA is a multivalent antibody, a single molecule of IgA can bind to several different antigenic sites, resulting in clumping of bacteria. However, binding of IgA to bacterial surface antigens does not kill the bacteria. Rather, clumping is believed to hinder the ability of bacteria to bind to tooth surfaces. Another drawback of this approach is that repeated administration of murine (i.e., heterologous) antibodies to

5 humans has the potential to evoke an immune response to the antibodies.

Unlike IgA antibodies, antibodies of the IgG and IgM classes have bacteriocidal effects. Binding of IgM or IgG antibodies to antigens present on the surface of cariogenic organisms may result in the destruction of the bacterial cells by either of two presently known separate mechanisms: complement mediated cell lysis and antibody-dependent cell-mediated cytotoxicity. In either case, antibodies that selectively bind to certain microbial organisms target just those cells for destruction by the immune system. Both complement mediated cell lysis and antibody-dependent cell mediated cytotoxicity are part of the humoral immune response that is mediated by antibodies of the IgG and IgM classes.

In order to elicit the desired cytotoxic effect of antibody binding, monoclonal antibodies to cariogenic organisms must be recognized by the human immune system. There are a number of different technologies by which antibodies that will trigger a response from the human immune system can be produced. One example is producing a chimeric antibody using a nucleic acid construct that codes for expression of a human antibody modified to incorporate sequences encoding the variable domain from a different source. Another method utilizes a phage display to determine the actual binding sites of the

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monoclonal antibody, the complimentarity determining regions (CDRs), and then grafting the CDRs onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis. Still other methods, known in the art, which allow the production of antibodies capable of engaging the humoral immune systems include: 1) Immunizing mice which have been 5 genetically altered to produce human antibodies; 2) Immunizing isolated human B cells in vitro and then going through a cell fusion procedure to produce a hybridoma that secretes the antibody; and 3) Isolating B cells from humans with 10 acute infection and producing an antibody generating hybridoma.

Production and administration of such genetically engineered humanized or human monoclonal antibodies to treat dental caries in man poses issues requiring innovative solutions. Prior art methods for production of monoclonal antibodies involve growing hybridomas in culture media, followed by extraction and purification of the desired antibody. These steps are 15 significantly simplified in a preferred embodiment of the invention by expressing the antibodies in edible plants or animals. The antibodies are administered upon oral ingestion of plant or animal products, such as fruits, vegetables or milk wherein the antibodies are not denatured. This mode of administration has the potential for obviating compliance issues in ameliorating tooth decay.

United States patent application Serial No. 09/378,247 discloses 20 three murine monoclonal antibodies specific to *S. mutans*: SWLA1, SWLA2 and SWLA3. Development of an effective immunological method for the treatment and prevention of dental caries requires preparation of monoclonal antibodies genetically engineered to both express monoclonal antibodies and 25 specific to *S. mutans* and engage the effector apparatus of the human immune system.

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Summary of the Preferred Embodiments

Dental caries may be prevented or treated by oral ingestion of human or humanized murine monoclonal IgG and IgM antibodies that bind to surface antigens of cariogenic organisms, such as *S. mutans*. The genetically engineered monoclonal antibodies engage the effector apparatus of the human immune system when they bind to cariogenic organisms, resulting in their destruction. In a preferred embodiment, monoclonal antibodies to cariogenic organisms are produced by edible plants, including fruits and vegetables, transformed by DNA sequences that code for expression of the desired antibodies. The genetically engineered monoclonal antibodies are applied by eating the transformed plants.

We have now isolated and sequenced the nucleotide sequences encoding the variable regions of monoclonal antibodies specific to *S. mutans*. When expressed, monoclonal antibodies encoded by these sequences bind specifically to *S. mutans*. Through the use of recombinant techniques, the variable regions of the monoclonal antibodies have been linked to the constant region of human antibodies thereby generating a chimeric monoclonal antibody that specifically binds *S. mutans*. This chimeric monoclonal antibody is directed specifically to surface antigens of cariogenic organisms which generates an effector response from the immune system upon binding to the target organism.

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Detailed Description of the Preferred Embodiments1. Preparation of Monoclonal Antibodies

5 The monoclonal antibody technique permits preparation of antibodies with extraordinary specificity. Monoclonal antibodies that bind to specific molecular structures can be produced using what are today considered standard techniques.

10 The monoclonal antibodies that may be used in this invention are those that are directed to surface antigens of cariogenic organisms. Surface antigens are substances that are displayed on the surface of cells. Such antigens are accessible to antibodies present in body fluids. In the context of the present invention, surface antigens of cariogenic organisms are present on the surface of organisms that cause dental caries. While the role of bacterial activity in the 15 genesis of carious lesions is well defined, establishing a cause and effect relationship between a particular organism and the occurrence of dental caries has not been completely successful. To date, only *S. mutans* has been definitively associated with dental caries. However, species of the *Lactobacilli* and *Actinomyces* are also believed to be involved, particularly with the active progression of carious lesions. Any organism that can produce a carious lesion is a potential target for the monoclonal antibodies prepared and used in accordance with this invention.

20 A further requirement of the monoclonal antibodies that may be used in the practice of the present invention is that they are selective for cariogenic organisms. Monoclonal antibodies directed to antigens present on cariogenic as well as non-cariogenic organisms may produce non-specific 25 alterations in the makeup of the flora within the oral cavity. The consequences of such changes are not understood.

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Accordingly, the preferred monoclonal antibodies selectively bind to surface antigens of cariogenic organisms. That is to say, the preferred monoclonal antibodies bind specifically to organisms that cause dental caries.

It should be clearly understood that the scope of the present invention is not limited to the prevention of tooth decay in man. Monoclonal antibodies in accordance with the present invention can be genetically engineered to engage the effector response of the immune system of other mammals, such as those that are domesticated as pets.

Monoclonal antibodies can be prepared by immunizing mice or other mammalian hosts with cell wall material isolated from cariogenic organisms. In a preferred embodiment, the cariogenic organisms are type c *S. mutans* (ATCC25175). The immunogenecity of molecules present in cell walls may be enhanced by a variety of techniques known in the art. In a preferred embodiment, immunogenecity of such molecules is enhanced by denaturation of the isolated cell material with formalin. Other techniques for modifying cell wall proteins to enhance immunogenecity are within the scope of this invention. Typically, hosts receive one or more subsequent injections of isolated bacterial cell fragments to increase the titer of antibodies prior to sacrifice and cloning.

Spleen cells from hosts are harvested. The NSI/Ag4.1 mouse myeloma cell line was used as the fusion partner and grown in spinner cultures in 5% CO<sub>2</sub> at 37° C and maintained in log phase of growth prior to fusion.

Hybridomas were produced according to the procedure reported by Kohler et al. *Nature*, 256:495-497, (1975). Hybrids were selected in media containing HAT (100 µg Hypoxanthine, 0.4 µM Aminopterin; 16 µM Thymidine). HT (100 µg Hypoxanthine; 16 µM Thymidine) was maintained in the culture medium for 2 weeks after aminopterin was withdrawn. OPI (1 mM oxaloacetate, 0.45 mM pyruvate and 0.2 U/ml bovine insulin) was added as additional growth factors to

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the tissue culture during cloning of the hybridomas. The hybridomas were further cloned by limiting dilution using techniques that have become standard since the pioneering work of Kohler and Milstein. In a preferred embodiment, surviving hybridomas were screened for antibody directed to cariogenic organisms by ELISA assay against microtiter plates coated with formalinized bacterial cell material. Positive supernatants were subjected to further screening to identify clones that secrete antibodies with the greatest affinity for the cariogenic organisms. In a preferred embodiment, clones with titers at least three times higher than background are screened again using immunoprecipitation with denatured cell wall material from *S. mutans*. In a preferred embodiment, three clones were identified which bound detectably only to *S. mutans* strains ATCC25175, LM7, OMZ175 and ATCC31377. These clones were deposited with the American Type Culture Collection, receiving Deposit Numbers HB 12599 (SWLA1), HB 12560 (SWLA2), and HB 12558 (SWLA3). United States patent application serial number 09/378,247.

There are various ways to obtain nucleic acid sequences that code for expression of human or humanized monoclonal antibodies specific for the surface antigens of cariogenic organisms: 1) Isolating murine hybridomas which produce monoclonal antibodies against cariogenic organisms and cloning murine genes that code for expression of those antibodies; 2) Using purified cariogenic organisms to screen a phage display random library made from human B lymphocytes to obtain genes that encode antibodies specific for cariogenic organisms; 3) Isolating human hybridomas that produce monoclonal antibodies against cariogenic organisms, using B lymphocytes recovered from heavily infected patients and cloning the human genes encoding these antibodies; or 4) Immunizing human B lymphocytes and spleen cells *in vitro* using purified cariogenic organisms, followed by fusion to form hybridomas to

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create immortal cell lines. The techniques required are known to those skilled in the art and are not limited to the methods described herein.

2. Preparation of Monoclonal Antibodies Capable Of Eliciting An Effector Response From Human Immune System

Previous efforts to develop an immunological method for the prevention of dental caries employed heterologous antibodies. For example, Lehner, United States patent 5,352,446, refers to use of monoclonal antibodies to *S. mutans* surface antigens raised in mice in inhibiting the proliferation of those bacteria in monkeys. More recently, Ma et al. *Nature Medicine*, 45(5) 601-6 (1998), reported similar results in humans, using a genetically engineered secretory monoclonal murine antibody to *S. mutans* expressed in tobacco plants. Drawbacks to this approach include 1) administration may aggregate the offending organisms, but not kill them because the non-human antibodies do not effectively engage the human immune response; and 2) repeated administration of the antibody may elicit an immune response from the patient to the antibody. A preferable approach is to use recombinant techniques to prepare chimeric antibody molecules directed specifically to surface antigens of cariogenic organisms, that will also elicit an effector response from the immune system of the mammal treated therewith upon binding to the target organism. This can be accomplished by inserting variable regions from murine monoclonal antibodies that are specific to cariogenic organisms into antibodies of the IgG and/or IgM classes from the mammal to be treated. It is also possible to generate antibodies that utilize just the complementarity determining regions (CDRs) of a murine monoclonal antibody specific to cariogenic organisms. Through known recombinant techniques, the CDRs are transferred into the immunoglobulin's variable domain.

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Methods are also known for generating the antibody directly, for example: 1) Immunizing mice which have been genetically altered to produce human antibodies; 2) Immunizing isolated human B lymphocytes in vitro and then going through a cell fusion procedure to produce a hybridoma that secretes the antibody; and 3) Isolating B lymphocytes from humans with acute infection and producing an antibody generating hybridoma. The techniques required are known to those skilled in the art. Because each method produces a human antibody, the antibodies are capable of engaging the humoral immune effector systems upon binding to their specific antigens.

In the presently preferred embodiment of the invention, chimeric antibodies specific to *S. mutans* were generated. Using PCR or Southern blot techniques, DNA fragments encoding the variable domains of murine hybridomas secreting antibody specific to cell surface antigens of cariogenic organisms were isolated. Using gene cloning techniques, the variable regions were joined to the constant regions of human immunoglobulins. The result of this genetic engineering is a chimeric antibody molecule with variable domains that selectively bind to surface antigens of cariogenic organisms, but which interacts with the human immune effector systems through its constant regions.

3. Administration of Monoclonal Antibodies

In order to prepare a sufficient quantity of monoclonal antibodies for clinical use, the desired cell line, transfected with sequences encoding the immunoglobulin, must be propagated. Existing technology permits large scale propagation of monoclonal antibodies in tissue culture. The transfected cell lines secrete monoclonal antibodies into the tissue culture medium. The secreted monoclonal antibodies were recovered and purified by gel filtration and related techniques of protein chemistry.

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In experimental studies, monoclonal antibodies to *S. mutans* have been applied directly to the surface of teeth. Application by ingestion of mouthwash, or by chewing gum has also been proposed. A presently preferred alternative is to express the chimeric monoclonal antibodies of the present invention in edible plants, such as banana or broccoli. Eating plants  
5 transformed in accordance with this invention will result in application of the antibodies to cariogenic organisms present on tooth surfaces, and elsewhere in the mouth. It is also contemplated that other organisms, both plant and animal, may be transformed to express the monoclonal antibodies described herein, so  
10 that such antibodies may be ingested, for example, by drinking milk.

