



(86) Date de dépôt PCT/PCT Filing Date: 2003/03/07
(87) Date publication PCT/PCT Publication Date: 2003/09/18
(85) Entrée phase nationale/National Entry: 2004/10/13
(86) N° demande PCT/PCT Application No.: US 2003/006962
(87) N° publication PCT/PCT Publication No.: 2003/075845
(30) Priorités/Priorities: 2002/03/07 (60/363,209) US;
2002/08/08 (60/402,483) US

(51) Cl.Int.⁷/Int.Cl.⁷ A61K 39/09, A61K 38/45, A61K 39/395,
A61P 1/02, A61K 38/16, A61P 31/04

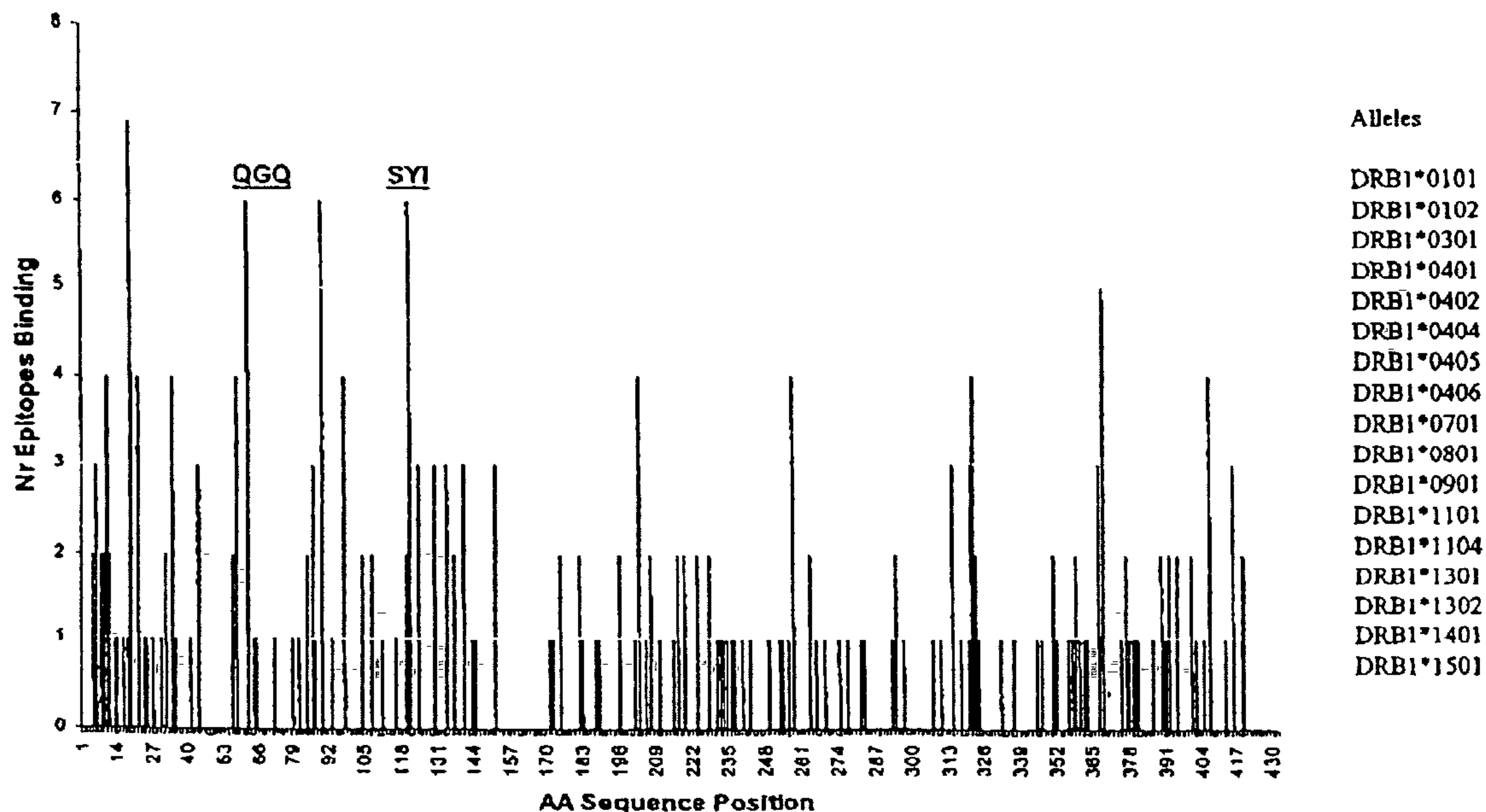
(71) Demandeur/Applicant:
THE FORSYTH INSTITUTE, US

(72) Inventeurs/Inventors:
SMITH, DANIEL J., US;
TAUBMAN, MARTIN A., US

(74) Agent: MBM & CO.

(54) Titre : IMMUNOGENICITE D'UNE PROTEINE DE LIAISON AU GLUCANEIMMUNOGENICITE D'UNE PROTEINE DE
LIAISON AU GLUCANE

(54) Title: IMMUNOGENICITY OF GLUCAN BINDING PROTEIN



(57) Abrégé/Abstract:

Immunogenic compositions and subunit vaccines for dental caries are described which comprise peptide subunits of glucan binding protein-B and peptide subunits of glucan binding protein-B in combination with peptide subunits of glucosyltransferase. Methods of provoking an immune response to *S. mutans* glucan binding protein-B or glucosyltransferase. Methods of immunizing a mammal against dental caries are also described, along with antibodies which bind particular epitopes of glucan binding protein-B or glucosyltransferase.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 September 2003 (18.09.2003)

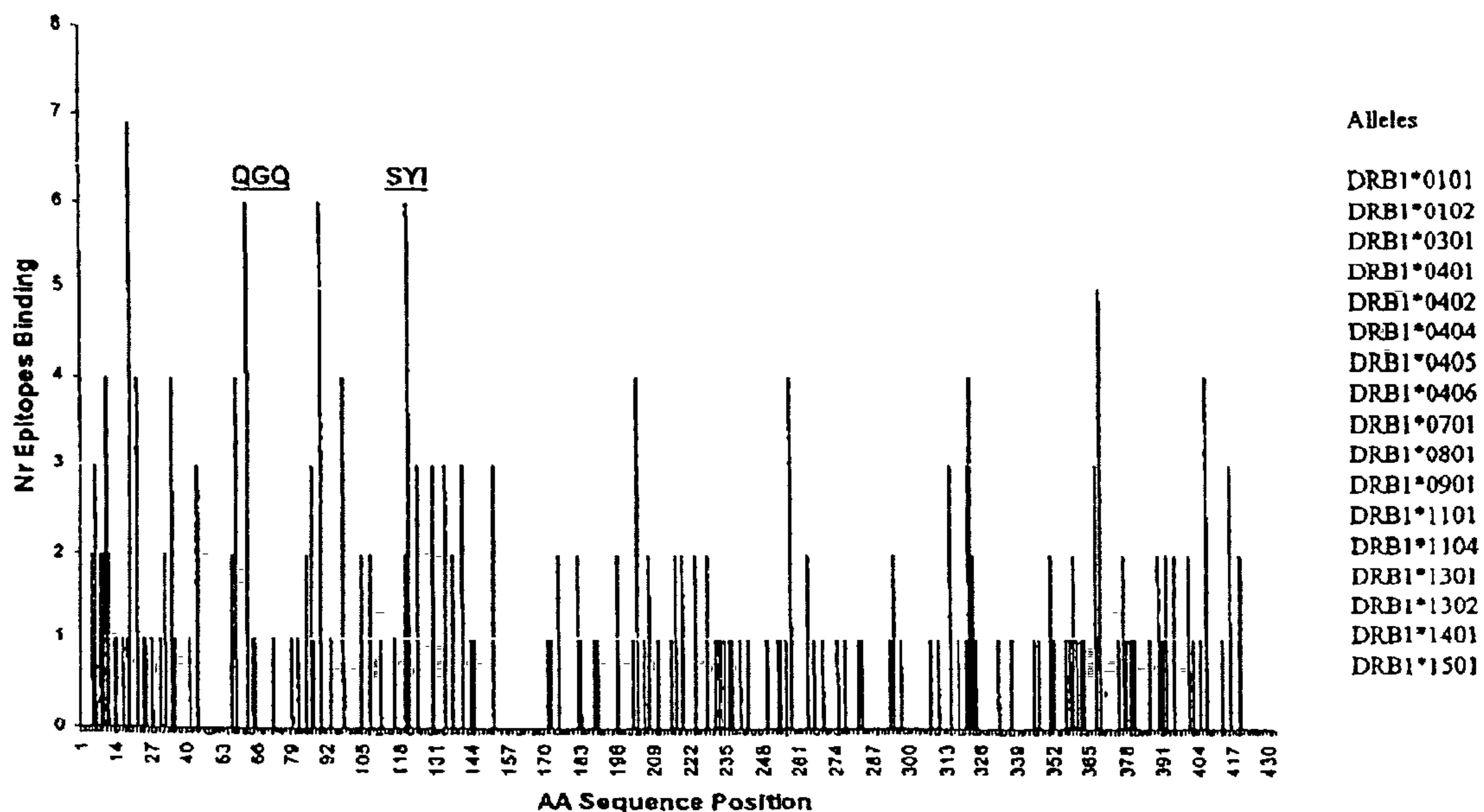
PCT

(10) International Publication Number
WO 03/075845 A2

- (51) International Patent Classification⁷: **A61K**
- (21) International Application Number: PCT/US03/06962
- (22) International Filing Date: 7 March 2003 (07.03.2003)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/363,209 7 March 2002 (07.03.2002) US
60/402,483 8 August 2002 (08.08.2002) US
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier applications:
US 60/363,209 (CIP)
Filed on 7 March 2002 (07.03.2002)
US 60/402,483 (CIP)
Filed on 8 August 2002 (08.08.2002)
- (71) Applicant (for all designated States except US): **THE FORSYTH INSTITUTE** [US/US]; 140 The Fenway, Boston, MA 02115-3799 (US).
- (72) Inventors; and
(75) Inventors/Applicants (for US only): **SMITH, Daniel, J.** [US/US]; 6 Deerfield Lane, Natick, MA 01760 (US).
TAUBMAN, Martin, A. [US/US]; 82 Otis Street, Newtonville, MA 02160 (US).
- (74) Agents: **BEATTIE, Ingrid, A.** et al.; Mintz, Levin, Cohn, Ferris, Glovsky and Popeo, P.C., One Financial Center, Boston, MA 02111 (US).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,

[Continued on next page]

(54) Title: IMMUNOGENICITY OF GLUCAN BINDING PROTEIN



(57) Abstract: Immunogenic compositions and subunit vaccines for dental caries are described which comprise peptide subunits of glucan binding protein-B and peptide subunits of glucan binding protein-B in combination with peptide subunits of glucosyltransferase. Methods of provoking an immune response to *S. mutans* glucan binding protein-B or glucosyltransferase. Methods of immunizing a mammal against dental caries are also described, along with antibodies which bind particular epitopes of glucan binding protein-B or glucosyltransferase.

WO 03/075845 A2



ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Published:

— *without international search report and to be republished
upon receipt of that report*

IMMUNOGENICITY OF GLUCAN BINDING PROTEIN

BACKGROUND OF THE INVENTION

Mutans streptococci have been implicated in the initiation of dental caries in humans. *Streptococcus mutans* have several virulence factors that allow the bacteria to accumulate within the dental biofilm and produce and tolerate the acids that cause dental caries. The ability of cariogenic *mutans streptococci* to accumulate in the dental biofilm is thought to be a consequence of the synthesis of glucans by glucosyltransferases, followed by the binding of the bacteria to these polymers via the cell-associated glucan binding proteins (Gbps). Biofilm development occurs in two distinct phases. During the first phase, bacterial surface proteins interact with host or bacterial products adsorbed on the tooth surface. In the second phase, a biofilm forms as bacteria accumulate by aggregation with the same or other species and produce an extracellular polysaccharide matrix.

Epitopes associated with these functions are thought to be primary targets for immunogenic attack, provided that the relevant sequences are located in molecular areas that can be accessible to antibody. Several *mutans streptococcal* proteins with glucan binding activity have been described (Russell, R.R., *J. Gen. Microbiol.*, 112:197-201 (1979); Smith D. J. *et al.*, *Infect. Immun.* 62:2545-2552 (1994); Sato, Y., *et al.*, *Infect. Immun.*, 65:668-675 (1997)). One of these components, glucan-binding protein- B (GbpB), has been shown to induce protective immune responses against experimental dental caries following systemic or mucosal immunization (Smith D. J. *et al.*, *Infect. Immun.* 64:3069-3073 (1996) and Smith D. J. *et al.*, *Oral Microbiol. Immunol.* 13:278-285(1998)). Furthermore, there is evidence that the expression of GbpB is directly related to biofilm formation (Mattos-Graner, R.O., *et al.*, *Infect. and Immun.* 69(11) 6931-6941(2001)). However, use of the intact GbpB protein in a vaccine may induce immunity to irrelevant or unwanted epitopes.

SUMMARY OF THE INVENTION

The invention provides improved immunogens and vaccine compositions for inducing antibody production against Streptococcal antigens. Accordingly, the invention features a composition containing a fragment of a glucan binding protein-B (GbpB), which binds to a

major histocompatibility complex (MHC) class II protein, e.g., an HLA protein selected from the group consisting of DRA, DRB1, DRB2, DQA1, DQB1, DPA1, DPB1, DMA, DMB, DOA, and DOB. The GbpB protein is preferably derived from a *Streptococcus mutans* strain. For example, the Streptococcal GbpB contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 29, 30, 31, 32, and 33. Preferably, the GbpB protein contains the amino acid sequence of SEQ ID NO: 29 (*S. mutans* strain SJ32)

The fragment is greater than 6 and less than 431 residues in length. For example, the fragment is less than 400 residues in length, less than 100 residues in length, or less than 50 residues in length. Preferably, the fragment is 10-25 residues in length. The fragment contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, and 22. Preferably, the fragment contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 1 and 3.

Also within the invention is a chimeric polypeptide containing a fragment of two or more streptococcal proteins. For example, the composition contains a GbpB polypeptide and a glucosyltransferase (GTF) polypeptide. The polypeptides are covalently linked. The chimeric polypeptide contains greater than two epitopes (diepitopic polypeptide), and may contain 3, 4, 5 or more epitopes (multiepitopic polypeptide). Optionally, the polypeptide contains two or more copies of a single epitope. The GbpB polypeptide preferably contains an amino acid sequence selected from the group consisting of SEQ ID NO's: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, and 22, and the glucosyltransferase polypeptide comprises a catalytic domain of SEQ ID NO: 34, 35, 36, 37, 38, 39, or 40. Preferably, the catalytic domain contains an amino acid sequence of SEQ ID NO: 24 or 25. Alternatively (or in addition), the glucosyltransferase polypeptide contains a glucan binding domain of SEQ ID NO: 34, 35, 36, 37, 38, 39, or 40. Preferably, the glucan binding domain comprises an amino acid sequence of SEQ ID NO: 23, and the glucosyltransferase polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 23, 24, 25, 26, 27, AND 28. For example, a diepitopic polypeptide construct includes a GbpB polypeptide containing SEQ ID NO: 1 and a glucosyltransferase polypeptide containing SEQ ID NO: 23 or a GbpB polypeptide containing SEQ ID NO: 1 and a glucosyltransferase polypeptide containing SEQ ID NO: 25. The di- or multi-epitopic constructs optionally contain a peptidyl core matrix. The matrix contains one or a plurality of lysine residues.

The compositions are used to elicit production of an antibody in a mammal. The method is carried out by administering to the mammal a composition containing a MHC class II-binding fragment of GbpB or a composition containing both a GbpB polypeptide and a glucosyltransferase polypeptide. In the latter case, the amount of an anti-GbpB antibody produced by the mammal is at least 10% greater than an amount produced by a mammal immunized with a composition comprising a GbpB peptide in the absence of a GTF peptide. Anti-GbpB titers in animals immunized with a di or multi-epitopic peptide constructs are preferably at least 20%, at least 50%, at least 75%, and at least 100% greater than titers achieved in animals immunized with a mono-epitopic peptide. Similarly, anti-GTF titers in animals immunized with a di or multi-epitopic peptide constructs are preferably at least 20%, at least 50%, at least 75%, and at least 100% greater than titers achieved in animals immunized with a mono-epitopic peptide. The immunization leads to production of mucosal immunity (IgA isotype) as well as systemic immunity (e.g., IgG isotype). Also, within the invention is a substantially pure antibody produced by any of the methods described above.

The polypeptides (including antibody molecules) within the invention are substantially pure. A polypeptide is substantially pure when it is separated from those contaminants, which accompany it in its natural state (proteins and other naturally-occurring organic molecules). In the case of an antibody preparation, the antibodies are purified from other blood components such as cells and other blood proteins. Typically, the polypeptide is substantially pure when it constitutes at least 60%, by weight, of the protein in the preparation. Preferably, the protein in the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, of the desired protein. Purity is measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis. Accordingly, substantially pure polypeptides include synthetic polypeptides, recombinant polypeptides derived from a eucaryote but produced in *E. coli* or another procaryote, or in a eucaryote other than that from which the polypeptide was originally derived.

The peptides are prepared synthetically or by recombinant DNA technology. The term peptide is used interchangeably with polypeptide in the present specification to designate a series of amino acids connected one to the other by peptide bonds between the alpha-amino and alpha-carboxy groups of adjacent amino acids. Optionally, one or more peptide bonds are replaced with an alternative type of covalent bond (a "peptide mimetic") which is less susceptible to

cleavage by peptidases compared to a peptide bond. Where proteolytic degradation of the peptides following injection into the subject is a problem, replacement of a particularly sensitive peptide bond with a noncleavable peptide mimetic yields a peptide mimetic, which is more stable and thus more useful as a therapeutic. Such mimetics, and methods of incorporating them into peptides, are well known in the art. Similarly, the replacement of an L-amino acid residue is a standard way of rendering the peptide less sensitive to proteolysis. Also useful are amino-terminal blocking groups such as t-butyloxycarbonyl, acetyl, theyl, succinyl, methoxysuccinyl, suberyl, adipyl, azelayl, dansyl, benzyloxycarbonyl, fluorenylmethoxycarbonyl, methoxyazelayl, methoxyadipyl, methoxysuberyl, and 2,4,-dinitrophenyl. The polypeptides or peptides are either in their neutral (uncharged) forms or in forms which are salts, and either free of modifications such as glycosylation, side chain oxidation, or phosphorylation or containing these modifications, subject to the condition that the modification not destroy the immune stimulatory activity of the polypeptides.

