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(54) Title: HUMAN MONOCLONAL ANTIBODIES FOR HUMAN NOROVIRUS AND EPITOPE DISCOVERY

(57) Abstract: Embodiments of the disclosure concern methods and compositions related to monoclonal antibodies for treatment or prevention of Norovirus infection, and methods of making the antibodies. Specific embodiments provide monoclonal antibodies that are antibodies that block the binding of histo-blood group antigens to Norovirus. Methods of treatment and prevention and diagnosis with the antibodies are disclosed, in addition to nucleic acid and polypeptide and other compositions for the methods or manufacture of the antibodies.



HUMAN MONOCLONAL ANTIBODIES FOR HUMAN NOROVIRUS AND EPI TOPE DISCOVERY

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 62/191,260, filed July 10, 2015, which is incorporated by reference herein in its entirety.

[0002] This invention was made with government support under PO1 AI080656 awarded by National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

[0003] Noroviruses are important human pathogens (FIG. 1). They are icosahedral viruses made up of 90 dimers of the major capsid protein VP1. The subunit structure is comprised of two principal domains: the S domain forms the shell and the P domain that projects out from the shell. The P domain is further divided into two subdomains, P1 and P2. The distally located P2 subdomain can be considered as a large insertion into the P1 domain. Several studies have shown that noroviruses use histo-blood group antigens (HBGA) as receptor/co-receptors for cell entry. These glycoconjugates are also susceptibility factors for these viruses. These HBGA bind to the P2 subdomain. Sequence-wise, the P2 subdomain is the least conserved region of the norovirus capsid protein leading to significant variations in the HBGA specificity and antigenicity. The interplay between variations in antigenicity and HBGA specificity are suggested to drive the norovirus evolution. Recent studies have shown that circulating serum antibodies that block HBGA binding correlate with protection. Based on the phylogenetic analysis of the capsid protein VP1, human noroviruses are classified into several genogroups and genotypes (FIG. 2). The HBGA binding site for several of these genogroups has been structurally well characterized. However, the structural basis of how ‘neutralizing’ antibodies block HBGA binding is not known.

BRIEF SUMMARY

[0004] Embodiments of the disclosure concern monoclonal antibodies to human norovirus and methods of their use. Monoclonal antibodies described herein can be used at least to analyze samples from animals, including biological fluids from individuals with or suspected of having Norovirus infection. The antibody can be a mouse antibody, a human antibody, or a humanized antibody, for example. In certain aspects the antibody is a mouse monoclonal antibody. In a further aspect the antibody is a human or humanized monoclonal

antibody. In other aspects, the antibody is formulated in a pharmaceutically acceptable formulation.

[0005] In certain aspects the compositions encompassed by the disclosure can be used as a novel treatment for Norovirus infection. In further aspects antibodies of the disclosure can be used to reduce or eliminate Norovirus infection. In yet another aspect, the disclosure provides methods for treating Norovirus infection or related sequelae comprising the step of administering an effective amount of an antibody (or multiple antibodies) encompassed by the disclosure or a peptide comprising or consisting of the amino acid sequence for a conformational epitope formed by surface-exposed loop clusters in the P domain in the capsid protein, including some or all of the residues N346, T348, D350, F352, S380, H381, S383, N394, and G396. Certain aspects are directed to a conformational antibody that specifically binds the surface-exposed loop cluster of the P domain of the capsid protein. In a further aspect the conformational epitope includes one or more amino acid residues selected from N346, T348, D350, F352, S380, H381, S383, N394, and G396 relative to SEQ ID NO: 61. In a further aspect the conformational antibody specifically binds an epitope comprising amino acid residues N346, T348, D350, F352, S380, H381, S383, N394, and G396 relative to SEQ ID NO: 61.

[0006] Embodiments of the disclosure concern the structural and mechanistic basis of HBGA binding with Noroviruses (NoVs). Embodiments of the disclosure provide the first crystal structure of the Norwalk virus P domain in complex with the FAB fragment of an HBGA blockade antibody, IgA 5I2. In specific embodiments, sequence differences and structural alterations in other genotypes play a role in the ability of other genotypes to escape from IgA 5I2 neutralization.

[0007] Described herein is a set of monoclonal antibodies made to human norovirus that are useful for therapy, prevention, diagnostic tests, and/or characterization of viral vaccines or VLPs prior to release. In addition, the disclosure provides the amino acid sequence(s) of an epitope recognized by inhibitory antibodies, for example that can be used in designing new vaccines. The disclosure provides a structural description of how an antibody binds to human norovirus and blocks binding of histo-blood group antigens (HBGAs), which are the initial receptors for these viruses. HBGA blocking by antibodies is a mechanism that can be used to neutralize the virus. Understanding the structural aspects of antibody-virus binding interface is useful in the structure-based design of potent broad-

spectrum vaccines and antiviral compounds against human noroviruses. The structural details relating how human antibody interacts with norovirus to block HBGA binding are useful for development of various therapeutic compounds. In certain aspects the monoclonal antibody is a human monoclonal antibody.

[0008] Certain embodiments are directed to methods of treating or preventing Norovirus infection in individuals in need thereof, including those susceptible to the infection or at risk for the infection. The individual may be at risk for the infection by having an impaired immune system or being exposed to large numbers of individuals, for example in a confined environment, such as in a school, transportation vessel (boat, plane, train), sports or entertainment venue, *etc.* The compositions may be provided to an individual as a precautionary measure or as a routine measure. Any individual of any age or gender may be exposed to methods and/or compositions of the disclosure.

[0009] Encompassed herein are antibodies and binding polypeptides comprising amino acid sequences that comprise or consist of or consist essentially of the amino acid sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or a combination thereof, or a functionally active derivative thereof. A functionally active derivative thereof is a polypeptide that can bind a Norovirus and block its binding to histoblood group antigens (glycans) and have a 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53 (percent identity is defined as the quantitative measurement of the similarity between two sequences). Any individual suspected of having or known to have

Norovirus infection or exposure or at risk thereof may be provided a therapeutically effective amount of one of the aforementioned antibodies or binding polypeptides. The polypeptides or antibodies may be delivered in any suitable manner, *e.g.*, liposomes.

[0010] Encompassed herein are nucleic acid sequences that comprise a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, and functional derivatives thereof that are at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, respectively. Any individual suspected of having or known to have Norovirus infection or exposure or at risk thereof may be provided a therapeutically effective amount of one of the aforementioned nucleic acids. The nucleic acids may be delivered in any manner, including in a vector or in the absence of a vector. Examples of vectors include liposomes, viral vectors, non-viral vectors, and so forth. Examples of non-viral vectors include plasmids. Examples of viral vectors include adeno-associated virus, adenoviral virus, vaccinia virus, retroviral virus, lentivirus, and so forth. The nucleic acid may be delivered as RNA, also.

[0011] Delivery of any composition for therapeutic purpose may occur by any suitable regimen, including being administered more than one time to the subject, and they may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more times. The route of administration of the compositions includes, but is not limited to oral, parenteral, subcutaneous and intravenous administration, rectal, or various combinations thereof, including inhalation or aspiration.

[0012] Certain embodiments are directed to diagnostic tests employing antibodies or binding polypeptides of the present disclosure. The diagnostic tests may include the HBGA-blocking monoclonal antibodies encompassed herein and may test whether or not the

individual is infected with Norovirus. The diagnostic tests may include more than one type of monoclonal antibody, for example in situations where an individual is being tested for more than one genotype of Norovirus. Any suitable sample from the individual may be employed, including at least stool and/or vomitus. In certain aspects, a monoclonal antibody is a human monoclonal antibody.

[0013] In other aspects, a peptide comprising surface-exposed loop clusters in the P domain in the capsid protein (and in some cases that can form a conformational epitope formed by surface-exposed loop clusters in the P domain from the capsid protein, including some or all of the residues N346, T348, D350, F352, S380, H381, S383, N394, and G396) can be used to induce antibodies that reduce Norovirus infection or detect Norovirus infection. In other aspects, a peptide comprising the epitope comprising amino acid residues 344, 346, 348, 351, 381, 383, and 385 of GenBank® Accession No. AAB50466 (SEQ ID NO: 61). In a further aspect the antibody binds a conformation-specific or conformational epitope or recognizes a conformational epitope located in a surface-exposed loop in the P2 subdomain.

[0014] Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE FIGURES

[0015] So that the matter in which the above-recited features, advantages and objects of the invention as well as others which will become clear are attained and can be understood in detail, more particular descriptions and certain embodiments of the invention briefly summarized above are illustrated in the appended drawings. These drawings form a part of the specification. It is to be noted, however, that the appended drawings illustrate certain embodiments of the invention and therefore are not to be considered limiting in their scope.

[0016] FIG. 1. General information about Noroviruses.

[0017] FIG. 2. Illustration of several genogroups and genotypes of Noroviruses.

[0018] FIG. 3. Example of a method of isolation and purification of anti-Norovirus human monoclonal antibodies.

[0019] FIG. 4. Demonstration that the monoclonal antibodies are specific to GI.1 and exhibit HBGA blockade.

[0020] FIG. 5. Structure of Norovirus P domain complexed with human IgA 5I2.

[0021] FIG. 6. Illustration that IgA 5I2 binds to P domain through CDRs H3, L1 and L3.

[0022] FIG. 7. Illustration of a close-up of Pdomain-5I2 interactions.

[0023] FIG. 8. Structure of Norovirus P domain bound to H type HBGA.

[0024] FIG. 9. IgA 5I2 blocks HBGA binding through steric hindrance.

[0025] FIG. 10. Sequence and structure and alterations mediate IgA 5I2 genotype specificity.

[0026] FIG. 11. IMGT analysis of particular antibodies (SEQ ID NOs. 90-108).

[0027] FIG. 12. Fab 5I2 binds tightly to NV P domain. Biolayer interferometry (BLI) analysis of Fab 5I2 binding to NV P domain. P domain- Fab 5I2 association- dissociation curves were obtained through serial two fold dilutions of Fab 5I2 (0.5-0.015 μ M) plus buffer controls using the Octet acquisition software. Sensograms for all concentrations are shown and labeled accordingly. The calculated KD, Kon, and Koff are shown in a tabular form.

[0028] FIG. 13. Fab 5I2 recognizes a conformational epitope on top of NV P domain. Cartoon representation of the overall structure of Fab 5I2-P domain complex showing one Fab 5I2 molecule bound to each subunit of the P domain dimer. The individual P dimer subunits are shown in blue and green with P1 and P2 subdomains labeled. The dashed black line indicates the two fold symmetry axis. Fab 5I2 is depicted with the heavy and light chains shown in cyan and magenta, respectively. The variable and constant domains of Fab 5I2 light chain and heavy chain are labeled VL-CL and VH- CH respectively. The N and C terminal are also indicated for both P domain and Fab 5I2.

[0029] FIGS. 14A and 14B. Detailed view of Fab 5I2-P domain interactions. FIG. 14A: Close up view of Fab 5I2 bound to P domain (blue) bound to Fab 5I2 (heavy chain: cyan, light chain: magenta) depicted in both surface and cartoon representation. All the six CDRs, three from the light chain (L1-L3) and three from the heavy chain (H1-H3), were identified and labeled respectively. Similarly six loop regions were identified on the P2 subdomain. Five of these loops have been previously identified in other genotypes and labeled according to convention (loops- A, B, P, T and U). The sixth loop labeled loop Q was identified in this disclosure. Three of these loops, loop T (yellow), Q (red) and U (green) form the conformational epitope recognized by the Fab 5I2. FIG. 14B. Molecular details of Fab 5I2-P domain interactions. Fab 5I2 binds P domain through CDRs L1, L2 and H3 that make a network of hydrogen bonding (black dashed lines) and hydrophobic interactions (red dotted line) with the loops U, Q and T of the P domain. CDRL1 makes the predominant interactions. All interacting loops are shown in cartoon representation with interacting residues shown as stick model as per above color convention with nitrogen and oxygen atoms in blue and red respectively.

[0030] FIGS. 15A, 15B, 15C, and 15D. Complementary surface residues involved in Fab 5I2-P domain interaction. Presentation of complementary surfaces is important to antigen- antibody recognition and binding; two pockets, one on the P domain surface (FIG. 15A) and one on the Fab surface (FIG. 15C), were identified in the disclosure and are shown to accommodate a complementary residue from the other molecule. FIG. 15A. A pocket on the P domain surface (blue) buries a lysine 32 residue (pink stick model) contributed by the CDRL1 of Fab 5I2. The P domain residues shown as sticks and labeled N394, G396 from loop U (green) and T346, F352 from loop Q (red) contribute to hydrogen bonding interactions with K32. FIG. 15C. A similar pocket on the surface of Fab 5I2 (magenta) is shown to accommodate a histidine residue (H381) (yellow stick model). H381 makes hydrophobic and stacking interactions with three tyrosine residues labeled Y31, Y38 and Y98 contributed by CDRs L1 and L3 of Fab 5I2. FIGS. 15B and 15D. Interestingly, superposition of the Fab 5I2 bound P domain structure and native NV VLP structure (PDB ID. 1IHM) (grey) shows that Fab binding induces local conformational changes on P domain to make favorable interactions. FIG. 15B. The loop U moves about 10Å to make favorable interactions with CDRL1 and forms one side of the pocket that buries residue K32. Loop U from VLP is labeled in grey and marked with an asterix the movement of loop U is indicated by an arrow. FIG. 15D. Similarly, Fab binding induces a flip in the orientation of the side

chain of a H381, allowing it to make favorable hydrophobic and stacking interactions. In the native VLP structure the sidechain of H381 (grey, indicated with asterisk) would sterically clash with Y98 residue of CDRL3.

[0031] FIGS. 16A, 16B, 16C, and 16D. Fab 5I2 blocks HBGA binding to P domain through steric hindrance. Superposition of HBGA bound P domain (PDB ID. 2ZL6) and the Fab 5I2 bound P domain structure reveals steric hindrance as the mechanism of HBGA blockade. FIG. 16A. Surface representation (grey) and cartoon representation of the P domain dimer (side view) bound to H type HBGA (yellow sticks). FIG. 16B. Superposition of Fab and HBGA bound P domain structures clearly shows that Fab 5I2 will sterically hinder binding of HBGA. FIG. 16C. Surface representation of the P domain dimer (top view), highlighting the footprint of the HBGA binding site (yellow) and labeled HBGA. Superposition of the Fab bound structure (FIG. 16D) onto this P domain structure shows that Fab binding limits access to the HBGA binding site, indicated by masking of the HBGA footprint (yellow).

[0032] FIGS. 17A and 17B. Sequence and structural changes mediate escape from Fab 5I2 neutralization in other genotypes. FIG. 17A. Amino acid sequence alignment of representative GI variants showing the poor conservation of residues at positions that correspond to residues from the three loop regions in GI.1 that are involved in interacting with Fab 5I2. The residues in loop Q, T and U are colored in red, yellow and green respectively. FIG. 17B. Superposition of P domain structures from GI genotypes; GI.7 (green) (PDB ID: 4P12), GI.8 (orange) (PDB ID: 4RDJ) and GI.1 (purple) (this disclosure) show that the loop regions (Q, T and U) involved in interacting with the Fab 5I2 (grey) in GI.1 undergo structural alterations that would disrupt the conformational epitope recognized by Fab 5I2 on GI.1 NV. The loops and CDRs from Fab 5I2 are respectively labeled.

[0033] FIGS. 18A and 18B. Mapping of neutralizing epitopes on NoVs. Surface representation of the P domains from GI (FIG. 18A) and GII (FIG. 18B) NoV genogroups. Based on this study and other biochemical studies, we hypothesize that the majority of the neutralizing epitopes on the surface of the NoV capsid protein lie in one of the three clusters identified in this study. Cluster 1 (red) comprises the evolving residues in the T loop. Cluster 2 (blue) comprises residues in the Q and U loops. Cluster 3 (green) comprises residues in the A and B loops. The identified clusters are in close proximity to the HBGA binding site

(yellow). NAb's can either bind to individual clusters or use a combination of these clusters to bind and neutralize NoVs. Epitope of Fab 5I2 is located in clusters 1 and 2 and is indicated by a dotted line and labeled respectively.

[0034] FIGS. 19A and 19B. Screening supernatants of EBV-transformed B cell cultures from two NoV-challenged subjects. B cell culture supernatants were added to replicate microtiter plates coated with NoV VLP and probed with a mixture of (i) a mixture of anti-human ($\kappa + \lambda$; to determine the total number of binders), or (ii) anti-human IgG (γ -specific; to determine IgG frequency), or (iii) anti human IgA (α -specific; to determine the IgA frequency) secondary antibodies. Blocking assay was done as described in Methods. The number of binding ($A_{450} > 1.5$) and blocking ($A_{450} < 2.1$) were counted and percent distribution among binders and blockers was calculated. Distribution of IgG (red) or IgA (blue) classes of antibodies that bound to NoV VLP (FIG. 19A) or blocked VLP - glycan interaction (FIG. 19B) is shown.

[0035] FIGS. 20A and 20B. Binding and blocking characteristics of purified monoclonal antibodies. Purified IgG (red) or IgA (blue) antibodies were tested for binding to NV VLP in ELISA (FIG. 20A) or for blocking VLP - glycan interaction (FIG. 20B). Each of the IgG antibodies bound to VLPs with lower EC₅₀ values than IgA antibodies, while in contrast the concentrations needed for blocking were similar for IgG and IgA. The blocking of murine mAb 8812 is shown in black.

[0036] FIGS. 21A, 21B, and 21C. Specificity of human mAbs. The binding (mean absorbance at 450 nm $\pm$ SD) of purified mAbs at 20 μ g/mL to VLPs representing homologous virus (NoV GI.I) or heterologous human NoVs of different genotypes (FIG. 21A) or antigens representing wild-type or mutant recombinant capsid proteins of homologous virus (FIG. 21B) were assessed by ELISA to evaluate genotype specificity and to infer the subdomain of major capsid protein bound by anti norovirus mAbs. The data shown in each figure summarizes the results from 2 independent experiments. (FIG. 21C) The ligand specificity of mAb-mediated inhibition of NoV VLP binding to a panel of its glycan ligands (H1, H2, H3, tri-A or Le(y)) was evaluated in HBGA blocking assay for three mAbs, 2L8, 3I23 and 5I2.