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Brief Description of the Drawings

The present invention is now described, by the way of illustration only, in the following examples which refer to the accompanying FIGS. 1-8, in which:

5 FIG. 1 shows the DNA sequences (SEQ ID NOS: 1 and 3) encoding the variable regions of the chimeric antibody (TEDW) specific to *S. mutans* derived from SWLA1 cells together with the predicted amino acid sequences (SEQ ID NOS: 2 and 4).

10 FIG. 2 shows the DNA sequences (SEQ ID NOS: 5 and 7) encoding the variable regions of the chimeric antibody (TEFE) specific to *S. mutans* derived from SWLA2 cells together with the predicted amino acid sequences (SEQ ID NOS: 6 and 8).

15 FIG. 3 shows the DNA sequences (SEQ ID NOS: 9 and 11) encoding the variable regions of the chimeric antibody (TEFC) specific to *S. mutans* derived from SWLA3 cells together with the predicted amino acid sequences (SEQ ID NOS: 10 and 12).

20 FIG. 4 shows the DNA sequence (SEQ ID NO: 13) encoding an aberrant light chain variable region derived from SWLA1 cells together with the predicted amino acid sequence (SEQ ID NO: 14).

25 FIG. 5 shows the DNA sequence (SEQ ID NO: 15) encoding a non-effective heavy chain variable region derived from SWLA1 cells together with the predicted amino acid sequence; (SEQ ID NO: 16).

FIG. 6 shows the DNA sequence (SEQ ID NO: 17) encoding an aberrant heavy chain variable region derived from SWLA1 cells together with the predicted amino acid sequence; (SEQ ID NO: 18).

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FIG. 7 shows the DNA sequence (SEQ ID NO: 19) encoding an aberrant heavy chain variable region derived from SWLA2 cells together with the predicted amino acid sequence; (SEQ ID NO: 20).

FIG. 8 shows light and fluorescent microscope images of chimeric antibody TEDW binding to *S. mutans*.

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Examples

5 1. Producing murine monoclonal antibodies against *S. mutans*

Type c *S. mutans* strain ATCC25175 were grown to log phase in BHI medium and washed twice with phosphate buffered saline, pH 7.2 (PBS), by centrifugation at 3000xg for 5 min. The pellet was resuspended in 1% 10 formalin/0.9% NaCl, mixed at room temperature for 30 min and washed twice with 0.9% NaCl. BALB/c mice (8-10 weeks) were immunized intraperitoneally with 100  $\mu$ l of the antigen containing approximately  $10^8$  whole cells of formalinized intact *S. mutans* bacteria emulsified with Freund's incomplete 15 adjuvant (FIA). After 3-5 weeks, mice received a second dose of antigen ( $10^8$  whole cells of bacteria in FIA). Three days prior to sacrifice, the mice were boosted intravenously with  $10^8$  whole cells of bacteria in saline.

Spleen cells from hosts were harvested. The tissue culture medium used was RPMI 1640 (Gibco) medium supplemented with 2 mM L-glutamine, 1mM sodium pyruvate, and 10 mM HEPES and containing 100  $\mu$ g/ml penicillin and 100  $\mu$ g/ml streptomycin with 10% fetal calf serum. The 20 NSI/Ag4.1 mouse myeloma cell line was used as the fusion partner and grown in spinner cultures in 5% CO<sub>2</sub> at 37° C and maintained in log phase of growth prior to fusion. Hybridomas were produced according to the procedure reported by Kohler et al. *Nature*, 256:495-497, (1975). Hybrids were selected in medium 25 containing HAT (100  $\mu$ g Hypoxanthine, 0.4  $\mu$ M Aminopterin; 16  $\mu$ M Thymidine). HT (100  $\mu$ g Hypoxanthine; 16  $\mu$ M Thymidine) was maintained in the culture medium for 2 weeks after aminopterin was withdrawn. OPI (1 mM oxaloacetate, 0.45 mM pyruvate and 0.2 U/ml bovine insulin) was added as additional growth factors to the tissue culture during cloning of the hybridomas.

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The hybridomas were further cloned by limiting dilution using techniques that have become standard since the pioneering work of Kohler and Milstein.

The following approach was used for screening for species-specific monoclonal antibodies against *S. mutans*. The initial screening was performed using an ELISA assay, which selects for the culture supernatants containing antibodies that bind to *S. mutans*. Formalinized bacteria were diluted in PBS to OD<sub>600</sub> = 0.5, and added to duplicate wells (100 µl) in 96 well PVC ELISA plates preincubated for 4 h with 100 µl of 0.02 mg/ml Poly-L-lysine. These antigen-coated plates were incubated overnight at 4°C in a moist box then washed 3 times with PBS and blocked with 0.5% fetal calf serum in PBS and stored at 4°C. 100 µl of mature hybridoma supernatants were added to the appropriate wells of the antigen plates, incubated for 1 h at room temperature, washed 3 times with PBS-0.05% Tween 20, and bound antibody was detected by the addition of polyvalent goat-anti-mouse IgG antibody conjugated with alkaline phosphatase diluted 1:1000 with PBS-1% fetal calf serum. After the addition of the substrate, 1 mg/ml p-nitrophenyl phosphate in carbonate buffer (15 mM Na<sub>2</sub>CO<sub>3</sub>, 35 mM NaH<sub>2</sub>CO<sub>3</sub>, 10 mM MgCl<sub>2</sub> pH 9.6), the color development after 15 min was measured in an EIA reader at 405 nm. The positive supernatants (3 fold higher than control) were then subjected to the immunoprecipitation assay (mixing 100 µl bacteria with 100 µl supernatant) to screen for those with strong positive reactivity with *S. mutans*. The deposited clones (ATCC HB 12599, HB 12560, and HB 12558) were prepared according to this method.

2. Preparation of Hybridoma lines for cloning of V regions.

A. Isotyping

The hybridoma supernatants were isotype with a Pharmigen Isotyping Kit (BD Pharmigen, San Deigo, CA). 200 µl of isotype specific rat

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anti-mouse antibody was diluted in 800  $\mu$ l of coating buffer and 50  $\mu$ l of each reagent was added to 10 wells of a 96 well polystyrene ELISA plate. Plates were incubated overnight at 4°C. The plate was washed four times with washing buffer, 0.05% Tween-20 in PBS, and the remaining contents shaken out and the plate blotted dry on a paper towel. 200  $\mu$ l of blocking solution, 1% BSA in PBS, was added to each well and the plate was incubated at room temperature for 30 minutes. Plates were again washed four times and the contents shaken out. 50  $\mu$ l of hybridoma supernatant was added to the appropriate wells. Positive controls from the kit were added to the appropriate wells. The plate was incubated at room temperature for one hour. The plate was washed five times with washing buffer and the plate was blotted dry. One phosphatase substrate tablet was dissolved in 5.0 ml of p-NNP substrate diluent. 50  $\mu$ l of substrate solution was added to each well and the plate was incubated for 40 minutes. The plate was read at 405nm on a Dynatech MR 700 microplate reader. SWLA1, SWLA2 and SWLA3 were all determined to be  $\gamma$ 2a, $\kappa$  (IgG).

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25 B. Biosynthetic labeling

Cells were washed twice in methionine-free, Dulbecco's modified Eagle's medium (DME, Irvine Scientific) supplemented with non-essential amino acids (Grand Island Biological) and glutamine (29.2 $\mu$ g/ml). Cells were labeled in 1 ml of DME with 15  $\mu$ Ci [ $^{35}$ S]methionine (Amersham, Arlington Heights, IL). All labels were done using  $3 \times 10^6$  cells.

For labeling of secretions,  $^{35}$ S-methionine was added to 15  $\mu$ Ci/ml and cells were labeled for 3 hours at 37°C. Cells were harvested on to ice and pelleted by centrifugation. To isolate secreted IgG, the radioactive medium was transferred to a clean tube.

For measurement of cytoplasmic IgG, the cell pellet was lysed in 0.5 ml of NDET (1 % NP40, 0.4% deoxycholate, 66mM EDTA and 10mM Tris,

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pH 7.4), nuclei were pelleted by centrifugation, and the cytoplasmic lysate transferred to a fresh tube. To immunoprecipitate the secreted or cytoplasmic IgG, rat anti-mouse kappa sepharose (prepared in the laboratory) was added. The samples were mixed overnight at 4°C, washed in NDET and then washed with dH<sub>2</sub>O. The precipitates were resuspended in sample buffer (25mM Tris, 5 pH 6.7, 2% SDS, 10% glycerol, 0.008% bromophenol blue), and the antibodies were eluted from the sepharose by boiling. The samples were analyzed by SDS-PAGE and autoradiography without reduction on 5% phosphate gels; samples treated with 2-mercaptoethanol were analyzed using 12% tris-glycine 10 gels. Results indicate that all three clones made the same size heavy chain but different size light chains. All three hybridomas were subcloned to ensure homogenous cell populations.

C. Subcloning

The hybridomas were subcloned on soft agar. A 60mm petri dish was coated with 5 ml of growth media plus 10% J774.2 (a murine 15 macrophage cell line) supernatant plus 0.24% agarose (Sigma). The agarose was allowed to harden and a single cell suspension of hybridoma cells mixed with agarose was layered on top. When colonies were about 64 cells in size, they were overlaid with rabbit anti-mouse  $\gamma$ 2a specific antiserum mixed with agarose. An immune precipitate forms over and partially obscures those clones secreting  $\gamma$ 2a, $\kappa$  antibody. Colonies making the most antibody, were identified 20 and moved up to bulk culture where they were once again biosynthetically labeled.

3. Cloning Variable Regions from SWLA Cells

The basic protocol for cloning the variable regions from SWLA 25 cells is outlined below followed by its application to specific SWLA hybridomas.

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- (i) Murine mRNA is made from about  $5 \times 10^6$  of both the original and subcloned cells using the Microfast Track Kit from Invitrogen.
- (ii) First strand cDNA is made using oligonucleotides that prime the 5' of the light or heavy chain constant region or that prime to the polyA tail of mRNA.

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Basic Protocol:

- (a) Half of the cDNA is resuspended in 20  $\mu$ l RNase free dH<sub>2</sub>O
- (b) 2  $\mu$ l of 0.5 mg/ml primer is added; the mixture is incubated @ 60°C for 10 minutes
- (c) The sample is cooled on ice; 8  $\mu$ l of 5X first strand cDNA buffer, 2  $\mu$ l of RNasin (Promega), 4  $\mu$ l of 5mM dNTP, and 0.5  $\mu$ l of AMV Reverse Transcriptase are then added
- (d) The sample is incubated @ 42°C for 1 hour

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- (iii) PCR amplification is done with a number of different light or heavy chain signal peptide primers and primers that hybridize 5' of the light or heavy chain constant region.

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PCR Conditions:

- (a) Denature @ 94°C for 40 sec.
- (b) Anneal @ 60°C for 40 sec.
- (c) Extend @ 72°C for 40 sec.
- (d) Amplify for 30 cycles
- (e) Final Extension at 72°C for 10 min.

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(iv) The resulting PCR products are cloned into Invitrogen's PCR2.1 vector via the TOPO Cloning Kit.

(v) Individual clones were sent out for sequencing. The results were analyzed for an open reading frame (ORF) and compared with the known database to ensure that the sequence cloned is a variable region.

5 (vi) To check for PCR induced nucleotide alterations in the sequence, steps III to V were repeated so that the sequence of different clones from independent PCR reactions can be compared to ensure the accuracy of the sequence. The sequence data are also used to determine the sequence of the J region primer that needs to be used.

10 (vii) The variable region was cloned into the proper light (human kappa) or heavy chain (human IgG1) expression vector.