Derivative peptide epitopes have an amino acid sequence, which differs from the amino acid sequence of a naturally-occurring receptor peptide. Such derivative peptides have at least 50% identity compared to a reference sequence of amino acids, e.g., a naturally-occurring glutamate receptor peptide. Preferably, a derivative is 90, 95, 98, or 99% identical to a naturally-occurring protein sequence. The derivative contains a conservative amino acid substitution. By conservative substitution is meant a replacement of an amino acid residue with another, which is biologically and/or chemically similar, e.g., one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Nucleotide and amino acid comparisons described herein are carried out using the Lasergene software package (DNASTAR, Inc., Madison, WI). The MegAlign module used is the Clustal V method (Higgins et al., 1989, CABIOS 5(2):151-153). The parameter used is gap penalty 10, gap length penalty 10.

In addition to eliciting active immunity by immunizing a mammal with streptococcal immunogens, the method encompasses methods of conferring passive immunity. For example, antibodies produced *in vitro* or *in vivo* are purified and administered to a mammal. The antibody preparation contains antibodies, which specifically bind to streptococcal antigens such as GbpB and/or GTF. For example, the antibodies used in a passive immunization regimen were raised by immunization of a first mammal with a composition containing a purified antibody which

specifically binds to an MHC class II binding fragment of GbpB or one or more of the multi-epitopic constructs described above. Following purification of the antibodies from the first animal, the antibodies are administered to a second animal. Alternatively, antibodies are produced in culture, purified, and administered to a mammal to confer passive immunity. Antibodies elicited by immunization or administered passively inhibit one or more activities, e.g., colonization, of oral Streptococci.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a chart depicting the results of an MHC class motif-matching algorithm used to compare GbpB primary sequence against a set of DBRI alleles represented as matches versus sequence.

FIG. 2 depicts box plots of serum IgG antibody to GbpB peptide constructs QGQ and SYI. Serum IgG antibody activity was measured in ELISA against QGQ (left panel) and SYI (middle panel) peptide constructs and GbpB protein (right panel). Sham-immunized and SYI, QGQ- and GbpB-immunized groups represent the immune experience of 6 rats per group 35 days after the initial of two subcutaneous immunizations. The absorbency was measured at 405nm.

FIG. 3 depicts box plots of serum IgG and IgA antibody to SYI in the protection experiment. IgG (left panel) and IgA (right panel) antibody activity was measured in ELISA against SYI in sera collected at the end of the protection experiment. Sham-immunized and SYI-immunized groups represent the immune experience of 13 rats per group three months after the initial of two subcutaneous immunizations. The absorbency was measured at 405nm.

FIG. 4 depicts box plots of serum IgG and IgA antibody to GbpB in the protection experiment. IgG (left panel) and IgA (right panel) antibody activity was measured in ELISA against GbpB in sera collected at the end of the protection experiment. Sham-immunized and SYI-immunized groups represent the immune experience of 13 rats/per group three months after the initial of two subcutaneous immunizations. The absorbency was measured at 405nm.

FIG. 5 depicts the level of infection after challenge with *S. mutans*. Each plot represents the number of *S. mutans* SJr cultivated after systematic swabbing of molar surfaces eight days

and 65 days after initial infection with *S. mutans* SJr. Bars indicate the mean colony forming units of *S. mutans* SJr in sham- or SYI-immunized groups. Open and closed circles indicate levels of infection of individual rats.

FIG. 6 depicts dental caries after 78 days of infection with *S. mutans* SJr. The buccal, lingual, occlusal and total molar caries scores for sham and SYI-immunized groups are shown in respective panels.

FIG. 7 is a box plot showing serum IgG antibody binding to SYI peptide depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 8 is a box plot showing serum IgG antibody binding to *S. mutans* GbpB depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 9 is a box plot showing serum IgG antibody binding to GLU peptide depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 10 is a box plot showing serum IgG antibody binding to CAT peptide depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 11 is a box plot showing serum IgG antibody binding to *S. sobrinus* GTF depicted as absorbency units at 405nm as measured in an ELISA assay. Sera was collected on day 63.

FIG. 12 is a pictorial representation of the peptide and protein antigens used for the immunization of rats in the ELISA binding assays (results of which are shown in FIGS. 7-11).

DETAILED DESCRIPTION OF THE INVENTION

Mutans streptococcus is the principle etiologic agent of the infectious disease dental caries. This oral pathogen infects the oral cavity during early childhood and normally remains associated with the host's dentition for life. The accumulation of bacteria within the dental biofilm is possible due to the effect of several virulence factors. The bacterial components associated with the accumulation phase of mutans streptococci include glucosyltransferases, their glucan products and glucan binding proteins. At least three *S. mutans* glucan binding proteins have been identified, GbpA, GbpB and GbpC. GbpA shares homology with the putative glucan binding domain of glucosyltransferase and the gbpA gene was found to encode a constitutively expressed secreted protein. Cell surface associated GbpC is related to the Spa family of streptococcal proteins and is only expressed during conditions of stress. GbpB is

immunogenically distinct from the other glucan binding proteins expressed by *S. mutans* and *Streptococcus sobrinus* and also differs in size and purification properties.

Glucan binding protein-B is a single polypeptide chain, which is 431-432 residues in length. Analysis of the primary sequence revealed a leucine zipper domain. However, GbpB bore no sequence homology with glucan binding domains of glucosyltransferases or *S. mutans* glucan binding protein A. This prevented the specific targeting of GbpB domains of putative glucan binding function using subunit vaccine approaches that had been employed successfully with synthetic peptide or recombinant construct derived from GTF glucan binding domains as described in U.S. Patent No. 5,686,075 and U.S. Application No. 09/290,049, the entire contents of which are herein incorporated by reference.

The GbpB sequence bears significant homology with peptidoglycan hydrolases from other gram positive microorganisms, and comparative genomic analysis of the *gbpB* region suggested a functional relationship between genes involved in cell shape and cell wall maintenance. Attempts to knock out the *gbpB* gene indicated that expression of GbpB is essential for the organism. Immunogenic interference with GbpB function reduces the adverse effects associated with the growth of cariogenic *S. mutans* in the oral cavity.

Tables 5-9 include the amino acid sequence of GbpB from various strains *S. mutans*.

Table 5 Deduced Amino Acid Sequence of <i>S. mutans</i> strain SJ32 GbpB GenBank accession number AY046410
MKKRILSAVLVSGVTLSSATTL SAVKADDFDAQIASQDSKINNLTAAQQQA AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM LQQQE QDKAAVEQKQQENQA AINTVAANQETIAQNTNALNTQQAQLEAAQ LNLQAELTTAQDQKATLVAQKAAAE EAARQAAAAQAAAEAKAAAEAKALQ EQAAQAQVAANNNTQATDASDQQA AADNTQAAQTGDSTEQSAAQAVNNS DQESTTATEAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVGN YWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQI QVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:29)

Table 6

Deduced Amino Acid Sequence of *S. mutans* strain 3VF4 GbpB
GenBank accession number AY046411

MKKRILSAVLVSGVTLSSATTLSAVKADDFDAQIASQDSKINNLTAAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LQQQEQDKAAVEQKQQENQAANTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEAAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQAAANNNTQATDASDQQAAAADNTQAAQTGDSTEQSAAQAVNNS
DQESTTATEAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVGN
YWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQI
QVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:30)

Table 7

Deduced Amino Acid Sequence of *S. mutans* strain 15JP2 GbpB
GenBank accession number AY046412

MKKRILSAVLVSGVTLSSATTLSAIKADDFDAQIASQDSKINNLTAAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LQQQEQDKAAVEQKQQENQAANTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEAAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQAAANNNTQATDASDQQAAAADNTQAAQTGDSTDQSAAQAVNN
SDQESTTATAAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVG
NYWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQ
IQVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:31)

Table 8

Deduced Amino Acid Sequence of *S. mutans* strain 3SN1 GbpB
GenBank accession number AY046413

MKKRILSAVLVSGVTLSSATTLSAVKADDFDAQIASQDSKINNLTAAQQQA
AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVSANEKM
LHQQEQDKAAVEQKHQENQAANTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEAAARQAAAAQAAAEAKAAAEAKALQ
EQAAQAQAAANNNTQATDASDQQAAAADNTQAAQTGDSTDQSAAQAVNN
SDQESTTATAAQPSASSASTAAVAANTSSANTYPAGQCTWGVKSLAPWVG
NYWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQ
IQVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:32)

Table 9

Deduced Amino Acid Sequence of *S. mutans* strain 5SM3 GbpB
GenBank accession number AY046414

MKKRILSAVLVSGVTLSSATTLSAVKADDFDAQIASQDSKINNLTAAQQQA

```

AQAQVNTIQGQVSALQTQQAELQAENQRLEAQSATLGQQIQTLSSKIVAR
NESLKQQARSAQKSNAATSYINAIINSKSVSDAINRVSAIREVVVSANEKM
LQQQEQDKAAVEQKQQENQAANTVAANQETIAQNTNALNTQQAQLEAAQ
LNLQAELTTAQDQKATLVAQKAAAEAAARQAAAQAAAEAKAAAEAKALQ
EQAAQAQAANNNTQATDASDQAAAADNTQAAQTGDSTEQSAAQAVNNS
DQESTTATEAQPSASSASTAVVTANTSSANTYPAGQCTWGVKSLAPWVGN
YWGNGGQWAASAAAAGYRVGSTPSAGAVAVWNDGGYGHVAYVTGVQGGQI
QVQEANYAGNQSIGNYRGWFNPGSVSYIYPN (SEQ ID NO:33)

```

The compositions described herein, *e.g.*, subunit vaccine compositions and immunogenic compositions, contain an amino acid sequence subunit of GbpB that is of sufficient length to raise an immune response in a mammal to which it is administered. As used herein, the terms "subunit" or "fragment" refer to a portion of the GbpB protein that is less than the whole naturally-occurring protein. For example, a fragment contains at least 5 contiguous amino acids of the full length naturally-occurring protein. Vaccines containing the peptide constructs described herein elicit antibodies, which bind specifically to functional domains of GbpB and/or GTF and have the additional advantage that such vaccines do not induce immunity to irrelevant or unwanted epitopes. Useful peptides are of sufficient length to raise an immune response in a mammal to which it is administered but will be less than the complete amino acid sequence of the intact GbpB. Typically, the peptide is at least 5-7 amino acids in length. Preferably the peptide is at least 12 amino acids in length; more preferable the peptide is at least 23 amino acids in length. GbpB polypeptides are derived from *S. mutans*. However, glucan binding proteins from the other strains of *S. mutans*, which share significant homology and/or function, can also be utilized. For example, a peptide in the immunogenic compositions and subunit vaccines of the invention typically comprise at least six amino acids with at least four matches to MHC Class II binding motifs. Preferably, the peptide has greater than five amino acid matches and most preferably the peptide has greater than six amino acid matches to an MHC class II binding motif. The matches are determined, for example, using a matrix-based algorithm for epitope prediction known in the art.

Peptides as shown in FIG.1 which have a significant peak resulting from a comparison of GbpB primary sequence against a set of DBRI alleles from MHC class II motif are also contemplated. For example, according to FIG. 1, peptides which extend at least 6 amino acid residues to the right in length from residues 16, 62, 90, 121, 322 and 369 are peptides having at least four matching residues to DBR1 alleles and are contemplated for use in the compositions of

the instant application. Suitable peptides may also encompass amino acid residues to the left of the indicated peak.

HLA-binding peptides of GbpB

GbpB peptides were synthesized and evaluated for immunogenicity, reactivity with the parent protein, and induction of caries-protective immunity. Exemplary peptides include the following fragments of glucan binding protein-B: KSNAATSYINAIINSKSVSD (the SYI peptide GbpB residues 113-132) (SEQ ID NO: 1); KHKLITIQQGVVSALQTQQAG (SEQ ID NO: 2); the SAS peptide TATEAQPSASSASTAAVAAN residues 306-325 (SEQ ID NO: 3); LSAVLVSGVTLSSATTL SAV residues 6-25 (SEQ ID NO: 4); LSSATTL SAVKADDFDAQIA residues 16-35 (SEQ ID NO: 5); QIASQDSKINNLT AQQQAAQ residues 33-52 (SEQ ID NO: 6); QDSKINNLT AQQQAAQAQVN residues 37-56 (SEQ ID NO: 7); QQAQAQVNTIQQGVVSALQT residues 48-67 (SEQ ID NO: 8); QAQVNTIQQGVVSALQTQQAQAE residues 52-71 (SEQ ID NO: 9); QQIQTLSKIVARNESLKQQ residues 88-107 (SEQ ID NO: 10); ATSYINAIINSKSVSDAINR residues 117-136 (SEQ ID NO: 11); VSAIREVVSANEKMLQQQEQ residues 137-156 (SEQ ID NO: 12); TVAANQETIAQNTNALNTQQ residues 174-193 (SEQ ID NO: 13); AQLEAAQLNLQAELTTAQDQ residues 194-213 (SEQ ID NO: 14); KATLVAQKAAAEAAARQAAA residues 214-233 (SEQ ID NO: 15); ALQEQAAQAQVAANNNTQAT residues 248-267 (SEQ ID NO: 16); TEQSAAQAVNNSDQESTTAT residues 289-308 (SEQ ID NO: 17); QPSASSASTAAVAANTSSAN residues 311-330 (SEQ ID NO: 18); GNYWGNNGGQWAASAAAAGYR residues 349-368 (SEQ ID NO: 19); AGYRVGSTPSAGAVAVWNDG residues 365-384 (SEQ ID NO: 20); DGGYGHVAYVTGVQGGQIQV residues 383-402 (SEQ ID NO: 21); QEANYAGNQSIGNYRGWFNP residues 403-422 (SEQ ID NO: 22); GNYWGNNGGQWAASAAAAGRY (SEQ ID NO: 41). Amino acid residue coordinates refer to full length GbpB (SEQ ID NO: 29). Equivalent peptides are intended to include equivalent sites (e.g., positions or residues) in other mutans streptococcal glucan binding proteins. For example, other glucan binding peptides can be found in *S. sobrinus* or other *S. mutans* strains. The

equivalents can be identified, for example, by aligning the amino acid sequences of other mutants streptococcal GbpB's, as is routinely done by one of skill in the art.

As used herein, a vaccine composition is a composition, which elicits an immune response in a mammal to which it is administered. Elicitation of GbpB-specific antibodies protects the immunized mammal against subsequent challenge by the immunizing agent or an immunogenically cross-reactive agent. For example, production of mucosal antibodies specific for GbpB reduces the amount of *S. mutans* in an immunized mammal. Protection can be complete or partial, such as a reduction or elimination of symptoms or infection as compared with an unvaccinated mammal. An immunogenically cross-reactive agent can be, for example the whole protein (GbpB) from which a subunit peptide used as the immunogen is derived. Alternatively, an immunogenically cross-reactive agent can be a different protein, which is recognized in whole or in part by the antibodies elicited by the immunizing agent.

As used herein, an immunogenic composition encompasses a composition, which elicits an immune response in a mammal to which it is administered and which may or may not protect the immunized mammal against subsequent challenge with the immunizing agent or an immunogenically cross-reactive agent.

The raised immune response is characterized by a B cell response, a T cell response or both a B cell and T cell response. The B cell response is associated with the appearance of mucosal antibody, which is predominately IgA, and systemic antibody, which is predominantly IgG. The antibodies elicited by immunization preferably recognize both the immunizing agent and an immunogenically cross-reactive agent (*e.g.*, the immunizing peptide and the intact GbpB protein). The antibody response protects the immunized mammal against subsequent challenge or infection with the immunizing agent or an immunogenically cross-reactive agent.

In addition to the peptides listed in Table 1, other immunogenic domains of GbpB, as well as domains of non-GbpB origin, which enhance adjuvanticity or produce an immunogenic response against other infectious agents are optionally included in the compositions of the invention. For example, the vaccine or immunogenic composition contains an additional immunogenic component which is an immunogenic portion of a pathogen including, but not limited to, diphtheria, pertussis, tetanus, measles, influenza, poliovirus, and retroviruses resulting in a multivalent composition raising an immune response to greater than one infectious disease

or agent. A multivalent vaccine includes immunogenic epitopes and appropriate adjuvant sequences targeting early childhood infections.

GbpB-GTF Chimeric Peptides

Immunogenic compositions containing one or more domains of GbpB combined with one or more domains of GTF were produced and evaluated for the ability to elicit antibody production. This strategy permits a combined attack on the molecular pathogenesis of *mutans streptococci* utilizing two or more epitopes. Synthetic peptides containing an amino acid sequence of one or more functional domains of *Streptococcus mutans* glucosyltransferases induce immune responses, which reduce recolonization of the bacteria. Chimeric peptides containing GTF sequences and GbpB sequences provide a more comprehensive attack on the colonization of the bacteria by increasing the enzyme inhibitory capacity of the immune response, eliminating responses to irrelevant epitopes and reducing the glucan binding capacity.

Tables 10-16 include the amino acid sequence of GTF isozymes from various *Streptococci*.