[0037] FIGS. 22A, 22B, and 22C. Nature of epitopes recognized by anti-norovirus mAbs. Norovirus VLPs were resolved on SDS-PAGE gels under (FIG. 22A) nonreducing,

nondenaturing, or (FIG. 22B) reducing, denaturing conditions and the membranes were probed with anti-norovirus mAbs. All the human antibodies, and the murine mAb 8812, bound to conformational epitopes, while denatured VLP were bound only by mAb 3901. Arrowhead in panel B indicates VP1. (FIG. 22C) Antibodies were binned into competition-binding groups in ELISA as described in Methods. Most of the antibodies seem to compete for the same or spatially proximate epitopes. The asymmetric nature of competition suggests subtle factors such as the angle of approach of the antibodies seem to have an effect on competition.

[0038] FIGS. 23A and 23B. Molecular assembly of hybridoma-derived antibodies obtained from Donor 1. IgA (blue) and IgG (red) antibodies were purified by affinity chromatography and resolved on SDS polyacrylamide gels under reducing, denaturing conditions (FIG. 23A) or non-reducing conditions (FIG. 23 B) and stained with Coomassie Blue. Monomeric (*) and dimeric (**) forms of IgA are evident.

[0039] FIGS. 24A and 24B. Average binding and blocking profiles of IgG and IgA antibodies. The dose-response curves for binding (FIG. 24A) or blocking (FIG. 24B) for all IgG and IgA antibodies were averaged to generate representative curves for each class using R software package. The binding curves for IgG and IgA are significantly different ($p < 0.001$), while there is insufficient evidence to show that IgG and IgA differ in blocking ($p = 0.39$).

[0040] FIG. 25. Variable heavy and light chain domains of anti-NoV mAbs were cloned into expression vectors containing γ or α constant domains for heavy chains, and κ or λ constant domains for the light chains. Antibodies were expressed transiently in HEK293 cells. For expression of dIgA, a plasmid coding for J chain was cotransfected with the heavy and light chains. Antibodies purified from supernatant by affinity chromatography were resolved on SDS-PAGE gels under nonreducing conditions and stained with Coomassie Blue reagent.

[0041] FIG. 26. Representative curves for blocking assays with isotype switch variants for each of the antibody clones. IgG or monomeric (mIgA) or dimeric (dIgA) forms of IgA were used in the HBGA blocking assay. Results are shown with concentration of Ab as log10 nM combining sites).

[0042] FIG. 27. Genetic characteristics of anti-norovirus mAbs (SEQ ID NOs. 109-127)

DETAILED DESCRIPTION

[0043] As used in this specification and claim(s), the words “comprising” (and any form of comprising, such as “comprise” and “comprises”), “having” (and any form of having, such as “have” and “has”), “including” (and any form of including, such as “includes” and “include”) or “containing” (and any form of containing, such as “contains” and “contain”) are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

[0044] The use of the term “or” in the claims is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and “and/or.” It is also contemplated that anything listed using the term “or” may also be specifically excluded.

[0045] Throughout this application, the term “about” is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0046] Following long-standing patent law, the words “a” and “an,” when used in conjunction with the word “comprising” in the claims or specification, denotes one or more, unless specifically noted.

[0047] The term “isolated” can refer to a nucleic acid or polypeptide that is substantially free of cellular material, bacterial material, viral material, or culture medium (when produced by recombinant DNA techniques) of their source of origin, or chemical precursors or other chemicals (when chemically synthesized). Moreover, an isolated compound refers to one that can be administered to a subject as an isolated compound; in other words, the compound may not simply be considered “isolated” if it is adhered to a column or embedded in an agarose gel. Moreover, an “isolated nucleic acid fragment” or “isolated peptide” is a nucleic acid or protein fragment that is not naturally occurring as a fragment and/or is not typically in the functional state.

I. Particular Embodiments of HBGA-Blocking Antibody and Related Compositions

[0048] Embodiments of the disclosure include molecules of any type that target a particular epitope to prevent binding thereto. In particular cases the molecules that target the epitope are peptide(s) or polypeptide(s), including antibodies or fragments thereof. In specific embodiments the targeting of a particular epitope includes direct binding to the epitope. In particular embodiments, the epitope comprises part or all of the histo-blood group antigens (HBGA) binding site on the P domain of VP1 of human Norovirus. Specific aspects provide for an antibody that binds to a site on Norovirus that sterically blocks HBGA from accessing the binding site. In specific embodiments the antibody is an IgA antibody, such as to facilitate mucosal immunity.

[0049] In specific cases the epitope is a conformational epitope formed by two surface-exposed loop clusters in the P domain of VP1 of human Norovirus. In particular embodiments, the epitope comprises H381 of GI.1 P domain and includes at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more residues on one or both sides of H381 of the polypeptide. The epitope may or may not have contiguous amino acids from a particular sequence involved. In specific embodiments, the amino acid sequence of an epitope of the disclosure comprises residues in the P domain in the capsid protein, including some or all of the residues N346, T348, D350, F352, S380, H381, S383, N394, and G396. Embodiments of the disclosure include antibodies or antibody fragments that recognize the epitope that comprises residues in the P domain in the capsid protein, including some or all of the residues N346, T348, D350, F352, S380, H381, S383, N394, and G396.. The antibody may be polyclonal or monoclonal. In specific embodiments, a monoclonal antibody or antibody fragment specifically binds an amino acid sequence comprising residues in the P domain in the capsid protein, including some or all of the residues N346, T348, D350, F352, S380, H381, S383, N394, and G396. The monoclonal antibody or antibody fragment may be a human monoclonal antibody, human monoclonal antibody fragment, a mouse monoclonal antibody, or a mouse antibody fragment. In some cases, the monoclonal antibody is a single chain antibody. The monoclonal antibody or antibody fragment may be a humanized monoclonal antibody or antibody fragment. In certain other embodiments, the antibody is a human antibody. In still further aspects the antibody is a recombinant antibody segment. An antibody may be isolated, chimeric, non-natural, and/or recombinant.

[0050] In particular embodiments, antibody binding occurs through one or more of complementarity determining loop (CDRL) 1 in light chain, CDRL3 in light chain, and complementarity determining region (CDR) loop (CDRH3) in heavy chain. In specific embodiments, the antibody binds the P2 subdomain through the CDRL1 and the histidine residue H381.

[0051] In particular aspects, any polypeptide of the disclosure targets a Norovirus of the genotype GI.1.

[0052] Embodiments of the disclosure include binding polypeptides that bind Norovirus particle P domain through one or more of complementarity determining loop 1 in the antibody light chain (CDRL1), CDRL3 in the light chain, and complementarity determining region (CDR) 3 loop in the antibody heavy chain (CDRH3). In specific embodiments, there is a binding polypeptide that binds Norovirus particle P domain at the H381 position of the P2 subdomain or the conformation that includes H381 of the P2 subdomain through the tyrosine residue CDRL1.

[0053] Embodiments of the disclosure include compositions that comprise the antibody, and in some cases the composition(s) comprise a monoclonal antibody or a mixture of two or more different monoclonal antibodies. When there is a mix of different monoclonal antibodies, the different antibodies may or may not target the same epitope or protein or genotype of Norovirus. In alternative cases, small molecule mimics that specifically target the HBGA binding site are utilized. In some cases, any composition encompassed by the disclosure is in a pharmaceutically acceptable formulation.

[0054] The antibodies described herein may block HBGA binding. The antibodies encompassed by the disclosure may block HBGA through direct competition for the HBGA binding site, through allosteric disruption of the HBGA binding site by inducing conformational changes in the P domain, or by steric masking of the HBGA binding site, or another mechanism. In specific embodiments, an antibody of the disclosure blocks HBGA predominantly by steric hindrance.

[0055] In some cases, antibodies with a prevalent involvement of CDRL1 in antigen recognition are employed. In particular embodiments, an antibody is utilized that comprises a length of the CDR of CDRL1 that is longer than typical lengths, including loop lengths longer than 11, 12, 13, 14, 15, 16, or 17 amino acids, for example. In certain aspects, an

antibody is utilized that has a length of CDRL1 that is longer than typical (in k chain human antibodies, the length of the CDRL1 varies between 10 and 17 residues, with the majority of the antibodies exhibiting a loop length of 11 residues) and in combination also has an H3 loop with fewer than typical amino acids involved in antigen specificity (in IgA 5I2, for example, the H3 loop is positioned slightly away from the P2 subdomain, with just two of its residues interacting with the P2 subdomain. Typically, in an antibody-antigen interaction, including those involving antiviral antibodies, CDRH3 encoded by the highly diverse D-J_H joining genes plays a dominant role because of the inherent sequence diversity and consequent conformational variability).

[0056] Embodiments of the disclosure include a crystal structure of a HBGA-blocking monoclonal antibody bound to Norovirus, revealing, in one embodiment, its mechanism of neutralization.

[0057] Certain embodiments are directed to a hybridoma cell and to a monoclonal antibody produced by a hybridoma.

[0058] Certain aspects include particular monoclonal antibodies, and sequences thereof. Sequences of the particular monoclonal antibody 5I2 are as follows (CDRs are underlined):

NORO-5I2 Fab Heavy Chain (SEQ ID NO:1):

QVQLVQSGAEVKKPGSSVKVSCRTSGDTFNTHAISWVRQAPGQGLEWMGGII
PIFATTNYANKFQGTVTISADESTSTAYLEVRSLRSEDVAVYYCASNRRANRADDYDY
YFDYWGQGTLLTVSSASFSGPSVFPPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
 SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPK
 KSC

NORO-5I2 Fab – Light chain (SEQ ID NO:2)

EIVMSQSPDSLAVSLGERATINCKSSQSVLYKSDKKNYLAWYQQKSGQPPKLL
 LIYWASTRESGVPDRFSGSGSGTDFTLTISSSLQAEDVAVYYCQQYYIPRTFGQGTKV
 DIKRTAAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQEQ
 SVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHXGLSSPVTKSFNRGEC

[0059] Specific sequences of the CDRs of monoclonal antibody 5I2 are as follows:

GDTFNTHA (SEQ ID NO:3)

IIPIFATTNY (SEQ ID NO:4)

ASNRANRADDYDYYFDY (SEQ ID NO:5)

QSVLYKSDKKNY (SEQ ID NO:6)

WASTR (SEQ ID NO:7)

QQYYSIPRT (SEQ ID NO:8)

[0060] Monoclonal antibodies other than 5I2 are encompassed in the disclosure. In specific embodiments, a monoclonal antibody is encoded by a particular nucleotide sequence. Nucleotide sequences of HBGA-blocking monoclonal antibodies other than 5I2 are as follows:

NV1A8 heavy:

GAATTCcagctgcaggagtcgggcccaggactggtgaagccttcggagaccctgtccctcacctgcagtgtctctgg
tggtccgtcaccaatattaatcactactggagttggatccggcagtcgccgaaaaggattgagtgattgggcgtattcataccag
ggggatcaccgattacaacccctccctcaagagtcgaatcatcctgtcaaccgactcgtccaagaatcagctctccctgacagtgagct
ctgtgaccgccgcagacacggcctttattactgtgcgagagagtctatgggggtcggggagttgttgactcctggggccagggaat
cctggtcaccgtctcctcaGCAAGCTTC (SEQ ID NO:9)

NV2J3 heavy:

GAATTCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGAC
CCTGTCCCTCACCTGCaCTGTCTCTGGTTACTCCATCAACAGTGGTTACTACTGGG
GCTGGATCCGGCAGGCCCCAGGGAAGGGGCTGGAGTGGATTGGGAGTATCTATC
ATACTGGGAGCACCTACAGAAGCCCGTCCCTCAAGAGTCGAGTCACCATATCAG
TAGACACGTCCAAGAACCAGTTCTCCCTGAAGCTGAGCTCTGTGACCGCCGCAG
ACACGGCCGTGTATTACTGTGCGAGAGATCGATCCGTAGTAGTGCCAGCTGCCCC
CCTCTACTACATGGACGTCTGGGGCAGAGGGACCACGGTCACCGTCTCCTCCGCA
AGCTTC (SEQ ID NO:10)

NV2J3 light:

CAGTCTGCCCTGACTCAGCCTGCCTCCGTGTCTGGGTCTCCTGGACAGTCG
ATCACCATCTCCTGCACTGGAACCATCAGTGATGTTGGTGGTTATAACTATGTCT
CCTGGTACCAACAACACCCAGGCAAAGCCCCCAAACATCATGATTTATGATGTCA
ATAAGCGGCCCTCAGGGGTTTCTAATCGCTTCTCTGGCTCCAAGTCTGGCAACAC
GGCCTCCCTGACAATCTCTGGGCTCCAGGCTGAGGACGAGGCTAATTATTACTGC
TGCTCATATGCAACTAGTACCAATTTGCTATTCGGCGGAGGGACCCAGCTGACCG
TCCTA (SEQ ID NO:11)

NV2L8 heavy

GAATTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTC
AGTGAAGGTCTCCTGCAAGGCGTCTGGATACACCTTCAGGAAATACTATATGCAC
TGGGTGCGACAGGCCCTGGACAAGGGCCTGAGTGGATGGGAATAATCAACCCT
AGTGGTGGTAACACAGGCTACGCACAGAAGTTCAGGGCAGAGTCACCGTGACC
AGGGACACGTCCACGAGCACAGTCTACATGGAGCTGAGCAGCCTGAGATCTGAG
GACACGGCCGTGTATTACTGTGCTAGAGGCGGAATCAGCTGGTACGTTACCGGCT
TTGACTACTGGGGCCAGGGGACCCTGGTACCGTCTCCTCAGCAAGCTTC (SEQ ID
NO:12)

NV2L8 light

AGATCTCAGTCTGTGCTGACTCAGCCTGCCTCCGtGTCTGGGTCTCCTGGAC
AGTCGATCACCATCTCCTGCACTGGAACCAGCAGTGACGTTGGTACTTATAACTA
TGTCTCCTGGTACCAACAACACCCAGGCAAAGCCCCCAGACTCATAATTTATGAT
GTCAGTAATCGGCCCTCAGGGGTTTCTGATCGCTTGTCTGGCTCCAAGTCTGGCA
ACACGGCCTCCCTGACAATCTCTGGGCTCCAGGCTGAGGACGAGGCTGATTATTA
CTGCAGCTCATATACAAGGAGCAGCACTTGGGTGTTTCGGCGGAGGGACCCAGCT
GACCGTCCTAGCGGCCGCA (SEQ ID NO:13)

NV3I3 heavy:

GAATTCcagctgcaggagtcgggcccaggactggtgaagcctTCGGAGACCCTGTCCCTCACC
TGCACTATTTCTGGTGGCTCCGTGAGCAGAGCTAGTTACTACTGGGGCTGGCTCC
GCCAGCCCCCAGGGAAGGGGCTGGAGTTTATTGGGAGTATCTATTATGGTGGGA
GCACCTACTACAACCCGTCCCTCAAGAGTCGAGTCACCATTTTCATAGACACGTC
CAAGAACCAGATCTCCCTGAAGCTGGCCTCTGTGACCGCCGCAGACACGGCTAT
GTATTACTGTGCGAGACACCCTAGTTGGGACAGGTCTTGTTTGACCCCTGGGGC
CCGGGAACCCAGGTCACCGTCTCCTCAGCAAGCTTC (SEQ ID NO:14)

NV3I3 light:

AGATCTCAGTCTGCCCTGACTCAGCCTCCCTCCGCGTCCGGGTCTCCTGGA
CAGTCAGTCACCATCTCCTGCACTGGAATCGCCAGTGACGTTGGTGGTCATAACT

CTGTCTCCTGGTACCAACAGCACCCAGGCAAAGCCCCCAAAGTCATTATTTATGA
GGTCACTCAGCGGCCCTCAGGGGTCCCTGATCGTTCTCTGGCTCCAAGTCGGA
AATACGGCCTCCCTGACCGTCTCTGGGCTCCAGGCTGACGATGAGGCTGATTATT
ACTGCAGCTCATATGTCTGGTAACAACAACCTTCGCATTTCGGCGGAGGGACCCAGCT
GACCGTCCTAGCGGCCGCA (SEQ ID NO:15)

NV3I23 heavy:

GAATTCCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCTC
CCTGAGACTCTCCTGTGCTGCCTCTGGATTACGTTTAGTAGGCATGCCATGACTT
GGGTCCGCCAGGCGCCAGGGAAGGGGCTTGAGTGGGTCTCAATTGTTAGTGGTA
GCGGCTATAAGACACTCTACGCAGACTCCGTGAGGGGCCGGTTCACCATGTCCA
GAGACAATTCCAAGGATACGATGTATTTGCAAATGAGCAGCCTGAGAGCTGAAG
ACACGGCCGTATATTACTGTGCGAAAGGAGTTGGCTCGGACTTTCGACTTCGCG
GATTCTTGACTCATGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCAAGCTTC
(SEQ ID NO:16)

NV3I23 light

AGATCTGAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCA
GGGGAAAGAGGCACCCTCTCCTGCAAGGCCAGTCAGATTGTTTACAGCAACTAC
TTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGATG
CATCCAGCAGGGCCACTGGCATCCCAGAAAGGTTTCAGTGGCAGTGGGTCTGGGA
CAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTA
CTGTCACCAATATGGAACCTCATTCACCTTCGGCCCTGGGACCAAGGTGGATATC
AAACGTACTGCGGCCGCT (SEQ ID NO:17)

NV4B19 heavy:

GAATTCCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGGGGTCTC
CCTGAGACTCTCCTGTGTAGCCTCTGGCTTCACCTTCAATAAGTATGCTATACACT
GGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTCGGTGGCAGTTATATCATATG
CTGGAGACCATAAATACTACGCAGACTCCGTGAAGGGCCGATTCACTATCTCCAG
AGACAATTCCAAGAACACCGTGTTTCTGCAAATGAGCAGCCTGAGACCCGACGA
CACGGCTGTCTATTTCTGTGCGAGAGACCTCAGTGCAAGTTTCGACTACTGGGGC
CAGGGAACCCTGGTCACCGTCTCCTCAGCAAGCTTC (SEQ ID NO:18)