Typical Variable Region Ligation Protocol:

15 (a) 1 µg each of vector and insert was cut with either NheI/EcoRV or SalI/EcoRV

(b) The relevant fragments were then isolated using Qiagen's Gel Extraction Kit

(c) The ligation reaction used 4 µl out of 30 µl of vector sample and 6 µl out of 30 µl of insert sample

20 (d) 4 to 5 units of T4 DNA ligase was then added for a 20 µl final reaction volume

Typical Transformation Protocol:

25 (a) An aliquot of HB101 chemically competent *E. coli* cells were thawed on ice

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- (b) The entire ligation reaction was then added to the cells and incubated on ice for 15 minutes
- (c) The cells were then heat shocked @ 42°C for 1 minute
- (d) 1 ml of LB was added to the cells and the tube was shaken @ 37°C for 1 hour
- 10 (e) The tube was spun down @ 8000 RPM for 2 minutes
- (f) All but 100 µl of supernatant was removed
- (g) The cells were resuspended, plated onto LB+AMP plates, and incubated @ 37°C overnight

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B. Cloning the Variable Regions from SWLA1 cells

In attempting to clone the light chain variable region (VL), PCR product was found using signal peptide primer 442 with constant region primer 450 as shown below. Previous studies have determined that 442 also primes to an endogenous aberrant or non-productive VL, SWLA1 Aberrant VL (SEQ ID NO: 13). Knowing this, attempts were made to enrich for non-aberrant transcripts by restriction digesting the PCR product with PflMI, which recognizes a specific sequence in the aberrant VL. Eventually, one variable region sequence was found to have an ORF. The final PCR product SWLA1 VL (SEQ ID NO: 1) was generated with primer 442 and J region primer 453 as shown below and inserted into the appropriate expression vector. The resulting human kappa expression vector carrying the VL from SWLA1 is named 5936 pAG.

20

25

See FIG. 1 Panel A which shows the sequence coding the VL domain and the predicted amino acid sequence (SEQ ID NOS: 1 and 2) and

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FIG. 4 which shows the sequence coding the aberrant VL and the predicted amino acid sequence (SEQ ID NOS: 13 and 14)

442 (SEQ ID NO: 21) 5' GGG GAT ATC CAC ATG GAG ACA GAC  
ACA CTC CTG CTA T 3'

5 450 (SEQ ID NO: 22) 5' GCG TCT AGA ACT GGA TGG TGG GAA  
GAT GG 3'

10 453 (SEQ ID NO: 23) 5' AGC GTC GAC TTA CGT TTK ATT TCC  
ARC TTK GTC CC 3'

15 The cloning of the heavy chain variable region (VH) resulted in finding two unique VHs both with ORFs. One VH uses signal peptide primer 440 and the other uses signal peptide primer 441 as shown below. In both reactions, the heavy chain constant region primer 451 was used. Two final PCRs were done. The first used J region primer 452 with primer 440 which generated SWLA1 VH (SEQ ID NO: 3) and the second used the same J region primer with primer 441 which produced SWLA1 2nd VH (SEQ ID NO: 15) and an aberrant non-productive VH, SWLA1 Aberrant VH (SEQ. ID NO: 17). The resulting human IgG1 expression vectors carrying the two different VHs generated are named 5937 pAH (SWLA1 VH) and 5943 pAH (SWLA1 2nd VH). Only vector 5937 pAH however was found to express an effective full length VH.

20

25 The DNA coding the VH domain and the predicted amino acid sequence are shown in FIG. 1 Panel B as SEQ ID NOS: 3 and 4. See FIG. 5 for the non-effective 2nd VH DNA and amino acid sequence (SEQ ID NOS: 15 and 16) and FIG. 6 for the DNA and amino acid sequence for the aberrant VH (SEQ ID NOS: 17 and 18).

- 23 -

440 (SEQ ID NO: 24) 5' GGG GAT ATC CAC ATG RAC TTC GGG  
YTG AGC TKG GTT TT 3'

441 (SEQ ID NO: 25) 5' GGG GAT ATC CAC ATG GCT GTC TTG  
GGG CTG CTC TTC T 3'

5 451 (SEQ ID NO: 26) 5' AGG TCT AGA AYC TCC ACA CAC AGG  
RRC CAG TGG ATA GAC 3'

10 452 (SEQ ID NO: 27) 5' TGG GTC GAC WGA TGG GGS TGT TGT  
GCT AGC TGA GGA GAC 3'

C. Cloning the Variable Regions from SWLA2 cells

Two PCR products were found in cloning the VL. One product came from primers 442 and 450. The other came from primer 443 and primer 450. A unique VL with an ORF was cloned from the 443 and 450 reaction. 15 The final PCR, which generated the SWLA2 VL (SEQ ID NO: 5), used J region primer 453 with primer 443. The resulting human kappa expression vector carrying the VL from SWLA2 is named 5938 pAG.

See FIG. 2 Panel A which shows the sequence coding the VL domain and the predicted amino acid sequence (SEQ ID NOS: 5 and 6).

20 443 (SEQ ID NO: 28) 5' GGG GAT ATC CAC ATG GAT TTT CAA  
GTG CAG ATT TTC AG 3'

Two PCR products were also found in cloning the VH. One product came from primers 439 and 451. The other product came from primers 440 and 451. The transcript from the former reaction turned out to be aberrant, 25 SWLA2 Aberrant VH (SEQ ID NO: 19). The transcript from the latter reaction was missing part of its 5' sequence. After aligning this sequence to several

- 24 -

similar known VHs, a new leader signal peptide primer 843 was designed as shown below. The final PCR product SWLA2 VH (SEQ ID NO: 7) was generated with primer 843 with J region primer 452. The resulting human IgG1 expression vector carrying the VH from SWLA2 is named 5939 pAH.

5 The DNA coding the VH domain and the predicted amino acid sequence are shown in FIG. 2 Panel B as SEQ ID NOS: 7 and 8. See FIG. 7 for the DNA and amino acid sequence for the aberrant VH (SEQ ID NOS: 19 and 20).

10

439 (SEQ ID NO: 29) 5' GGG GAT ATC CAC ATG GRA TGS AGC  
TGK GTM ATS CTC TT 3'

843 (SEQ ID NO: 30) 5' GGG ATA TCC ACC ATG GRC AGR CTT  
ACW TYY TCA TTC CTG 3'

15

#### D. Cloning the Variable Regions from SWLA3 cells

The only VL PCR product came from primer combination 442 and 450. Once again the PCR product was digested with PflMI to enrich for non-aberrant transcripts. This procedure didn't help. Another enzyme Eco0109I was used similarly and one transcript was found with the 5' end missing. The sequence was compared to the known database and a new signal peptide primer 826 was designed as shown below. This primer 826 was then used with J region primer 835 shown below to yield the final PCR product SWLA3 VL (SEQ ID NO: 9). It was cloned into a human kappa expression vector and named 5940 pAG.

20

25 See FIG. 3 Panel A which shows the sequence coding the VL domain and the predicted amino acid sequence (SEQ ID NOS: 9 and 10).

- 25 -

826 (SEQ ID NO: 31) 5' GGG GAT ATC CAC ATG ATG AGT CCT  
GCC CAG TTC C 3'

835 (SEQ ID NO: 32) 5' GGT CGA CTT AGC TTT CAG CTC CAG  
CTT GGT 3'

5 The only VH PCR product was obtained from primer combination 440 and 451. The final PCR reaction used primer 440 and J region primer 452 to generate SWLA3 VH (SEQ ID NO: 11). The VH was cloned into 10 a human IgG1 expression vector and named 5941 pAH.

The DNA coding the VH domain and the predicted amino acid sequence are shown in FIG. 3 Panel B as SEQ ID NOS: 11 and 12.

4. Generating murine/human chimeric genes which encode humanized monoclonal antibodies against *S. mutans*.

15 (i) DNA was prepared from the expression vectors and from the plasmid containing the correct V regions. See *Current Protocols in Immunology*, Section 2.12.1 (1994) for detailed information about the vectors that express the light and heavy chain constant regions.

(ii) The expression vector was digested with the appropriate restriction enzyme. The digests were then electrophoresed on an agarose gel to isolate the appropriate sized fragment.

20 (iii) The plasmid containing the cloned V region was also digested and the appropriate DNA fragment containing the V region was isolated from an agarose gel.

(iv) The V region and expression vector were then mixed together, T4 DNA ligase was added and the reaction mixture was incubated at 16°C over night.

25 (v) Competent cells were transfected with the ligation mixture and the clones expressing the correct ligation

- 26 -

sequence were selected. Restriction mapping was used to confirm the correct structure.

5. Transfected eukaryotic cells

10 micrograms of DNA from each expression vector was linearized by BSPC 1 (Stratagene, Pvul isoschizomer) digestion and 1 X 10<sup>7</sup> myeloma cells (Sp2/0 or NSO/1) were cotransfected by electroporation. Prior to transfection the cells were washed with cold PBS, then resuspended in 0.9 ml of the same cold buffer and placed in a 0.4 cm electrode gap electroporation cuvette. 960 microF and 200V were used for electroporation. The shocked cells were then incubated on ice in IMDM medium (Gibco, NY) with 10% calf serum.

15 The transfected cells were plated into 96 well plates at a concentration of 10<sup>4</sup> cells/well. Selective medium including selective drugs such as histidinol or mycophenolic acid were used to select the cells which contain expression vectors. After 12 days, the supernatants from growing clones were tested for antibody production.

20 6. Analyses of recombinant antibodies

25 ELISA assay was used to identify transfectomas that secrete human IgG antibodies. 100  $\mu$ l of 5  $\mu$ g/ml goat anti-human IgG was added to each well of a 96-well ELISA plate and incubated overnight. The plate was washed several times with PBS and blocked with 3% BSA. Supernatants from above growing clones were added to the plate for 2 hours at room temperature. Plates were then washed and anti-human kappa antibody labeled with alkaline phosphatase diluted 1:10,000 in 1% BSA was added for 1 hour at 37° C. Plates were washed with PBS and p-NPP in diethanolamine buffer (9.6% diethanolamine, 0.24 mM MgCl<sub>2</sub>, pH 9.8) was added. Color development at OD<sub>405</sub> was indicative of cells producing H<sub>2</sub>L<sub>2</sub>.

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For the supernatants that produce humanized IgG constant regions, their reactivity with *S. mutans* was tested as described in Shi et al., *Hybridoma* 17:365-371 (1998). Briefly, bacteria strains listed in Table 1 were grown in various media suggested by the American Type Culture Collection. The anaerobic bacteria were grown in an atmosphere of 80% N<sub>2</sub>, 10% CO<sub>2</sub>, and 10% H<sub>2</sub> at 37° C. The specificity of antibodies to various oral bacteria was assayed with ELISA assays. Bacteria were diluted in PBS to OD<sub>600</sub>=0.5, and added to duplicate wells (100 µl) in 96 well PVC ELISA plates preincubated for 4 h with 100 µl of 0.02 mg/ml Poly-L-lysine. These antigen-coated plates were incubated overnight at 4° C in a moist box then washed 3 times with PBS and blocked with 0.5% fetal calf serum in PBS and stored at 4° C. 100 µl of chimeric antibodies at 50 µg/ml were added to the appropriate wells of the antigen plates, incubated for 1 h at RT, washed 3 times with PBS-0.05% Tween 20, and bound antibody detected by the addition of polyvalent goat-anti-human IgG antibody conjugated with alkaline phosphatase diluted 1:1000 with PBS-1% fetal calf serum. After the addition of the substrate, 1 mg/ml p-nitrophenyl phosphate in carbonate buffer (15 mM Na<sub>2</sub>CO<sub>3</sub>, 35 mM NaH<sub>2</sub>CO<sub>3</sub>, 10 mM MgCl<sub>2</sub> pH 9.6), the color development after 15 min was measured in a EIA reader at 405 nm. "+" means OD405>1.0; "-" means OD405<0.05. The negative control is <0.05. Chimeric antibodies used are TEDW (derived from SWLA1), TEFE (derived from SWLA2) and TEFC (derived from SWLA3). The results are given in Table 1.

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**TABLE 1**

Reactivity of Chimeric Antibodies to Various Oral Bacterial Strains

	Oral Bacteria	Strains	Chimeric antibodies
5	<i>S. mutans</i>	AATCC25175	+
		LM7	+
		OMZ175	+
	<i>S. Mitis</i>	ATCC49456	-
	<i>S. ratus</i>	ATCC19645	-
	<i>S. sanguis</i>	ATCC49295	-
	<i>S. sobrinus</i>	ATCC6715-B	-
	<i>S. sobrinus</i>	ATCC33478	-
	<i>L. acidophilus</i>	ATCC4356	-
	<i>L. casei</i>	ATCC11578	-
	<i>L. plantarum</i>	ATCC14917	-
	<i>L. salivarius</i>	ATCC11742	-
	<i>A. actinomycetemcomitans</i>	ATCC33384	-
	<i>A. naeslundi</i>	ATCC12104	-
10	<i>A. viscosus</i>	ATCC19246	-
	<i>Fusobacterium nucleatum</i>	ATCC25586	-
15	<i>Porphyromonas gingivalis</i>	ATCC33277	-

FIG. 8 shows fluorescent microscopy images generated using the chimeric TEDW antibody derived from SWLA1. *S. mutans* ATCC25175 was grown in Brain-Heart Infusion medium in an atmosphere of 80% N<sub>2</sub>, 10% CO<sub>2</sub>, and 10% H<sub>2</sub> at 37°C. Bacteria were then washed and resuspended in PBS buffer, mixed with various antibodies and examined with light microscopy or fluorescent microscopy. Referring to FIG. 8: Left, chimeric antibodies bind and agglutinate *S. mutans* cells; middle, chimeric antibodies interact with goat, FITC conjugated anti-human IgG (Fc specific) antibody (Sigma F9512) to give fluorescent image of *S. mutans*; right, chimeric antibodies do not react with goat, FITC conjugated anti-mouse IgG (Fc specific) antibody (Sigma F5387) and give no fluorescent image of *S. mutans*. Chimeric antibodies TFE and

- 29 -

TEFC were also used and produced results consistent with the TEDW chimeric antibody.