Table 10
Deduced Amino Acid Sequence of *S. mutans* GTF-B

```
MDKKVRYKLRKVKKRWVTVSVASAVMTLTTLSGGLVKADSNESEK
SQISNDSNTSVVTANEESNVITEATSKQEAASSQTNHTVTTSSSSTSVVNPKEVVSNP
YTVGETASNGEKLQNQTTTVDKTSEAAANNISKQTTEADTDVIDDSNAANLQILEKLP
NVKEIDGKYYYDNNKGVRTNFTLIADGKILHFDDETGA YTDTSIDTVNKDIVTTRSNI
YKKYNQVYDRSAQSFEHVDHYLTAESWYRPKYILKDGKTWTQSTEKDFRPLLMTWWPD
QETQRQYVNYMNAQLGINKTYDDTSNQLQLNIAAATIQA KIEAKITTLKNTDWLRQTI
SAFVKTQSAWNSDSEKPFDDHLQNGAVLYDNEGKLT PYANSNYRILNRTPTNQTGKKD
PRYTADNTIGGYEFLLANDVDNSNPVVQAEQLNWLHFLMNF GNIYANDPDANFDSIRV
DAVDNVDADLLQIAGDYLKAAKGIHKNDKAANDHLSILEAWS DNDTPYLHDDGDNMIN
MDNKLRLSLLFSLAKPLNQRSGMNPLITNSLVNRTDDNAETA AVPSYSFIRAH DSEVQ
DLIADI IKA E INPNVVGYSFTMEEIKKAFEIYNKDLLATEKKYTHYNTALS YALLLTN
KSSVPRVYYGDMFTDDGQYMAHKTINYEAIETLLKARIKYVSGGQAMRNQQVGNSEII
TSVRYGKGALKATDTGDRTRTSGVAVIEGNNPSLRLKASDRVVNMGA AHKNQAYRP
LLLTTDNGIKAYHSDQEAAGLVRYTNDRGELIFTAADIKGYANPQVSGYLG VWPVGA
ALIKMFALRLARPHQQMASVHQNAALDSRVMFEGFSNFQAFATKKEEYTNVVI AKNVD
KFAEWGVTD FEMAPQYVSSTDG SFLDSVIQNGYAFTDRYDLGISKPNKYGTADDLVKA
IKALHSKGIKVMADWVPDQMYAFPEKEVVTATRVDKYGTPVAGSQIKNTLYVVDGKSS
GKDQQA KYGGAFLEELQAKYPELFARKQISTGVPM DPSVKIKQWSAKYFNGTNILGRG
AGYVLKDQATNTYFNISDNKEINFLPKTLLNQDSQVGFSYDGKG YVYYSTSGYQAKNT
FISEGDKWYYFDNNGYMTGAQSINGVNY YFLSNGLQLRDAILKNEDGTYAYYGNDGR
RYENGY YQFM SGVWRHFNNGEMSVGLTVIDGQVQYFDEMGYQAKGKFVTTADGKIRYF
DKQSGNMYRNRFIENEEGKWLYLGEDGAAVTGSQTINGQHLYFRANGVQVKGEFVTDH
```

HGRISYYDGNSSGDQIRNRFVRNAQGGQWFYFDNNGYAVTGARTINGQLLYFRANGVQVK
 GEFVTDYGRISYYDGNSSGDQIRNRFVRNAQGGQWFYFDNNGYAVTGARTINGQHLYFR
 ANGVQVKGEFVTDYHGRISYYDGNSSGDQIRNRFVRNAQGGQWFYFDNNGYAVTGARTIN
 GQHLYFRANGVQVKGEFVTDYGRISYYDANSGERVRIN (SEQ ID NO:34)

Table 11

Deduced Amino Acid Sequence of *S. mutans* GTF-C

MEKKVRFKLRKVKKRWTVSIAVVTLTSLSGSLVKADSTDDR
 QQAVTESQASLVTTSEAAKETLTATDTSTATSATSQPTATVTDNVSTTNQSTNTTANT
 ANFVVKPTTTTSEQAKTDNSDKIITTSKAVNRLTATGKFVPANNNTAHPKTVTDKIVPI
 KPKIGKLGKQPSSLSQDDIAALGNVKNIRKVNGKYYYYKEDGTLQKNYALNINGKTEFF
 DETGALSNNLTLPSSKGNITNNDNTNSFAQYNQVYSTDVANFEHVDHYLTAESWYRPKY
 ILKDGKTWTQSTEKDFRPLLMTWWPDQETQRQYVNYMNAQLGIHQTYNTATSPLQLNL
 AAQTIQTKIEEKITAENNTNWLRTISAFVKTQSAWNSDSEKPFDDHLQKGALLYSN
 SKLTSQANSNYRILNRTPTNQTGKKDPRYTADRTIGGYEFLLANDVDNSNPVVQAEQL
 NWLHFLMNFNGNIYANDPDANFDSIRVDAVDNVDADLLQIAGDYLKAAKGHKNDKAAN
 DHLSELEAWSYNDTPYLHDDGDNMINMDNRLRLSLLYSLAKPLNQRSGMNPLITNSLV
 NRTDDNAETAAPVPSYSFIRAHDSVQDLIRNIIRTEINPNVVGYSFTTEEIKKAFEIY
 NKDLLATEKKYTHYNTALSALLTNKSSVPRVYGYDMFTDDGQYMAHKTINYEALET
 LLKARIKYVSGGQAMRNQOVGNSEIITSVRYGKGALKATDTGDRTRTSGVAVIEGNN
 PSLRLKASDRVVNMGAHKNQAYRPLLLTTDNGIKAYHSDQEAAGLVRYTNDRGELI
 FTAADIKGYANPQVSGYLGWVVPVGAADQDVRVAASTAPSTDGKSVHQNAALDSRVM
 FEGFSNFQAFATKKEEYTNVVIKKNVDKFAEWGVTDFEMAPQYVSSTDGSLDSVIQN
 GYAFTDRYDLGISKPNKYGTADDLVKAIKALHSGIKVMADWVPDQMYALPEKEVVTA
 TRVDKYGTPVAGSQIKNTLYVVDGKSSGKDQQAQYGGAFLEELQAKYPELFARKQIST
 GVPMDPSVKIKQWSAKYFNGTNILGRGAGYVLKDQATNTYFSLVSDNTFLPKSLVNP
 HGTSSSVTGLVFDGKGYVYYSTSGNQAKNAFISLGNNWYFYFDNNGYMTGAQSINGAN
 YYFLSNGIQLRNAIYDNGNKVLSYYGNDGRRYENGYYLFGQQWRYFQNGIMAVGLTRV
 HGAVQYFDASGFQAKGQFITTADGKLRYFDRDSGNQISNRFVRNSKGEWFLFDHNGVA
 VTGTVTFNQRLYFKPNGVQAKGEFIRDANGYLRYYDPNSGNEVRNRFVRNSKGEWFL
 FDHNGIAVTGARVVNGHASILSLMVFRLLRESSLQSVKVVSNMILIPMKFVIVM
 (SEQ ID NO:35)

Table 12

Deduced Amino Acid Sequence of *S. mutans* GTF-D

METKRRYKMHKVKKHWTVAVASGLITLGTTLGSSVSAETEQQ
 TSDKVVTQKSEDDKAASESSQTDAPKTKQAQTEQTQAQSQANVADTSTSITKETPSQN
 ITTQANSDDKTVTNTKSEEAQTSEERTKQSEEAQTTASSQALTQAKAELTKQRQTAAQ
 ENKNPVDLAAIPNVKQIDGKYYYIGSDGQPKKNFALTVNKNVLYFDKNTGALTDTSQY
 QFKQGLTKLNNDYTPHNQIVNFENTSLETIDNYVTADSWYRPKDILKNGKTWTASSES
 DLRPLLMSWWPDQQTQIAYLNYMNQQGLGTGENYTADSSQESLNLAQTVQVKIETKI
 SQTQQTQWLRDIINSFVKTPQPNWNSQTESDTSAGEKDHLLQGGALLYSNSDKTAYANS
 YRLNRTPTSQTGKPKYFEDNSSGGYDFLLANDIDNSNPVVQAEQLNWLHYLMNYGSI

VANDPEANFDGVRVDAVDNVNADLLQIASDYLKAHYGVDKSEKNAINHLSILEAWSDN
 DPQYNKDTKGAQLPIDNKLRLSLLYALTRPLEKDASNKNEIRSGLEPVITNSLNNRSA
 EGKNSEARMANYIFIRAHDSVQTVIAKIIKAQINPKTDGLTFTLDELKQAFKIYNEDM
 RQAKKKYTQSNIPTAYALMLSNKDSITRLYYGDMYSDDGQYMATKSPYYDAIDTLLKA
 RIKYAAGGQDMKITYVEGDKSHMDWDYTGVLTSVRYGTGANEATDQGSEATKTQGMAV
 ITSNNPSLKLNNQNDKVIIVNMGAHKNQEYRPLLLTTKDGLTSYTSDAAAKSLYRKTND
 KGELVFDASDIQGYLNPQVSGYLAVWVPVGASDNQDVRVAASNKANATGQVYESSAL
 DSQLIYEGFSNFQDFVTKDSDYTNKKIAQNVQLFKSWGVTSEMAPQYVSSEDGSLD
 SIIQNGYAFEDRYDLAMSKNNKYGSQQDMINAVKALHKSQIQVIADWVPDQIYNLPKG
 EVVTATRVNDYGEYRKDSEIKNTLYAANTKSNGKDYQAKYGGAFLELAAYPSIFNR
 TQISNGKKIDPSEKITAWKAKYFNGTNILGRGVGYVLKDNASDKYFELKGNQTYLPKQ
 MTNKEASTGFVNDGNGMTFYSTSGYQAKNSFVQDAKGNWYYFDNNGHVMVYGLQQLNGE
 VQYFLSNGVQLRESFLENADGSKNYFGHLGNRYSNGYYSFDNDSKWRYFDASGVMAVG
 LKTINGNTQYFDQDGYQVKGAWITGSDGKKRYFDDGSGNMAVNRFANDKNGDWYYLNS
 DGIALVGVQTINGKTYFYGQDGKQIKGKIITDNGKLKYFLANSSELARNIFATDSQNN
 WYYFGSDGVAVTGSQTIAGKKLYFASDGKQVKGSFVTYNGKVHYYHADSGELQVNRFE
 ADKDGWYYLDSNGEALTGSQRINDQRVFFFTREGKQVKGDVAYDERLLVYR (SEQ ID NO:36)

Table 13

Deduced Amino Acid Sequence of *S. sobrinus* GTF-I

MEKNVRFKMHKVKRWTLSVASATMLASALGASVASADTDTS
 DDSNQAVVTGDQTTNNQATDQTSIAATATSEQSASTDAATDQASAAEQTGTTASTDT
 AAQTTTNANEAKWVPTENENQGFTDEMLAEAKNVATAESDSIPSDLAKMSNVKQVDGK
 YYYDQDGNVKKNFVSVGDKIYFDETGAYKDTSKVDADKSSSAVSQNAITFAANNR
 AYSTSAKNFEAVDNYLTADSWYRPKSILKDGKTWTESGKDDFRPLLMAWWPDTETKRN
 YVNYMKNKVVGIDKTYTAETSQADLTAAAEVLQARIEQKITSENNTKWLREAI SAFVKT
 QPQWNGESEKPYDDHLQNGALLFDNQTDLTPDTQSNYRLNRTPTNQTGSLDSRFTYN
 PNDPLGGYDFLLANDVDNSNPVQAEQLNWLHYLLNFGSIYANDADANFDSIRVDAVD
 NVDADLLQISSDYLKAAYGIDKNNKNANNHVSIVEAWSNDTPYLHDDGDNLMNMDNK
 FRLSMLWSLAKPLDKRSGLNPLIHNSLVDREVDDREVETVPSYSFARAHDSVQDIIR
 DIIKAEINPNSFGYSFTQEEIEQAFKIYNEDLKKTKKYTHYNVPLSYTLLLTNKGSI
 PRVYYGDMFTDDGQYMANKT VNYDAIESLLKARMKYVSGGQAMQNYQIGNGEILTSVR
 YGKGALKQSDKGDATTRTSGVGVVMGNQPNFSLDGKVVALNMGAAHANQEYRALMVST
 KDG VATYATDADASKAGLVKRTDENG YLYFLNDDLKGVANPQVSGFLQVWVPVGAADD
 QDIRVAASDTASTDGKSLHQDAAMDSRVMFEGFSNFQS FATKEEEYTNVVIANNVDKF
 VSWGITDFEMAPQYVSSTDGQFLDSVIQNGYAFTRDYDLGMSKANKYGTADQLVKAIAK
 ALHAKGLKVMADWVPDQMYTFPKQEVTVTRTDKFGKPIAGSQINHSLYVTDTKSSGD
 DYQAKYGGAFLEDELKEKYPELEFTKKQISTGQAIDPSVKIKQWSAKYFNNGSNILGRGAD
 YVLSQVSNKYFNVASDTLFLPSSLLGKVVESGIRYDGKGYIYNSSATGDQVKASFIT
 EAGNLYYFGKDGMYMTGAQTINGANYFFLENGTALRNTIYTDAQGN SHYYANDGKRYE
 NGYQQFGNDWRYFKDGNMAVGLTTVDGNVQYFDKDG VQAKDKIIVTRDGKVRYFDQHN
 GNAATNTFIADKTGHWYYLGKDGVAVTGAQTVGKQKLYFEANGQQVKGDFVTSDEGKL
 YFYDVDSGDMWTDTFIEDKAGNWFYLGKDGA AVTGAQTIRGQKLYFKANGQQVKGDIV
 KGTGDKIRYYDAKSGEQVFNKTVKAADGKTYVIGNDGVAVDPSVVKGQTFKDASGALR
 FYNLKGQLVTGSGWYETANHDWVYIQSGKALTGEQTINGQHLYFKEDGHQVKGQLVTG
 TDGKVRYDANS GDQAFNKSVTVNGKTYFYGNDGTAQTAGNPKGQTFKDGSDIRFYSM
 EGQLVTGSGWYENAGQQLVYVKNKGVLTLGLQTVGSQRVYFDENGIQAKGKAVRTSDGK
 IRYFDENSGSMITNQWKFVYGQYYYFGNDGARIYRGWN (SEQ ID NO:37)

Table 14

Deduced Amino Acid Sequence of *S. sobrinus* GTF-U

MEKKLHYKLHKVKKHWVTIAVASIGLVSLVGAGTVSAEDKVAND
TTAQATVGVDTGQDQATTNDANTNTTDTDTADQSANTNQDQAGSDQSNNQDQAKQDTA
NTDRNQADNSQTDNNQATDQATSPATDGTSVQRRDAANVATAADQEGQTAPSEQEKS
ALSLDNVKLIDGKYVYVQADGSYKKNFAITVNGQMLYFDSDTGALSSTSTYSFSQGT
NLVDDFSSHNKAYDSTAKSFELVNGYLTANSWYRPAGILRNGQTWEASNENDLRPVL
SWWPKDQTQVAYVNYMKNKLSANETEVNETSQVDLNKEAQSIQTKIEQKITSNDSTQ
WLRTAMEAFVAAQPKWNMSTENFNKGDHLQGGALLYTNSDLTPWANS DYRLNRTPTQ
QDGTKKYFTEGGEGGYEFLLSNDVDNSNPVQAEQLNQLHYLMNWGDI VMGDKDANFD
GVRVDAVDNVNADLLQVYSNYFKDNYKVT DSEANALAHISILEAWSLNDNQYNEDTNG
TALSIDNSSRLTSLAVLTKQPGQRI DLSNLISESVNKERANDTAYGDTIPTYSFVRAH
DSEVQTVIAKIVKEKIDTNSDGYTFTLDQLKDAFKIYNEDMAKVNKTYTHYNI PAAYA
LLLSNMESVPRVYYGDLYTDDGQYMAKKS PYDAIATMLQGRIAYVSGGQSEEVHKVN
GNNQILSSVRYGQDLMSADDTQGTDL SRTSGLVTLVSNDPNLDLGGDSLTVNMGRAHA
NQAYRPLILGTDGVSYLKDSDTNIVKYTDANGNLTFTADDIKGYSTVDM SGYLAVW
VPVGAKDGDVRVAADTNQKADGKSLKTS AALDSQVIYEGFSNFQDFANNDADYTNKK
IAENADFFKKLGITSFEMAPQYVSATDGSFLDSIIQNGYAFSDRYDLAMSKNNKYGSK
DDLANKALHANGIQAIADWVPDQIYQLPGEEVVTAKRTNSYGNPTFDAYINNALYA
TNTKSSGSDYQAQYGGAFLELAKAYPDMFTVNMISTGKPIDPSTKIKQWEAKYFNGT
NVLGKGAGYVLSDDATGKYFTVNENGDFLPASFTGDQNAKTGFYYDGTGMAYYSTSGN
KAVNSFIYEGGHYYYFDKDGHMVTGSYKAEDGNDYYFLPNGIQMRDAIYQDAQGNSYY
YGRGTGILYKGDNWYPFVDPNNANKTVFRYFDANNVMAIGYRNMYGQTYYFDENGFOAK
GQLLTDDKGTHYFDEDNGAMAKNKFN VVGDDWYMDGNNGNAVKGQYPVNNQILYFNPE
TGVQVKGQFITDAQGRTSYYDANS GALKSSGFFT PNGSDWYYAENGYVYKGFQVAEN
QDQWYYFDQTTGKQAKGA AKVDGRDLYFNPD SGVQVKGFATDESGNTSFYHGDNGDK
VVGGFFTTGNNAWYYADNNGNLVKGFQEIDGKWHYFDEV TGQQA KGAALVNGQQLYFD
VDSGIQVKGDFVTDGQGNTSY YDVNSGDKKVNGFFTGDNAWYYADGQGNLAKGRKSI
DNQDLYFDPATGKQVKGQLVSI DGRNYYFDSGSGNMAKNRFVRIGDQWIYFGNDGAAT
NL (SEQ ID NO:38)