NV4B19 light

AGATCTGATGTTGTGATGACTCAGTCTCCGGTTTCCCTGCCCCGTCACCCCT
GGACAGCCGGCCTCCATCTCCTGCAGGTCTAGTCAAAGCCTCGTATACAGTGATG
GAAACACCTACTTGAGTTGGTATCACCAGAGGCCAGGCCAATCTCCAAGGCGCC
TAATTTATCAGGTTTCTAACCAGGACTCTGGGGTCCCAGACAGATTTCAGCGGCAG
TGGGTGAGGCACTGATTTACACTGAAAATCAGCAGGGTGGAGGCTGAGGATGT
TGGGGTTTATTACTGCATGCAAGGTACACACTGGCCCATGTTCACTTTTGGCCAG
GGGACACGACTGGAGATTAAACGTACTGCGGCCGCT (SEQ ID NO:19)

NV4C10 heavy

GAATTCCAGCTGGTGGAGTCTGGGGGAGGTGTGGTACGGCCTGGGGGGTCTC
CCTGAGACTCTCCTGTGCAGCCCTGGATTACCTTTGAAGATTTTGGCATGAGCT

GGGTCCGCCAAGCTCCAGGGAAGGGGCTGGAGTGGGTCTCTGCTATCAATTGGG
CTGGTTACACCAGAGGGTATGCAGACTCTGTGAAGGGCCGATTCACCATCTCCAG
AGACAACGCCAAGAACACCCTGTTTCTGGAGATGAACAATCTGCAAGTCGAGGA
CACGGCCTTGTATTACTGTGCGAGAGATAACCGTGGACAGAGGGGCTCCAGTTTC
GGATGGTTCTGACTCCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCAAGCT
TC (SEQ ID NO:20)

NV4C10 light

AGATCTGaCATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCTTCTGTAG
GAGACAGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTAACTATTTAA
ATTGGTATCAGCAGAAACCAGGGAAAGCCCCTAACCTCCTGATCTATGGGGCAT
CCAGTTTGCAAGGTGGGGTCCCATCAAGGTTCACTGGCAGTGGATCTGGGACAG
ATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGT
CAACAGGATTACATTACCCCGAGGACTTTCGGCCCTGGGACCAAGGTGGATATC
AAACGTACTGCGGCCGct (SEQ ID NO:21)

NV4E7 heavy

GAATTCCAGCTGCAGGAGTCGGGGCCAGGACTGGTGAAGCCTTCGGGGAC
CCTGTCCCTCACCTGCGGTGTCTCTGGTGGCTCCATCAGCAGTACTAACTGGTGG
AGTTGGGTCCGCCAGCCCCAGGGAAAGGGGCTGGAGTGGATTGGGGAAATCTAT
CATAGTGGCGACACCAACTACAACCCGTCCCTTAAGAGTCGAGTCACCATTTCAC
TGGACAAGTCCACGAACCAGTTCTCCCTGAAGTTGAGCTCTGTGACCGCCGCGGA
CACGGCCGTGTATTACTGTGCGATAGGGGGCAGCGCCTCAGTTCCGACCAAGTAC
TGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCAAGCTTC (SEQ ID NO:22)

NV4E7 light

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTGGGAGAC
AGAGTCACCATCACTTGCCGGGCAAGTCAGAGCATTGGCAGCTATTTAAATTGGT
ATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATGTATGCTGCATCCCGTTT
GCAAAGTGGGGTCCCATCCAGGTTCACTGGCAGTGGATCTGGGACAGATTTCACT
CTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTACTGTCAACAGA
GTTACAGTTCCCCGTACACTTTTGGCCAGGGGACCAAGCTGGAAATCAAACGA
(SEQ ID NO:23)

NV4I23 heavy:

GAATTCCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTTC
CCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTTAGCAGCTATGCCATGAGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCCGGCATTGGTGGT
AGTGGTGGTAGCACATACTACGCAGACGCCGCGAAGGGCCGGTTCACCATCTCC
AGAGACAATTCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAG
GACGCGGCCGTATATTATTGTGCGAAAAATGCAGGTGACTACGCCCCGTGCGCTG
CTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCAGCAAGCTTC (SEQ
ID NO:24)

NV4I23 light

GAAATTGTGCTGACTCAGTCTCCAGACTTTCAGTCTGTGACTCCTAAGGAG
ACAGTCACCATCACCTGCCGGGCCAGTCAGAGCATTGGTAGTAGCTTACACTGGT
ACCAGCAGAAACCAGGTCAGTCTCCAAAGCTCCTCATCAAGTATGCTTCCCAGTC
CTTCTCAGGGGTCCCCTCGAGGTTCAAGTGGCAGTGGATCTGGGACAGATTTCACC
CTCACCATCAATAGCCTGGAAGCTGAAGATGGTGCAACGTATTACTGTCATCAGA
GTAGTACTTTACCGGGGACGTTTCGGCCAAGGGACCAAGGTGGAAATCAAACGA
(SEQ ID NO:25)

NV5I2 heavy

CAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTC
GGTGAAGTCTCCTGCAGGACTTCTGGAGACACTTTCAACACCCACGCTATCAGC
TGGGTGCGACAGGCCCTGGACAGGGGCTTGAGTGGATGGGAGGGATCATCCCC
ATCTTTGCTACAACAAATTACGCAAACAAGTTCCAGGGCACAGTCACAATTAGCG
CGGACGAGTCCACGAGCACAGCCTACTTAGAGGTGCGCAGCCTGAGATCTGAGG
ACACGGCCGTCTATTACTGTGCGAGTAATCGGGCCAATCGTGCAGACGATTACGA
CTACTACTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA (SEQ ID
NO:26)

NV5I2 light

AGATCTGAGATCGTGATGAGCCAGTCTCCAGACTCCCTGGCTGTGTCTCTG
GGCGAGAGGGCCACCATCAACTGCAAGTCCAGCCAGAGTGTTTTATACAAGTCC
GACAAGAAGAACTACTTAGCTTGGTACCAGCAAAAATCAGGGCAGCCTCCTAAG
CTGCTCATTTACTGGGCATCTACCCGGGAATCCGGGGTCCCTGACCGATTCAAGT
GCAGCGGGTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAAG
ATGTGGCAGTTTATTACTGTCAACAGTATTATAGTATTCTCGGACGTTTCGGCCA
AGGGACCAAGGTGGATATCAAACGTACTGCGGCCGCT (SEQ ID NO:27)

NORO 105 Heavy

Tctctgttcaggetcaggattcaactttcatgaatatggcatgagctgggtccgccaagttccagggaaggggctggagt
gggtctctcttattaattggaatggcgatagtagctttatagactctgtgaaggccgattcaccatctccagagacaacgccaaga
actccctgtatctgcaaatgaacagctctgagagccaggacacggcctgtattactgtgtgagagaaccaagagtggcgggctacta
ctattacggtatggacgtctggggcca (SEQ ID NO: 54)

NORO 105 Light

Cagtctccatcctcctgtctgcatctgtcggagacagagtcaccatcacttgcgggcaagtcagaagtttagcagctattt
gaattggtatcagcagacaccaggagagccctaaactcctgatctatgctgcatccagggtgcaagttgggtcccatccagggtca
gtggcagtggtatctgggacagatttcaactttaccatcagcagctctgcaacctgaagactttgcaacttactactgtcaacagagttacttt
atccctcgaacgttcggccaagggaccaaggtggaattcaaac (SEQ ID NO: 55)

NORO 115 Heavy

Caggtgcagctggtggagctctgggggaggcttggtcaagcctggggggtccctgagactctcctgtacagcctctggaat
caccatcagtggtactacatgagttggatccgaggtccagggaagggactggaatggattgtatacataatacaagtggtagaa
ccatactacgcagactctgtgaaggccggttctccgtctccagggacaacgccaaggagtcgctgtatttgcaaatggacagcct
gacggtcgtatgacacgggcatatatttgtgcgagagatcgattaccagcatctggttccactggttccacctggggccaggga
acctggtcaccgtctcctcag (SEQ ID NO: 56)

NORO 115 Light

Ggctgtgtctctgggcccggaggccaccatcaactgcaagtccagccagagtgtttatacacctccgacaataagaacta
cttagcctgtgtaccagcagaaccaggacagcctcctaagctgtcatttctctgggcttctactcgggaatccgggtccctgaccgat
tcagtggcagcgggtctgggacagatttcactctcaccatcagcaacctgcaggctgaagatgtggcagtttattactgtcagcagatt
ataatagctctctcgtttcggcggagggaaccaaggtggagatcaaac (SEQ ID NO: 57)

NORO 118 Heavy

Caggtgcagctggtggagtctgggggagggcgtgggtccagcctggggggtccctgagactctcctgtacagcgtctggatt
caccttcagtgggtcatggcatgcactgggtccgccaggctccaggcaaggggctggaatgggtgacatttatcatatgatggaagta
ataaattctatcgggactcagtgaagggccgattcatctcagagacaattccgagaacacgttgtttctgcagatgaacagcctga
gaccggaagacacgggagtctattggtgtgcgagagatgggttacagaaatttggtcctcgttgggtgggtacttcgatctctggggccgt
ggcacctggtcaccgtctcctcag (SEQ ID NO: 58)

NORO 123 Heavy

Caggtgcagctggtggagtctgggggagggcgtgggtccagcctgggaggtccctgagactctcctgttcaacctctggatt
caccttcagtcaatatctatgcactgggtccgccaggctccaggcaaggggctggagtgggtggcacttatcctatgatggaatga
ataaatactacgcagactccgtgaggggccgattcaccatctccagagacaattccgagaacacgcagtatctgcaaatgaacagcct
gagaggtgacgacacgggtgtctattattgtgcgagagtcacgggcgattgtactggtaatagatgctcatattgggcatactactacta
cggctctggacgtctggggccaaggaccct (SEQ ID NO: 59)

NORO 123 Light

Cagtctgcgctgactcagcctccctccgcgtccgggtctcctggacagtcagtcaccatctcctgcactggaaccagtagt
gacgttgggtggtataagtatgtctcctggtaccaacagcaccaggcaagccccaaactcatgatttatgaggtcactaggcggcc
ctcaggggtccctgatcgttctctgggtccaagtctggcaacacggccttctgaccgtctctgggtccaggctgaggatgaggctg
attattactcgggtcatatgcaggcagcaccacttccgggtatgtcttcggaactgggaccaaggtcatcgtcctag (SEQ ID
NO: 60)

[0061] Polypeptide sequences of the corresponding HBGA-blocking monoclonal antibodies referred to above are as follows:

NV1A8 HEAVY:

EFQLQESGPGPLVKPSETLSLTCSVSGGSVTNINHYWSWIRQSAEKGFEWIGRIH
TRGITDYNPSLKSRIILSTDSSKNQLSLTVSSVTAADTAFFYYCAREFYGGRGVVDSWG
QGILVTVSSASF (SEQ ID NO:28)

NV2J3 HEAVY

EFQLQESGPGPLVKPSETLSLTCTVSGYSINSGYYWGWIRQAPGKGLEWIGSIY
HTGSTYRSPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARDRSVVVPAAPLY
YMDVWGRGTTVTVSSASF (SEQ ID NO:29)

NV2J3 LIGHT:

QSALTQPASVSGSPGQSITISCTGTISDVGGYNYVSWYQQHPGKAPKLMYIDV
NKRPSGVSNRFSKSGNTASLTISGLQAEDEANYCCSYATSTNLLFGGGTQLTVL
(SEQ ID NO:30)

NV2L8 HEAVY

EFQLVQSGAEVKKPGASVKVSCKASGYTFRKYYMHWVRQAPGQGPEWMGI
INPSGGNTGYAQKFQGRVTVTTRDTSTSTVYMESSLRSEDNAVYYCARGGISWYVT
GFDYWGQGTLVTVSSASF (SEQ ID NO:31)

NV2L8 LIGHT

RSQSVLTQPASVSGSPGQSITISCTGTSSDVGTNYVSWYQQHPGKAPRLIIYD
VSNRPSGVSDRLSGSKSGNTASLTISGLQAEDADYYCSSYTRSSTWVFGGGTQLTV
LAAA (SEQ ID NO:32)

NV3I3 HEAVY

EFQLQESGPGLVKPSETLSLTCTISGGSVSRASYWGWLRQPPGKGLEFIGSIY
YGGSTYYNPSLKSRTIFIDTSKNQISLKLASVTAADTAMYYCARHPSWDRSWFDPW
GPGTQVTVSSASF (SEQ ID NO:33)

NV3I3 LIGHT

RSQSALTQPPSASGSPGQSVTISCTGIASDVGGHNSVSWYQQHPGKAPKVIIYE
VTQRPSGVDPDRFSGSKSGNTASLTVSGLQADDEADYYCSSYVGNNNFAFGGGTQLT
VLAAA (SEQ ID NO:34)

NV3I23 HEAVY

EFQLLESGGGLVQPGGSLRLSCAASGFTFSRHAMTWVRQAPGKGLEWVSIVS
GSGYKTLYADSVRGRFTMSRDNSKDTMYLQMSSLRAEDNAVYYCAKGVGSDFPTS
RILDSWGQGTLVTVSSASF (SEQ ID NO:35)

NV3I23 LIGHT

RSEIVLTQSPGTLSPGERGTLSCKASQIVYSNYLAWYQQKPGQAPRLLIYD
ASSRATGIPERFSGSGSGTDFTLTISRLEPEDFAVYYCHQYGTSTFTFGPGTKVDIKRTA
AA (SEQ ID NO:36)

NV4B19 HEAVY

EFQLVESGGGVVQPGGSLRLSCVASGFTFNKYAIHWVRQAPGKGLESVAVIS
YAGDHKYYADSVKGRFTISRDNKNTVFLQMSSLRPDDTAVYFCARDLSASFDYWG
QGTLVTVSSASF (SEQ ID NO:37)

NV4B19 LIGHT

RSDVVMVTQSPVSLPVTGQPASISCRSSQSLVYSDGNTYLSWYHQRPGQSPRR
LIYQVSNRDSGVDPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQGTHWPMFTFGQG
TRLEIKRTAAA (SEQ ID NO:38)

NV4C10 HEAVY

EFQLVESGGGVVVRPGGSLRLSCAASGFTFEDFGMSWVRQAPGKGLEWVSAIN
WAGYTRGYADSVKGRFTISRDNKNTLFLEMNNLQVEDTALYYCARDNRGQRGSS
FGWFDSWGQGTLVTVSSASF (SEQ ID NO:39)

NV4C10 LIGHT

RSDIQMTQSPSSLSASVGDRVTITCRASQSINTYLNWYQQKPGKAPNLLIYGA
SSLQGGVPSRFTGSGSGTDFTLTISLQPEDFATYYCQQDYITPRTFGPGTKVDIKRTA
AA (SEQ ID NO:40)

NV4E7 HEAVY

EFQLQESGPGLVKPSGTLSTCGVSGGSISSTNWWWSWVRQPPGKGLEWIGEY
HSGDTNYPNPSLKSRTISLDKSTNQFSLKLSSVTAADTAVYYCAIGGSASVPTKYWG
QGTLVTVSSASF (SEQ ID NO:41)

NV4E7 LIGHT

DIQMTQSPSSLSASVGDRVTITCRASQSIGSYLNWYQQKPGKAPKLLMYAAS
RLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQSYSSPYTFGQGTKLEIKR
(SEQ ID NO:42)

NV4I23 HEAVY

EFQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGIG
GSGGSTYYADAAGKRTISRDNKNTLYLQMNSLRAEDAAVYYCAKNAGDYAPSP
ADYWGQGTLVTVSSASF (SEQ ID NO:43)

NV4I23 LIGHT

EIVLTQSPDFQSVTPKETVTITCRASQSIGSSLHWYQQKPGQSPKLLIKYASQSF
SGVPSRFSGSGSGTDFTLTINSLEAEDGATYYCHQSSTLPQTFGQGTKVEIKR (SEQ
ID NO:44)

NV5I2 HEAVY

QVQLVQSGAEVKKPGSSVKVSCRTSGDTFNTHAISWVRQAPGQGLEWMGGII
PIFATTNYANKFQGTVTISADESTSTAYLEVRSLRSEDVAVYYCASNRANRADDYDY
YFDYWGQGTLVTVSS (SEQ ID NO:45)

NV5I2 LIGHT

RSEIVMSQSPDSLAVSLGERATINCKSSQSVLYKSDKKNYLAWYQQKSGQPP
KLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQQYYISIPRTFGQGT
KVDIKRTAAA (SEQ ID NO:46)

NORO 105 Heavy

SCSGSGFNHFHEYGMSWVRQVPGKGLEWVSLINWNGDSTAYIDSVKGRFTISR
DNAKNSLYLQMNSLRAEDTALYYCVREPRVAGYYYYGMDVWG (SEQ ID NO: 47)

NORO 105 Light

QSPSSLSASVGDRVTITCRASQKFSSYLNWYQQTPGRAPKLLIYAASRLQVGV
PSRFSGSGSGTDFTLTISLQPEDFATYYCQSYFIPRTFGQGTKVEFK (SEQ ID NO:
48)

NORO 115 Heavy

QVQLVESGGGLVKPGGSLRLSCTASGITISGYMSWIRQAPGKGLEWIVYINT
SGRTIYYADSVKGRFSVSRDNAKESLYLQMDSLTVDDTGIYYCARDRLPASGSHWF
HPWGQGTTLVTVSS (SEQ ID NO: 49)

NORO 115 Light

AVSLGGRATINCKSSQSVLYTSDNKNYLAWYQQQPGQPPKLLISWASTRESG
VPDRFSGSGSGTDFTLTISNLQAEDVAVYYCQQYYNSPLAFGGGKVEIK (SEQ ID
NO: 50)

NORO 118 Heavy

QVQLVESGGGVVQPGGSLRLSCTASGFTFSGHGMHWVRQAPGKGLEWVTFI
SYDGSNKFYADSVKGRFIISRDNSENTLFLQMNSLRPEDTGVYWCARDGYRNLVLV
GWYFDLWGRGTLVTVSS (SEQ ID NO: 51)

NORO 123 Heavy

QVQLVESGGGVVQPGRSLRLSCSTSGFTFSQYPMHWVRQAPGKGLEWVALIS
YDGMNKYYADSVRGRFTISRDNSENTQYLQMNSLRGDDTAVYYCARVTGDCTGNR
CSYWAYYYYGLDVWGQGT (SEQ ID NO: 52)

NORO 123 Light

QSALTQPPSASGSPGQSVTISCTGTSSDVGGYKYVSWYQQHPGKAPKLMIYE
VTRRPSGVPDRFSGSKSGNTAFLTVSGLQAEDADYYCGSYAGSTTSGYVFGTGTKV
IVL (SEQ ID NO: 53)

[0062] Thus, in specific embodiments there is a monoclonal antibody that comprises one or more sequences selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53.