Results from both the flow cytometry and fluorescent microscopy experiments indicate that each chimeric antibody (TEDW, TEFE, and TEFC) contained both a human IgG constant region and a variable region capable of specifically recognizing *S. mutans*.

5           7. Expressing Monoclonal Antibodies to *S. mutans* In Transformed Organisms

10           A. Producing human or humanized monoclonal antibodies in animal cells

The heavy and light chain of a human IgG gene are separately introduced or cotransfected into an animal cell line (such as Sp2/0) using electroporation. The transfected cells are plated onto a microtiter plate and incubated at 37° C in a 5% CO<sub>2</sub> atmosphere in medium containing 10% fetal bovine serum. After a 48 h incubation, the cells are grown in selection medium containing histidinol or mycophenolic acid. The supernatants of drug-resistant cells are collected and screened for immuno-reactivity against *S. mutans* using the ELISA or precipitation assays mentioned above.

15           B. Producing human or humanized monoclonal antibodies in edible plants

20           Transgenic plants have been recognized as very useful systems to produce large quantities of foreign proteins at very low cost. Expressing human or humanized monoclonal antibodies against *S. mutans* in edible plants (vegetables or fruits) allows direct application of plant or plant extracts to the mouth to treat existing dental caries and to prevent future bacterial infection. The choice of transgenic, edible plants includes, but is not limited to, potato, tomato, broccoli, corn, and banana.

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Presented here are the procedures to produce transgenic *Arabidopsis*, an edible plant closely related to *Brassica* species including common vegetables such as cabbage, cauliflower and broccoli. It is chosen because many genetic and biochemical tools have been well developed for this plant. There are several strategies to express IgG in this plant. One strategy is to first introduce the human IgG genes encoding the heavy chain and light chain to two separate transgenic lines. The two genes are brought together by genetic crossing and selection. Other methods involve sequential transformation, in which transgenic lines transformed with one IgG gene are re-transformed with the second gene. Alternatively, genes encoding the heavy chain and light chain are cloned into two different cloning sites in the same T-DNA transformation vector under the control of two promoters, and the expression of both genes can be achieved by the transformation of a single construct to plant. Technically, the separate transformation method is the simplest one and it usually results in higher antibody yield. Therefore, we present this strategy here. It is possible to transform other plants using similar techniques.

The DNA fragments encoding the heavy and light chains of a human IgG gene are separately cloned into a Ti plasmid of *Agrobacterium tumefaciens*. The plasmid contains a promoter to express human heavy and light chains of IgG in *Arabidopsis thaliana*, an antibiotic marker for selection in *Agrobacterium tumefaciens* and an herbicide resistance gene for transformation selection in *Arabidopsis*. An *Agrobacterium tumefaciens* strain is transformed with these plasmids, grown to late log phase under antibiotic selection, and resuspended in infiltration medium described by Bethold et al. (C.R. Acad. Sci. Paris Life Sci. 316:1194-1199, 1993).

Transformation of *Arabidopsis* by Ti-plasmid containing *Agrobacterium tumefaciens* is performed through vacuum infiltration. Entire

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plants of *Arabidopsis* are dipped into the bacterial suspension. The procedure is performed in a vacuum chamber. Four cycles of 5 min vacuum (about 40 cm mercury) are applied. After each application, the vacuum is released and reapplied immediately. After infiltration, plants are kept horizontally for 24 h in a growth chamber. Thereafter, the plants are grown to maturity and their seeds  
5 are harvested. The harvested seeds are germinated under unselective growth condition until the first pair of true leaves emerged. At this stage, plants are sprayed with the herbicide Basta at concentration of 150 mg/l in water. The  
10 *arabidopsis* plants containing transformed Ti plasmids are resistant to the herbicide while the untransformed plants are bleached and killed. Such a selection continues to the second generation of the plants. For the resistant plants, total genomic DNA is isolated and probed with the DNA fragments encoding heavy and light chains of the IgG gene. The plant extracts from the positive transformants are prepared and screened for the expression of human  
15 IgG protein with Western blot using antibodies against heavy and light chains of constant regions of human IgG.

The plants expressing human IgG heavy chain are sexually crossed with plants expressing human IgG light chain to produce progeny expressing both chains. Western blotting is used to screen the both heavy and light chains. Extracts from positive transformants are collected and screened for immuno-reactivity against *S. mutans* using the ELISA or precipitation assays  
20 mentioned above.

8. Using human or humanized monoclonal antibodies against *S. mutans* to treat or prevent human dental caries

With the successful completion of the above studies, humanized monoclonal antibodies against *S. mutans* are obtained. The plant tissue is tested  
25 for efficacy.

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Plant tissue extracts containing monoclonal antibodies to *S. mutans* are mixed with various concentrations of *S. mutans* in the presence and absence of purified human complement components or purified human polymorphonuclear neutrophilic leukocytes. After a two hour incubation, the mixtures are plated onto BHI plates to examine the bactericidal activity.

5           Using the artificial plaque formation system developed by Wolinsky et al., *J. Dent. Res.* 75:816-822 (1996), plant tissue extracts containing monoclonal antibodies are used to examine the ability of the expressed monoclonal antibodies to kill *S. mutans* in saliva or in existing dental 10 plaques on artificial dental enamel. Analogous techniques are used to examine the ability to prevent the formation of dental plaques.

15           Human clinical trials are performed using these monoclonal antibodies produced through animal cells or plants. Human volunteers are treated with or without these human monoclonal antibodies against *S. mutans*. Then the level of *S. mutans* in saliva and in dental plaques is examined. The correlation between present and future dental caries in relationship with treatment of monoclonal antibodies is also examined.

20           It should be understood that the foregoing examples are for illustrative purposes only, and are not intended to limit the scope of applicants' invention which is set forth in the claims appearing below.

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Claims

What is claimed is:

1. A method for treatment and prevention of dental caries in a mammal comprising oral administration of a genetically engineered antibody, wherein the variable region of the antibody specifically binds to a cariogenic organism and the constant region of the antibody engages the humoral immune effector systems.

5 2. The method for treatment and prevention of dental caries of claim 1 wherein the cariogenic organism is *Streptococcus mutans*.

10 3. The method for treatment and prevention of dental caries of claim 2 wherein the variable region of the light chain of the antibody comprises the nucleic acid sequence of SEQ ID NO: 1.

4. The method for treatment and prevention of dental caries of claim 2 wherein the variable region of the heavy chain of the antibody comprises the nucleic acid sequence of SEQ ID NO: 3.

15 5. The method for treatment and prevention of dental caries of claim 2 wherein the variable region of the light chain of the antibody comprises the nucleic acid sequence of SEQ ID NO: 5.

6. The method for treatment and prevention of dental caries of claim 2 wherein the variable region of the heavy chain of the antibody comprises the nucleic acid sequence of SEQ ID NO: 7.

7. The method for treatment and prevention of dental caries of claim 2 wherein the variable region of the light chain of the antibody comprises the nucleic acid sequence of SEQ ID NO: 9.

20 8. The method for treatment and prevention of dental caries of claim 2 wherein the variable region of the heavy chain of the antibody comprises the nucleic acid sequence of SEQ ID NO: 11.

25 9. The method for treatment and prevention of dental caries wherein the variable region of the light chain of the antibody of claim 2 comprises the amino acid sequence of SEQ ID NO: 2.

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10. The method for treatment and prevention of dental caries wherein the variable region of the heavy chain of the antibody of claim 2 comprises the amino acid sequence of SEQ ID NO: 4.

11. The method for treatment and prevention of dental caries wherein the variable region of the light chain of the antibody of claim 2 comprises the amino acid sequence of SEQ ID NO: 6.

5 12. The method for treatment and prevention of dental caries wherein the variable region of the heavy chain of the antibody of claim 2 comprises the amino acid sequence of SEQ ID NO: 8.

10 13. The method for treatment and prevention of dental caries wherein the variable region of the light chain of the antibody of claim 2 comprises the amino acid sequence of SEQ ID NO: 10.

14. The method for treatment and prevention of dental caries wherein the variable region of the heavy chain of the antibody of claim 2 comprises the amino acid sequence of SEQ ID NO: 12.

15 15. A method for treatment and prevention of dental caries in a mammal comprising oral administration of a purified antibody, wherein the variable region of the antibody specifically binds to a cariogenic organism and the constant region of the antibody engages the humoral immune effector systems.

16. The method for treatment and prevention of dental caries of claim 15 wherein the cariogenic organism is *Streptococcus mutans*.

17. The method for treatment and prevention of dental caries of claim 15 wherein the mammal is a human.

20 18. The method for treatment and prevention of dental caries of claim 17 wherein the purified antibody is produced through the steps of:

- a) immunizing mice which have been genetically altered to produce human antibodies with at least one cariogenic organism;
- b) generating hybridomas which secrete antibodies specific to at least one cariogenic organism; and

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c) isolating the antibodies of step b).

19. The method for treatment and prevention of dental caries of  
claim 17 wherein the purified antibody is produced through the steps of:

- a) immunizing isolated human B lymphocytes in vitro with at least one cariogenic organism;
- 5 b) generating hybridomas which secrete antibodies specific to at least one cariogenic organism;
- c) isolating the antibodies of step b).

10 20. The method for treatment and prevention of dental caries of  
claim 17 wherein the purified antibody is produced through the steps of:

- a) isolating B lymphocytes from humans with an acute infection of at least one cariogenic organism;
- b) generating hybridomas which secrete antibodies specific to at least one cariogenic organism; and
- 15 c) isolating the antibodies of step b).

21. The method for treatment and prevention of dental caries of  
claim 17 wherein the purified antibody is produced through the steps of:

- a) isolating the genetic sequence that codes for the expression of said variable region;
- b) cloning the genetic sequence that codes for the expression of said variable region;
- 20 c) linking the genetic sequence that codes for the expression of said variable region to the genetic sequence that codes for the expression of said constant region;
- d) expressing said linked sequence; and
- e) isolating the expressed antibodies of step d).

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22. The method for treatment and prevention of dental caries of claim 21 wherein step a) is accomplished by screening a phage display random library.

23. The method for treatment and prevention of dental caries of claim 21 wherein the genetic sequence that codes for the expression of said constant region in step c) is derived from IgG or IgM antibodies.

5 24. The method for treatment and prevention of dental caries of claim 21 wherein the expression of said linked sequence in step d) is conducted in an expression system selected from a group comprising animal, human, chicken egg, or plant.