Table 15

Deduced Amino Acid Sequence of *S. downei* GTF-S

MEKNLRYKLHKVKKQWVAIGVTTVTLSFLAGGQVVAADTNNNDG
TSVQVNKMVPSDPKFDAQAQNGQLAQAMFKAANQADQTATSQVSPATDGRVDNQVTPA
ANQPAANVANQDVANPATDAGALNRQSAADTSTDGKAVPQTS DQPGHLETVDGKTYV
DANGQRLKNYSMVIDGKTYYFDGQTGEAQTDLPKTGQANQDNVPDSYQANNQAYSNEA
SSFETVDNYLTADSWYRPRKILKNGQSWQASSEGDLRPILMTWWPDAATKAAYANFWA
KEGLISGSYRQNSANLDAATQNIQSAIEKKIASEGNTNWLRDKMSQFVKSQNQWSIAS
ENETVYPNQDHMQGGALLFSNSKDTEHANS DWRLNLRNPTFQTGKQKYFTTNYAGYEL
LLANDVDNSNPVQAEQLNHLHYLMNWGDI VMGDKDANFDGVRVDAVDNVNADLLQIQ
RDYYKAKYGTQNEKNAIDHLSILEAWSGNDNDYVKDQNNFSLSIDNDQRS GMLKAFG

YASAYRGNLSNLATAGLKNRSANPDSDPVPNYVFIRAHDSVQTRIAKIIREKLGKTN
 ADGLTNLTLDLNLKAFDIYNQDMNATDKVYYPNNLPMAYAWMLQNKDTVTRVYYGDMY
 TDNGQYMATKTPFYNAIETLLKGRIKYVAGGQAVSYKQDWSSGILTSVRYGKGANSAS
 DAGNTETRNSGMALLINNRPNFRAYRNLTLMGAAHKSQAYRPLLSTKDGIIATYLND
 SDVDSRQYKYTDSQGNLSFSASELQSVANAQVSGMIQVWVPVGAADNQDVRTSPSTQA
 TKDGNIYHQSDALDSQVIYEGFSNFQAFAQSPDQYTNNAVIKNGDLFKSWGITQFEMA
 PQYVSSSEDGTFLDSVILNGYAFSDRYDLAMSKNNKYGSKQDLANAIKGLQSAGIKVLS
 DLVPNQLYNLPGKEVVTATRVNQYGQAKSGATINKTPYVANTRSYGDYQEYGGKFLD
 DLQKLYPRLFSTKQISTGKPIDPSVKITNWSAKYFNGSNILGRGAKYVLSEGNKYLN
 ADGKLFPLPTVLNNTYGPQVSANGFISKNGGIHYLDKNGQEVKNRFKEISGSWYYFDS
 DGKMATGKTKIGNDTYLFMPNGKQLKEGVWYDGGKAYYYDDNGRTWTNKGFEFRVDG
 QDKWRYFNGDGTIAIGLVSLDNRTLYFDAYGYQVKGQTVTINGKSYTFDADQGDVQV
 DNANPAPQGQAGWKL LGDNQWGYRKDGQLLTGEQTIDGQKVFFQDNGVQVKGGTATDA
 SGVLRFYDRDQGHQVGKGWYSTSDDNWVYVNESGQVLTGLQTIDGQTVYFDDKGIQAK
 GKAVWDENGNLRYFDADSGNMLRDRWKNVDGNWYYFNRNGLATRW (SEQ ID NO: 39)

Table 16

Deduced Amino Acid Sequence of *S. salivarius* GTF-I

MENKIHKLHKVKKQWVTIAVASVALATVLGGLSVTTSSVSAD
 TQDKTVTQSNSTASLVTSPPEATKEADKRTNTKEADVLTPAKETNAVETATTTNTQA
 TAEAATTATTADVAVAAVPNKEAVVTDDAPAVTTEKAEQPATVKAEEVNTEVKAPEA
 ALKDSEVEAALSLKNIDGKYYYVNEDGSHKENFAITVNGQLLYFGKDGALTSSST
 YSFTPGTTNIVDGFSINNRAYDSSEASFELIDGYLTADSWYRPASIIKDGVTWQASTA
 EDFRPLLMAWWPNVDTQVNYLNYMSKVFNLDKYSSTDKQETLKVAAKDIQIKIEQKI
 QAEKSTQWLRETISAFVKTQPQWNKETENYSKGGGEDHLQGGALLYVNDSTRTPWANS
 YRRLNRTATNQTGTIDKSILDEQSDPNHMGGFDFLLANDVDLSNPVVQAEQLNQIHYL
 MNWGSIVMGDKDANFDGIRVDAVDNVDADMLQLYTNYFREYYGVNKSEANALAHISVL
 EAWSLNDNHYNDKTDGAALAMENKQRLALLFSLAKPIKERTPAVSPLYNNTFNTTQRD
 EKTDWINKDGSKAYNEDGTQVQSTIGKYNEKYGDASGNVVFIRAHDNVQDIIAEIIK
 KEINPKSDGFTITDAEMKQAFEIYNKDMLSSDKKYTLNNIPAAAYAVMLQNMETITRVY
 YGDLYTDDGHYMETKSPYYDTIVNLMKSRIKYVSGGQAQRSYWLPTDGKMDNSDVELY
 RTNEVYTSVRYGKDIMTANDTEGSKYSRTSGQVTLVANNPKLNLDQSAKLVNEMGKIH
 ANQKYRALIVGTADGIKNFTSDADAIAAGYVKETDSNGVLTFGANDIKGYETFDMSGF
 VAVWVPVGASDNQDIRVAPSTEAKKEGELTLKATEAYDSQLIYEGFSNFQTIPDGSDP
 SVYTNRKIAENVDLFKSWGVTSFEMAPQFVSADDGTFLDSVIQNGYAFADRYDLAMSK
 NNKYGSKEDLRDALKALHKAGIQAIADWVPDQIYQLPGKEVVTATRTDGAGRKIADAI
 IDHSLYVANSKSSGKDYQAKYGGEFLAELKAKYPEMFKVNMI STGKPIDDSVKLKQWK
 AEYFNGTENVLERGVGYVLSDEATGKYFTVTKEGNFIPLQLTGKEKVITGFSSDGKGIT
 YFGTSGTQAKSAFVTENGNTYYFDARGHMVTNSEYSPNGKDVYRFLPNGIMLSNAFYI
 DANGNTYLYNSKGQMYKGGYTKFDVSETDKDGKESKVVKFRYFTNEGVMAGVTVIDG
 FTQYFGEDGFQAKDKLVTFKGKTYFDDAHTGNGIKDTRWNINGKWYYFDANGVAATGA
 QVINGQKLYFNEDGSQVKGGVVKNAADGTYSKYKEGFGELVTNEFFTTDGNVWYYAGAN
 GKTVTGAQVINGQHLYFNADGSQVKGGVVKNAADGTYSKYNASTGERLTNEFFTTGDNN
 WYYIGANGKSVTGEVKIGDDTYFFAKDGKQVKGQTVSAGNGRISYYYGDSGKRAVSTW
 IEIQPGVYVYFDKNGLAYPPRVLN (SEQ ID NO: 40)

Exemplary GTF peptides are shown in Tables 17-19.

Table 17
Catalytic Domain Peptides of GTF
DANFDSIRVDAVDNVDADLLQI (SEQ ID NO: 25)
PLDKRSGLNPLIHNSLVDREVDDRE (SEQ ID NO: 26)

Table 18
Glucan-binding Domain Peptides of GTF
TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO: 23)
DGKLRYDANSQDQAFNKS (SEQ ID NO: 27)

Table 19
Surface Domain Peptide of GTF
QWNGESEKPYDDHL (SEQ ID NO: 28)

Diepitopic peptide constructs, which induce protective antibody to both *S. mutans* GbpB, and GTF of *mutans streptococci* are made using known methods. One peptide is drawn from the GbpB sequence and the other peptide is drawn from the GTF sequence. Useful GTF sequences are described in U.S. Patent No. 5,686,075 and U.S. Patent Application 09/290,049. The multiepitopic sequences are placed on a multiple antigenic peptide (MAP) backbone, expressed recombinantly or in an attenuated expression vector or by other methods known in the art. For example, diepitopic immunogenic and vaccine compositions include *S. mutans* GbpB peptide KSNAATSYINAIINSKSVSD (SEQ ID NO: 1) combined with *S. sobrinus* GTF-B residues 1303 to 1324; TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO: 23) or *S. mutans* GTF-B residues 442 to 462; DANFDSIRVDAVDNVDADLLQ (SEQ ID NO: 24).

The peptides are directly linked to one another or are separated by intervening residues (e.g., one or more lysines). The compositions optionally contain an immunogenicity-enhancing agent, such as a bacterially-derived adjuvant. For example, a GbpB or GTF peptide is conjugated to a known protein, (such as tetanus toxoid) or a carrier (such as a synthetic polymer carrier) to give a macromolecular structure to the composition, which enhances immunogenicity. The compositions contain at least two different peptides, and the peptides are synthesized and

covalently attached to a peptidyl core matrix to yield a macromolecule with a high density of peptides in a single structure. Each peptide in such a structure comprises a GbpB peptide, which is of sufficient length to raise an immune response in a mammal to which it is administered. The composition optionally contains a plurality of copies of a GbpB or GTF peptide. Synthetic peptide vaccine design was carried out using a MAP construct using known methods, *e.g.*, Tam *et al.*, PNAS USA 85:5409-5413 (1988).

A peptidyl core matrix contains of amino acids such as lysine, arginine and histidine. In particular, at least 2 peptides are synthesized on a core matrix of at least one lysine to yield a macromolecular vaccine composition. Particularly, at least 2 peptides are synthesized on a core matrix of 3 lysines. In another example, the vaccine composition is made by covalently attaching 4 peptides to a core matrix of 3 lysines yielding a radially branched peptide with four dendritic arms. The four peptides present can be the same or different. Such multiepitopic peptide constructs induced enhanced immune responses. Moreover, the combination of sequences from several strains into a synthetic or recombinant multi-epitopic construct increases the protective potential of subunit vaccines for dental caries.

Vaccine Formulations

Suitable peptide(s) are incorporated into a microparticle, *e.g.*, a PGLA microparticle, for improved delivery and immune response. Such bioadhesive microparticles can facilitate primary and secondary mucosal antibody formation. Additionally, other ways of enhancing immune responses to mucosally applied peptides (antigens) include use of mucosal adjuvants such as detoxified versions of cholera toxin or *E. coli* heat-labile toxins (Smith *et al.*, *Infect. Immunity* 69(8):4767-4773 (2002)).

Peptides are formulated with a physiologically acceptable medium. The physiological medium may include, but is not limited to, water, buffered saline, polyols (*e.g.*, glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to in the art, and will depend on the ultimate pharmaceutical formulation desired. Methods of introduction of exogenous peptides at the site of treatment include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, oral, sublingual, intraocular, rectal and intranasal. Other suitable methods of introduction can also include rechargeable or biodegradable devices and slow release polymeric

devices. The compositions of this invention can also be administered as part of a combinatorial therapy with other agents.

Peptides are administered at an intravenous dosage of approximately 1 to 100 μ moles of the polypeptide per kg of body weight per day. Administration is typically parenteral. For example, the peptides are administered intravenously, subcutaneously, intramuscularly, intraperitoneally, orally, or intranasally.

Antibody Production

The peptides of the invention are used to raise antibodies or to elicit an immune response. The term "antibody" as used herein refers to immunoglobulin molecules and immunogenically active portions of immunoglobulin molecules, *i.e.*, molecules that contain an antigen binding site that specifically binds an antigen. A molecule that specifically binds to a peptide of the invention is a molecule that binds to that peptide or a fragment thereof, but does not substantially bind other molecules in a sample, *e.g.*, a biological sample, which naturally contains the peptide. Examples of immunogenically active portions of immunoglobulin molecules include F(ab) and F(ab')₂ fragments which can be generated by treating the antibody with an enzyme such as pepsin. The invention provides polyclonal, monoclonal, and transgenic antibodies that bind to a peptide of the invention. The term "monoclonal antibody" or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one species of an antigen binding site capable of immunoreacting with a particular epitope of a peptide of the invention. A monoclonal antibody composition thus typically displays a single binding affinity for a particular peptide of the invention with which it immunoreacts.

Polyclonal antibodies are prepared as described above by immunizing a suitable subject with a desired immunogen, *e.g.*, the whole glucan binding protein-B, a peptide of the invention or fragment thereof. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized peptide or protein. If desired, the antibody molecules directed against the peptide can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction. Also, antibodies can be isolated from the yolks of immunized chicken eggs (IgY) (Svendsen *et al.*, *Lab. Anim. Sci.* 45:89-93 (1995)) or transgenic antibodies can be prepared in plants or other hosts and extracted

for human use (Ma *et al.*, *Eur. J. Immunol.*, 24:131-138 (1994)). At an appropriate time after immunization, *e.g.*, when the antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler and Milstein, *Nature*, 256:495-497 (1975), the human B cell hybridoma technique (Kozbor *et al.*, *Immunol. Today*, 4:72 (1983)), the EBV-hybridoma technique (Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96 (1985)) or trioma techniques. The technology for producing hybridomas is well known (see generally Ausubel, *et al.* (Eds.), *Current Protocols in Immunology*, John Wiley & Sons, Inc., New York, NY (2001)). Briefly, an immortal cell line (typically a myeloma) is fused to lymphocytes (typically splenocytes) from a mammal immunized with an immunogen as described above, and the culture supernatants of the resulting hybridoma cells are screened to identify a hybridoma producing a monoclonal antibody that binds a peptide of the invention.

Any of the many well known protocols used for fusing lymphocytes and immortalized cell lines can be applied for the purpose of generating a monoclonal antibody to a peptide of the invention (see, *e.g.*, *Current Protocols in Immunology*, *supra*; Galfre *et al.*, *Nature*, 266:55052 (1977); R.H. Kenneth, in *Monoclonal Antibodies: A New Dimension In Biological Analyses*, Plenum Publishing Corp., New York, New York (1980); and Lerner, *Yale J. Biol. Med.*, 54:387-402 (1981)). Moreover, the ordinarily skilled worker will appreciate that there are many variations of such methods that also would be useful.

Alternative to preparing monoclonal antibody-secreting hybridomas, a monoclonal antibody to a peptide of the invention can be identified and isolated by screening a recombinant combinatorial immunoglobulin library (*e.g.*, an antibody phage display library) with the peptide to thereby isolate immunoglobulin library members that bind the peptide. Kits for generating and screening phage display libraries are commercially available (*e.g.*, the Pharmacia *Recombinant Phage Antibody System*, Catalog No. 27-9400-01; and the Stratagene *SurfZAP™* Phage Display Kit, Catalog No. 240612). Additionally, examples of methods and reagents particularly amenable for use in generating and screening antibody display library can be found in, for example, U.S. Patent No. 5,223,409; PCT Publication No. WO 92/18619; PCT Publication No. WO 91/17271; PCT Publication No. WO 92/20791; PCT Publication No. WO 92/15679; PCT Publication No. WO 93/01288; PCT Publication No. WO 92/01047; PCT

Publication No. WO 92/09690; PCT Publication No. WO 90/02809; Fuchs *et al.*, *Bio/Technology*, 9:1370-1372 (1991); Hay *et al.*, *Hum. Antibod. Hybridomas*, 3:81-85 (1992); Huse *et al.*, *Science*, 246:1275-1281 (1989); Griffiths *et al.*, *EMBO J.*, 12:725-734 (1993).

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are within the scope of the invention. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art. The invention also is intended to cover human antibodies. Methods for production, isolation purification and use are known to those skilled in the art using standard methodologies.

Active immunization with *Streptococcus mutans* has been shown to induce protection against experimental dental caries. Protection results from continuous secretion of salivary antibody to Gbp-B. Also contemplated by the present application is the use of the peptides described herein for conferring passive immunity to a mammal by administration of antibodies directed to the peptides of the invention using the methods described by Smith *et al.*, *Infect. and Immun.* 69(5):3135-3142 (2001). Passive immunity and protection from *Streptococcus mutans* can result from administration of antibodies specific to Gbp-B peptides (*e.g.*, SEQ ID NOS. 1-22) as described herein. Routes for administration include intravenous, intranasal, topical, and dietary, including the inclusion of the antibody in mouthwash, toothpaste or chewing gum. The administration of Gbp-B peptide antibodies can have an immunotherapeutic efficacy for dental caries by interfering with the accumulation of *S. mutans* in the biofilm and the subsequent events that cause dental caries.

The present invention further relates to a method of provoking an immune response to glucan binding protein or to glucosyltransferase in a mammal by administering an immunogenic or vaccine composition of the invention. Preferably, the immune response results in interference with glucan binding in the biofilm in mammals after administration of the vaccine composition. Alternatively, the immune response results in interference with the enzymatic activity of glucosyltransferase in mammals. The immune response elicited by the compositions and methods of the invention can be humoral or systemic; for example, the immune response can be a mucosal response. The immune response elicited by the method of the present invention results in reduction of the colonization or accumulation of mutans streptococcal strains in the mammal to which the vaccine or immunogenic composition is administered.

The compositions of the present invention are administered to any mammal in which the prevention and/or reduction of dental caries is desired. Suitable mammals include primates, humans, cats, dogs, mice, rats and other mammals in which it is desirable to inhibit dental caries. The present invention provides a vaccine that is useful for preventing, halting or reducing the progression of dental caries in a mammal to which the vaccine is administered.

In the method of the present invention of provoking an immune response to GbpB, mammals in which an immune response to GbpB is desired are given the vaccine or immunogenic compositions described herein. The compositions can be included in a formulation, which is administered to an individual being treated; such a formulation can also include a physiologically compatible carrier (*e.g.*, a physiological buffer), stabilizers, flavorants, adjuvants and other components. The vaccine can be administered by a variety of routes (*e.g.*, parenterally, mucosally, intranasally, intraocularly, intravenously, rectally, orally) and the components of the formulation will be selected accordingly. The amount to be administered and the frequency of administration can be determined empirically and will take into consideration the age and size of the mammal being treated and the stage of the dental caries disease (*e.g.*, prior to colonization of *mutans streptococci*, soon after colonization of *mutans streptococci* or in later stages of colonization).