[0063] Embodiments are directed to monoclonal antibody polypeptides, polypeptides having one or more segments thereof, and polynucleotides encoding the same. In certain aspects a polypeptide can comprise all or part of the heavy chain variable region and/or the light chain variable region of Norovirus-specific antibodies. In a further aspect, a polypeptide can comprise an amino acid sequence that corresponds to a first, second, and/or third complementary determining regions (CDRs) from the light variable chain and/or heavy variable chain of an antibody, *e.g.*, a Norovirus-specific antibody. Additionally an antibody

or binding polypeptide may have a binding region comprising an amino acid sequence having, having at least, or having at most 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% identity or homology (substitution with a conserved amino acid) (or any range derivable therein) with any sequence encompassed herein, including any of SEQ ID NOs: 1-8 and 28-46, for example. In specific embodiments, an antibody having all or part of one or more CDRs disclosed herein has been humanized in non-CDR regions. In further embodiments, the CDR regions disclosed herein may be changed by 1, 2, 3, 4, 5, 6, 7 or 8 amino acids per CDR, which may be instead of or in addition to humanization. In some embodiments, a change may be a deletion or addition of 1, 2, or 3 amino acids, or it may be a substitution of any amino acid, which may or may not be with an amino acid that is a conserved amino acid.

[0064] In some embodiments, a Norovirus-binding polypeptide or antibody has one, two, three, four, five, or six CDRs that have or have at least 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 100% identity with a consensus sequence identified for that CDR. It is contemplated that in some embodiments, a Norovirus-binding polypeptide or antibody has an amino acid sequence corresponding to CDR1, CDR2, and CDR3 of a light chain variable region and a CDR1, CDR2, and CDR3 of a heavy chain variable region. As discussed herein the amino acid sequence corresponding to a CDR may have a percent identity to a CDR encompassed herein.

[0065] In still further aspects, a polypeptides described herein comprise one or more amino acid segments of any of the amino acid sequences disclosed herein. For example, a polypeptide can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid segments comprising about, at least or at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 to 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199 or 200 amino acids in length, including

all values and ranges there between, that are at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identical to any of the amino acid sequences disclosed herein. In certain aspects the amino segment(s) are selected from one of the amino acid sequences provided herein. Embodiments of the disclosure include an antigen comprising the sequence of a peptide designed to mimic the P domain in the capsid protein, including some or all of the residues N346, T348, D350, F352, S380, H381, S383, N394, and G396.

[0066] In further aspects, a nucleic acid molecule of the embodiments comprises one or more nucleic acid segments of the any of the nucleic acid sequences disclosed herein. For example, a nucleic acid molecule can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nucleic acid segments comprising about, at least or at most 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 to 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more nucleotides in length, including all values and ranges there between, that are at least 80, 85, 90, 95, 96, 97, 98, 99, or 100% identical (or any range derivable therein) to any of the nucleic acid sequences disclosed herein.

[0067] In additional embodiments, there are pharmaceutical compositions comprising one or more polypeptides or antibodies or antibody fragments that are encompassed herein. Such a composition may or may not contain additional active ingredients.

[0068] In certain embodiments there is a pharmaceutical composition comprising, consisting of, or consisting essentially of a polypeptide comprising one or more antibody fragments encompassed herein. It is contemplated that the composition may contain non-active ingredients.

[0069] In any use for antibodies encompassed by the disclosure, the antibody may comprise one or more detectable agents, such as a radioactive marker, a nucleic acid, a fluorescent label, or an enzymatic label, and so forth.

II. Examples of Methods of Use

[0070] Compositions or antibodies described herein may be utilized in the treatment, prevention, and/or detection of Norovirus infection for a mammal, including at least a human, dog, cat, horse, pig, sheep, goat, and so forth, and/or an environment. In some cases, the compositions or antibodies are useful for the treatment, prevention, and/or detection of any genotype of Norovirus, although in some cases the compositions or antibodies are useful for the treatment, prevention, and/or diagnosis of a particular genotype or subcombination of genotypes. In specific embodiments, the compositions or antibodies are useful for Norovirus genotype GI.1, for example.

[0071] In at least some cases, compositions or antibodies, or mixtures thereof (including mixtures of different antibodies each of which may or may not target a different Norovirus genotype), are delivered prior to and/or following exposure of an individual to large populations of individuals or environments prone to Norovirus infection, including confined environments. Such environments include passenger vessels, including cruise ships, airplanes, and trains; schools; arenas; military environments, such as military encampments; health care facilities, including nursing homes, hospitals, and long-term care facilities; food service settings, such as restaurants and catered events; child care centers; prisons; recreational water settings; and so forth. The compositions or antibodies may be additionally or alternatively provided to an individual in the course of routine preventative measures.

[0072] In some embodiments of the disclosure an individual is provided antibody composition(s) for the prevention of Norovirus infection. In specific embodiments, individual antibodies are effective for one genotype, and therefore an individual is given a plurality of antibodies, each specific for a Norovirus genotype. In specific embodiments, an individual is given the antibody composition(s) in multiple administrations, such as through booster deliveries.

[0073] The antibodies encompassed herein can be used in immunohistochemical and biochemical methods for qualitative and/or quantitative analysis of samples from an individual suspected of having Norovirus infection.

[0074] Further aspects are directed to methods for evaluation of an individual suspected of having Norovirus. In certain aspects the method of evaluating an individual suspected of or having Norovirus comprises the step of detecting binding of an antibody that specifically binds to a particular Norovirus epitope in a biological sample from the individual, wherein the detection in the biological sample is indicative of the presence of Norovirus. In certain aspects the detection may be by immunoassay, for example.

[0075] A biological sample from an individual for analysis in methods of the disclosure may comprise stool, vomitus, saliva, serum, plasma, or tissue specimens for histopathology such as intestinal biopsy specimens.

[0076] In some embodiments, food (for example, shellfish, including mollusks such as oysters, clams, mussels and scallops), water, and/or environment samples (including swabs of environmental surfaces) are tested for the presence of Norovirus using antibodies encompassed by the disclosure.

[0077] A subject being treated or in need of treatment will typically have (*e.g.*, diagnosed with a Norovirus infection), be suspected of having, or be at risk of developing a Norovirus infection. Compositions include Norovirus-binding polypeptides in amounts effective to achieve the intended purpose – treatment or protection of Norovirus infection. The term “binding polypeptide” refers to a polypeptide that specifically binds to a target molecule, such as the binding of an antibody to an antigen. Binding polypeptides may but need not be derived from immunoglobulin genes or fragments of immunoglobulin genes. More specifically, an effective amount means an amount of active ingredients necessary to provide resistance to, amelioration of, or mitigation of infection. In more specific aspects, an effective amount prevents, alleviates or ameliorates symptoms of disease or infection, or prolongs the survival of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any preparation used in the methods described herein, an effective amount or dose can be estimated initially from *in vitro*, cell culture, and/or animal model assays. For example, a dose can be formulated in animal models to achieve a desired response. Such information can be used to more accurately determine useful doses in humans.

[0078] Any of the compositions and methods of using these compositions can treat a subject having, suspected of having, or at risk of developing a Norovirus infection or related disease. One use of the compositions is to prevent infections by inoculating a subject prior to exposure to Norovirus.

[0079] In certain embodiments, there is a method for treating a Norovirus infection comprising the step of administering an effective amount of antibody that specifically binds an epitope comprising a conformational epitope formed by surface-exposed loop clusters in the P domain in the capsid protein, including some or all of the residues N346, T348, D350, F352, S380, H381, S383, N394, and G396 to an individual having or suspected of having a Norovirus infection. In some embodiments, an individual may also be treated for dehydration, including oral rehydration fluids and/or intravenous fluids. In some cases, the dose of antibody delivered to the individual a dose of 0.1, 0.5, 1, 5, 10, 50, 100 mg or $\mu\text{g/kg}$ to 5, 10, 50, 100, 500 mg or $\mu\text{g/kg}$. In specific embodiments, the antibody is delivered to the individual *via* a route that is intravenous, intramuscular, and/or oral.

[0080] Certain aspects are directed to methods of preventing or treating Norovirus infection comprising administering to an individual having or suspected of having a Norovirus infection an effective amount of one or more purified polypeptides or proteins that specifically bind the P2 subdomain of the Norovirus. In certain aspects, the polypeptides bind a conformational epitope that includes the H381 residue of the P2 subdomain of the protein sequence of GenBank® Accession No. AAB50466 or a corresponding residue(s) define corresponding. It is contemplated that this polypeptide (or protein) may be referred to as an antibody by virtue of it being a polypeptide or protein with amino acid sequences of or derived from one or more CDR regions of an antibody. Any embodiment discussed herein in the context of an antibody may be implemented with respect to a polypeptide or protein so long as the polypeptide or protein has one or more amino acid regions that has at least 70%, 75%, 80%, 85%, 90%, 95%, or greater identity to a CDR from an antibody that is capable of specifically binding the P2 subdomain. The binding polypeptide can be a purified polyclonal antibody, a purified monoclonal antibody, a recombinant polypeptide, or a fragment thereof. In certain aspects the polypeptide is an antibody that is humanized, which means the non-variable portion of the antibody has been altered in order to simulate the constant regions found in human antibodies. Thus, it is contemplated that a humanized antibody is one that has the CDR sequences of a non-human antibody (or at least amino acid sequences that are

derived from such sequences, *i.e.*, are at least 70%, 75%, 80%, 85%, 90%, 95%, or greater in identity).

[0081] Certain aspects are directed to methods of treating a subject having or suspected of having a Norovirus infection comprising administering to a patient having or suspected of having a Norovirus infection an effective amount of a purified antibody or binding polypeptide that specifically binds Norovirus, including the P2 subdomain, including at or near H381 residue or a residue as noted in FIG. 10. In a further aspect the antibody or binding peptide binds a conformation epitope that includes the H381 residue of GenBank® Accession No. AAB50466 (SEQ ID NO:61) or a residue as noted in FIG. 10.

[0082] In a further aspect methods are directed to treating a subject at risk of a Norovirus infection comprising administering to a patient at risk of a Norovirus infection an effective amount of an antibody or binding polypeptide that binds a Norovirus P2 polypeptide prior to infection with Norovirus.

[0083] The subject typically will have (*e.g.*, diagnosed with a Norovirus infection), will be suspected of having, or will be at risk of developing a Norovirus infection. Compositions include antibodies in amounts effective to achieve the intended purpose – treatment or protection of Norovirus infection. More specifically, an effective amount means an amount of active ingredients necessary to provide resistance to, amelioration of, or mitigation of infection. In more specific aspects, an effective amount prevents, alleviates or ameliorates symptoms of disease or infection, or prolongs the survival of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any preparation used in the methods described herein, an effective amount or dose can be estimated initially from *in vitro*, cell culture, and/or animal model assays. For example, a dose can be formulated in animal models to achieve a desired response. Such information can be used to more accurately determine useful doses in humans.

[0084] Any of the compositions and methods of using these compositions can treat a subject having, suspected of having, or at risk of developing a Norovirus infection or related disease. One use of the compositions is to prevent infections by inoculating a subject prior to exposure to Norovirus.

[0085] There is encompassed herein a method of treating an individual for Norovirus infection or preventing Norovirus infection in an individual, comprising the step of providing to the individual a therapeutically effective amount of a binding polypeptide or an antibody that blocks the binding of the virus to the HBGA.

[0086] In certain aspects the binding polypeptide or an antibody binds the exposed loops in the P2 subdomain of the Norovirus particle. In a further aspect the binding polypeptide or an antibody binds an epitope comprising residue H381 of a Norovirus particle. In still a further aspect the binding polypeptide or antibody binds through one or more of complementarity determining loops in the light chain (*e.g.*, CDRL1 or CDRL3); and CDRH3 in the heavy chain. In certain aspects the binding polypeptide or an antibody binds Norovirus particle P domain at the H381 position of the P2 subdomain through the tyrosine residue. In a further aspect the Norovirus P2 domain binding polypeptide specifically binds the P2 domain to the partial or complete exclusion of the binding of HBGA to the P2 domain. In certain aspects the purified Norovirus P2 domain binding polypeptide competes for binding to the P2 domain with one or more HBGA.

[0087] One use of the immunogenic compositions of the disclosure is to prophylactically treat a subject for Norovirus, such as in the early or late stages of infection, by inoculating an individual, particularly once a risk of developing disease from Norovirus infection has been indicated. In certain aspects, a “risk” means symptoms being presented or the individual having been present environment where Norovirus has been detected or is suspected of being present.

[0088] Certain methods utilize a vaccine specifically targeting GenBank® Accession No. AAB50466. Furthermore, the anti-Norovirus compositions can be provided as a passive immunotherapy, intrabodies, and/or as humanized mAb agents for the detection and/or treatment of Norovirus related diseases. The present disclosure provides for Norovirus therapeutics that can induce a specific immune response against Norovirus or provide passive immunity to Norovirus. As used herein, the term “antigen” is a molecule capable of being bound by an antibody or T-cell receptor. An antigen is additionally capable of inducing a humoral immune response and/or cellular immune response leading to the production of B- and/or T- lymphocytes. B-lymphocytes respond to foreign antigenic determinants via antibody production, whereas T-lymphocytes mediate cellular immunity. The structural aspect of an antigen, *e.g.*, three dimensional conformation or modification (*e.g.*,

phosphorylation), which gives rise to a biological response is referred to herein as an “antigenic determinant” or “epitope.” Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and usually at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include those methods described in Epitope Mapping Protocols (1996). T cells that recognize the epitope can be identified by in vitro assays that measure antigen-dependent proliferation, as determined by ^3H -thymidine incorporation by primed T cells in response to an epitope (Burke *et al.*, 1994), by antigen-dependent killing (cytotoxic T lymphocyte assay, Tigges *et al.*, 1996) or by cytokine secretion.

[0089] As used herein the phrase “immune response” or its equivalent “immunological response” refers to a humoral (antibody mediated), cellular (mediated by antigen-specific T cells or their secretion products) or both humoral and cellular response directed against a protein, peptide, or polypeptide of the disclosure in a recipient patient. Treatment or therapy can be an active immune response induced by administration of immunogen or a passive therapy effected by administration of antibody, antibody containing material, or primed T-cells.

[0090] As used herein “passive immunity” refers to any immunity conferred upon a subject by administration of immune effectors including cellular mediators or protein mediators (*e.g.*, an polypeptide that binds to Norovirus protein). An antibody composition may be used in passive immunization for the prevention or treatment of infection by organisms that carry the antigen recognized by the antibody. An antibody composition may include antibodies or polypeptides comprising antibody CDR domains that bind to a variety of antigens that may in turn be associated with various organisms. The antibody component can be a polyclonal antiserum. In certain aspects the antibody or antibodies are affinity purified from an animal or second subject that has been challenged with an antigen(s). Alternatively, an antibody mixture may be used, which is a mixture of monoclonal and/or polyclonal antibodies.

[0091] Passive immunity may be imparted to a patient or subject by administering to the patient immunoglobulins (Ig) or fragments thereof and/or other immune factors obtained

from a donor or other non-patient source having a known immunoreactivity. In other aspects, an antigenic composition can be administered to a subject who then acts as a source or donor for globulin, produced in response to challenge from the composition (“hyperimmune globulin”), that contains antibodies directed against Norovirus or other organism. A subject thus treated would donate plasma from which hyperimmune globulin would then be obtained, *via* conventional plasma-fractionation methodology, and administered to another subject in order to impart resistance against or to treat Norovirus infection. Hyperimmune globulins are particularly useful for immune-compromised individuals, for individuals undergoing invasive procedures or where time does not permit the individual to produce their own antibodies in response to vaccination. See U.S. Patents 6,936,258, 6,770,278, 6,756,361, 5,548,066, 5,512,282, 4,338,298, and 4,748,018, each of which is incorporated herein by reference in its entirety, for exemplary methods and compositions related to passive immunity.

[0092] For purposes of this specification and the accompanying claims the terms “epitope” and “antigenic determinant” are used interchangeably to refer to a site on an antigen to which B and/or T cells respond or recognize. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include those methods described in Epitope Mapping Protocols (1996). T cells recognize continuous epitopes of about nine amino acids for CD8 cells or about 13-15 amino acids for CD4 cells. T cells that recognize the epitope can be identified by *in vitro* assays that measure antigen-dependent proliferation, as determined by ³H-thymidine incorporation by primed T cells in response to an epitope (Burke *et al.*, 1994), by antigen-dependent killing (cytotoxic T lymphocyte assay, Tigges *et al.*, 1996) or by cytokine secretion.

[0093] The presence of a cell-mediated immunological response can be determined by proliferation assays (CD4 (+) T cells) or CTL (cytotoxic T lymphocyte) assays. The relative contributions of humoral and cellular responses to the protective or therapeutic effect of an immunogen can be distinguished by separately isolating IgG and T-cells from an immunized syngeneic animal and measuring protective or therapeutic effect in a second

subject. As used herein and in the claims, the terms “antibody” or “immunoglobulin” are used interchangeably.

[0094] Optionally, an antibody or preferably an immunological portion of an antibody, can be chemically conjugated to, or expressed as, a fusion protein with other proteins. For purposes of this specification and the accompanying claims, all such fused proteins are included in the definition of antibodies or an immunological portion of an antibody.

[0095] In one embodiment a method includes treatment for a disease or condition caused by a Norovirus pathogen. In certain aspects embodiments include methods of treatment of Norovirus infection, such as hospital acquired nosocomial infections. In some embodiments, the treatment is administered in the presence of Norovirus antigens. Furthermore, in some examples, treatment comprises administration of other agents commonly used against bacterial infection, such as one or more antibiotics.

[0096] The therapeutic compositions are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. Suitable regimens for initial administration and boosters are also variable, but are typified by an initial administration followed by subsequent administrations.