10

15

20

25

FIG. 1

# A SWLA1: LIGHT CHAIN SEQUENCE *DNA AND AMINO ACID SEQUENCE OF THE VL DOMAIN OF CHIMERIC ANTIBODY TEDW*

**EcoRV (424)**  
GGGGATATCCACCATGGAGACAGACACACTCCTGCTATGGGTGCTGCTGGTTCCAGGTTCCACAGGTGACATTGT  
► M E T D T L L L W V L L L W V P G S T G D I V  
**PstI (377)**  
GCTGACCCAATCTCCAGTTCTTGGCTGTCTCTAGGGCAGAGGGCCACCATATCCTGCAGAGCCAGTGAAAGTGTGA  
► L T Q S P V S L A V S L G Q R A T I S C R A S E S V D  
**KpnI (427)**  
TAGTTATGGCAATAGTTTATGAACCTGGTACCGAGCAGAAACCAGGACAGCCACCCAACTCCTCATCTCGTCATCCAA  
► S Y G N S F M N W Y Q Q K P G Q P P Q L L I Y R A S N  
**XbaI (482)**  
TCTAGAATACTGGATCCCTGCCAGGTTCACTGGCAGTGGTCTAGGACAGACTCACCCCTACCATTAATCCTGTGGAGGC  
► L E Y G I P A R F S G S G S R T D F T L T I N P V E A  
TGATGATGTTGCAACCTATTACTGTCAGCAAAATAATGCGGATCTCCACGTTGGAGGGGGACCAAGTTGAAATCAA  
► D D V A T Y Y C Q Q N N A D P P T F G G G T K L E I K  
**SalI (650)**  
ACGTAAGTCGACGCT  
► R K S

# **B** SWLA1: HEAVY CHAIN SEQUENCE *DNA AND AMINO ACID SEQUENCE OF THE VH DOMAIN OF CHIMERIC ANTIBODY TEDW*

EcoRV (242)  
 GGGGATATCCACCATGGCTGCTTGGGGCTGCTCTGCCTGGTGACATTCCAAGCTGTGCTCTGCCAGGTGC  
 ▶ M A V L G L L F C L V T F P S C V L S Q V  
 AGCTGAAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCATCACATGCACTGTCTCAGGGTTCTCA  
 ▶ Q L K E S G P G L V A P S Q S L S I T C T V S G F S  
 TTAACCAACTATGATATAAATTGGGTCGCCAGCCTCCAGGAAAGGGTCTGGAGTGGCTGGAAATAATATGGGTTGA  
 ▶ L T N Y D I N W V R Q P P G K G L E W L G I I W G D  
 CGGGAGCACAAATTATCATTAGCTCTCATATCCAGACTGAGCATCAGCAAGGATAACTCCAAGAGGCCAAATTCTC  
 ▶ G S T N Y H S A L I S R L S I S K D N S K S Q I F  
 TAAAAACTGAAACAGTCTGCAAACGTGATGACACAGCCACGTTACTACTGTAACTACCCGTGTTATATTCATGGTATG  
 ▶ L K L N S L Q T D D T A T Y Y C N Y P C L Y F Y G M  
 NheI (663) Sall (684)  
 GACTACTGGGTCAAGGAACCTCAGTCACCGTCTTCAGCTAGCACAAACAGCCCCATCAGTCGACCCA  
 ▶ D Y W G Q G T S V T V S S A S

**FIG. 2****A****SWLA2: LIGHT CHAIN SEQUENCE**  
*DNA AND AMINO ACID SEQUENCE OF THE VL DOMAIN OF CHIMERIC ANTIBODY TEEF*

EcoRV (243)

```

GGGGATATCCACCATGGATTTCAAGTGCAGATTTCAGCTTCTGCTAATCAGTGTACAGTCATATTGACCAATGGAGAAA
▶ M D F Q V Q I F S F L L I S V T V I L T N G E
                                         BstEII (372)    PstI (384)
TTTGCTCACCCCGTCTCCAGCAATCATAGCTGCATCTCCTGGGAAAAGGTACCCATCACCTGCAGTGCAGCTCAAGTGTGTT
▶ I L L T P S P A I I A A S P G E K V T I T C S A S S S V
                                         KpnI (419)
AGTTACATGAACTGGTACCGAGCAGAAACCCAGGATCTTCCCCAAAATCTGGATTATGGTGTATCCAACCTGGCTTCTGGAGT
▶ S Y M N W Y Q Q K P G S S P K I W I Y G V S N L A S G V
  TCCCTGCTCGCTTCAGTGGCAGTGGTCTGGACATCTTCTCTTCACAATCAACAGCATGGAGGCTGAAGATGTTGCCACTT
▶ P A R F S G S G S G T S F S F T I N S M E A E D V A T
                                         Sall (642)
ATTACTGTCAGCAAAGGAGTAGTTACCCATTACGTTGGCTCGGGACCAAGCTGGAAATAAACGTAAGTCGACGCT
▶ Y Y C Q Q R S S Y P F T F G S G T K L E I K R K S

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**B****SWLA2: HEAVY CHAIN SEQUENCE***DNA AND AMINO ACID SEQUENCE OF THE VH DOMAIN OF CHIMERIC ANTIBODY TEEF*

EcoRV (242)

NdeI (295)

```

GGGATATCCACCATGGACAGGCTTACTCTTCATTCTGCTACTGATTGTTCTGCATATGTCCTCTCCCAGGGTACTCT
▶ M D R L T S S F L L L I V P A Y V L S Q V T L
GAAAGAGTCTGGCCCTGGGATATTGCAAGCCCTCCCAGACCCCTCAGTCTGACTTGTCTTCTCTGGGTTTCACTGAGAA
▶ K E S G P G I L Q P S Q T L S L T C S F S G F S L R
CTTATGGTATAGGAGTAGGCTGGATTGTCAGCCCTCAGGGAGGGGTCTGGAGTGGCTGGCACACATTGGTGGAAATGAT
▶ T Y G I G V G W I R Q P S G R G L E W L A H I W W N D
                                         Scal (484)
AATAAGTACTATAACACAGTCCTGAAGAGCCGGCTACAATCTCAAGGATAACCTCCAACAACCAGGTATTCTCAAGAT
▶ N K Y Y N T V L K S R L T I S K D T S N N Q V F L K I
  CGCCAGTGTGGACACTGCAGATACTGCCACATACTACTGTGCGCGAATAGACGGGGCTCGGCTACGATGTTATGGACT
▶ A S V D T A D T A T Y Y C A R I E G G S G Y D V M D
                                         NheI (675)    Sall (696)
ACTGGGGTCAAGGAATCTCAGTCACCGTCTTCAGCTAGCACAACACCCCATCTGTCGACCCA
▶ Y W G Q G I S V T V S S A S

```

**FIG. 3**

**A SWLA3: LIGHT CHAIN SEQUENCE**  
*DNA AND AMINO ACID SEQUENCE OF THE  
 VL DOMAIN OF CHIMERIC ANTIBODY TEFC*

EcoRV (242)  
 GGGATATCCACCATGATGAGTCCTGCCAGTTCTGTTAGTGCTCTGGATTGGAAACCAACGGTGTGTTG  
 ▶ M M S P A Q F L F L L V L W I R E T N G D V V  
 BstEI (347)  
 ATGACCCAGACTCCACTCACTTTGCGGTACCATGGACAACCAAGCCTCCATCTCTGCAAGTCAGAGCCTCTA  
 ▶ M T Q T P L T L S V T I G Q P A S I S C K S S Q S L L  
 GATCGTGATGGAAGGACATATTGAGTTGGTTACAGAGGCCAGGCAAGTCCTCAAAGGCCATAATCTATCTGGTGTCT  
 ▶ D R D G R T Y L S W L L Q R P G Q S P K R L I Y L V S  
 AAACCTGGACTCTGGAGTCCCTGACAGGGTCACTGGCAGTGGATCAGGGACAGATTTCACACTGAAAATCAGCAGAGTGGAG  
 ▶ K L D S G V P D R F T G S G S G T D F T L K I S R V E  
 GCTGAGGATTGGGAGTTATTATGCTGGCAAGGTACACATTTCGGCTCACGGTGGCTGGGACCAAGCTGGAGCTG  
 ▶ A E D L G V Y Y C W Q G T H F P L T F G A G T K L E L  
 Sfi (653)  
 AAACGTAAGTCGACC  
 ▶ K R K S

**B SWLA3: HEAVY CHAIN SEQUENCE**  
*DNA AND AMINO ACID SEQUENCE OF THE  
 VH DOMAIN OF CHIMERIC ANTIBODY TEFC*

EcoRV (1425)  
 GATATCCACCATGGACTTGGGTTGAGCTGGTTTCTGCTTACTTTAAAGGTGTCAGTGTGACGTGAAGCTGGT  
 ▶ M D F G L S L V F L V L T L K G V Q C D V K L V  
 CGAGTCTGGGGAGGCTTAGTGAACCTGGAGGGTCCCTGAAACTCTCTGTCAGCCTCTGGATTCACTTCAGTAGCTA  
 ▶ E S G G G L V N P G G S L K L S C A A S G F T F S S Y  
 BspEI (1611)  
 TACCATGTCCTGGGTTGCCAGACTCOGGAGAAGAGGCTGGAGTGGTGGCATCCATTAGTAGTGGTGGTACTACACCTA  
 ▶ T M S W V R Q T P E K R L E W V A S I S S G G T Y T Y  
 CTATCCAGACAGTGTGAAGGGCGATTCAACCCTCCAGAGACAATGCCAAGAACACCCCTGTACCTGCCAAATGACCAAGCT  
 ▶ Y P D S V K G R F T I S R D N A K N T L Y L Q M T S L  
 GAAGTCTGAGGACACAGCCATGTAATTACTGTTCAAGAGAGTACGGCTCTAOGGCTCCTATTACTATGCTATGGACTACTG  
 ▶ K S E D T A M Y Y C S R D D G S Y G S Y Y Y A M D Y W  
 NheI (1861)  
 GGGTCAAGGAACCTCAGTCACCGTCTCTCAGCTAGCTCAACACCCCCATCAGTOGACCCA  
 ▶ G Q G T S V T V S S A S

FIG. 4

# SWLA1: LIGHT CHAIN SEQUENCE DNA AND AMINO ACID SEQUENCE OF THE ABERRANT VL DOMAIN

FIG. 5

# SWLA1: HEAVY CHAIN SEQUENCE *DNA AND AMINO ACID SEQUENCE OF THE NON-EFFECTIVE 2ND VH DOMAIN*

## EcoRV (242)

```

GGGGATATCCACCATGAACTTGGGTGAGCTGGGTTCTTCTTCTTCTTCAAGGTGCAATTGAGGTGCA
▶ M N F G L S W V F F V V F Y Q G V H C E V Q
GCTTGTGAGACTGGTGGAGGATTGGTGCAGCCTAAAGGGTCATGAAACTCTCATGTCAGCCTCTGGATTCACTT
▶ L V E T G G G L V Q P K G S L K L S C A A S G F T F
CAATACCAATGCCATGAACTGGTCGCCAGGCTCCAGGAAAGGGTTGGAATGGTGTGCTGCATAAGAAGTAAAAG
▶ N T N A M N W V R Q A P G K G L E W V A R I R S K S
TAATAACTATGCAACATATTATGCCGATTCACTGGAAGACAGGTCACCATCTCCAGAGATGATTCAAAAGCATGCT
▶ N N Y A T Y Y A D S V E D R F T I S R D D S Q S M L
CTATCTGCAAATGAACAACCTGAAAACCTGAGGACACAGCCATGTTATTACTGTTGAGAAACTACTATGATTACGACGC
▶ Y L Q M N N L K T E D T A M Y Y C V R N Y Y D Y D A
                                         NheI (675)
CTGGTCCGCTTACTGGGGCCAAGGGACTGTTGTCACIGTCCTTCAGCTAGCACAACACCCCCCATCAGTCACCCA
▶ W S A Y W G Q G T V V T V S S A S

```

FIG. 6

# SWLA1: HEAVY CHAIN SEQUENCE DNA AND AMINO ACID SEQUENCE OF THE ABERRANT VH DOMAIN

```

EcoRI           EcoRV
CAGAATTGCCCTGGGATATCCACCATGGAGACAGACACACTCCTGCTATGGGTACTGCTGCTCTGGGTCAGGT
                                         ▶ M E T D T L L W V L L W V P G
TCCACTGGTACATTTGCTGACACAGTCTCCTGCTTCTAGCTGTATCTCTGGGGCAGAGGGCCACCATCTCATACT
▶ S T G D I V L T Q S P A S L A V S L G Q R A T I S Y
AGGGCCAGCAAAGTGTCACTGACATCTGGCTATAGTTATGCACGGAAACCAACAGAAACCAGGACAGCCACCCAGA
▶ R A S K S V S T S G Y S Y M H W N Q Q K P G Q P P R
                                         EcoO109I
CTCCTCATCTATCTTGTATCCAACCTAGAAATCTGGGGTCCCTGCCAGGTTAGTGGCAGTGGCTCTGGGACAGACTTC
▶ L L I Y L V S N L E S G V P A R F S G S G S G T D F
                                         PM
ACCCCTCAACATCCATCTGTGGAGGAGGAGGATGCTGCAACCTATTACTGTCAGCACATTAGGGAGCTAACCGTTCG
▶ T L N I H P V E E E D A A T Y Y C Q H I R E L T R S
GAGGGGGGACCAAGCTGAAATAAAACGGNCTATGCTGCACCAACTGTATCCATCTTAAAANCATCAGTTCTAGAG
▶ E G G P S W K .