Example 1: Identification of Immunogenic Regions

Peptides presented in conjunction with class II MHC molecules were derived from GbpB that has been processed in the phagosome of the antigen processing cell. The peptides bind to MHC molecules on the surface of these cells in a linear fashion. The binding was determined by the interaction of the peptide's amino acid side chains with the binding pockets in the MHC molecule. The characteristics of peptides that are likely to bind to a given MHC were directly deduced from pooled sequencing data of MHC alleles, resulting in an estimated binding probability. Thus, in order to identify potential B cell epitopes within GbpB sequence, which can be used for design of subunit vaccines, a matrix-based algorithm for epitope prediction (EpiMatrix; EpiVax, Inc, Providence, RI) was used to search the primary amino acid sequence of GbpB for known MHC class II binding motifs.

The motif-matching algorithms analyzed the GbpB sequence against each MHC class II allele to indicate regions of sequence that contain clusters of binding motifs. Those sequences

with sufficiently high estimated binding probabilities (EBP) were used to predict MHC ligands. FIG. 1 illustrates regions of predicted epitopes as the number of motif matches associated with a given sequence. Four regions within the expressed protein sequence were identified which had at least 6 matches within the N terminal region of the expressed protein sequence. One of these regions, beginning at residue 16, fell within the 27 residue signal peptide. Three other regions in the N terminal third of the molecule began at residue 62, residue 90 and residue 121. One peptide in the C terminal region, beginning with residue 369, had 5 matches. Independent analysis of other sets of known alleles also identified these as regions with higher estimated binding probabilities. These latter analyses also showed the 10-mer region following residue 322 to have more binding potential than predicted from the results shown in FIG. 1. Table 1 indicates the peptide sequences that were identified by this method.

Table 1

Amino Acid Sequence of GbpB Peptide	SEQ ID NO:
113 KSNAATSYINAIINSKSVSD 132	SEQ ID NO: 1
KHKLITIQQQVSALQTQQAG	SEQ ID NO: 2
306 TATEAQPSASSASTAAVAAN 325	SEQ ID NO: 3
6 LSAVLVSGVTLSSATTLSAV 25	SEQ ID NO: 4
16 LSSATTLSAVKADDFDAQIA 35	SEQ ID NO: 5
33 QIASQDSKINNLTAAQQAAQ 52	SEQ ID NO: 6
37 QDSKINNLTAAQQAAQAQVN 56	SEQ ID NO: 7
48 QQAAQAQVNTIQQGQVSALQT 67	SEQ ID NO: 8
52 QAQVNTIQQGQVSALQTQQAE 71	SEQ ID NO: 9
88 QQIQTLSKIVARNESLKQQ 107	SEQ ID NO: 10
117 ATSYINAIINSKSVSDAIR 136	SEQ ID NO: 11
137 VSAIREVVSANEKMLQQQEQ 156	SEQ ID NO: 12
174 TVAANQETIAQNTNALNTQQ 193	SEQ ID NO: 13
194 AQLEAAQLNLQAELTTAQDQ 213	SEQ ID NO: 14
214 KATLVAQKAAAEAAARQAAA 233	SEQ ID NO: 15
248 ALQEQAQAQVAANNNTQAT 267	SEQ ID NO: 16

289 TEQSAAQAVNNSDQUESTTAT 308	SEQ ID NO: 17
311 QPSASSASTAAVAANTSSAN 330	SEQ ID NO: 18
349 GNYWGNNGGQWAASAAAAGYR 368	SEQ ID NO: 19
365 AGYRVGSTPSAGAVAVWNDG 384	SEQ ID NO: 20
383 DGGYGHVAYVTGVQGGQIQV 402	SEQ ID NO: 21
403 QEANYAGNQSIGNYRGWFNP 422	SEQ ID NO: 22

Peptide Constructs

MAP constructs of three 20-mer peptides (SYI, QGQ, SAS), which included the predicted binding epitopes following residues 62, 121 and 322 were synthesized using the following peptides:

SYI (KSNAATSYINAIINSKSVSD; GbpB residues 113-132) (SEQ ID NO: 1)
 QGQ (QAQVNTIQGQVSALQTQQAQ; GbpB residues 52-71) (SEQ ID NO: 9); and SAS
 (TATEAQPSASSASTAAVAAN; residues 306-325) (SEQ ID NO: 3).

The constructs (SYI, QGQ and SAS; SEQ ID NOs. 1, 3 and 9) were selected for synthesis and further analysis based on the estimated high MHC Class II binding probability identified in the matrix-based approach described above. Peptides were synthesized (Applied Diagnostics, Foster City, CA) using the stepwise solid phase method of Merrifield R.B., *J. Amer. Chem. Soc.* 85:2149-2154 (1963) on a core matrix of lysines to yield macromolecules with four peptides per molecule, after the method of Tam *et al.*, *PNAS USA* 85:5409-5413 (1988). Synthesis was successful with two of the three peptides (SYI and QGQ). Purity (>90%) was assessed using HPLC, amino acid analysis, and molecular weight determination by mass spectrometry.

Glucan Binding Protein (GbpB)

GbpB was purified from *S. mutans* strain SJr by ion exchange chromatography on MONO-Q HR 5/5 (Pharmacia) in the presence of urea. Bacteria were cultivated in sucrose-free defined medium as previously described by Navarre and Schneewind in *Mol. Microbiol.*

14:115-121 (1994). GbpB prepared in this manner migrates as a single protein band in SDS-polyacrylamide gel electrophoresis.

ELISA

Serum IgG and salivary IgA antibodies were tested by enzyme-linked immunosorbent assay (ELISA). Polystyrene microtiter plates (Flow Laboratories) were coated with 2.5 mg/ml of SYI or QGQ or 0.5 mg/ml of *S. mutans* GbpB. Antibody activity was then measured by incubation with 1:400 and 1:4000 dilutions of sera, or 1:4 dilutions of saliva. Plates were then developed for IgG antibody with rabbit anti-rat IgG, followed in sequence by alkaline phosphatase goat anti-rabbit IgG (Biosource Inc.) and p-nitrophenylphosphate (Sigma Chemical Co., St. Louis, MO). A mouse monoclonal reagent to rat α chain (Zymed, South San Francisco, CA) was used with biotinylated goat anti-mouse IgG (Zymed), followed by avidin-alkaline phosphatase (ICN Biomedicals, Inc., Aurora, OH), followed by p-nitrophenylphosphate to reveal levels of salivary IgA antibody to peptides. Reactivity was recorded as absorbance (A405 nm) in a micro plate reader (Biotek Instruments, Winooski, VT). Data are reported as ELISA units (EU), which were calculated relative to the levels of appropriate reference sera or salivas from Sprague Dawley rats twice immunized with the respective peptide construct. Dilutions of sera producing an A405 nm of approximately 1.0 were considered 100 EU for serum IgG antibody measurements. Dilutions of saliva producing an A405nm of approximately 0.8 were considered 100 EU for salivary IgA antibody.

Immunogenicity of Peptides

Sprague Dawley CD strain 45 day-old female rats (Charles River Laboratories, Wilmington, MA) were used for injection. Four groups of 6 rats/group were injected subcutaneously in the vicinity of the salivary glands with 50 μ g each of SYI or QGQ peptide constructs, or 10 μ g of GbpB, or sham-immunized with buffer alone. The initial injection included complete Freund adjuvant (CFA; Difco Laboratories, Detroit, MI); one subsequent injection 21 days later included incomplete FA. Animals were bled prior to injection and 14 days after the second injection. In this experiment, rats were first momentarily anesthetized with a gas mixture of 50% carbon dioxide and 50% oxygen, and then anesthetized by intra-peritoneal injection of a mixture (0.65 ml/kg) of 3 parts ketamine (Ketaset, 100 mg/ml, Fort Dodge Lab, Ft.

Dodge, Iowa) and seven parts xylazine (Rompun, 20 mg/ml, Bayer Corp., Shawnee Mission, Kansas). Saliva secretion was stimulated by subcutaneous injection of 0.6 ml carbachol (containing 0.1 mg/ml in saline; Sigma Chemical Co., St. Louis, MO) per kilogram of rat weight. After fluid collection, rats were injected subcutaneously first with 0.1 ml/kg of atropine sulfate (0.4 mg/ml; American Pharmaceutical Partners, Inc., Los Angeles, CA) and then with yohimbine (yobine, 2.0 mg/ml; Lloyd Laboratories, Shenandoah, IO) at a volume equal to 1.4 times that used for anesthesia. Sera from coagulated and centrifuged blood were stored frozen at -20°C until measurement of antibody activity. Serum taken thirty-five days after the first injection was analyzed in ELISA for serum IgG antibody levels to each peptide construct and to GbpB (FIG. 2).

All rats injected with the QGQ peptide responded with high levels of serum antibody to the QGQ peptide, whereas no significant response to QGQ epitopes were seen in sham immunized rats or rats injected with SYI. Interestingly, the sera from two of the four rats injected with GbpB protein also reacted with QGQ.

All rats injected with the SYI peptide also demonstrated elevated levels of serum IgG antibody to the inciting SYI MAP peptide construct, in contrast to sham- or QGQ-injected rats. Again, serum IgG from one of the four rats injected with the parent GbpB protein also showed a significant reaction with the SYI peptide.

All sera were also evaluated in ELISA using plates coated with GbpB. Rats from SYI (6/6) or QGQ (4/6) peptide-injected groups reacted with the parent GbpB protein. Although the levels of serum IgG antibody from peptide-injected rats that were reactive with GbpB did not achieve levels from protein-injected rats, the overall response in the SYI-injected rats to native GbpB epitopes was significant. Taken together, these results supported the immunogenicity of these peptides predicted using the bioinformatics approach. Furthermore, they also suggested that the linear epitope(s) found especially on the SYI peptide construct were shared with those on the intact parent GbpB protein.

Protective Immunity

Peptides and multiple-epitope peptide constructs were tested in an art-recognized rat model for human dental caries. The SYI peptide was selected to test this assumption since this

peptide induced more consistent immune responses reactive with GbpB than did the QGQ peptide.

Two groups (n=13/group) of 25 day-old Sprague-Dawley female rats were singly caged. Rats were subcutaneously (sc) injected in the salivary gland vicinity with 50 µg of SYI MAP peptide construct or phosphate buffered saline (control animals). Antigen was incorporated with complete Freund's adjuvant (CFA). Nine days later, rats were reinjected with PBS or with SYI at the same dose in incomplete FA. Six days after the second injection, blood and saliva was collected under anesthesia described above. About fifteen days after the second injection, rats were placed in tubs (6 rats/ tub), given diet 2000, and orally infected with approximately 10^8 *S. mutans* SJ32 for 3 consecutive days. Rats were again singly caged after the infection protocol was completed and continued on diet 2000 for the duration of the experiment. Blood and saliva were collected 78 days after initial infection, followed by sacrifice. In preparation for the scoring of dental caries, rat skulls were defleshed by dermaphagic beetles, followed by a rinse with 70% ethanol.

Sera collected at the end of the 78 day infection period were analyzed for IgG and IgA antibody to both the peptide construct (FIG. 3) and to GbpB (FIG. 4). As expected, immunization with the peptide induced serum antibody in both isotypes to the inciting SYI peptide. Also, consistent with the previous experiment, SYI immunization also induced IgG antibody to intact GbpB in all rats, although some rats did not demonstrate serum IgA antibody levels to GbpB, at least at the dilutions tested. Saliva was collected prior to infection and at the end of the experiment was analyzed in ELISA for IgA antibody to SYI and GbpB (Table 2). Several (5/13) SYI-immunized rats demonstrated induction of salivary IgA antibody to both the peptide and the intact protein at either time point, although group levels were not significantly different under the conditions of measurement.

Table 2

Group	Test Antigen	Mean EU	SE
Sham-immunized	GbpB	7.1	5.1
SYI-immunized	GbpB	31.1	16.5
Sham-immunized	SYI	3.6	2.4

SYI-immunized	SYI	12.5	5.9
---------------	-----	------	-----

The protective response of the SYI immunization was evaluated by systematic swabbing of molar teeth for *S. mutans* infection (FIG. 5) and measurement of caries on molar surfaces (FIG. 6).

Bacterial Recoveries

The mutans streptococcal flora was assessed at 70 days after infection. After systematic swabbing of teeth, sonication, and plating appropriate dilutions on *mitis salivarius* agar (MS; total streptococci), and MS agar with 0.2 mg/ml streptomycin sulfate (MSS; *Streptococcus mutans* strain SJr), plates were incubated for 48 hours at 37°C in 90% N₂, 10% CO₂. *S. mutans* colony forming units (CFU) were then enumerated microscopically on MSS agar.

Caries Assessment

The extent and depth of carious lesions in all rat molar teeth (caries score) were microscopically evaluated by standard methods. Caries scores were determined separately on smooth and on occlusal dental surfaces. Thus, measurements of protective influence of immunization support the conservation of at least one epitope on SYI capable of inducing a caries-protective response in this model. The mean levels of infecting (streptomycin tolerant) *S. mutans* SJr recovered from SYI-immunized groups were lower than sham-immunized group recoveries both eight and 65 days after infection was initiated, although these differences did not achieve statistical significance at the $p < 0.05$ level because of the variation in bacterial recoveries. The trend in the infection data was supported by measurements of dental caries. Caries scores on smooth (buccal) and occlusal surfaces as well as total caries scores of SYI-peptide immunized rats were significantly lower than those of sham-immunized and infected rats (FIG. 6).

Example 2: Diepitopic Immunization Studies

GTF and glucan binding protein B (GbpB) from *mutans streptococci* have each been implicated in the molecular pathogenesis of dental caries caused by these organisms. Native GTF

and GbpB, as well as synthetic peptides derived from each protein, have been shown to induce protective immune responses to infection with cariogenic mutans streptococci in experimental models.

Two diepitopic synthetic peptide constructs were synthesized in a MAP format. Both peptides contained SYI, a 20-mer sequence from GbpB that bioinformatic analyses indicated was similar in sequence to an MHC class II binding peptide. One diepitopic peptide (SYI-CAT) also contained a 22-mer sequence from the catalytic domain of GTF. The other diepitopic construct (SYI-GLU) contained a 22-mer sequence from the glucan binding domain of GTF.

Diepitopic and monoepitopic MAP constructs were synthesized by AnaSpec, Inc. (San Jose, CA). Eight groups of Sprague-Dawley rats (n=4-8/group) were initially injected subcutaneously with one the following, together with complete Freund adjuvant: (1) buffer alone, (2) MAP-CAT, (3) MAP-GLU, (4) MAP-SYI, (5) MAP-CATGTF-SYIGbpB, (6) MAP-GLUGTF-SYIGbpB, (7) *S. mutans* GbpB, or (8) *S. sobrinus* GTF. On day 21, the 8 groups were again injected with the same contents, except the second injections substituted incomplete Freund adjuvant. Animals were then bled and salivated on days 42 and 63. Sera were tested for antibody activity against peptides and proteins using an alkaline phosphatase enzyme-linked immunosorbent assay (ELISA). Antibody levels were compared using one and two way ANOVA, followed by Dunn's multiple comparison test.

Sera from blood taken 42 days after the second injection were examined for IgG antibody activity against constituent peptides or native proteins. The serum IgG response to GLU was similar whether SYI-GLU or GLU alone was used for injection. In contrast, SYI-CAT induced an IgG response to CAT that was significantly higher than that induced by CAT alone. Both diepitopic peptide constructs induced IgG antibody that reacted with GTF and GbpB native proteins. Sera from SYI-CAT-immunized animals reacted with GTF to a significantly greater degree than SYI-GLU. These results indicate that diepitopic synthetic peptides, especially SYI-CAT, induce an immune response that provides a broader range of protective antibody epitopes in a subunit dental caries vaccine. Furthermore, these results indicate that the combination of SYI with CAT potentiated the immune response to this important GTF catalytic domain.

Sequences Used in Diepitopic Immunization Studies:

1. GTF-derived catalytic (CAT) peptide: DANFDSIRVDAVDNVDADLLQI (SEQ ID NO:25)
2. GTF-derived glucan binding (GLU) peptide: TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO:23)
3. GbpB-derived MHC class II (SYI) peptide: KSNAATSYINAIINSKSVSD (SEQ ID NO:1)
4. Diepitopic SYI-CAT peptide, two copies of each in multiple antigenic peptide (MAP) format:
KSNAATSYINAIINSKSVSD (SEQ ID NO:1) – DANFDSIRVDAVDNVDADLLQI (SEQ ID NO:25)
5. Diepitopic SYI-GLU peptide, two copies of each in multiple antigenic peptide (MAP) format:
KSNAATSYINAIINSKSVSD (SEQ ID NO:1) – TGAQTIKGQKLYFKANGQQVKG (SEQ ID NO:23)

Results of Diepitopic Immunization Studies:

Sera were tested at a 1:200 dilution in groups of 4-7 rats.

Both diepitopic constructs induce significant antibody to Gbp-B (Table 3).

Table 3: Serum IgG responses to GBP-b (glucan binding protein B)

Group	Serum IgG antibody to GbpB Mean $\pm$ SE
Sham	0.027 $\pm$ 0.014
SYI-CAT	0.783 $\pm$ 0.268
SYI-GLU	0.847 $\pm$ 0.186
SYI	0.599 $\pm$ 0.201
CAT	0.029 $\pm$ 0.009
GLU	0.022 $\pm$ 0.009
GluB	1.838 $\pm$ 0.052

The SYI-GLU diepitopic construct enhances anti-peptide (SYI) and anti-glucan binding protein responses (FIG. 7). Rats immunized with either diepitopic construct develop antibody to the parent GbpB protein (FIG. 8).

The SYI-CAT diepitopic construct significantly enhances the anti-peptide (CAT) responses over the mono-epitopic MAP (FIG. 10). Only the SYI-CAT diepitopic construct induced significant IgG antibody to the parent GTF protein in all rats (FIG. 11), and thus was the most efficient stimulus for antibody to both virulence antigens. Furthermore, the SYI-CAT

diepitopic construct alone induced a significant serum IgG immune response to GTF in all animals (Table 4).