[0097] The manner of application may be varied widely. Any of the conventional methods for administration of a polypeptide therapeutic are applicable. These are believed to include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection and the like. The dosage of the composition will depend on the route of administration and will vary according to the size and health of the subject.

[0098] In certain instances, it will be desirable to have multiple administrations of the composition, *e.g.*, 2, 3, 4, 5, 6 or more administrations. The administrations can be at 1, 2, 3, 4, 5, 6, 7, 8, to 5, 6, 7, 8, 9, 10, 11, 12 twelve week intervals, including all ranges there between.

[0099] Certain aspects are directed to methods of preparing an antibody for use in prevention or treatment of Norovirus infection comprising the steps of immunizing a recipient with a vaccine and isolating antibody from the recipient, or producing a recombinant antibody. An antibody prepared by these methods and used to treat or prevent a Norovirus infection is a further aspect. A pharmaceutical composition comprising antibodies that specifically bind Norovirus and a pharmaceutically acceptable carrier is a further aspect that could be used in the manufacture of a medicament for the treatment or prevention of Norovirus disease. A method for treatment or prevention of Norovirus infection comprising a step of administering to a patient an effective amount of the pharmaceutical preparation is a further aspect.

[0100] Although in particular embodiments the antibody is a monoclonal antibody, in alternative embodiments the antibody is a polyclonal antibody. Inocula for polyclonal antibody production are typically prepared by dispersing the antigenic composition (*e.g.*, a peptide or antigen or epitope of Norovirus or a consensus thereof) in a physiologically tolerable diluent such as saline or other adjuvants suitable for human use to form an aqueous composition. An immunostimulatory amount of inoculum is administered to a mammal and the inoculated mammal is then maintained for a time sufficient for the antigenic composition to induce protective antibodies. The antibodies can be isolated to the extent desired by well-known techniques such as affinity chromatography (Harlow and Lane, *Antibodies: A Laboratory Manual* 1988). Antibodies can include antiserum preparations from a variety of commonly used animals *e.g.*, goats, primates, donkeys, swine, horses, guinea pigs, rats or man. The animals are bled and serum recovered.

[0101] An antibody can include whole antibodies, antibody fragments or subfragments. Antibodies can be whole immunoglobulins of any class (*e.g.*, IgG, IgM, IgA, IgD or IgE), chimeric antibodies, human antibodies, humanized antibodies, or hybrid antibodies with dual specificity to two or more antigens. They may also be fragments (*e.g.*, F(ab')₂, Fab', Fab, Fv and the like including hybrid fragments). An antibody also includes natural, synthetic or genetically engineered proteins that act like an antibody by binding to specific antigens with a sufficient affinity.

[0102] A vaccine can be administered to a recipient who then acts as a source of antibodies, produced in response to challenge from the specific vaccine. A subject thus treated would donate plasma from which antibody would be obtained via conventional

plasma fractionation methodology. The isolated antibody would be administered to the same or different subject in order to impart resistance against or treat Norovirus infection. Antibodies are particularly useful for treatment or prevention of Norovirus disease in infants, immune compromised individuals or where treatment is required and there is no time for the individual to produce a response to vaccination.

[0103] An additional aspect is a pharmaceutical composition comprising two or more antibodies or monoclonal antibodies (or fragments thereof; preferably human or humanized) reactive against at least two constituents of the immunogenic composition, which could be used to treat or prevent infection by Norovirus.

[0104] The compositions and related methods, particularly administration of an antibody that binds Norovirus or a peptide or consensus peptide thereof to a patient/subject, may also be used in combination with the administration of traditional therapies. These include, but are not limited to, the administration of one or more other antivirals.

[0105] In one aspect, it is contemplated that a therapy is used in conjunction with antiviral treatment. Alternatively, the therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agents and/or a proteins or polynucleotides are administered separately, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the therapeutic composition would still be able to exert an advantageously combined effect on the subject. In such instances, it is contemplated that one may administer both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for administration significantly, however, where several days (2, 3, 4, 5, 6 or 7) to several weeks (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

[0106] Administration of the antibody compositions to a patient/subject will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the composition. It is expected that the treatment cycles would be repeated as necessary. It is also contemplated that various standard therapies, such as hydration, may be applied in combination with the described therapy.

III. Pharmaceutical Compositions

[0107] In some embodiments, pharmaceutical compositions are administered to a subject. Different aspects may involve administering an effective amount of a composition to a subject. In some embodiments, an antibody that binds Norovirus or a peptide or consensus peptide thereof may be administered to the patient to protect against or treat infection by Norovirus. Alternatively, an expression vector encoding one or more such antibodies or polypeptides or peptides may be given to a patient as a preventative treatment. Additionally, such compositions can be administered in combination with an antibiotic. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[0108] The phrases “pharmaceutically acceptable” or “pharmacologically acceptable” refer to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal or human. As used herein, “pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in immunogenic and therapeutic compositions is contemplated. Supplementary active ingredients, such as other anti-infective agents and vaccines, can also be incorporated into the compositions.

[0109] The active compounds can be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, or even intraperitoneal routes. Typically, such compositions can be prepared as either liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified.

[0110] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil, or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage

and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0111] The proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0112] A pharmaceutical composition can include a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0113] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization or an equivalent procedure. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0114] Administration of the compositions will typically be *via* any common route. This includes, but is not limited to oral, nasal, or buccal administration. Alternatively,

administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intranasal, or intravenous injection. In certain embodiments, a vaccine composition may be inhaled (*e.g.*, U.S. Patent 6,651,655, which is specifically incorporated by reference). Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients.

[0115] An effective amount of therapeutic or prophylactic composition is determined based on the intended goal. The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the protection desired.

[0116] Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition.

[0117] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above.

IV. Antibodies and Antibody-Like Molecules

[0118] In certain aspects, one or more antibodies or antibody-like molecules (*e.g.*, polypeptides comprising antibody CDR domains) may be obtained or produced which have a specificity for a Norovirus. These antibodies may be used in various diagnostic or therapeutic applications described herein.

[0119] As used herein, the term “antibody” is intended to refer broadly to any immunologic binding agent such as IgG, IgM, IgA, IgD and IgE as well as polypeptides comprising antibody CDR domains that retain antigen binding activity. Thus, the term “antibody” is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab', Fab, F(ab')₂, single domain antibodies (DABs),

Fv, scFv (single chain Fv), and polypeptides with antibody CDRs, scaffolding domains that display the CDRs (*e.g.*, anticalins) or a nanobody. For example, the nanobody can be antigen-specific VHH (*e.g.*, a recombinant VHH) from a camelid IgG2 or IgG3, or a CDR-displaying frame from such camelid Ig. The techniques for preparing and using various antibody-based constructs and fragments are well known in the art. Means for preparing and characterizing antibodies are also well known in the art (See, *e.g.*, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988; incorporated herein by reference).

[0120] Mini-antibodies” or “minibodies” are also contemplated for use with embodiments. Minibodies are sFv polypeptide chains which include oligomerization domains at their C-termini, separated from the sFv by a hinge region. Pack *et al.* (1992). The oligomerization domain comprises self-associating α -helices, *e.g.*, leucine zippers, that can be further stabilized by additional disulfide bonds. The oligomerization domain is designed to be compatible with vectorial folding across a membrane, a process thought to facilitate *in vivo* folding of the polypeptide into a functional binding protein. Generally, minibodies are produced using recombinant methods well known in the art. See, *e.g.*, Pack *et al.* (1992); Cumber *et al.* (1992).

[0121] Antibody-like binding peptidomimetics are also contemplated in embodiments. Liu *et al.* (2003) describe “antibody like binding peptidomimetics” (ABiPs), which are peptides that act as pared-down antibodies and have certain advantages of longer serum half-life as well as less cumbersome synthesis methods.

[0122] Alternative scaffolds for antigen binding peptides, such as CDRs are also available and can be used to generate Norovirus-binding molecules in accordance with the embodiments. Generally, a person skilled in the art knows how to determine the type of protein scaffold on which to graft at least one of the CDRs arising from the original antibody. More particularly, it is known that to be selected such scaffolds must meet the greatest number of criteria as follows (Skerra, 2000): good phylogenetic conservation; known three-dimensional structure (as, for example, by crystallography, NMR spectroscopy or any other technique known to a person skilled in the art); small size; few or no post-transcriptional modifications; and/or easy to produce, express and purify.

[0123] The origin of such protein scaffolds can be, but is not limited to, the structures selected among: fibronectin and preferentially fibronectin type III domain 10, lipocalin,

anticalin (Skerra, 2001), protein Z arising from domain B of protein A of *Staphylococcus aureus*, thioredoxin A or proteins with a repeated motif such as the “ankyrin repeat” (Kohl *et al.*, 2003), the “armadillo repeat”, the “leucine-rich repeat” and the “tetratricopeptide repeat”. For example, anticalins or lipocalin derivatives are a type of binding proteins that have affinities and specificities for various target molecules and can be used as Norovirus-binding molecules. Such proteins are described in US Patent Publication Nos. 20100285564, 20060058510, 20060088908, 20050106660, and PCT Publication No. WO2006/056464, incorporated herein by reference.

[0124] Scaffolds derived from toxins such as, for example, toxins from scorpions, insects, plants, mollusks, etc., and the protein inhibitors of neuronal NO synthase (PIN) may also be used in certain aspects.

[0125] Monoclonal antibodies (MAbs) are recognized to have certain advantages, *e.g.*, reproducibility and large-scale production. Embodiments include monoclonal antibodies of the human, murine, monkey, rat, hamster, rabbit and chicken origin.

[0126] “Humanized” antibodies are also contemplated, as are chimeric antibodies from mouse, rat, or other species, bearing human constant and/or variable region domains, bispecific antibodies, recombinant and engineered antibodies and fragments thereof. As used herein, the term “humanized” immunoglobulin refers to an immunoglobulin comprising a human framework region and one or more CDR's from a non-human (usually a mouse or rat) immunoglobulin. The non-human immunoglobulin providing the CDR's is called the “donor” and the human immunoglobulin providing the framework is called the “acceptor”. A “humanized antibody” is an antibody comprising a humanized light chain and a humanized heavy chain immunoglobulin. In order to describe antibodies of some embodiments, the strength with which an antibody molecule binds an epitope, known as affinity, can be measured. The affinity of an antibody may be determined by measuring an association constant (K_a) or dissociation constant (K_d). Antibodies deemed useful in certain embodiments may have an association constant of about, at least about, or at most about 10^6 , 10^7 , 10^8 , 10^9 or 10^{10} M or any range derivable therein. Similarly, in some embodiments antibodies may have a dissociation constant of about, at least about or at most about 10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} or 10^{-10} M or any range derivable therein. These values are reported for antibodies discussed herein and the same assay may be used to evaluate the binding properties of such antibodies.

[0127] Methods for generating antibodies (*e.g.*, monoclonal antibodies and/or monoclonal antibodies) are known in the art. Briefly, a polyclonal antibody is prepared by immunizing an animal with a Norovirus polypeptide or a portion thereof in accordance with embodiments and collecting antisera from that immunized animal.

[0128] A wide range of animal species can be used for the production of antisera. Typically the animal used for production of antisera is a rabbit, a mouse, a rat, a hamster, a guinea pig or a goat. The choice of animal may be decided upon the ease of manipulation, costs or the desired amount of sera, as would be known to one of skill in the art. It will be appreciated that antibodies can also be produced transgenically through the generation of a mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, antibodies can be produced in, and recovered from, the milk of goats, cows, or other mammals. See, *e.g.*, U.S. Pat. Nos. 5,827,690, 5,756,687, 5,750,172, and 5,741,957.

[0129] As is also well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Suitable adjuvants include any acceptable immunostimulatory compound, such as cytokines, chemokines, cofactors, toxins, plasmodia, synthetic compositions or vectors encoding such adjuvants.

[0130] Adjuvants that may be used in accordance with embodiments include, but are not limited to, IL-1, IL-2, IL-4, IL-7, IL-12, γ -interferon, GMCSF, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion is also contemplated. MHC antigens may even be used. Exemplary adjuvants may include complete Freund's adjuvant (a non-specific stimulator of the immune response containing killed *Mycobacterium tuberculosis*), incomplete Freund's adjuvants and/or aluminum hydroxide adjuvant.

[0131] In addition to adjuvants, it may be desirable to coadminister biologic response modifiers (BRM), which have been shown to upregulate T cell immunity or downregulate suppressor cell activity. Such BRMs include, but are not limited to, Cimetidine (CIM; 1200

mg/d) (Smith/Kline, PA); low-dose Cyclophosphamide (CYP; 300 mg/m²) (Johnson/ Mead, NJ), cytokines such as γ -interferon, IL-2, or IL-12 or genes encoding proteins involved in immune helper functions, such as B-7.

[0132] The amount of immunogen composition used in the production of antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen including but not limited to subcutaneous, intramuscular, intradermal, intraepidermal, intravenous and intraperitoneal. The production of antibodies may be monitored by sampling blood of the immunized animal at various points following immunization.

[0133] A second, booster dose (*e.g.*, provided in an injection), may also be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate MAbs.

[0134] MAbs may be readily prepared through use of well-known techniques, such as those exemplified in U.S. Patent 4,196,265, incorporated herein by reference. Typically, this technique involves immunizing a suitable animal with a selected immunogen composition, *e.g.*, a purified or partially purified protein, polypeptide, peptide or domain, be it a wild-type or mutant composition. The immunizing composition is administered in a manner effective to stimulate antibody producing cells. The methods for generating monoclonal antibodies (MAbs) generally begin along the same lines as those for preparing polyclonal antibodies. In some embodiments, Rodents such as mice and rats are used in generating monoclonal antibodies. In some embodiments, rabbit, sheep or frog cells are used in generating monoclonal antibodies. The use of rats is well known and may provide certain advantages (Goding, 1986, pp. 60 61). Mice (*e.g.*, BALB/c mice) are routinely used and generally give a high percentage of stable fusions. The animals are injected with antigen, generally as described above. The antigen may be mixed with adjuvant, such as Freund's complete or incomplete adjuvant. Booster administrations with the same antigen or DNA encoding the antigen may occur at approximately two-week intervals.

[0135] Following immunization, somatic cells with the potential for producing antibodies, specifically B lymphocytes (B cells), are selected for use in the MAb generating protocol. These cells may be obtained from biopsied spleens, tonsils or lymph nodes, or from

a peripheral blood sample. Generally, spleen cells are a rich source of antibody-producing cells that are in the dividing plasmablast stage. Typically, peripheral blood cells may be readily obtained, as peripheral blood is easily accessible.

[0136] In some embodiments, a panel of animals will have been immunized and the spleen of an animal with the highest antibody titer will be removed and the spleen lymphocytes obtained by homogenizing the spleen with a syringe. Typically, a spleen from an immunized mouse contains approximately 5×10^7 to 2×10^8 lymphocytes.

[0137] The antibody producing B lymphocytes from the immunized animal are then fused with cells of an immortal myeloma cell, generally one of the same species as the animal that was immunized. Myeloma cell lines suited for use in hybridoma producing fusion procedures preferably are non antibody producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas).

[0138] The culturing provides a population of hybridomas from which specific hybridomas are selected. Typically, selection of hybridomas is performed by culturing the cells by single-clone dilution in microtiter plates, followed by testing the individual clonal supernatants (after about two to three weeks) for the desired reactivity. The assay should be sensitive, simple and rapid, such as radioimmunoassays, enzyme immunoassays, cytotoxicity assays, plaque assays, dot immunobinding assays, and the like.

[0139] The selected hybridomas would then be serially diluted and cloned into individual antibody producing cell lines, which clones can then be propagated indefinitely to provide MAbs. The cell lines may be exploited for MAb production in two basic ways. First, a sample of the hybridoma can be injected (often into the peritoneal cavity) into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion (*e.g.*, a syngeneic mouse). Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. The injected animal develops tumors secreting the specific monoclonal antibody produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide MAbs in high concentration. Second, the individual cell lines could be cultured *in vitro*, where the MAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations.

[0140] MAbs produced by either means may be further purified, if desired, using filtration, centrifugation and various chromatographic methods such as HPLC or affinity chromatography. Fragments of the monoclonal antibodies can be obtained from the monoclonal antibodies so produced by methods which include digestion with enzymes, such as pepsin or papain, and/or by cleavage of disulfide bonds by chemical reduction. Alternatively, monoclonal antibody fragments can be synthesized using an automated peptide synthesizer.

[0141] Alternatively, monoclonal antibody fragments can be synthesized using an automated peptide synthesizer, or by expression of full-length gene or of gene fragments in *E. coli*.

[0142] some embodiments, fragments of a whole antibody can perform the function of binding antigens. Examples of binding fragments are (i) the Fab fragment constituted with the VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment constituted with the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, 1989; McCafferty *et al.*, 1990; Holt *et al.*, 2003), which is constituted with a VH or a VL domain; (v) isolated CDR regions; (vi) F(ab')₂ fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.*, 1988; Huston *et al.*, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; Holliger *et al.*, 1993). Fv, scFv or diabody molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter *et al.*, 1996). Minibodies comprising a scFv joined to a CH3 domain may also be made (Hu *et al.* 1996). The citations in this paragraph are all incorporated by reference.

[0143] Antibodies also include bispecific antibodies. Bispecific or bifunctional antibodies form a second generation of monoclonal antibodies in which two different variable regions are combined in the same molecule (Holliger, P. & Winter, G. 1999 Cancer and metastasis rev. 18:411-419, 1999). Their use has been demonstrated both in the diagnostic field and in the therapy field from their capacity to recruit new effector functions or to target several molecules on the surface of tumor cells. Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of

ways (Holliger et al, PNAS USA 90:6444-6448, 1993), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. These antibodies can be obtained by chemical methods (Glennie *et al.*, 1987 J. Immunol. 139, 2367-2375; Repp *et al.*, J. Hemat. 377-382, 1995) or somatic methods (Staerz U. D. and Bevan M. J. PNAS 83, 1986; *et al.*, Method Enzymol. 121:210-228, 1986) but likewise by genetic engineering techniques which allow the heterodimerization to be forced and thus facilitate the process of purification of the antibody sought (Merchand *et al.* Nature Biotech, 16:677-681, 1998). Examples of bispecific antibodies include those of the BiTE™ technology in which the binding domains of two antibodies with different specificity can be used and directly linked via short flexible peptides. This combines two antibodies on a short single polypeptide chain. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction. The citations in this paragraph are all incorporated by reference.