EcoRI
AAGGGCGAATTCC

```

FIG. 7

# SWLA2: HEAVY CHAIN SEQUENCE DNA AND AMINO ACID SEQUENCE OF THE ABERRANT VH DOMAIN

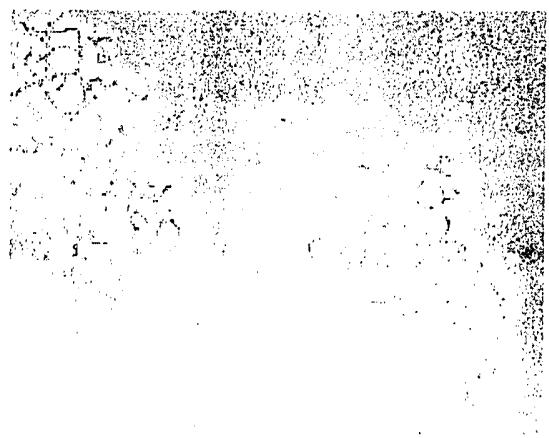
```

EcoRI          EcoRV
GGAATTGCCCTGGGATATCCACCATGGGATGGAGCTGGTCATGCTCTTCCTGGCAGGAACTCAGGTGTCC
                                ▶ M G W S W V M L F L L A G T A G V L
EcoRV
CTCTGAGGTCCAGCTGCAACAGTCTGGACCTGAGCTGGTAAGCCTGGGCTTCAGTGAAGATATCCTGCAAGACTTCT
▶ S E V Q L Q Q S G P E L V K P G A S V K I S C K T S
GGATACACATTCACTGAATACAACATGCACTGGGTAAACAGAGCCATGGAAAGAGCCTTGAGTGGATTGGAGGTATTA
▶ G Y T F T E Y N M H W V K Q S H G K S L E W I G G I
ATCCTAACAAATGGGGTACTAGTTACAACAGAAGTTCAAGGCCAAGGCCACATTGACTGTAGACAAGCCTCCAGCAC
▶ N P N N G G T S Y N Q K F K A K A T L T V D K S S S T
AGCCTACATGGAGCTCCGAAACCTGACATCTGAGGATTCTGCAGTCTATTACTGTGCAAGGGGGTTATGATGGTTA
▶ A Y M E L R N L T S E D S A V Y Y C A R G V Y D G Y
CTCCCTTTGACTACTGGGGCCAAGGCACACTCTCACAGTCTCCTCAGCCAAAACAACAGCCCCATCGGTCTATCCAC
▶ S L L T T G A K A P L S Q S P Q P K Q Q P H R S I H
TGGCCCCCTG
▶ W P L

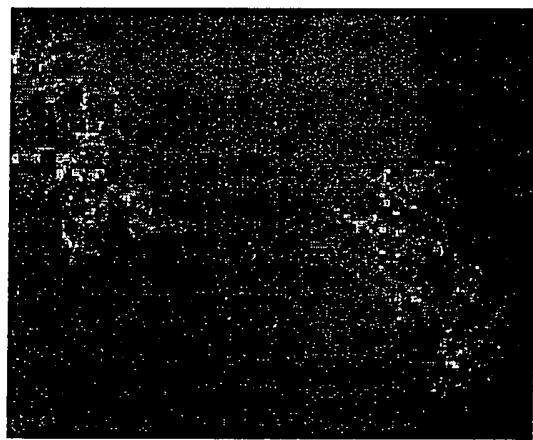
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**FIG. 8**

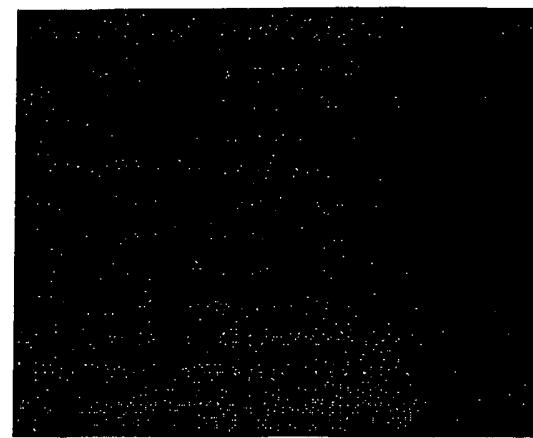
**LIGHT AND FLUORESCENT MICROSCOPE IMAGES**  
*CHIMERIC ANTIBODY TEDW BINDING TO S. MUTANS*



S. mutans  
+TEDW  
+Sigma F9512  
Light microscope



S. mutans  
+TEDW  
+Sigma F9512  
Fluorescent microscope



S. mutans  
+TEDW  
+Sigma F9512  
Fluorescent microscope

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
SEQUENCE LISTING

<110> SHI, WENYUAN  
ANDERSON, MAXWELL  
MORRISON, SHERIE  
TRINH, RYAN  
WIMS, LETITIA  
CHEN, LI

<120> Method for the Treatment and Prevention of Dental Caries

<130> 22851-032

<150> US 07/378,577  
<151> 1999-08-20

<160> 32

<170> PatentIn version 3.0

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<212> DNA  
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<220>

<221> CDS  
<222> (14)..(415)

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49

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu

1 5 10

ctc tgg gtt cca ggt tcc aca ggt gac att gtg ctg acc caa tct cca

97

Leu Trp Val Pro Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro

15 20 25

gtt tct ttg gct gtg tct cta ggg cag agg gcc acc ata tcc tgc aga  
45

Val Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg

1

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
30 35 40

gcc agt gaa agt gtt gat agt tat ggc aat agt ttt atg aac tgg tac 1  
93  
Ala Ser Glu Ser Val Asp Ser Tyr Gly Asn Ser Phe Met Asn Trp Tyr  
45 50 55 60

cag cag aaa cca gga cag cca ccc caa ctc ctc atc tat cgt gca tcc 2  
41  
Gln Gln Lys Pro Gly Gln Pro Pro Gln Leu Leu Ile Tyr Arg Ala Ser  
65 70 75

aat cta gaa tac ggg atc cct gcc agg ttc agt ggc agt ggg tct agg 2  
89  
Asn Leu Glu Tyr Gly Ile Pro Ala Arg Phe Ser Gly Ser Arg  
80 85 90

aca gac ttc acc ctc acc att aat cct gtg gag got gat gat gtt gca 3  
37  
Thr Asp Phe Thr Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val Ala  
95 100 105

acc tat tac tgt cag caa aat aat gcg gat cct ccc acg ttc gga ggg 3  
85  
Thr Tyr Tyr Cys Gln Gln Asn Asn Ala Asp Pro Pro Thr Phe Gly Gly  
110 115 120

ggg acc aag ttg gaa atc aaa cgt aag tcg acgct 4  
20  
Gly Thr Lys Leu Glu Ile Lys Arg Lys Ser  
125 130

<210> 2  
<211> 134

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

<212> PRT  
<213> Murine

<400> 2

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
1 5 10 15

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Val Ser Leu Ala  
20 25 30

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser  
35 40 45

Val Asp Ser Tyr Gly Asn Ser Phe Met Asn Trp Tyr Gln Gln Lys Pro  
50 55 60

Gly Gln Pro Pro Gln Leu Leu Ile Tyr Arg Ala Ser Asn Leu Glu Tyr  
65 70 75 80

Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Arg Thr Asp Phe Thr  
85 90 95

Leu Thr Ile Asn Pro Val Glu Ala Asp Asp Val Ala Thr Tyr Tyr Cys  
100 105 110

Gln Gln Asn Asn Ala Asp Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu  
115 120 125

Glu Ile Lys Arg Lys Ser  
130

<210> 3  
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<220>  
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Method for the Treatment and Prevention of Dental Caries.ST25.txt  
<222> (14)..(430)

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49

Met Ala Val Leu Gly Leu Leu Phe Cys Leu Val Thr

1 5 10

ttc cca agc tgt gtc ctg tcc cag gtg cag ctg aag gag tca gga cct  
97  
Phe Pro Ser Cys Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro

15 20 25

ggc ctg gtg gcg ccc tca cag agc ctg tcc atc aca tgc act gtc tca 1  
45  
Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser

30 35 40

ggg ttc tca tta acc aac tat gat ata aat tgg gtt cgc cag cct cca 1  
93  
Gly Phe Ser Leu Thr Asn Tyr Asp Ile Asn Trp Val Arg Gln Pro Pro

45 50 55 60

gga aag ggt ctg gag tgg ctg gga ata ata tgg ggt gac ggg agc aca 2  
41  
Gly Lys Gly Leu Glu Trp Leu Gly Ile Ile Trp Gly Asp Gly Ser Thr

65 70 75

aat tat cat tca gct ctc ata tcc aga ctg agc atc agc aag gat aac 2  
89  
Asn Tyr His Ser Ala Leu Ile Ser Arg Leu Ser Ile Ser Lys Asp Asn

80 85 90

tcc aag agc caa att ttc tta aaa ctg aac agt ctg caa act gat gac 3  
37  
Ser Lys Ser Gln Ile Phe Leu Lys Leu Asn Ser Leu Gln Thr Asp Asp

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

95	100	105	
aca gcc acg tac tac tgt aac tac ccg tgt tta tat ttc tat ggt atg 85	Thr Ala Thr Tyr Tyr Cys Asn Tyr Pro Cys Leu Tyr Phe Tyr Gly Met		3
110	115	120	
gac tac tgg ggt caa gga acc tca gtc acc gtc tct tca gct agc 30	Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser		4
125	130	135	
acaacagccc catcagtcga cccca 54			4
<210> 4			
<211> 139			
<212> PRT			
<213> Murine			
<400> 4			
Met Ala Val Leu Gly Leu Leu Phe Cys Leu Val Thr Phe Pro Ser Cys 1 5 10 15			
Val Leu Ser Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala 20 25 30			
Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu 35 40 45			
Thr Asn Tyr Asp Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu 50 55 60			
Glu Trp Leu Gly Ile Ile Trp Gly Asp Gly Ser Thr Asn Tyr His Ser 65 70 75 80			

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

Ala Leu Ile Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln  
85 90 95

Ile Phe Leu Lys Leu Asn Ser Leu Gln Thr Asp Asp Thr Ala Thr Tyr  
100 105 110

Tyr Cys Asn Tyr Pro Cys Leu Tyr Phe Tyr Gly Met Asp Tyr Trp Gly  
115 120 125

Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser  
130 135

<210> 5  
<211> 411  
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<220>  
<221> CDS  
<222> (14)..(406)

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49 Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu  
1 5 10

atc agt gtc aca gtc ata ttg acc aat gga gaa att ttg ctc acc ccg  
97 Ile Ser Val Thr Val Ile Leu Thr Asn Gly Glu Ile Leu Leu Thr Pro

15 20 25

tct cca gca atc ata gct gca tct cct ggg gaa aag gtc acc atc acc 1  
45 Ser Pro Ala Ile Ile Ala Ala Ser Pro Gly Glu Lys Val Thr Ile Thr

30 35 40

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
tgc agt gcc agc tca agt gtt agt tac atg aac tgg tac cag cag aaa 1  
93  
Cys Ser Ala Ser Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys  
45 50 55 60

cca gga tct tcc ccc aaa atc tgg att tat ggt gta tcc aac ctg gct 2  
41  
Pro Gly Ser Ser Pro Lys Ile Trp Ile Tyr Gly Val Ser Asn Leu Ala  
65 70 75

tct gga gtt cct gct cgc ttc agt ggc agt ggg tct ggg aca tct ttc 2  
89  
Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Thr Ser Phe  
80 85 90

tct ttc aca atc aac agc atg gag gct gaa gat gtt gcc act tat tac 3  
37  
Ser Phe Thr Ile Asn Ser Met Glu Ala Glu Asp Val Ala Thr Tyr Tyr  
95 100 105

tgt cag caa agg agt agt tac cca ttc acg ttc ggc tcg ggg acc aag 3  
85  
Cys Gln Gln Arg Ser Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys  
110 115 120

ctg gaa ata aaa cgt aag tcg acgct 4  
11  
Leu Glu Ile Lys Arg Lys Ser  
125 130

<210> 6  
<211> 131  
<212> PRT  
<213> Murine

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
<400> 6

Met Asp Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Val Thr  
1 5 10 15

Val Ile Leu Thr Asn Gly Glu Ile Leu Leu Thr Pro Ser Pro Ala Ile  
20 25 30

Ile Ala Ala Ser Pro Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser  
35 40 45

Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Ser Ser  
50 55 60

Pro Lys Ile Trp Ile Tyr Gly Val Ser Asn Leu Ala Ser Gly Val Pro  
65 70 75 80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Phe Ser Phe Thr Ile  
85 90 95

Asn Ser Met Glu Ala Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Arg  
100 105 110

Ser Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys  
115 120 125

Arg Lys Ser  
130

<210> 7  
<211> 465  
<212> DNA  
<213> Murine

<220>  
<221> CDS  
<222> (13)...(441)

<400> 7

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
gggatatcca cc atg gac agg ctt act tct tca ttc ctg cta ctg att gtt  
51

Met Asp Arg Leu Thr Ser Ser Phe Leu Leu Leu Ile Val

1 5 10

cct gca tat gtc ctc tcc cag gtt act ctg aaa gag tct ggc cct ggg  
99

Pro Ala Tyr Val Leu Ser Gln Val Thr Leu Lys Glu Ser Gly Pro Gly

15 20 25

ata ttg cag ccc tcc cag acc ctc agt ctg act tgt tct ttc tct ggg 1  
47

Ile Leu Gln Pro Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly

30 35 40 45

ttt tca ctg aga act tat ggt ata gga gta ggc tgg att cgt cag cct 1  
95

Phe Ser Leu Arg Thr Tyr Gly Ile Gly Val Gly Trp Ile Arg Gln Pro

50 55 60

tca ggg agg ggt ctg gag tgg ctg gca cac att tgg tgg aat gat aat 2  
43

Ser Gly Arg Gly Leu Glu Trp Leu Ala His Ile Trp Trp Asn Asp Asn

65 70 75

aag tac tat aac aca gtc ctg aag agc cgg ctc aca atc tcc aag gat 2  
91

Lys Tyr Tyr Asn Thr Val Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp

80 85 90

acc tcc aac aac cag gta ttc ctc aag atc gcc agt gtg gac act gca 3  
39

Thr Ser Asn Asn Gln Val Phe Leu Lys Ile Ala Ser Val Asp Thr Ala

95 100 105

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

gat act gcc aca tac tac tgt gcg cga ata gag ggg ggc tcg ggc tac 3  
87 Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Ile Glu Gly Gly Ser Gly Tyr

110 115 120 125

gat gtt atg gac tac tgg ggt caa gga atc tca gtc acc gtc tct tca 4  
35 Asp Val Met Asp Tyr Trp Gly Gln Gly Ile Ser Val Thr Val Ser Ser

130 135 140

gct agc acaacacccc catctgtcga ccc 4  
65 Ala Ser

<210> 8  
<211> 143  
<212> PRT  
<213> Murine

<400> 8

Met Asp Arg Leu Thr Ser Ser Phe Leu Leu Leu Ile Val Pro Ala Tyr  
1 5 10 15

Val Leu Ser Gln Val Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln  
20 25 30

Pro Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu  
35 40 45

Arg Thr Tyr Gly Ile Gly Val Gly Trp Ile Arg Gln Pro Ser Gly Arg  
50 55 60

Gly Leu Glu Trp Leu Ala His Ile Trp Trp Asn Asp Asn Lys Tyr Tyr  
65 70 75 80

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

Asn Thr Val Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Asn  
85 90 95

Asn Gln Val Phe Leu Lys Ile Ala Ser Val Asp Thr Ala Asp Thr Ala  
100 105 110

Thr Tyr Tyr Cys Ala Arg Ile Glu Gly Gly Ser Gly Tyr Asp Val Met  
115 120 125

Asp Tyr Trp Gly Gln Gly Ile Ser Val Thr Val Ser Ser Ala Ser  
130 135 140

<210> 9

<211> 420

<212> DNA

<213> Murine

<220>

<221> CDS

<222> (13)...(417)

<400> 9

gggatatcca cc atg atg agt cct gcc cag ttc ctg ttt ctg tta gtg ctc  
51

Met Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu

1

5

10

tgg att cgg gaa acc aac ggt gat gtt gtg atg acc cag act cca ctc  
99

Trp Ile Arg Glu Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu

15

20

25

act ttg tcg gtt acc att gga caa cca gcc tcc atc tct tgc aag tca  
47

Thr Leu Ser Val Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser

30

35

40

45

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

agt cag agc ctc tta gat cgt gat gga agg aca tat ttg agt tgg ttg 1  
 95  
 Ser Gln Ser Leu Leu Asp Arg Asp Gly Arg Thr Tyr Leu Ser Trp Leu

50 55 60

tta cag agg cca ggc cag tct cca aag cgc cta atc tat ctg gtg tct 2  
 43  
 Leu Gln Arg Pro Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser

65 70 75

aaa ctg gac tct gga gtc cct gac agg ttc act ggc agt gga tca ggg 2  
 91  
 Lys Leu Asp Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly

80 85 90

aca gat ttc aca ctg aaa atc agc aga gtg gag gct gag gat ttg gga 3  
 39  
 Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly

95 100 105

gtt tat tat tgc tgg caa ggt aca cat ttt ccg ctc acg ttc ggt gct 3  
 87  
 Val Tyr Tyr Cys Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala

110 115 120 125

ggg acc aag ctg gag ctg aaa cgt aag tcg acc 4  
 20  
 Gly Thr Lys Leu Glu Leu Lys Arg Lys Ser

130 135

<210> 10  
 <211> 135  
 <212> PRT  
 <213> Murine

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

&lt;400&gt; 10

Met Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg  
1 5 10 15

Glu Thr Asn Gly Asp Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser  
20 25 30

Val Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser  
35 40 45

Leu Leu Asp Arg Asp Gly Arg Thr Tyr Leu Ser Trp Leu Leu Gln Arg  
50 55 60

Pro Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp  
65 70 75 80

Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe  
85 90 95

Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Leu Gly Val Tyr Tyr  
100 105 110

Cys Trp Gln Gly Thr His Phe Pro Leu Thr Phe Gly Ala Gly Thr Lys  
115 120 125

Leu Glu Leu Lys Arg Lys Ser  
130 135

<210> 11  
<211> 466  
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<213> Murine

<220>  
<221> CDS  
<222> (11) . . (442)

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

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gatatccacc atg gac ttc ggg ttg agc ttg gtt ttc ctt gtc ctt act  
49

Met Asp Phe Gly Leu Ser Leu Val Phe Leu Val Leu Thr

1

5

10

tta aaa ggt gtc cag tgt gac gtg aag ctg gtg gag tct ggg gga ggc  
97

Leu Lys Gly Val Gln Cys Asp Val Lys Leu Val Glu Ser Gly Gly

15

20

25

tta gtg aac cct gga ggg tcc ctg aaa ctc tcc tgt gca gcc tct gga  
45

Leu Val Asn Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly

30

35

40

45

ttc act ttc agt agc tat acc atg tct tgg gtt cgc cag act ccg gag  
93

Phe Thr Phe Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu

50

55

60

aag agg ctg gag tgg gtc gca tcc att agt agt ggt act tac acc  
41

Lys Arg Leu Glu Trp Val Ala Ser Ile Ser Ser Gly Gly Thr Tyr Thr

65

70

75

tac tat cca gac agt gtg aag ggc cga ttc acc atc tcc aga gac aat  
89

Tyr Tyr Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn

80

85

90

gcc aag aac acc ctg tac ctg caa atg acc agt ctg aag tct gag gac  
37

Ala Lys Asn Thr Leu Tyr Leu Gln Met Thr Ser Leu Lys Ser Glu Asp

95

100

105

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

aca gcc atg tat tac tgt tca aga gat gac ggc tcc tac ggc tcc tat 3  
85

Thr Ala Met Tyr Tyr Cys Ser Arg Asp Asp Gly Ser Tyr Gly Ser Tyr

110 115 120 125

tac tat gct atg gac tac tgg ggt caa gga acc tca gtc acc gtc tct 4  
33

Tyr Tyr Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser

130 135 140

tca gct agc tcaacacccc catcagtcga ccca 4  
66

Ser Ala Ser

<210> 12

<211> 144

<212> PRT

<213> Murine

<400> 12

Met Asp Phe Gly Leu Ser Leu Val Phe Leu Val Leu Thr Leu Lys Gly  
1 5 10 15

Val Gln Cys Asp Val Lys Leu Val Glu Ser Gly Gly Leu Val Asn  
20 25 30

Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe  
35 40 45

Ser Ser Tyr Thr Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu  
50 55 60

Glu Trp Val Ala Ser Ile Ser Ser Gly Gly Thr Tyr Thr Tyr Tyr Pro

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
65 70 75 80

Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn  
85 90 95

Thr Leu Tyr Leu Gln Met Thr Ser Leu Lys Ser Glu Asp Thr Ala Met  
100 105 110

Tyr Tyr Cys Ser Arg Asp Asp Gly Ser Tyr Gly Ser Tyr Tyr Tyr Ala  
115 120 125

Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser  
130 135 140

<210> 13  
<211> 481  
<212> DNA  
<213> Murine

<220>  
<221> CDS  
<222> (28)..(411)

<220>  
<221> variation  
<222> (420)..(420)  
<223> atgc

<220>  
<221> variation  
<222> (449)..(449)  
<223> atgc

<220>  
<221> variation  
<222> (454)..(454)  
<223> atgc

<220>  
<221> variation

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
<222> (423)..(423)  
<223> atgc

<400> 13  
cagaattcgc ccttggggat atccacc atg gag aca gac aca ctc ctg cta tgg  
54 Met Glu Thr Asp Thr Leu Leu Leu Trp

1 5

gta ctg ctg ctc tgg gtt cca ggt tcc act ggt gac att gtg ctg aca 1  
02 Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp Ile Val Leu Thr

10 15 20 25

cag tct cct gct tcc tta gct gta tct ctg ggg cag agg gcc acc atc 1  
50 Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile

30 35 40

tca tac agg gcc agc aaa agt gtc agt aca tct ggc tat agt tat atg 1  
98 Ser Tyr Arg Ala Ser Lys Ser Val Ser Thr Ser Gly Tyr Ser Tyr Met

45 50 55

cac tgg aac caa cag aaa cca gga cag cca ccc aga ctc ctc atc tat 2  
46 His Trp Asn Gln Gln Lys Pro Gly Gln Pro Pro Arg Leu Leu Ile Tyr

60 65 70

ctt gta tcc aac cta gaa tct ggg gtc cct gcc agg ttc agt ggc agt 2  
94 Leu Val Ser Asn Leu Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser

75 80 85

ggg tct ggg aca gac ttc acc ctc aac atc cat cct gtg gag gag gag 3

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

42

Gly Ser Gly Thr Asp Phe Thr Leu Asn Ile His Pro Val Glu Glu Glu  
90 95 100 105

gat gct gca acc tat tac tgt cag cac att agg gag ctt aca cgt tcg 3  
90

Asp Ala Ala Thr Tyr Tyr Cys Gln His Ile Arg Glu Leu Thr Arg Ser  
110 115 120

gag ggg gga cca agc tgg aaa taaaacggnc tnatgctgca ccaactgtat 4  
41

Glu Gly Gly Pro Ser Trp Lys

125

ccatcttnaa aancatcaagt tctagagaag ggcgaattcc 4  
81

&lt;210&gt; 14

&lt;211&gt; 128

&lt;212&gt; PRT

&lt;213&gt; Murine

&lt;400&gt; 14

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
1 5 10 15

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala  
20 25 30

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Tyr Arg Ala Ser Lys Ser  
35 40 45

Val Ser Thr Ser Gly Tyr Ser Tyr Met His Trp Asn Gln Gln Lys Pro  
50 55 60

Gly Gln Pro Pro Arg Leu Leu Ile Tyr Leu Val Ser Asn Leu Glu Ser

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
65 70 75 80

Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
85 90 95

Leu Asn Ile His Pro Val Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
100 105 110

Gln His Ile Arg Glu Leu Thr Arg Ser Glu Gly Gly Pro Ser Trp Lys  
115 120 125

<210> 15

<211> 466

<212> DNA

<213> Murine

<220>

<221> CDS

<222> (14)..(442)

<400> 15  
ggggatatcc acc atg aac ttc ggg ttg agc tgg gtt ttc ttt gtt gtt  
49

Met Asn Phe Gly Leu Ser Trp Val Phe Phe Val Val

1

5

10

ttt tat caa ggt gtg cat tgt gag gtg cag ctt gtt gag act ggt gga  
97  
Phe Tyr Gln Gly Val His Cys Glu Val Gln Leu Val Glu Thr Gly Gly