Table 4: Serum IgG responses to GTF

Group	Serum IgG antibody to GTF Mean + SE
Sham	0.114 $\pm$ 0.029
SYI-CAT	0.955 $\pm$ 0.200
SYI-GLU	0.083 $\pm$ 0.018
SYI	0.073 $\pm$ 0.018
CAT	0.088 $\pm$ 0.018
GLU	0.108 $\pm$ 0.031
GTF	1.728 $\pm$ 0.098

The SYI-CAT construct induces significant levels of serum IgG antibody to both GbpB and GTF virulence antigens of *mutans streptococci*. In addition, the diepitopic construct enhanced the immune response to the CAT epitopes over that observed when monoepitopic CAT construct is used. Thus the SYI-CAT construct reduces the pathogenicity of *Streptococcus mutans* by inhibiting enzymatic activity (glucan formation) and inhibiting activity of glucan binding protein B.

All references cited herein are incorporated by reference in their entirety. While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

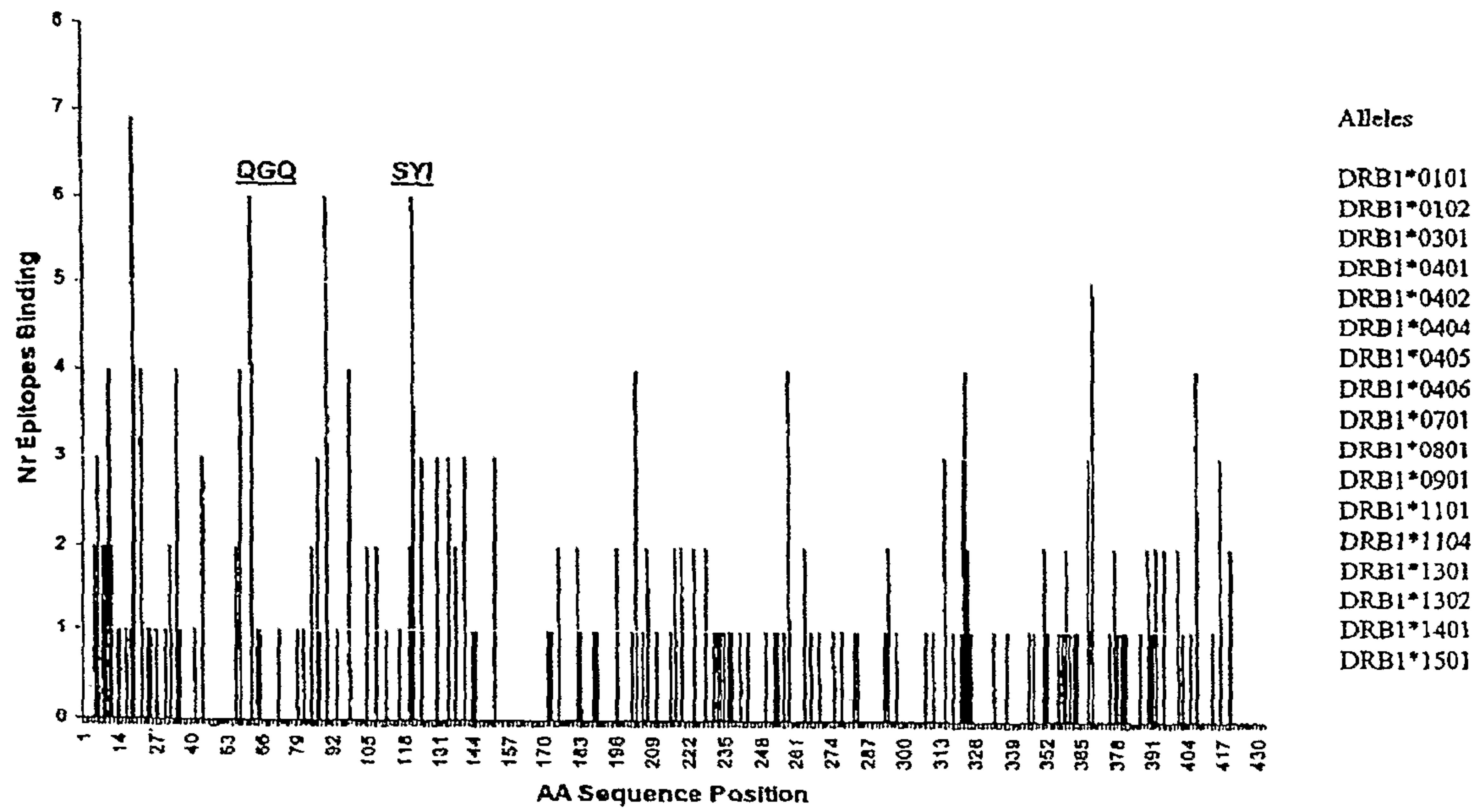
What is claimed is:

1. A composition comprising a fragment of a glucan binding protein-B (GbpB), wherein said fragment binds to a major histocompatibility complex (MHC) class II protein.
2. The composition of claim 1, wherein said fragment binds to an HLA protein.
3. The composition of claim 1, wherein said HLA protein is selected from the group consisting of DRA, DRB1, DRB2, DQA1, DQB1, DPA1, DPB1, DMA, DMB, DOA, and DOB.
4. The composition of claim 1, wherein said HLA protein is DRB1.
5. The composition of claim 1, wherein said GbpB protein comprises an amino acid sequence selected from the group consisting of SEQ ID NO's: 29, 30, 31, 32, and 33.
6. The composition of claim 1, wherein said GbpB protein comprises the amino acid sequence of SEQ ID NO: 29.
7. The composition of claim 6, wherein said fragment is greater than 6 and less than 431 residues in length.
8. The composition of claim 1, wherein said fragment is less than 400 residues in length.
9. The composition of claim 1, wherein said fragment is less than 100 residues in length.
10. The composition of claims 1, wherein said fragment is 10-25 residues in length.
11. The composition of claim 1, wherein said fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO's: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 41.

12. The composition of claim 1, wherein said fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NO's: 1 and 3.
13. A composition comprising a GbpB polypeptide and a glucosyltransferase polypeptide.
14. The composition of claim 13, wherein said GbpB polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO's: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, and 41.
15. The composition of claim 13, wherein said GbpB polypeptide comprises an amino acid sequence of SEQ ID NO: 1 or 3.
16. The composition of claim 13, wherein said glucosyltransferase polypeptide comprises a catalytic domain of SEQ ID NO: 34, 35, 36, 37, 38, 39, or 40.
17. The composition of claim 16, wherein said domain comprises an amino acid sequence of SEQ ID NO: 24 or 25.
18. The composition of claim 13, wherein said glucosyltransferase polypeptide comprises a glucan binding domain of SEQ ID NO: 34, 35, 36, 37, 38, 39, or 40.
19. The composition of claim 18, wherein said domain comprises an amino acid sequence of SEQ ID NO: 23.
20. The composition of claim 13, wherein said glucosyltransferase polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 23, 24, 25, 26, 27, AND 28.
21. The composition of claim 13, wherein said GbpB polypeptide comprises SEQ ID NO: 1 and said glucosyltransferase polypeptide comprises SEQ ID NO: 23.

22. The composition of claim 13, wherein said GbpB polypeptide comprises SEQ ID NO: 1 and said glucosyltransferase polypeptide comprises SEQ ID NO: 25.
23. The composition of claim 13, wherein said composition further comprises a peptidyl core matrix.
24. The composition of claim 23, wherein said core matrix comprises at least one lysine.
25. The composition of claim 23, wherein said core matrix comprises a plurality of lysine residues.
26. A method of eliciting production of an antibody in a mammal, comprising administering to said mammal a composition comprising a fragment of a glucan binding protein-B (GbpB), wherein said fragment binds to a major histocompatibility complex (MHC) class II protein.
27. A method of eliciting production of an antibody in a mammal, comprising administering to said mammal a composition comprising a GbpB polypeptide and a glucosyltransferase polypeptide.
28. The method of claim 27, wherein an amount of an anti-GbpB antibody produced by said mammal is at least 10% greater than an amount produced by a mammal immunized with a composition comprising a GbpB peptide in the absence of a GTF peptide.
29. The method of claims 26 or 27, wherein said antibody comprises an IgA isotype.
30. The method of claims 26 or 27, wherein said antibody comprises an IgG isotype.
31. A substantially pure antibody produced by the method of claim 26 or 17.

32. A method of conferring passive immunity to oral Streptococci, comprising administering to a mammal a composition comprising a purified antibody which specifically binds to an MHC class II binding fragment of GbpB.