[0144] Bispecific antibodies can be constructed as entire IgG, as bispecific Fab'2, as Fab'PEG, as diabodies or else as bispecific scFv. Further, two bispecific antibodies can be linked using routine methods known in the art to form tetravalent antibodies.

[0145] Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E. coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against Norovirus, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by alternative engineering methods as described in Ridgeway et al, (Protein Eng., 9:616-621, 1996), which is hereby incorporated by reference.

V. Administration and Formulation

[0146] As discussed above, the compositions can be administered to a subject having, suspected of having, or at risk of developing a Norovirus related disease. Therapeutic compositions are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective. The quantity to be administered depends on the subject to be treated. Precise amounts of active ingredient to be administered depend on the judgment of the practitioner. Suitable regimes for initial administration and boosters are

also variable, but are typified by an initial administration followed by subsequent administrations.

[0147] The manner of application may be varied widely. Any of the conventional methods for administration of a polypeptide as a therapeutic are applicable. These are believed to include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection and the like. The dosage of the composition will depend on the route of administration and will vary according to the size and health of the subject. Administration of the compositions according to the present disclosure will typically be *via* any common route. This includes, but is not limited to oral, nasal, or buccal administration. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal, intranasal, or intravenous injection. In certain aspects a Norovirus-specific antibody that specifically binds an oligomer comprising a peptide having an amino acid sequence of SEQ ID NO:1 can be administered into the cerebrospinal fluid of the brain or spine. In certain embodiments, an immunogenic composition of the disclosure may be inhaled (*e.g.*, U.S. Patent 6,651,655, which is specifically incorporated by reference). Such compositions would normally be administered as pharmaceutically acceptable compositions that include physiologically acceptable carriers, buffers or other excipients.

[0148] In certain instances, it will be desirable to have multiple administrations of the composition, *e.g.*, 2, 3, 4, 5, 6 or more administrations. The administrations can be at 1, 2, 3, 4, 5, 6, 7, 8 to 5, 6, 7, 8, 9, 10, 11, 12 day or week intervals, including all ranges there between.

[0149] Administration of the antibody or immunogenic compositions of the present disclosure to a patient/subject will follow general protocols for the administration of such compositions, taking into account the toxicity, if any, of the composition. It is expected that the treatment cycles would be repeated as necessary. It is also contemplated that various standard therapies, such as hydration, may be applied in combination with the described therapy.

[0150] In some embodiments, pharmaceutical compositions are administered to a subject to treat Norovirus-related disease or condition. Alternatively, an expression vector encoding one or more such antibodies or polypeptides or peptides may be given to a patient

as a treatment. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[0151] The phrases “pharmaceutically acceptable” or “pharmacologically acceptable” refer to molecular entities and compositions that do not produce an adverse, allergic, or other untoward reaction when administered to an animal or human. As used herein, “pharmaceutically acceptable carrier” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in immunogenic and therapeutic compositions is contemplated. Supplementary active ingredients, such as other anti-infective agents and vaccines, can also be incorporated into the compositions.

[0152] The active compounds of the present disclosure can be formulated for parenteral administration, *e.g.*, formulated for injection *via* the intravenous, intramuscular, sub-cutaneous, or even intraperitoneal routes. Typically, such compositions can be prepared as either liquid solutions or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared; and, the preparations can also be emulsified. The form should be sterile and should be fluid to the extent that it may be easily injected. It also should be stable under the conditions of manufacture and storage and can be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0153] The proteinaceous compositions may be formulated into a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0154] A pharmaceutical composition can include a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The

proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0155] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by sterilization (e.g., filter sterilization) or an equivalent procedure. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques, which yield a powder of the active ingredient, plus any additional desired ingredient from a previously sterilized solution thereof.

[0156] A pharmaceutical composition comprising antibodies that specifically bind an oligomer comprising a peptide having an amino acid sequence of the disclosure and a pharmaceutically acceptable carrier is a further aspect of the disclosure that can be used in the manufacture of a medicament for the treatment or prevention of a Norovirus-related disease or condition.

[0157] An additional aspect of the disclosure is a pharmaceutical composition comprising one of more antibodies or monoclonal antibodies (or fragments thereof; preferably human or humanized) generated by using peptides having an amino acid sequence of the disclosure that specifically bind Norovirus. It is contemplated that in compositions of the disclosure, there is about 0.001, 0.01, 0.1, 1, 5, μ g or mg to about 0.01, 0.1, 1, 5, 10 μ g or mg of total polypeptide, peptide, and/or protein per ml. The concentration of protein in a composition can be about, at least about or at most about 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg /ml, including all values and ranges there between. In certain aspects the

dose range is 0.01 to 500 mg/kg, 10 to 300 mg/kg, or 0.01 to 10 mg/kg. About, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% may be a peptide having the amino acid sequence of the disclosure or antibody that specifically binds the same.

[0158] An effective amount of therapeutic or prophylactic composition is determined based on the intended goal, *i.e.*, treatment or amelioration of a Norovirus-related disease. The term “unit dose” or “dosage” refers to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the composition calculated to produce the desired responses discussed above in association with its administration, *i.e.*, the appropriate route and regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the protection desired.

[0159] Precise amounts of the composition also depend on the judgment of the practitioner and are peculiar to each individual. Factors affecting dose include physical and clinical state of the subject, route of administration, intended goal of treatment (alleviation of symptoms versus cure), and potency, stability, and toxicity of the particular composition.

[0160] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically or prophylactically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above.

VI. Polypeptides and Polypeptide Production

[0161] Embodiments involve polypeptides, peptides, and proteins and immunogenic fragments thereof for use in various aspects described herein. For example, specific antibodies are assayed for or used in neutralizing or inhibiting Norovirus infection. In specific embodiments, all or part of proteins described herein can also be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. See, for example, Stewart and Young, (1984); Tam *et al.*, (1983); Merrifield, (1986); and Barany and Merrifield (1979), each incorporated herein by reference.

[0162] Certain embodiments are related to peptides, antibodies, and antibody fragments for use in various embodiments of the present disclosure. For example, antibodies generated to a peptide comprising an amino acid sequence in a conformational epitope formed by surface-exposed loop clusters in the P domain in the capsid protein are utilized for specific binding to Norovirus.

[0163] Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, with or without the loss of other functions or properties. Substitutions may be conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine. Alternatively, substitutions may be non-conservative such that a function or activity of the polypeptide is affected. Non-conservative changes typically involve substituting a residue with one that is chemically dissimilar, such as a polar or charged amino acid for a nonpolar or uncharged amino acid, and vice versa.

[0164] Proteins may be recombinant, or synthesized *in vitro*. Alternatively, a non-recombinant or recombinant protein may be isolated from bacteria. It is also contemplated that a bacteria containing such a variant may be implemented in compositions and methods. Consequently, a protein need not be isolated.

[0165] The term “functionally equivalent codon” is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids.

[0166] It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids, or 5' or 3' sequences, respectively, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the

maintenance of biological protein activity where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5' or 3' portions of the coding region.

[0167] It is contemplated that in compositions there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. Thus, the concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein). Of this, about, at least about, or at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100% may be an antibody that binds Norovirus, and may be used in combination with other Norovirus proteins or protein-binding antibodies described herein.

VII. Nucleic Acids

[0168] certain embodiments, there are recombinant polynucleotides encoding the proteins, polypeptides, or peptides described herein. Polynucleotide sequences contemplated include those encoding antibodies to Norovirus, such as the P2 subdomain binding portions thereof.

[0169] As used in this application, the term “polynucleotide” refers to a nucleic acid molecule that either is recombinant or has been isolated free of total genomic nucleic acid. Included within the term “polynucleotide” are oligonucleotides (nucleic acids 100 residues or less in length), recombinant vectors, including, for example, plasmids, cosmids, phage, viruses, and the like. Polynucleotides include, in certain aspects, regulatory sequences, isolated substantially away from their naturally occurring genes or protein encoding sequences. Polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be RNA, DNA (genomic, cDNA or synthetic), analogs thereof, or a combination thereof. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide.

[0170] In this respect, the term “gene,” “polynucleotide,” or “nucleic acid” is used to refer to a nucleic acid that encodes a protein, polypeptide, or peptide (including any sequences required for proper transcription, post-translational modification, or localization). As will be understood by those in the art, this term encompasses genomic sequences, expression cassettes, cDNA sequences, and smaller engineered nucleic acid segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. A nucleic acid encoding all or part of a polypeptide may contain a contiguous nucleic acid sequence encoding all or a portion of such a polypeptide. It also is contemplated that a particular polypeptide may be encoded by nucleic acids containing variations having slightly different nucleic acid sequences but, nonetheless, encode the same or substantially similar protein (see above).

[0171] In particular embodiments, there are isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a polypeptide (*e.g.*, an antibody or fragment thereof) that binds to Norovirus. The term “recombinant” may be used in conjunction with a polypeptide or the name of a specific polypeptide, and this generally refers to a polypeptide produced from a nucleic acid molecule that has been manipulated *in vitro* or that is a replication product of such a molecule.

[0172] The nucleic acid segments, regardless of the length of the coding sequence itself, may be combined with other nucleic acid sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant nucleic acid protocol. In some cases, a nucleic acid sequence may encode a polypeptide sequence with additional heterologous coding sequences, for example to allow for purification of the polypeptide, transport, secretion, post-translational modification, or for therapeutic benefits such as targeting or efficacy. As discussed above, a tag or other heterologous polypeptide may be added to the modified polypeptide-encoding sequence, wherein “heterologous” refers to a polypeptide that is not the same as the modified polypeptide.

A. Vectors

[0173] Polypeptides may be encoded by a nucleic acid molecule. The nucleic acid molecule can be in the form of a nucleic acid vector. The term “vector” is used to refer to a carrier nucleic acid molecule into which a heterologous nucleic acid sequence can be inserted for introduction into a cell where it can be replicated and expressed. A nucleic acid sequence can be “heterologous,” which means that it is in a context foreign to the cell in which the vector is being introduced or to the nucleic acid in which is incorporated, which includes a sequence homologous to a sequence in the cell or nucleic acid but in a position within the host cell or nucleic acid where it is ordinarily not found. Vectors include DNAs, RNAs, plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (*e.g.*, YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (for example Sambrook *et al.*, 2001; Ausubel *et al.*, 1996, both incorporated herein by reference). Vectors may be used in a host cell to produce an antibody that binds Norovirus.

[0174] The term “expression vector” refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. Expression vectors can contain a variety of “control sequences,” which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described herein.

[0175] A “promoter” is a control sequence. The promoter is typically a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at which regulatory proteins and molecules may bind such as RNA polymerase and other transcription factors. The phrases “operatively positioned,” “operatively linked,” “under control,” and “under transcriptional control” mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and expression of that sequence. A promoter may or may not be used in conjunction with an “enhancer,” which refers to a *cis*-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

[0176] The particular promoter that is employed to control the expression of a peptide or protein encoding polynucleotide is not believed to be critical, so long as it is capable of expressing the polynucleotide in a targeted cell, preferably a bacterial cell. Where a human cell is targeted, it is preferable to position the polynucleotide coding region adjacent to and under the control of a promoter that is capable of being expressed in a human cell. Generally speaking, such a promoter might include either a bacterial, human or viral promoter.

[0177] A specific initiation signal also may be required for efficient translation of coding sequences. These signals include the ATG initiation codon or adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals.

[0178] Vectors can include a multiple cloning site (MCS), which is a nucleic acid region that contains multiple restriction enzyme sites, any of which can be used in conjunction with standard recombinant technology to digest the vector. (See Carbonelli *et al.*, 1999, Levenson *et al.*, 1998, and Cocea, 1997, incorporated herein by reference.)

[0179] In order to propagate a vector in a host cell, it may contain one or more origins of replication sites (often termed “ori”), which is a specific nucleic acid sequence at which replication is initiated. Alternatively an autonomously replicating sequence (ARS) can be employed if the host cell is yeast.

B. Host Cells

[0180] As used herein, the terms “cell,” “cell line,” and “cell culture” may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, “host cell” refers to a prokaryotic or eukaryotic cell, and it includes any transformable organism that is capable of replicating a vector or expressing a heterologous gene encoded by a vector. A host cell can, and has been, used as a recipient for vectors or viruses. A host cell may be “transfected” or “transformed,” which refers to a process by which exogenous nucleic acid, such as a recombinant protein-encoding sequence, is

transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny.

[0181] Some vectors may employ control sequences that allow it to be replicated and/or expressed in both prokaryotic and eukaryotic cells. One of skill in the art would further understand the conditions under which to incubate all of the above described host cells to maintain them and to permit replication of a vector. Also understood and known are techniques and conditions that would allow large-scale production of vectors, as well as production of the nucleic acids encoded by vectors and their cognate polypeptides, proteins, or peptides.

C. Expression Systems

[0182] Numerous expression systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with an embodiment to produce nucleic acid sequences, or their cognate polypeptides, proteins and peptides. Many such systems are commercially and widely available.

[0183] The insect cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Patents 5,871,986, 4,879,236, both herein incorporated by reference, and which can be bought, for example, under the name MAXBAC[®] 2.0 from INVITROGEN[®] and BACPACK[™] BACULOVIRUS EXPRESSION SYSTEM FROM CLONTECH[®].

[0184] In addition to the disclosed expression systems, other examples of expression systems include STRATAGENE[®]'s COMPLETE CONTROL Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible receptor, or its pET Expression System, an *E. coli* expression system. Another example of an inducible expression system is available from INVITROGEN[®], which carries the T-REX[™] (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. INVITROGEN[®] also provides a yeast expression system called the *Pichia methanolica* Expression System, which is designed for high-level production of recombinant proteins in the methylotrophic yeast *Pichia methanolica*. One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

VIII. Kits of the Disclosure

[0185] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, a Norovirus antibody or immunogenic composition may be comprised in a kit in suitable container means. The kit may be utilized for the treatment of Norovirus infection and/or for the prevention of Norovirus infection and/or for detection of Norovirus, including from a mammalian sample(s) and/or one or more environments. In specific embodiments, the kit comprises certain monoclonal antibodies encompassed by the disclosure.

[0186] The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present disclosure also will typically include a means for containing the Norovirus antibody or immunogenic composition and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[0187] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. The Norovirus antibody or immunogenic compositions may also be formulated into a syringeable composition. In which case, the container means may itself be a syringe, pipette, and/or other such like apparatus, from which the formulation may be applied to an infected area of the body, injected into an animal, and/or even applied to and/or mixed with the other components of the kit.

[0188] However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

[0189] The kits of the present disclosure will also typically include a means for containing the vials in close confinement for commercial sale, such as, *e.g.*, injection and/or blow-molded plastic containers into which the desired vials are retained.

[0190] In certain embodiments, the kit comprises one or more apparatuses and/or reagents for obtaining a sample from an individual and/or processing thereof.

EXAMPLES

[0191] The following examples are given for the purpose of illustrating various embodiments and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

EXAMPLE 1

CRYSTAL STRUCTURE OF A HBGA-BLOCKING MONOCLONAL ANTIBODY BOUND TO NOROVIRUS REVEALS ITS MECHANISM OF NEUTRALIZATION

Generation of HBGA blocking human monoclonal antibodies

[0192] Starting from the serum sample of an individual infected with Norwalk virus (NV), we isolated a panel of monoclonal antibodies (mAbs) using hybridoma technology and screened for the ability of these mAbs to bind recombinant Norwalk virus particles and block HBGAs (FIG. 3). The purified mAbs were tested for binding to Norwalk virus and other Noroviruses using ELISA. All these antibodies were genotype specific and showed binding only to GI.1 (FIG. 4). Binding analysis using biolayer interferometry showed that mAbs bound to NV P domain with high affinity. One of these antibodies, mAbs 5I2, an IgA subtype, was selected for crystallographic studies.

Crystallographic structure of 5I2 IgA in complex with NV P domain

[0193] For crystallographic analysis, we mixed recombinant NV P domain and the Fab of the 5I2 in a 1:2 molar ratio and crystallized the complex. The crystals diffracted to 2.4

Å resolution producing excellent quality diffraction data (FIG. 5). Structure of the complex was determined by molecular replacement and refined further with excellent refinement statistics. Structural analyses show the 5I2 recognizes a conformational epitope in the NV P2 subdomain. 5I2 interacts with the residues in the exposed loops of the P2 subdomain through two of the complementarity determining loops (CDRL1 and CDRL3) in light chain and CDR loop (CDRH3) in the heavy chain (FIG. 6). The contact area with several hydrogen bonds and hydrophobic interactions is quite extensive with a buried surface area of $\sim 700 \text{ Å}^2$ (FIG. 7). A prominent stabilizing interaction is the stacking interaction between the tyrosine residue CDRL1 and the histidine residue (H381) of the P2 subdomain.

Structural basis for how 5I2 IgA blocks HBGA binding.

[0194] Previous crystallographic studies have provided structural details of how HBGA interacts with the NV P2 subdomain (FIG. 8). From these studies it has been possible to clearly map the HBGA binding site in the P domain (FIG. 9, top right). Superposition of the HBGA-P domain complex with that 5I2-P domain complex clearly shows that 5I2 IgA masks the HBGA binding site (FIG. 9, bottom right) indicating the mechanism of ‘neutralization’ by this antibody is by steric hindrance.

Structural basis for why 5I2 IgA is genotype specific

[0195] The crystal structure of the 5I2 IgA with P domain clearly shows which P2 subdomain residues interact with the IgA. Sequence comparisons of the GI.1 NV with other GI genotypes show significant variation in the residues that interact with 5I2 IgA (FIG. 10, bottom). Particularly interesting is the variation in the position 381. In the case of GI.1 NV, this residue, H381, is involved in stabilizing stacking interactions with a tyrosine residue of the IgA. None of the other GI genotypes have this residue. Sequence differences translate local structural changes in the 5I2 binding region as shown clearly by superimposing the known P domain structures of other genotypes (FIG. 10, top right). Thus, 5I2 specifically recognizes GI.1 NV and not the other genotypes, not only because of sequence variations but also because of local structural alterations caused by these sequence changes.