15

20

25

gga ttg gtg cag cct aaa ggg tca ttg aaa ctc tca tgt gca gcc tct  
45  
Gly Leu Val Gln Pro Lys Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser

30

35

40

gga ttc acc ttc aat acc aat gcc atg aac tgg gtc cgc cag gct cca  
93

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
Gly Phe Thr Phe Asn Thr Asn Ala Met Asn Trp Val Arg Gln Ala Pro

45 50 55 60

gga aag ggt ttg gaa tgg gtt gct cgc ata aga agt aaa agt aat aac 2  
41

Gly Lys Gly Leu Glu Trp Val Ala Arg Ile Arg Ser Lys Ser Asn Asn

65 70 75

tat gca aca tat tat gcc gat tca gtg gaa gac agg ttc acc atc tcc 2  
89

Tyr Ala Thr Tyr Tyr Ala Asp Ser Val Glu Asp Arg Phe Thr Ile Ser

80 85 90

aga gat gat tca caa agc atg ctc tat ctg caa atg aac aac ttg aaa 3  
37

Arg Asp Asp Ser Gln Ser Met Leu Tyr Leu Gln Met Asn Asn Leu Lys

95 100 105

act gag gac aca gcc atg tat tac tgt gtg aga aac tac tat gat tac 3  
85

Thr Glu Asp Thr Ala Met Tyr Tyr Cys Val Arg Asn Tyr Tyr Asp Tyr

110 115 120

gac gcc tgg tcc gct tac tgg ggc caa ggg act gtg gtc act gtc tct 4  
33

Asp Ala Trp Ser Ala Tyr Trp Gly Gln Gly Thr Val Val Thr Val Ser

125 130 135 140

tca gct agc acaacacccc catcagtcta cccca 4  
66

Ser Ala Ser

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

<210> 16  
<211> 143  
<212> PRT  
<213> Murine

<400> 16

Met Asn Phe Gly Leu Ser Trp Val Phe Phe Val Val Phe Tyr Gln Gly  
1 5 10 15

Val His Cys Glu Val Gln Leu Val Glu Thr Gly Gly Gly Leu Val Gln  
20 25 30

Pro Lys Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe  
35 40 45

Asn Thr Asn Ala Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu  
50 55 60

Glu Trp Val Ala Arg Ile Arg Ser Lys Ser Asn Asn Tyr Ala Thr Tyr  
65 70 75 80

Tyr Ala Asp Ser Val Glu Asp Arg Phe Thr Ile Ser Arg Asp Asp Ser  
85 90 95

Gln Ser Met Leu Tyr Leu Gln Met Asn Asn Leu Lys Thr Glu Asp Thr  
100 105 110

Ala Met Tyr Tyr Cys Val Arg Asn Tyr Tyr Asp Tyr Asp Ala Trp Ser  
115 120 125

Ala Tyr Trp Gly Gln Gly Thr Val Val Thr Val Ser Ser Ala Ser  
130 135 140

<210> 17  
<211> 518  
<212> DNA  
<213> Murine

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

<220>  
<221> CDS  
<222> (29)..(376)

<220>  
<221> variation  
<222> (459)..(459)  
<223> atgc

<220>  
<221> variation  
<222> (482)..(482)  
<223> atgc

<220>  
<221> variation  
<222> (490)..(491)  
<223> atgc

<400> 17  
gcagaattcg cccttgggga tatccacc atg gct gtc ttg ggg ctg ctc ttc  
52 Met Ala Val Leu Gly Leu Leu Phe  
1 5

tgc ctg gtg aca ttc cca agc tgt gtc ctg tcc cag gtg cag ctg aag 1  
00 Cys Leu Val Thr Phe Pro Ser Cys Val Leu Ser Gln Val Gln Leu Lys

10 15 20

gac tca gga cct ggc ctg gtg gcg ccc tca cag agc ctg tcc atc aca 1  
48 Asp Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile Thr

25 30 35 40

tgc act gtc tca ggg ttc tca tta acc aac tat gat ata aat tgg gtt 1  
96 Cys Thr Val Ser Gly Phe Ser Leu Thr Asn Tyr Asp Ile Asn Trp Val

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
45 50 55

cgc cag cct cca gga aag ggt ctg gag tgg ctg gga ata ata tgg ggt 2  
44 Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp Leu Gly Ile Ile Trp Gly  
60 65 70

gac ggg agc aca aat tat cat tca gct ctc ata tcc aga ctg agc atc 2  
92 Asp Gly Ser Thr Asn Tyr His Ser Ala Leu Ile Ser Arg Leu Ser Ile  
75 80 85

agc aag gat aac tcc aag agc caa att ttc tta aaa ctg aac agt ctg 3  
40 Ser Lys Asp Asn Ser Lys Ser Gln Ile Phe Leu Lys Leu Asn Ser Leu  
90 95 100

caa act gat gac aca gcc acg tac tac tgt tta ttg taactacccg 3  
86 Gln Thr Asp Asp Thr Ala Thr Tyr Tyr Cys Leu Leu  
105 110 115

tgttatatt tctatggat ggactactgg ggtcaaggaa cctcagtcac cgtctcctca 4  
46

gccaaaacaa canccccatc ggtctatcca ctggcncctg tganngagat tctaaaccta 5  
06

agggcgaatt cc 5  
18

<210> 18  
<211> 116  
<212> PRT  
<213> Murine  
<400> 18

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
Met Ala Val Leu Gly Leu Leu Phe Cys Leu Val Thr Phe Pro Ser Cys  
1 5 10 15

Val Leu Ser Gln Val Gln Leu Lys Asp Ser Gly Pro Gly Leu Val Ala  
20 25 30

Pro Ser Gln Ser Leu Ser Ile Thr Cys Thr Val Ser Gly Phe Ser Leu  
35 40 45

Thr Asn Tyr Asp Ile Asn Trp Val Arg Gln Pro Pro Gly Lys Gly Leu  
50 55 60

Glu Trp Leu Gly Ile Ile Trp Gly Asp Gly Ser Thr Asn Tyr His Ser  
65 70 75 80

Ala Leu Ile Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln  
85 90 95

Ile Phe Leu Lys Leu Asn Ser Leu Gln Thr Asp Asp Thr Ala Thr Tyr  
100 105 110

Tyr Cys Leu Leu  
115

<210> 19  
<211> 482  
<212> DNA  
<213> Murine

<220>  
<221> CDS  
<222> (27) .. (482)

<400> 19  
ggaattcgcc cttggggata tccacc atg gga tgg agc tgg gtc atg ctc ttt  
53 Met Gly Trp Ser Trp Val Met Leu Phe

1 5

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

ctc	ctg	gca	gga	act	gca	ggt	gtc	ctc	tct	gag	gtc	cag	ctg	caa	cag	1
01																
Leu	Leu	Ala	Gly	Thr	Ala	Gly	Val	Leu	Ser	Glu	Val	Gln	Leu	Gln	Gln	
10																25
tct	gga	cct	gag	ctg	gtg	aag	cct	ggg	gct	tca	gtg	aag	ata	tcc	tgc	1
49																
Ser	Gly	Pro	Glu	Leu	Val	Lys	Pro	Gly	Ala	Ser	Val	Lys	Ile	Ser	Cys	
30																40
aag	act	tct	gga	tac	aca	ttc	act	gaa	tac	aac	atg	cac	tgg	gtg	aaa	1
97																
Lys	Thr	Ser	Gly	Tyr	Thr	Phe	Thr	Glu	Tyr	Asn	Met	His	Trp	Val	Lys	
45																55
cag	agc	cat	gga	aag	agc	ctt	gag	tgg	att	gga	ggt	att	aat	cct	aac	2
45																
Gln	Ser	His	Gly	Lys	Ser	Leu	Glu	Trp	Ile	Gly	Gly	Ile	Asn	Pro	Asn	
60																70
aat	ggt	ggt	act	agt	tac	aac	cag	aag	ttc	aag	gcc	aag	gcc	aca	ttg	2
93																
Asn	Gly	Gly	Thr	Ser	Tyr	Asn	Gln	Lys	Phe	Lys	Ala	Lys	Ala	Thr	Leu	
75																85
act	gta	gac	aag	tcc	tcc	agc	aca	gcc	tac	atg	gag	ctc	cgc	aac	ctg	3
41																
Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	Met	Glu	Leu	Arg	Asn	Leu	
90																105
aca	tct	gag	gat	tct	gca	gtc	tat	tac	tgt	gca	agg	ggg	gtt	tat	gat	3
89																
Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	Ala	Arg	Gly	Val	Tyr	Asp	
110																120

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

ggt tac tcc ctt ttg act act ggg gcc aag gca cca ctc tca cag tct 4  
37  
Gly Tyr Ser Leu Leu Thr Thr Gly Ala Lys Ala Pro Leu Ser Gln Ser

125 130 135

cct cag cca aaa caa cag ccc cat cgg tct atc cac tgg ccc ctg 4  
82  
Pro Gln Pro Lys Gln Gln Pro His Arg Ser Ile His Trp Pro Leu

140 145 150

<210> 20  
<211> 152  
<212> PRT  
<213> Murine

<400> 20

Met Gly Trp Ser Trp Val Met Leu Phe Leu Leu Ala Gly Thr Ala Gly  
1 5 10 15

Val Leu Ser Glu Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys  
20 25 30

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Thr Ser Gly Tyr Thr Phe  
35 40 45

Thr Glu Tyr Asn Met His Trp Val Lys Gln Ser His Gly Lys Ser Leu  
50 55 60

Glu Trp Ile Gly Gly Ile Asn Pro Asn Asn Gly Gly Thr Ser Tyr Asn  
65 70 75 80

Gln Lys Phe Lys Ala Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser  
85 90 95

Method for the Treatment and Prevention of Dental Caries.ST25.txt  
Thr Ala Tyr Met Glu Leu Arg Asn Leu Thr Ser Glu Asp Ser Ala Val  
100 105 110

Tyr Tyr Cys Ala Arg Gly Val Tyr Asp Gly Tyr Ser Leu Leu Thr Thr  
115 120 125

Gly Ala Lys Ala Pro Leu Ser Gln Ser Pro Gln Pro Lys Gln Gln Pro  
130 135 140

His Arg Ser Ile His Trp Pro Leu  
145 150

<210> 21  
<211> 37  
<212> DNA  
<213> synthetic construct

<400> 21  
ggggatatcc acatggagac agacacactc ctgctat  
37

<210> 22  
<211> 29  
<212> DNA  
<213> synthetic construct

<400> 22  
gcgtctagaa ctggatggtg ggaagatgg  
29

<210> 23  
<211> 35  
<212> DNA  
<213> synthetic construct

<400> 23  
agcgtcgact tacgtttkat ttccarcttk gtccc  
35

<210> 24  
<211> 38

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

<212> DNA  
<213> synthetic construct

<400> 24  
ggggatatcc acatgractt cgggytgagc tkggtttt  
38

<210> 25  
<211> 37  
<212> DNA  
<213> synthetic construct

<400> 25  
ggggatatcc acatggctgt cttggggctg ctcttct  
37

<210> 26  
<211> 39  
<212> DNA  
<213> synthetic construct

<400> 26  
aggtctagaa yctccacaca caggrccag tggatagac  
39

<210> 27  
<211> 39  
<212> DNA  
<213> synthetic construct

<400> 27  
tgggtcgacw gatggggstg ttgtgctagc tgaggagac  
39

<210> 28  
<211> 38  
<212> DNA  
<213> synthetic construct

<400> 28  
ggggatatcc acatggattt tcaagtgcag attttcag  
38

## Method for the Treatment and Prevention of Dental Caries.ST25.txt

<210> 29  
<211> 38  
<212> DNA  
<213> synthetic construct

<400> 29  
ggggatatcc acatggratg sagctgkgtm atsctctt  
38

<210> 30  
<211> 39  
<212> DNA  
<213> synthetic construct

<400> 30  
gggatatcca ccatggrcag rcttacwtyy tcattcctg  
39

<210> 31  
<211> 34  
<212> DNA  
<213> synthetic construct

<400> 31  
ggggatatcc acatgatgag tcctgcccag ttcc  
34

<210> 32  
<211> 30  
<212> DNA  
<213> synthetic construct

<400> 32  
ggtcgactta cgtttcagct ccagcttgggt  
30