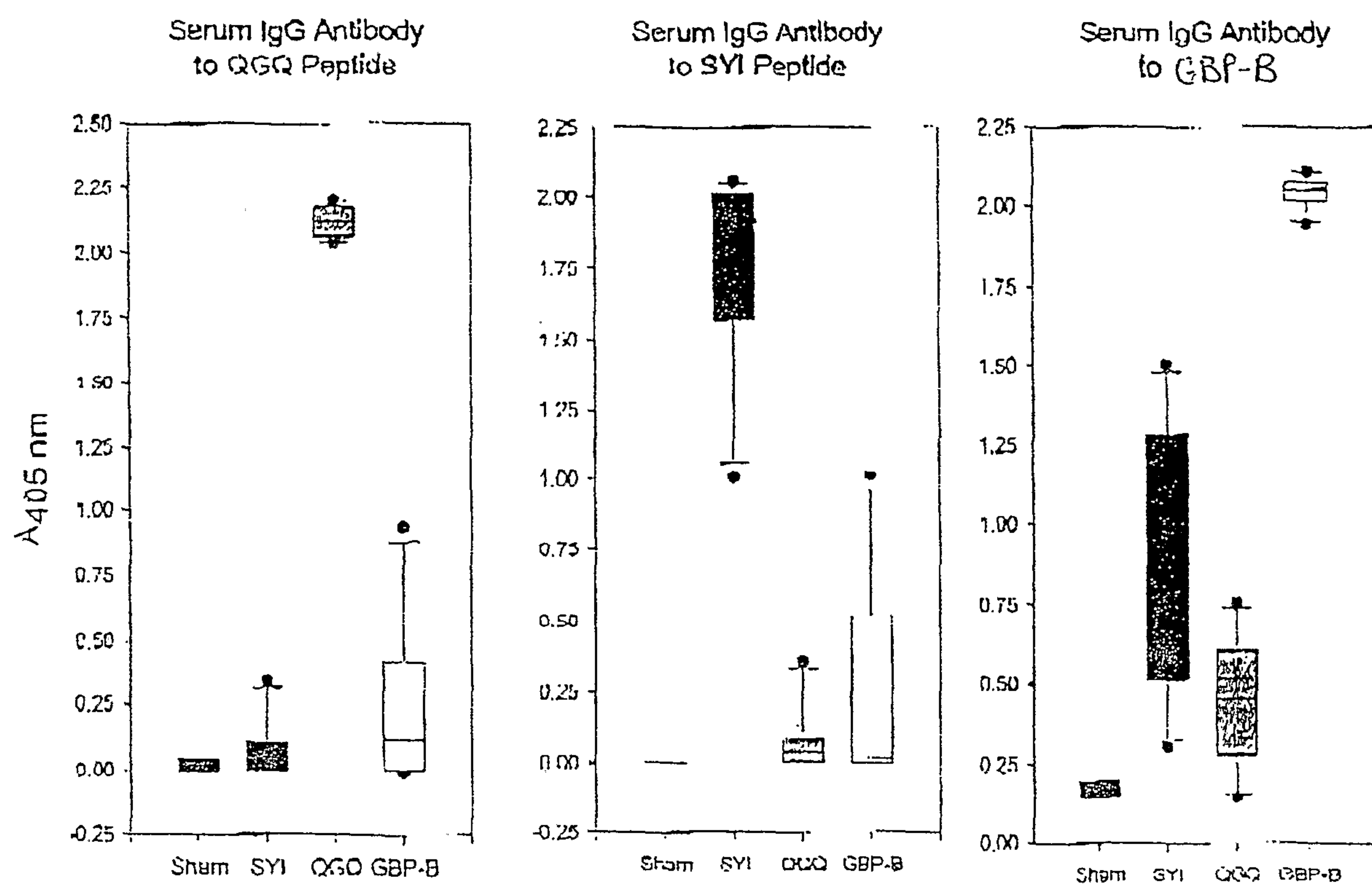


FIG. 2

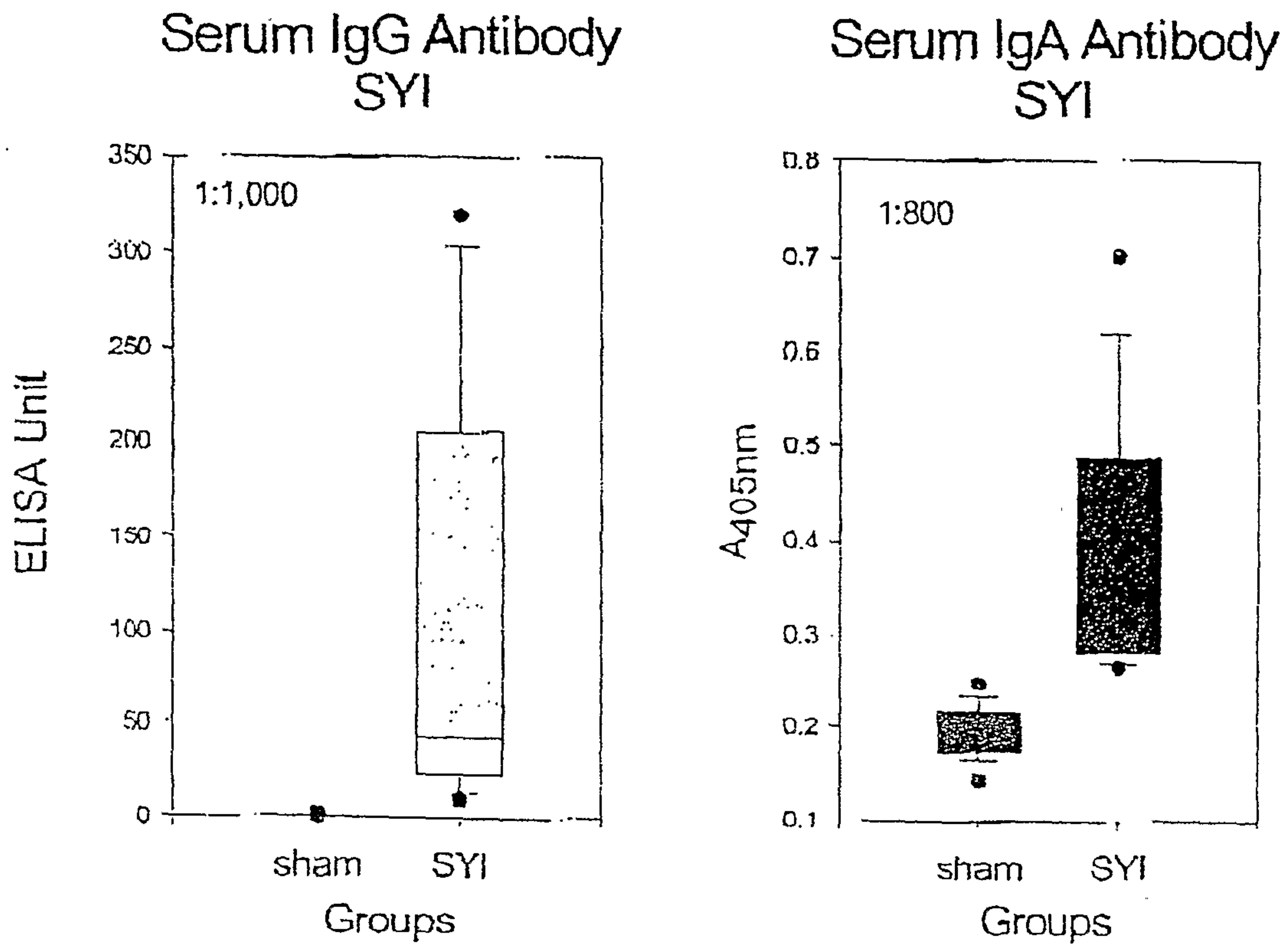


FIG. 3

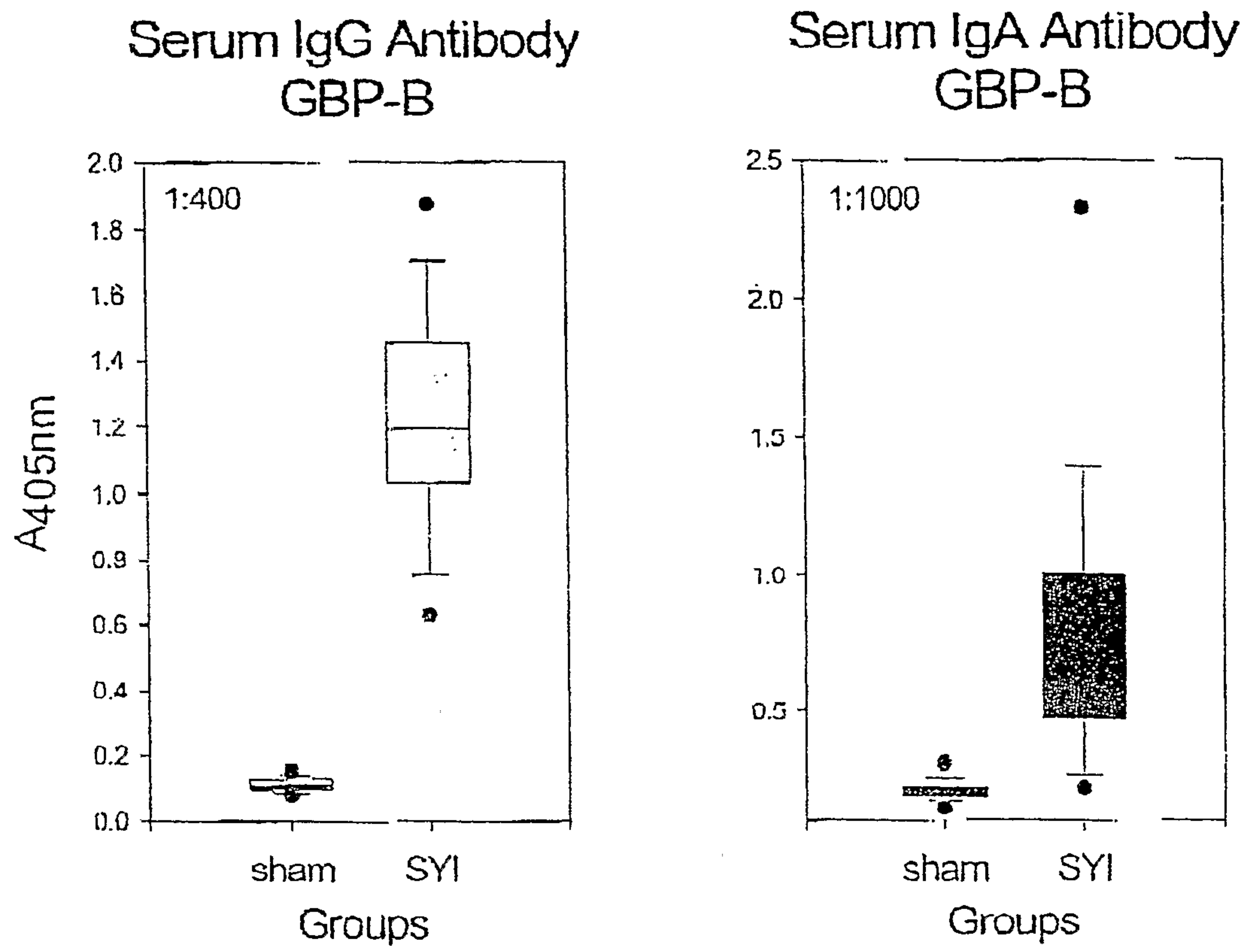


FIG. 4

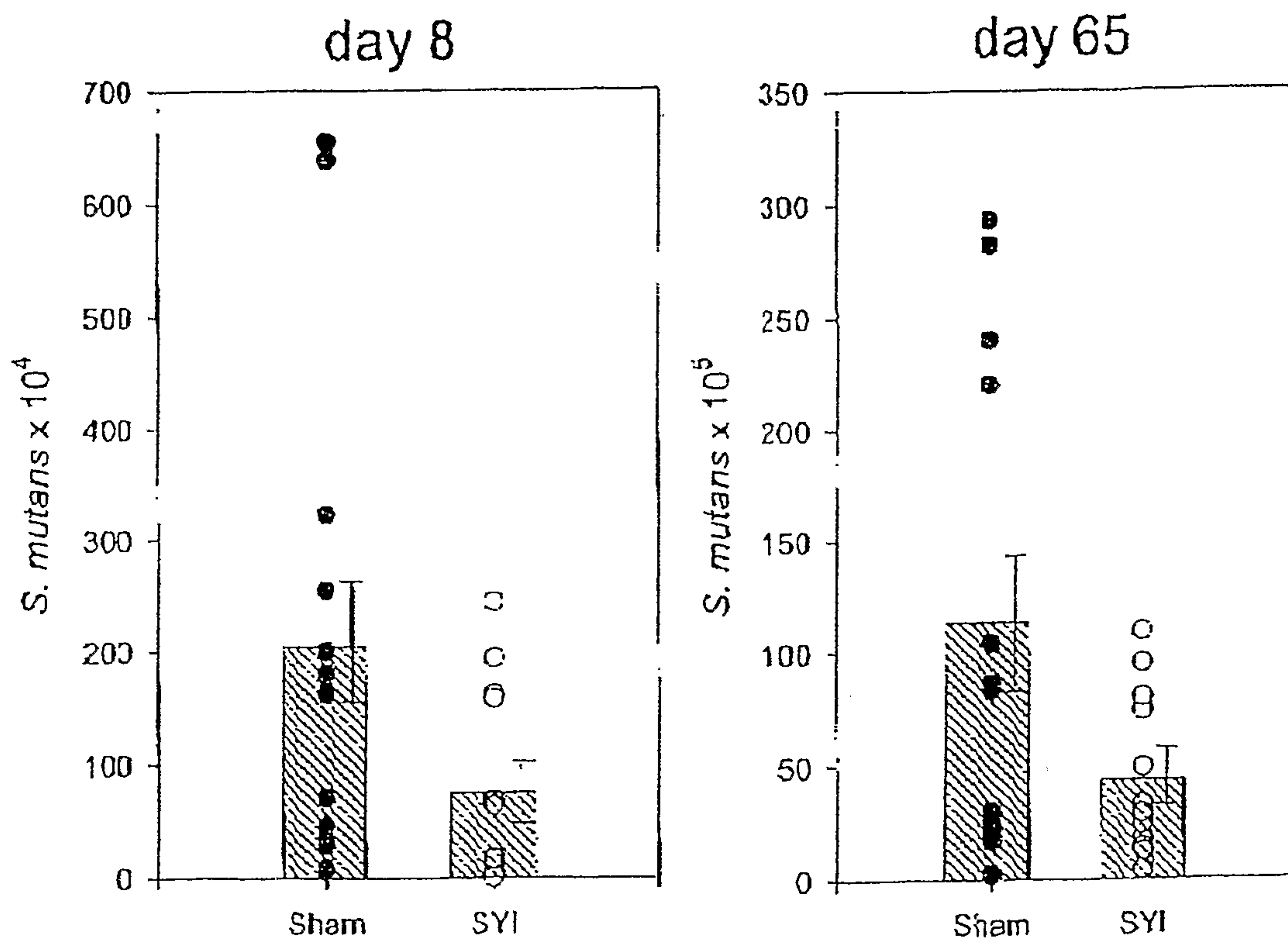
***S. mutans* infection**

FIG. 5

Caries Protective Effect of Immunization with SYI, an *S. mutans* GBP-B-derived Peptide Construct