Conclusions

[0196] The inventors have successfully isolated a panel of human monoclonal antibodies from an individual infected with NV, and characterized their ability to block

HBGA binding. Several of these antibodies clearly interact with NV with high affinity and block HBGA binding effectively.

[0197] Selecting one of these mAbs, 5I2 IgA, by determining the crystal structure of the 5I2 in complex with the NV P domain, we have provided here the first atomic details of how ‘neutralizing’ antibody recognizes a norovirus. These studies clearly show that the mechanism by which 5I2 antibody ‘neutralizes the NV is by sterically blocking the HBGA binding. The structural basis for the genotype specificity is also clearly evident from our crystallographic studies.

[0198] Knowledge of the detailed atomic level structural description of the antibody-Norovirus interacting surface is useful for the design and development of genotype-specific and broadly reactive immuno-therapeutic agents in the form of antibody scaffolds that can effectively neutralize norovirus by blocking the receptor interaction. Novel synthetic vaccines can also be designed to elicit antibodies that block this critical neutralizing epitope.

EXAMPLE 2

CRYSTAL STRUCTURE OF NOROVIRUS P DOMAIN IN COMPLEX WITH HBGA-BLOCKING HUMAN MONOCLONAL ANTIBODY REVEALS MECHANISM OF NEUTRALIZATION

[0199] Human noroviruses (HuNoVs) cause acute gastroenteritis worldwide. They evolve with the periodic emergence of new epidemic strains based on antigenic variations and differential glycan binding specificities that lead to sequence and structural changes in the P domain of the NoV capsid protein. Histo blood group antigens (HBGAs) serve as susceptibility and cell attachment factors to HuNoVs. Recent studies show that the presence of antibodies that block virus-HBGA interactions is associated with protection against illness and thus act as putative neutralization antibodies (NAb)s. Although the structural basis of HBGA binding is well characterized, there are no structural studies to explain the basis of antigenic variation and how these NAb)s block HBGA binding in HuNoVs. Do NAb)s directly compete for the HBGA binding site or do they induce conformational changes in the P domain that disrupt the HBGA binding site? How do the emerging strains escape from NAb)s? In this disclosure B cells were isolated from a person challenged with the prototype Norwalk virus (NV) and screened for antibodies that block HBGA interactions with NV VLPs. Fab fragments from one of the HBGA-blocking monoclonal antibodies (5I2) were generated for crystallographic studies of NV P domain-Fab 5I2 complex. The structural studies reveal that

Fab binds to the loops located on the top of the P domain without inducing any significant conformational changes in the P domain. The Fab binding site is in close proximity to the HBGA binding site thereby blocking access to the HBGAs. Further, the identified Fab binding loops are known to undergo structural variations among other NoVs genotypes, which could explain the molecular mechanism underlying antigenic variation. The complementarity determining regions (CDRs) of the Fab that interact with the P -domain are also clearly delineated which could be used to for the structure-based design and optimization of scaffolds that can block HBGA binding across the genotypes. Such scaffolds in tum can be used in the development of vaccines and/or antivirals against noroviruses. Overall the studies have major implications for understanding the structural basis of neutralization and role of antigenic variation in the evolution of HuNoVs.

EXAMPLE 3

STRUCTURAL BASIS FOR NOROVIRUS NEUTRALIZATION BY A HBGA BLOCKING HUMAN IGA ANTIBODY

Introduction

[0200] Human noroviruses (HuNoVs) are the leading cause of viral gastroenteritis. They are associated with almost a fifth of all cases of acute gastroenteritis worldwide (Ahmen, *et al.*, 2014). It is estimated that approximately 200,000 children under the age of 5 years die annually from HuNoV infections (Patel, *et al.*, 2008). Currently there are no licensed vaccines or antivirals to treat the disease, although vaccine candidates are in the pipeline (Treanor, *et al.*, 2014; Atmar, *et al.*, 2011). Development of efficient vaccines is limited by lack of understanding of the immune correlates of protection and rapid evolution of NoVs based on antigenic variations and differential glycan binding.

[0201] Noroviruses (NoVs) are non-enveloped positive strand RNA viruses belonging to the family Caliciviridae. They are phylogenetically classified into at least six genogroups (GI-GVI) with each genogroup divided into several genotypes. Genogroups GI, GII and GIV contain human pathogens (Green, *et al.*, 2000; Ramani, *et al.*, 2014). The prototype Norwalk virus (NV) is classified as genogroup I genotype 1 (GI.1). NoVs belonging to genotype GII.4 are the most prevalent and are associated with ~70% of all HuNoV infections (Lindesmith, *et al.*, 2011). HuNoVs recognize and bind to histo-blood group antigens (HBGAs) as receptor/co-receptors for cell entry. These glycoconjugates are also associated with susceptibility to HuNoV infection (Hutson, *et al.*, 2002; Lindesmith, *et*

al., 2003; Nordgren, *et al.*, 2016). HuNoVs bind HBGAs through their major capsid protein VP1, which as 90 dimers forms the T=3 icosahedral capsid (Prasad, *et al.*, 1999). VP1 when expressed by itself in insect cells self assembles to form virus like particles (VLPs) that are structurally and antigenically similar to the native virus. VP1 is composed of two principal domains, the shell (S) domain, which is involved in the formation of icosahedral shell, and the protruding (P) domain that projects out from the shell (Prasad, *et al.*, 1999). The P domain is further divided into P1 and P2 subdomains, with the latter being an insertion in the P1 subdomain. Evolutionarily, the P2 subdomain is the least conserved and is implicated in strain diversity, differential HBGA binding and antigenicity (Singh, *et al.*, 2015; Shanker, *et al.*, 2014).

[0202] HuNoVs are suggested to evolve through a coordinated interplay between differential HBGA binding specificities and antigenic variations that allow emerging strains to escape host immunity. Differential HBGA binding has been previously well characterized in both GI and GII HuNoVs (Huang, *et al.*, 2005; Shanker, *et al.*, 2011). These studies show that both genogroups have evolved distinct HBGA binding sites localized on the outermost hypervariable P2 subdomain of VP1 (Shanker, *et al.*, 2011; Lindesmith, *et al.*, 2008; Choi, *et al.*, 2008; Cao, *et al.*, 2007; Tan, *et al.*, 2008; Hansman, *et al.*, 2011; Kubota, *et al.*, 2012; Singh, *et al.*, 2015). Human challenge studies show circulating serum antibodies that block HBGA binding correlate with protection from clinical disease and infection, and these antibodies have been proposed to serve as surrogate neutralizing antibodies (NAbs) (Atmar, *et al.*, 2011; Reeck, *et al.*, 2010; Ramani, *et al.*, 2015; Lindesmith, *et al.*, 2015). The presence of HBGA-blocking serum antibodies have also been associated with protection from infection in an intravenous challenge model in chimpanzees (Chen, *et al.*, 2013) and in resolution of diarrhea in an immunocompromised patient with chronic gastroenteritis (Knoll, *et al.*, 2016). Surrogate neutralization or HBGA blockade assays have allowed identification of critical residues on VP1 that may be involved in NAb recognition (Lindesmith, *et al.*, 2012). However, the lack of an efficient cell culture or small animal model systems (Lay, *et al.*, 2010; Herbst-Kralovetz, *et al.*, 2013)) for HuNoVs has restricted the ability to define neutralization epitopes. This lack of information is in contrast with the field of study of other viruses such as influenza virus, human immunodeficiency virus (HIV) and dengue virus wherein immune correlates of protection and neutralization are better understood (Kwong, *et al.*, 2011; Wei, *et al.*, 2010; Pierson, *et al.*, 2008).

[0203] In the absence of any structural studies of HuNoV in complex with HBGA blockade antibodies, many critical questions remain unanswered including: how do NAb recognize and bind to VP1 and what are the structural determinants of such binding, what is the mechanism of HBGA blockade, does binding induce conformational changes, and how does antigenic variation allow escape from host immunity. Understanding the molecular basis of HuNoV-antibody interactions is critical for the design and development of genotype-specific and broadly reactive immuno-therapeutic agents in the form of antibody scaffolds and can also facilitate development of vaccine candidates that elicit blockade antibodies. In this study, we determined the first crystal structure of the Fab fragment of a potentially neutralizing human monoclonal antibody, IgA 5I2 in complex with the P domain of VP1 from NV. Our studies reveal that Fab 5I2 binds to a conformational epitope on the P2 subdomain and elucidates the molecular determinants of NV P domain-Fab 5I2 interactions. The work further provides structural insights into the mechanism of HBGA blockade and how sequence and structural variations among the different GI genotypes could allow escape from recognition by IgA 5I2.

Examples of Results

[0204] Interaction of Fab 5I2 with P domain of Norwalk virus – Among HuNoVs, the surface exposed P2 subdomain in the P domain of capsid protein VP1 is implicated in differential HBGA binding and antigenicity. Although HBGA binding to P domain has been characterized extensively, information about antigenicity, neutralization and how these HuNoVs evolve to escape host immunity remains limited. In order to understand the basis of antibody binding and neutralization among HuNoVs, we purified the P domain of GI.1 NV and the Fab fragment of IgA 5I2 (see Examples of Materials and Methods below). IgA 5I2 was produced from a hybridoma generated with B cells isolated from a person previously challenged with GI.1 NV. Using ELISA-based surrogate neutralization assays, IgA 5I2 was shown to be genotype specific; it binds to the P domain of GI.1 NV, and effectively blocks HBGA binding to NV VLP. To assess the suitability of Fab 5I2 and NV P domain complex for crystallographic studies, we first carried out binding studies using bio-layer interferometry (BLI). In these studies, biotinylated P domain was immobilized on a streptavidin biosensor and titrated against serial dilutions of Fab 5I2. Data analysis showed that Fab 5I2 binds to the P domain with an affinity constant K_D of 20.5 nM, and rate constants K_{on} of $2.04 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ and K_{off} of $4.01 \times 10^{-3} \text{ s}^{-1}$ for association and dissociation respectively (Fig. 12), indicating a tight interaction between Fab 5I2 and NV P domain.

[0205] Crystallographic structure of Fab 5I2 in complex with NV P domain – To understand the molecular details of how Fab 5I2 recognizes the NV P domain and what the mechanism of HBGA blockade is, we carried out crystallographic studies of the recombinant NV P domain (amino acids 229-519) in complex with the Fab 5I2. The P domain-Fab 5I2 complex crystals diffracted to $\sim 2.3\text{\AA}$ and the structure was determined in the space group $P6_522$, with one P domain-Fab complex in the crystallographic asymmetric unit. The structure of the complex was determined using molecular replacement techniques and refined with a final R_{fac} and R_{free} values of 18% and 21% respectively (Table 1). The P domains related by crystallographic 2-fold symmetry associate to form a dimer, as typically found in the NV capsid and other NoV P domain structures, with each of the dimeric subunits interacting separately with a Fab 5I2 molecule (Fig. 13). The Fab recognizes and interacts with a conformational epitope in the P2 subdomain. Superposition of the unbound and Fab 5I2-bound P domain structures showed that Fab binding does not alter the overall structure of the P domain (r.m.s.d. of $0.5\text{\AA}$) but Fab binding does induce local conformation changes in some of the loop regions.

[0206]

Table 1: Data processing and Refinement statistics

	P domain-Fab 5I2
Space group	P6522
Cell dimensions(Å)	162, 162, 146.81
α, β, γ (°)	90, 90, 120
Resolution (Å)	80.97 – 2.3 (2.38– 2.3)
Wavelength (Å)	0.97935
No. of Reflections	101568
Unique Reflections	50814
R merge (%)	10.9 (27)
I/ σ I	15.08 (2.88)
Completeness (%)	100 (100)
Redundancy	2.0 (2.0)
R work (%) ^a	18.40
R free (%)	21.40
Bond lengths (Å)	.009
Bond angles (°)	1.3

[0207] The overall structure of the bound Fab 5I2 is similar to other structurally characterized Fabs. The constant (CH and CL) and variable (VH and VL) domains of the heavy and light chains exhibit a typical immunoglobulin fold. The CH and CL interact closely with one another as do the VL and VH. As expected, the three hypervariable complementarity determining regions (CDRs) from each heavy (CDR- H1, H2 and H3) and light chains (CDR- L1, L2, L3) in the variable domains are oriented facing the P2 subdomain. The CDR loops of Fab 5I2 are of varying lengths, with CDRH3 and CDRL1 being the longest, each consisting of 17 residues. Although the length of CDRH3 with 17 residues is typical, the 17-residue length of CDRL1 is unusual, and analysis of the interfacial interactions between P domain and Fab shows that CDRL1 plays a dominant role in antigen recognition.

[0208] Molecular determinants of antigen recognition – The epitope on the P2 subdomain that is recognized by Fab 5I2 is formed by residues from three surface exposed loops - T (377-386), U (394-405) and Q (345-354) (FIG. 14A). Some of these loop regions have been observed to have sequence and structural changes in other genogroups and within

genotypes contributing to variations of HBGA binding specificities (Shanker, *et al.*, 2014; Shanker, *et al.*, 2011; Kubota, *et al.*, 2012)). The paratope of Fab 5I2 comprises three of the six CDRs including CDRL1 (residues 24-40) and CDRL3 (residues 96 -103) in the light chain and CDRH3 (residues 97-113) in the heavy chain (FIG. 14A). The P domain-Fab 5I2 interaction is achieved through several hydrogen bonding, electrostatic, and hydrophobic interactions involving a total buried surface area of $\sim 735 \text{ \AA}^2$ (FIG. 14B), consistent with the low nanomolar affinity indicated by binding assays (FIG. 12).

[0209] Of the three CDRs, CDRL1 makes the most extensive interaction with the P domain. Its residues Q27, S28, L30, K32 and K35 contribute to eight hydrogen bonding interactions with the P domain residues N346, T348, D350 and F352 in loop Q and residues N394, G396 and S398 in loop U. CDRL1 also contributes to several water-mediated hydrogen bonding interactions. CDRL3 participates in the epitope recognition through its residues Y98 and I100. Y98 makes two hydrogen bonds, one with residue T348 in loop U and another with H381 in loop T. I100 is involved in water-mediated hydrogen bonding interaction with residue S380 in loop T. CDRH3 is the lone CDR from the heavy chain of Fab 5I2 that interacts with the P domain. Interactions involve its residues, Y107 and D108. Y107 is involved in hydrophobic and water mediated hydrogen bond interaction with residue S383 in loop T of P domain and D108 makes two direct hydrogen bonds with residue S383 on the T loop of P domain (FIG. 14B).

[0210] The P domain-Fab complex structure also exhibits interactions that contribute significantly to surface complementarity. First, the side chain of K32 from CDRL1 buries itself into a narrow, $\sim 8 \text{ \AA}$ deep pocket on the surface of P domain, contributing to a network of hydrogen bonding interactions. While the main chain amide group of K32 hydrogen bonds with the main chain carbonyl group of T348 on the rim of the pocket, its side chain hydrogen bonds with the main chain carbonyl groups of F352, N394 and G396 lying at the bottom of the pocket (FIG. 15A). Second, the side chain of H381 from the P domain buries itself in a hydrophobic pocket on the surface of Fab 5I2 (Fig 15c) making π - π stacking and cation- π interactions with the aromatic side chains of residues Y31 (CDRL1), Y38 (CDRL1), and Y98 (CDRL3) and hydrogen bonding interaction with the main chain carbonyl group of Y98. This surface complementarity is enhanced by subtle conformational alterations in the P2 subdomain in response to Fab 5I2 binding. A comparison of Fab 5I2 bound P domain structure with the P domain structure of NV VLP (PDB ID 1IHM) (Prasad, *et al.*, 1999)

reveals that Fab binding induces conformational changes in loop U. This loop shifts by as much as 10Å (at maximum Cα divergence) to make favorable interactions with CDRL1 including hydrogen bonding interactions described above involving K32 of CDRL1 (FIG. 15B). Similarly, the orientation of the H381 sidechain is flipped as compared to its orientation in the unbound P domain structure (FIG. 15D). This flipping ensures the H381 side chain does not sterically hinder binding of Fab 5I2 and allows it to participate in favorable intramolecular stacking interactions with the side chain of P382 of the P domain.

[0211] Structural basis for how IgA 5I2 blocks HBGA binding – To gain insight into the mechanism of HBGA blockade or neutralization by IgA 5I2, the inventors superimposed the Fab 5I2-P domain complex structure with an HBGA bound NV P domain structure (FIG. 16A, 16B). This comparison shows that Fab 5I2 binds in close proximity to the primary HBGA binding site (FIG. 16B) but does not alter the structural integrity of the HBGA binding site, including the sidechain orientations of the residues that participate in HBGA binding. It is clear 172 that binding of Fab 5I2 sterically blocks HBGA from accessing the binding site (FIG. 16C, 16D). Thus HBGA blockade or neutralization by IgA 5I2 antibody is principally through steric hindrance as opposed to direct competition or disruption of the HBGA binding site.

[0212] Structural basis for why IgA 5I2 is genotype specific – To understand the basis of the genotype specificity exhibited by IgA 5I2 and how other genotypes escape neutralization by IgA 5I2, we aligned the P domain sequences of various GI genotypes focusing on the residues that are involved in Fab 5I2 binding (FIG. 17A). Sequence alignment of these residues clearly shows that these residues are poorly conserved and might not be able to participate in the observed hydrogen bonding and hydrophobic interactions with Fab 5I2. Particularly interesting is residue H381 that in our structure is involved in highly stabilizing stacking interactions with residues from Fab 5I2. This histidine residue is not conserved and varies significantly between other GI genotypes (FIG. 17A). Superposition of our Fab bound GI.1 P domain structure with other available P domain structures in the GI genogroup including GI.7 and GI.8 shows that in addition to sequence changes the loop regions involved in Fab 5I2 binding are susceptible to significant conformational changes (FIG. 17B). These structural alterations among the other GI genotypes lead to disruption of the conformational epitope recognized by Fab 5I2 on the GI.1 P domain. Thus both sequence

changes and structural changes allow other genotypes to escape Fab 5I2 mediated HBGA blockade.