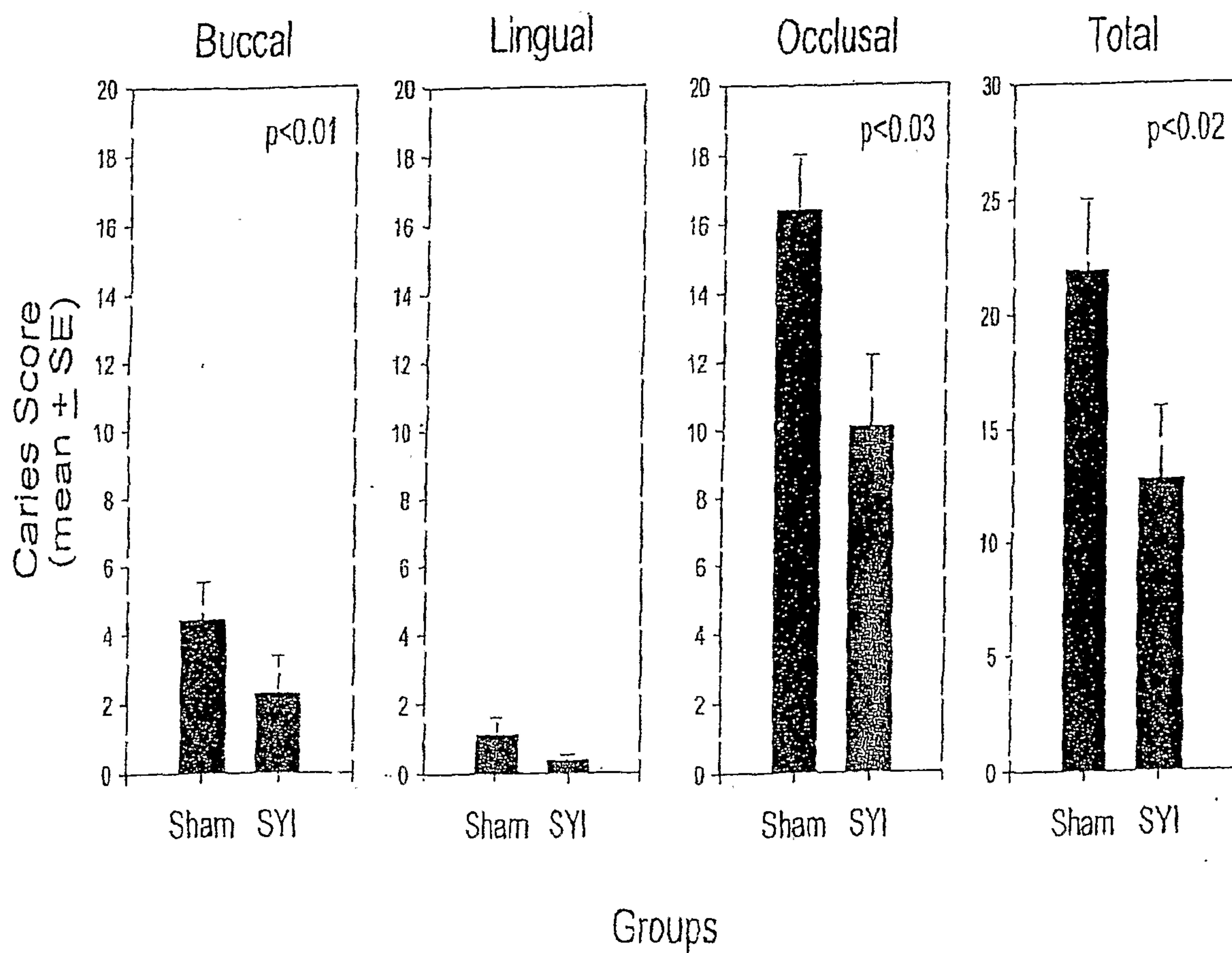


FIG. 6

FIG. 7

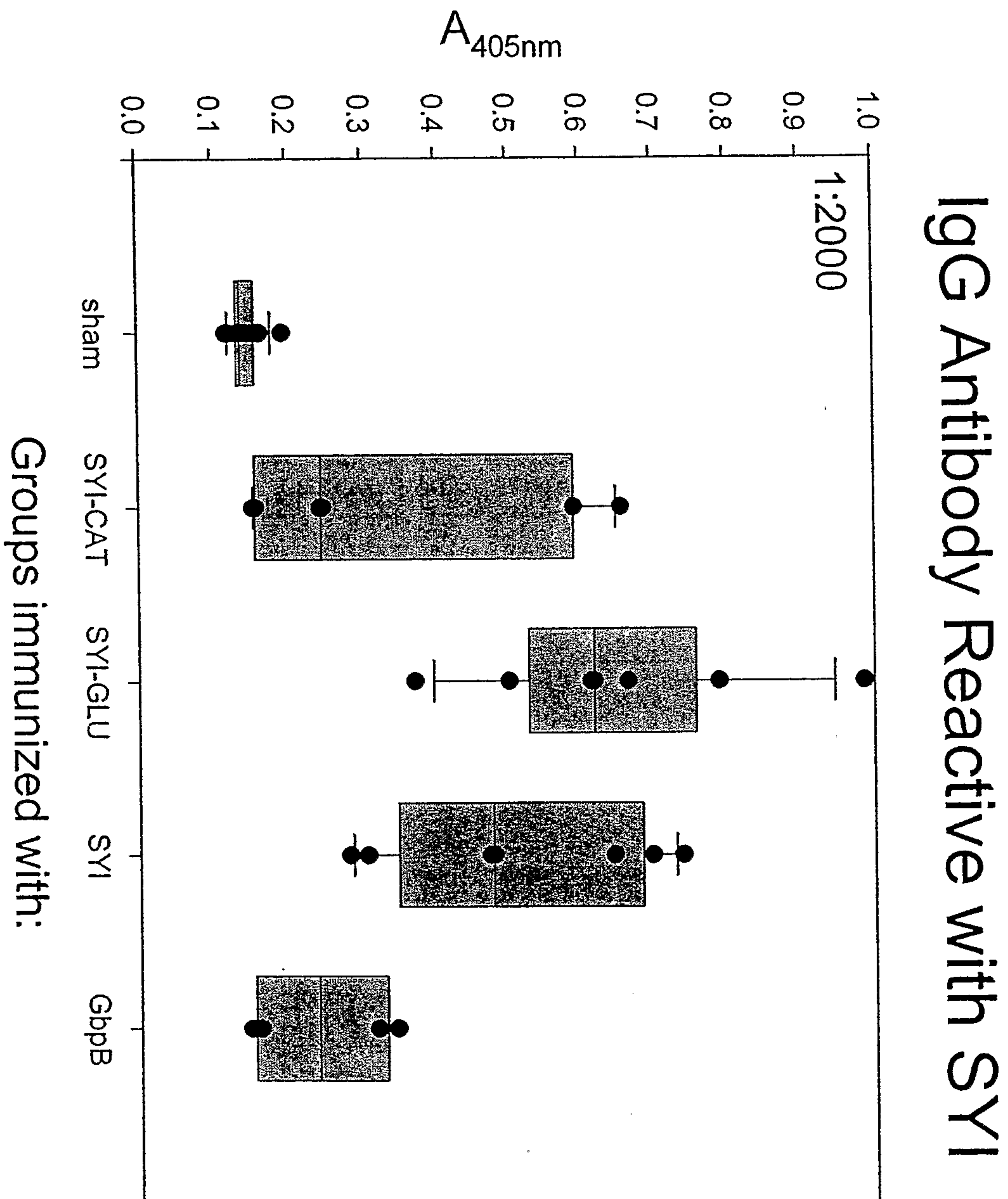


FIG. 8

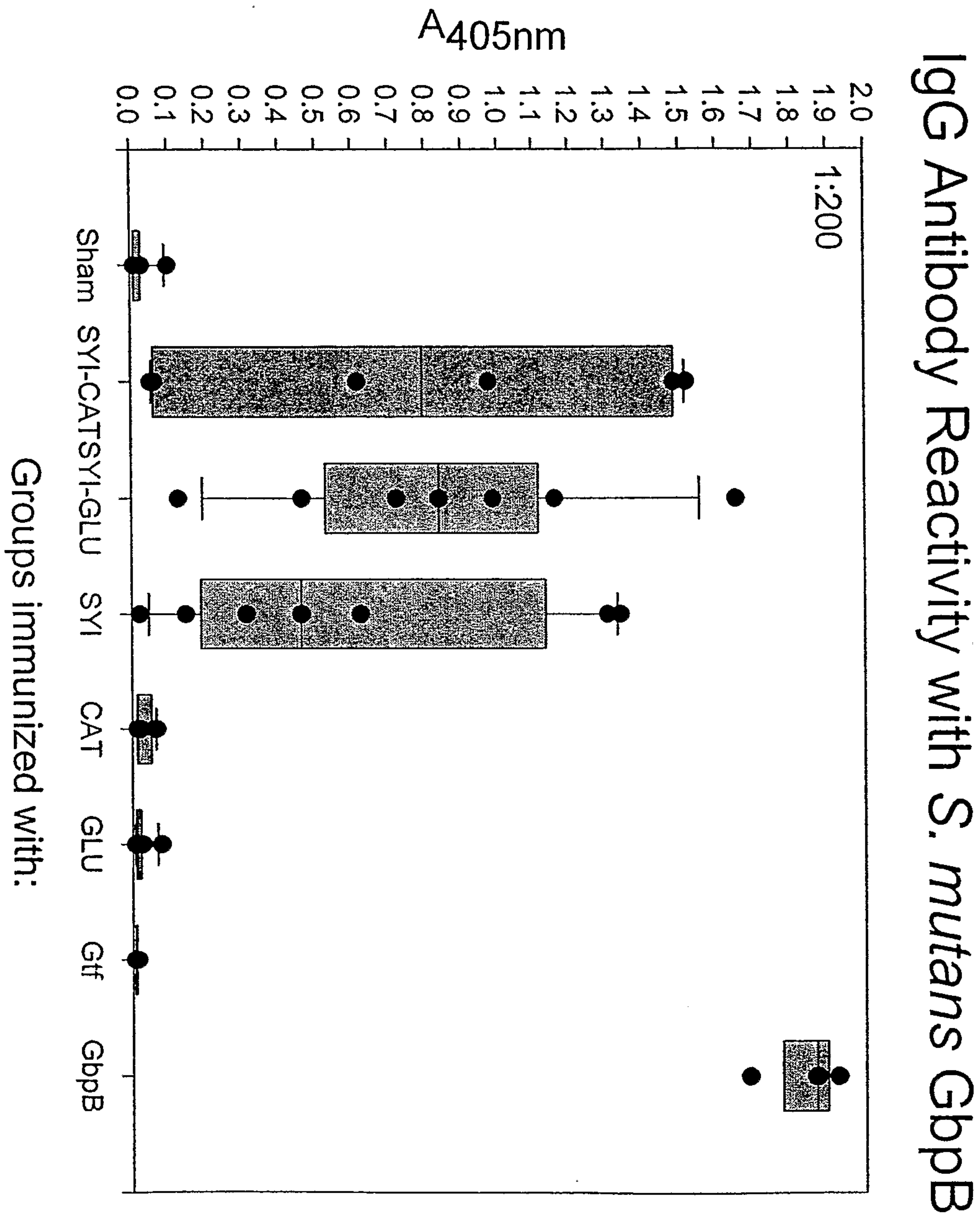


FIG. 9

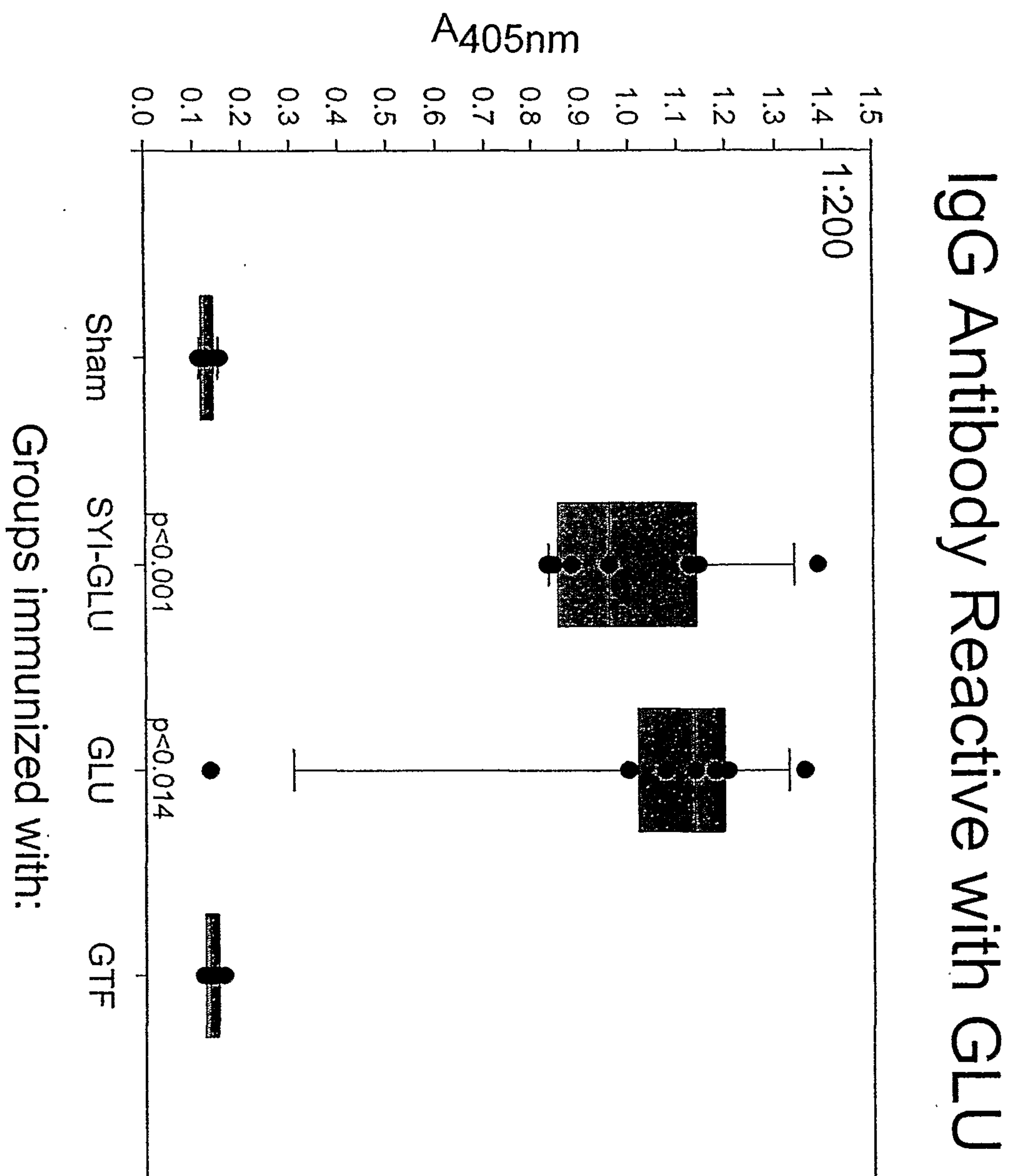


FIG. 10

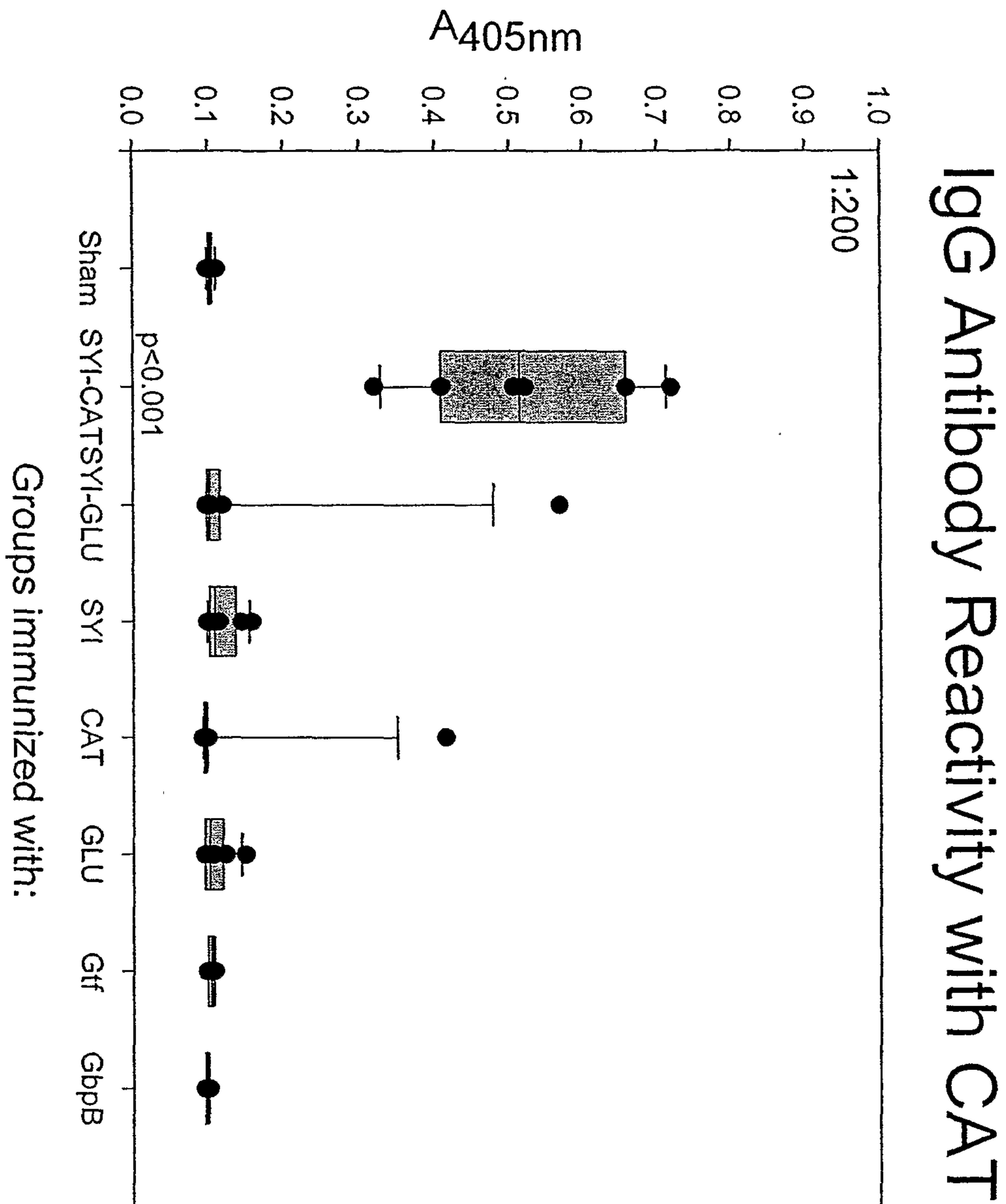
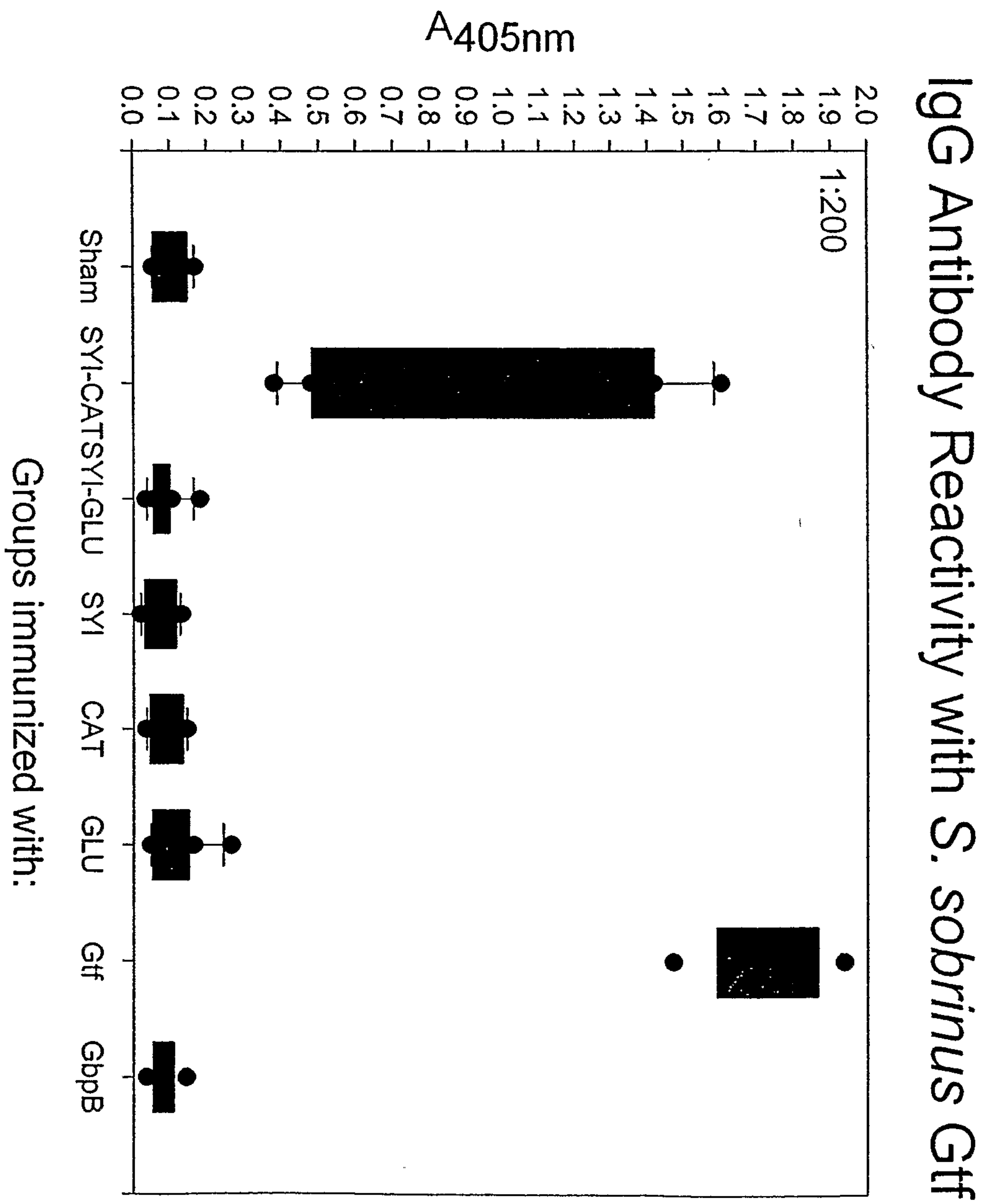
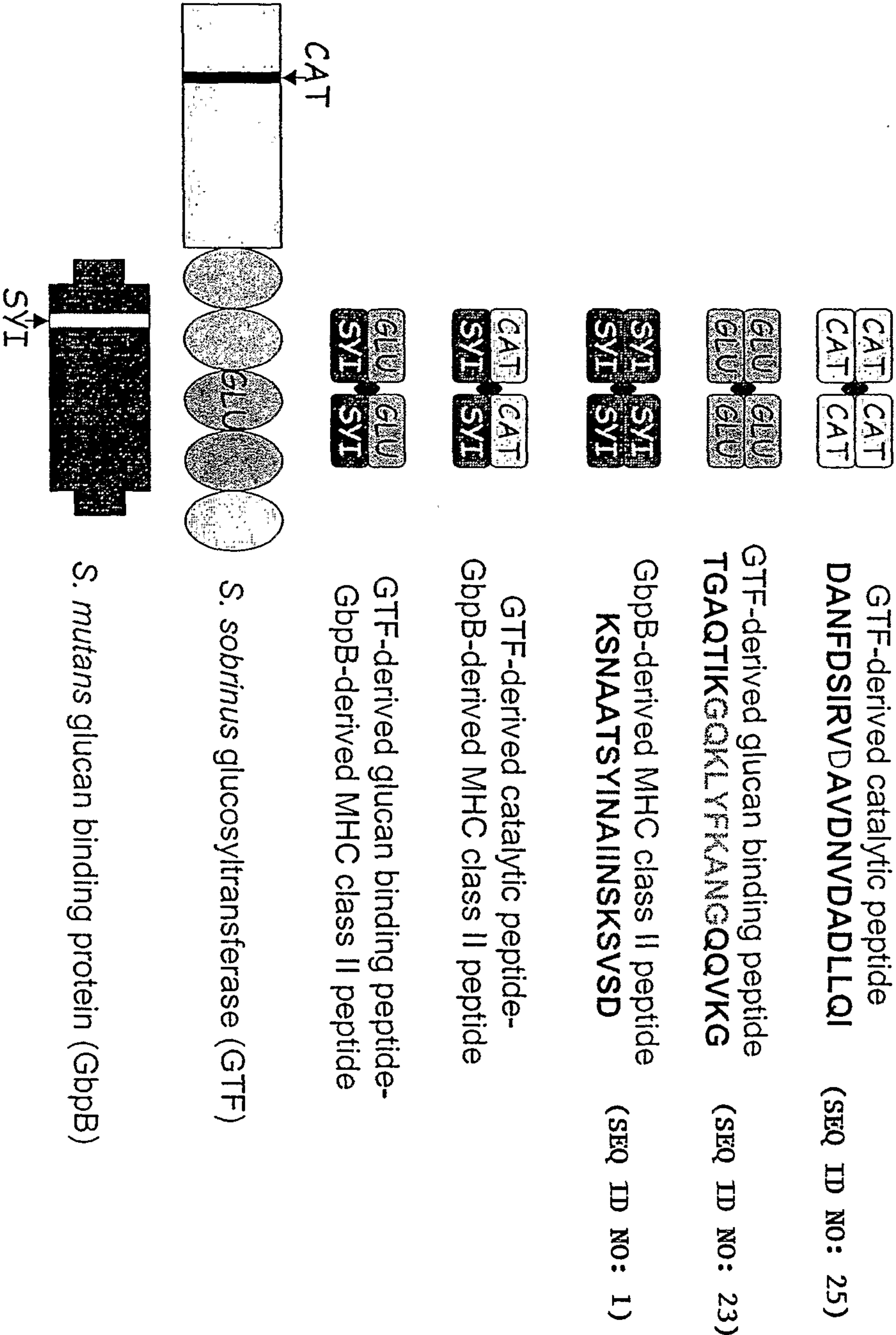


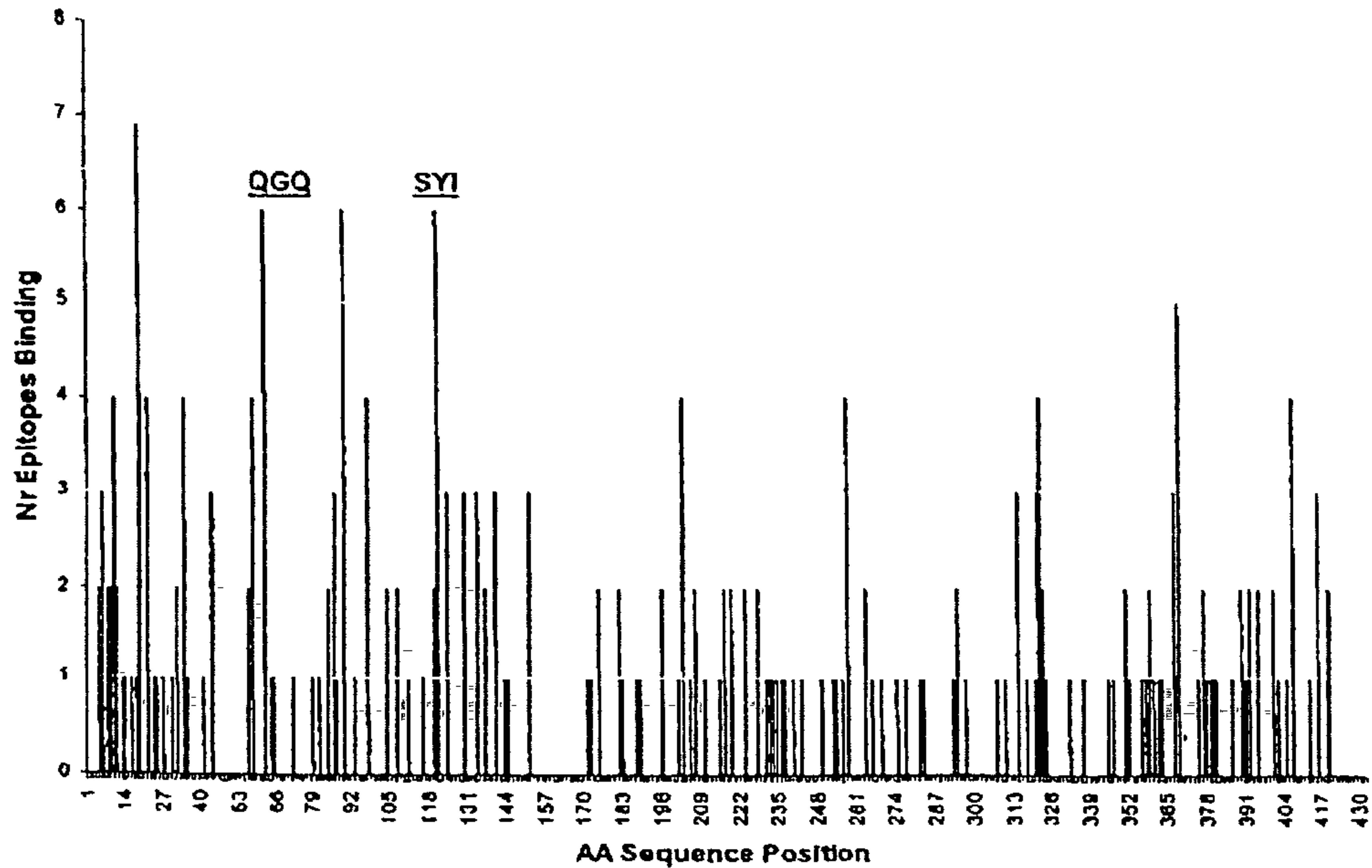
FIG. 11



MAP Mono- & Di-epitopic Peptide and Protein Antigens

FIG. 12





Alleles

DRB1*0101
DRB1*0102
DRB1*0301
DRB1*0401
DRB1*0402
DRB1*0404
DRB1*0405
DRB1*0406
DRB1*0701
DRB1*0801
DRB1*0901
DRB1*1101
DRB1*1104
DRB1*1301
DRB1*1302
DRB1*1401
DRB1*1501