Significance of Certain Embodiments

[0213] HuNoVs are unique among viral pathogens in exploiting the genetically-controlled polymorphic nature of the HBGAs among host populations for their sustained evolution (Huang, *et al.*, 2005; Lindesmith, *et al.*, 2008)). In response to adaptive immunity, the distally located P2 subdomain can evolve to escape neutralization and differentially interact with HBGAs as underscored by recent studies that show HBGA-blocking antibodies confer protection against HuNoV infection (Reeck, *et al.*, 2010; Bok, *et al.*, 2011). Although there are numerous studies delineating HuNoV interactions with HBGAs, the understanding of the mechanism by which a human antibody blocks HBGA binding is limited. The crystallographic structure of the NV (GI.1) P domain in complex with the Fab of a human IgA 5I2 monoclonal antibody addresses key questions such as how a blockade antibody recognizes HuNoV, what is the mechanism of HBGA blockade, and how sequence alterations allow other genotypes to escape neutralization. The IgA 5I2 was selected from a panel of HBGA blocking antibodies obtained by generating hybridomas from B cells isolated from an individual challenged with GI.1 NV. Both IgG and IgA antibodies were identified. An IgA antibody was chosen for the structural studies because of the important role of IgA, compared to IgG, in conferring mucosal immunity. The subnanomolar binding affinity of this antibody together with its HBGA blockade activity *in vitro*, is suggestive of its high potency in virus neutralization.

[0214] IgA 5I2 recognizes a conformational epitope formed by the P2 subdomain loops – The crystal structure of the Fab 5I2 in complex with the NV P domain shows that Fab recognizes a conformational epitope comprised of residues from the solvent-exposed loops in the distal portion of the P2 subdomain. Involvement of the surface loops in antibody recognition is a common feature as observed in many antigen-antibody structures. The distal surface of the P2 subdomain consists of 6 loops that project out into the solvent, which can be grouped into three clusters 1-3 (FIG. 18A). Despite sequence changes, differences in their lengths and orientations, these loops are similarly clustered in GII.4 (FIG. 18B) as well as in murine NoVs. Residues from clusters 1 and 2 in GI.1 constitute the antigenic site recognized by IgA 5I2 (FIG. 18A). Although IgA 5I2 specifically recognizes clusters 1 and 2, it is possible that other blockade antibodies can recognize residues in other clusters as suggested

by previous biochemical studies characterizing such antibodies in GI and GII.4. In GI, residues in the A and B loops in cluster 3 have been identified as important for binding blockade antibodies (Chen Z *et al.* J Virol 2013;87:9547-57). In GII.4, three blockade epitopes (A, D, and E) have been suggested; residues in epitope A map to loops A and B (cluster 3), whereas epitopes D and E map to residues in T and U loops (clusters 1 and 2), respectively (Lindesmith, *et al.*, 2012; Debbink, *et al.*, 2012; Allen, *et al.*, 2009; Parra, *et al.*, 2012). In murine NoVs and rabbit hemorrhagic disease virus, an animal calicivirus, neutralization epitopes have been mapped to A and B loops (Taube, *et al.*, 2010; Kolavole, *et al.*, 2014). Together with the structural studies and available epitope mapping on other NoVs, the data indicate that these loops allow for differential antigenic presentations contributing to serotypic differences in HuNoVs.

[0215] CDRL1 plays a dominant role in antigen recognition – A rather unusual feature of IgA 5I2 is the dominant involvement of CDRL1 in antigen recognition, providing a unique perspective into antibody diversity and antigen interactions. Typically, in an antibody-antigen interaction, including those involving antiviral antibodies, CDRH3 encoded by the highly diverse D-J_H joining genes plays a dominant role because of the inherent sequence diversity and consequent conformational variability. The H3 loop is also a common site for somatic hypermutations, allowing affinity maturation of the antibodies (Tsuchiya, *et al.*, 2016; Shirai, *et al.*, 1999). In the case of IgA 5I2, five out of eight residues in the CDRs that interact with P domain are from CDRL1. In k chain human antibodies, the length of the CDRL1 varies between 10 and 17 residues, with the majority of the antibodies exhibiting a loop length of 11 residues. In IgA 5I2, CDRL1 is 17 residues long. Despite its unusual length, it exhibits the expected canonical conformation. In IgA 5I2, the CDRH3 is also 17 residues long and is within the expected range of 10-30 residues. The general expectation is that H3 loops with longer lengths (>14) play a predominant role in antigen specificity, whereas in those with shorter lengths, antigen interactions involve other loops. In IgA 5I2, H3 loop is positioned slightly away from the P2 subdomain, with just two of its residues interacting with the P2 subdomain. The other non-H3 CDRs are of normal lengths with canonical conformations as observed in other antibody structures. With the exception of two residues in the CDRL3, residues from other CDRs do not participate in antigen recognition. CDRL1, together with L3 and H3 residues, provide the complimentary residues for optimal hydrogen bond and hydrophobic interactions with the loop residues of the P2 subdomain, as well as appropriate topographical features to enhance the surface complementarity with the P2

subdomain consistent with the observed binding affinity in the low nanomolar range. It remains to be seen whether the dominant role of CDRL1 observed with IgA 5I2 is a common feature in HuNoV blockade antibodies.

[0216] HBGA blockade by Fab 5I2 is by steric hindrance – HBGA blockade by an antibody potentially can occur in a number of ways, including directly competing for the HBGA binding site, allosterically disrupting the HBGA binding site by inducing conformational changes in the P domain, or by sterically masking the HBGA binding site. The crystallographic studies show that in the case of IgA 5I2, the mechanism of HBGA blockade is principally through steric hindrance. The Fab 5I2 binds to the NV P domain without affecting either the dimeric conformation of the P domain or the structural integrity of the HBGA binding site. In NV, and generally in other HuNoVs, the HBGA binding site is located in a shallow depression on the distal surface of the P2 subdomain surrounded by clusters of loop regions. Given the considerably larger size of the Fab compared to HBGA, its direct access to the HBGA binding site for optimal interactions is perhaps restricted. This is clearly evident from the structure of the complex; that despite the Fab entirely engulfing the primary HBGA binding site, none of the residues in the HBGA binding site make contact with the Fab. Their side chain orientation also remains unaltered by antibody binding. It is interesting to hypothesize that the steric hindrance could be a common mechanism employed by blockade antibodies. Several observations support such a hypothesis. First, as noted, the HBGA binding sites in both GI and GII are surrounded by loop regions. Second, the majority of the residues mapped by biochemical studies as being critical for blockade antibody binding are outside of the primary HBGA binding site, whether it is the β Gal binding site in the case of GI or the α Fuc site, as seen in GII.4 HuNoVs. Third, most of the HBGA blocking mAbs characterized thus far are genotype specific and do not cross react, even within the same genogroup, similar to IgA 5I2, suggesting that these mAbs also primarily interact with the loop regions that are prone to genotypic alterations. Although HBGA blocking polyclonal antibodies from the HuNoV-infected individuals have shown cross-reactivity (Czako, *et al.*, 2015), many derived mAbs, such as IgA 5I2, are genotype-specific (Lindesmith, *et al.*, 2013; Payne, *et al.*, 2015). Inaccessibility of the HBGA binding site, which represents the most conserved region of the P2 subdomain and is highly genogroup-specific, is consistent with the observation that IgA 5I2 is highly specific for GI.1 HuNoV (Sapparapu *et al.*, submitted).

[0217] Sequence and structural changes allow other GI genotypes to escape neutralization – The structural studies show that the observed genotype specificity of IgA 5I2 is mainly because the conformational epitope it recognizes is formed by residues in the loop regions that are prone to sequence and structural alterations. The residues in GI.1 P domain that interact with the Fab 5I2 are poorly conserved and some of the loop regions involved in antibody binding show significant conformational variations, including changes in their orientations. Although it has not been well documented in the case of GI genotypes, in the case of GII.4 several studies have suggested that some of the residues in the corresponding loop regions represent evolutionary hotspots contributing to epochal strain diversity (Lindesmith, *et al.*, 2013; Bok, *et al.*, 2009; Donaldson, *et al.*, 2010). These changes, likely in response to adaptive immunity, alter HBGA binding profiles of these epochal variants. The structural studies have previously indicated how a small change in the T loop of GII.4 2004 alters the HBGA binding profile (Shanker, *et al.*, 2011). The current studies suggest a similar phenomenon in the case of GI genotypes. The H381 residue in the T loop of NV P domain is critical for IgA 5I2 binding as it is involved in multiple stabilizing interactions with the antibody and is not conserved in other GI genotypes. While this residue in GI.1 is far removed from the HBGA binding site, because of the conformational changes, the structurally corresponding residue S391 in GI.7 becomes a part of the primary HBGA binding site (Shanker, *et al.*, 2014) clearly illustrating a coordinated interplay between antigenic variation and HBGA binding in the evolution of NoVs.

[0218] In conclusion, by determining the crystal structure of a human HBGA blockade antibody in complex with the NV P domain the inventors have provided here the first atomic details of how a potentially neutralizing HBGA blockade antibody recognizes and binds to HuNoV. The structural study shows that the mechanism by which 5I2 antibody neutralizes the NV is by sterically blocking the HBGA binding. Further, the structural basis for the genotype specificity is also clearly evident from our crystallographic studies. Based on the observation from our structural studies as well as from epitope mapping studies by others that the antigenic sites in HuNoVs is mainly composed of residues from loop regions that are hypervariable raises a question whether it will be possible to obtain broadly reactive HBGA blocking antibodies for therapeutic intervention. While the naturally occurring human HBGA blockade mAbs tend to be genotype-specific, one possibility is to use a cocktail of such antibodies, or to design antibody scaffolds with a smaller footprint such as single-chain

antibodies or even small molecule mimics that specifically target highly conserved HBGA binding site. Further studies are clearly required to explore such a possibility.

Examples of Materials and Methods

[0219] Expression and Purification of the P domain and Fab 5I2: The P domain was expressed and purified as described previously (Shanker, *et al.*, 2011). In brief we cloned the P domain construct (aa 216–519) of GL1 NV into the pMal-C2E expression vector, overexpressed the protein in *E. coli* cells and purified the P domain using basic chromatography techniques. The purified P domain was concentrated to ~10mg/ml in a buffer containing 25 mM Tris·HCl (pH 7.5), 150 mM NaCl, and 5 mM MgCl₂ and stored at -80°C till further use.

[0220] Determination of variable domain sequences of IgA 5I2 and synthesis of expression-optimized genes was done as described previously (Sapparapu *et al.* submitted). The VH domain was cloned as an *EcoRI* / *HindIII* fragment into pHc-huCg1Fab expression vector. The VL domain was cloned as a *BglII* / *NotI* fragment into pML-huCk kappa expression vector (McLean, *et al.*, 2000). Recombinant antibodies were expressed transiently in Expi293F cells by cotransfection of equal amount of heavy and light chain plasmid DNA using ExpiFectamine 293 transfection reagent (Life Technologies). After 7 days of culture, the supernatants were clarified by centrifugation and filtered using 0.4 µm pore size filter devices. Antibodies were harvested from the supernatants by affinity chromatography on CaptureSelect™ IgG-CH1 columns (Life Technologies) as previously described (Aiyegbo, *et al.*, 2013). Antibodies eluted from affinity columns were concentrated using Amicon centrifugal filters (Millipore).

[0221] P domain-Fab 5I2 complex formation and crystallization: Purified P domain (mw 32kd) and Fab 5I2 (mw 50kd) proteins were mixed in a 1:1 molar ratio in the P domain storage buffer and incubated for 2-4 hours at 4°C. The mixture was run through the S75pg 16/60 gel filtration column and the peak corresponding to the complex (assessed by peak shift compared to P domain by itself) was collected. The complex eluted at an mw of approximately 160kd corresponding to a P domain dimer bound to two Fab molecules. SDS-PAGE confirmed the presence of both the proteins in the complex peak. The peak fractions were then pooled and concentrated to 10mg/ml for crystallization trials. Crystallization screening using hanging-drop vapor diffusion method at 20°C was set up by nanoliter handling system Mosquito (TTP LabTech) with commercially available crystal screens. P

domain-Fab complex crystallized in a buffer containing using 0.2M sodium formate, 0.1M Bis Tris propane, pH 6.5 and 20% w/v PEG3350. Initial crystals were small and diffracted to $\geq 3.5\text{\AA}$. The initial crystallization conditions were further optimized based on ionic strength, pH and precipitant concentrations, and microseeding technique was employed to obtain larger well diffracting crystals. Crystals measuring 0.1-0.2 mm were obtained in 1- 2 weeks. The crystals were soaked in the reservoir solution containing 20% glycerol as cryoprotectant followed by flash freezing in liquid nitrogen.

[0222] Diffraction, data collection and structure determination: Diffraction data for the P domain-Fab 5I2 crystals were collected on the 5.0.1 beamline at Advance Light Source Berkeley. Diffraction data were processed using IMOSFLM (Battye, *et al.*, 2011). Space group was confirmed using POINTLESS program incorporated in the PHENIX suite (Adams, *et al.*, 2002). Initial electron density map was obtained by molecular replacement (MR) using the previously published GL1 P domain structure (PDB ID: 2ZL5) as the starting model using program PHASER (McCoy, *et al.*, 2007) in the CCP4i suite (Collaborative Computational Project, *et al.*, 1994). The solution from PHASER clearly showed extra electron density for the bound Fab molecule. PHASER was then rerun using the P domain structure (PDB ID: 2ZL5) and an additional neutralizing Fab structure (PDB ID 4RQQ) (Sok, *et al.*, 2014) as starting models to resolve the Fab density. This was followed by ab-initio automated model building and solvent addition using AUTOBUILD (Terwilliger, *et al.*, 2008) to reduce model bias. Further model building was carried out using iterative cycles of refinement and model building based on the FO-FC difference maps. The programs phenix.refine and Coot (Emsley & Cowtan, 2004) were used throughout structure determination and refinement. Data collection and refinement statistics are provided in Table. 1. Program PyMOL was used for generating the final figures. Fab 5I2-P domain interactions were analyzed using COOT and LIGPLOT (Wallace, *et al.*, 1995) with donor to acceptor distances between 2.6 Å and 3.3 Å for hydrogen bonding interactions. The buried surface area of the interaction was calculated using PISA server.

[0223] Protein structure, accession code: The coordinates and structure factors for the Fab 5I2-P domain complex structure determined in this study has been deposited in the Protein Data Bank (PDB) under the accession code _____.

EXAMPLE 4

FREQUENT USE OF THE IGA ISOTYPE IN HUMAN B CELLS ENCODING POTENT NOROVIRUS-SPECIFIC MONOCLONAL ANTIBODIES THAT BLOCK HBGA BINDING

Introduction

[0224] Norwalk virus, the prototype of human noroviruses (NoVs), was the first virus identified in 1972 as a causative agent for acute gastroenteritis (Kapikian, 2000). NoVs are the leading cause of epidemic acute and sporadic cases of gastroenteritis responsible for about 19-21 million cases of infection leading to >70,000 hospitalizations and about 800 deaths annually in the U.S. (Hall, *et al.*, 2013). NoVs recently surpassed rotaviruses as the leading cause of pediatric non-bacterial gastroenteritis after the introduction of vaccines against rotaviruses (Payne, *et al.*, 2013). The infection is typically self-limiting, lasts for 1-3 days, and is characterized by diarrhea, vomiting, nausea, stomach pain and fever, with more severe complications and chronic disease in the immunocompromised. Therapy involves rest and rehydration, and no specific therapeutic agent is currently available.

[0225] NoVs, members of the *Caliciviridae* family, are non-enveloped and contain a positive-sense, non-segmented single stranded RNA genome enclosed by a protein capsid. The genome codes for three open reading frames (ORF), with the first ORF coding for six non-structural proteins involved in viral transcription and replication. The second and third ORFs encode virus protein (VP1) and VP2, respectively. VP1 is a major capsid ~60 kDa protein and can self-assemble into virus-like particles (VLP) that resemble native virions both morphologically and antigenically (Jiang, *et al.*, 1992). The viruses are classified into at least six genogroups (GI, GII, GIII, GIV, GV and GVI), based upon the sequence of VP1 (Ramani, *et al.*, 2014). The genogroups are further subdivided into genotypes, with GI and GII accounting for the most diversity with 9 and 22 genotypes, respectively. GI and GII NoVs are responsible for the majority of human infections, with the genotype GII.4 responsible for most. Human susceptibility to 58 NoVs depends on the expression of histo-blood group antigens (HBGAs) on the intestinal epithelial cells (Marionneau, *et al.*, 2002; Lindesmith, *et al.*, 2003; Hutson, *et al.*, 2003). These blood group carbohydrates are thought to play a role as receptors or co-receptors based on recent studies of correlations between susceptibility, HBGA profile and secretor status (expression of secretor enzyme α 1,2 fucosyltransferase) (Hall, *et al.*, 2013; Donaldson, *et al.*, 2008; Tan, *et al.*, 2014).

[0226] The correlates of protection in NoV infections are not completely understood. Almost all infected persons seroconvert, but epidemiological observations and clinical studies suggest that serum antibody measurable by traditional ELISA assays may not be long-lived or is otherwise insufficient to protect individuals from re-infection. Instead, the presence of anti-NoV Abs that block binding of virus to HBGA *in vitro* can protect from NoV gastroenteritis in the context of experimental challenge, suggesting a potential correlate (Reeck, *et al.*, 2010; Atmar, *et al.*, 2015). We showed previously that a serum HBGA blocking antibody titer >200 (Atmar, *et al.*, 2011) or a serum hemagglutination inhibition titer of >40 (Czakó, *et al.*, 2012) is associated with protection of susceptible individuals from an experimental challenge. Deeper understanding of the immune response to human norovirus infection is hampered by the lack of a robust *in vitro* culture model and immunological reagents.

Examples of Materials and methods

[0227] Donors – Persons previously challenged experimentally with norovirus Hu/NoV/GI.1/Norwalk/68/US (Norwalk virus [NV]) were recruited to donate peripheral blood mononuclear cell (PBMC) samples for study. We obtained PBMCs that were isolated from heparinized blood by density gradient centrifugation using Ficoll-Histopaque from donors 1-2 years following oral challenge with a GI.1 NV inoculum (Atmar, *et al.*, 2013). The donors from whom the panel of antibodies were isolated had been challenged 26 or 12 months prior. Infection was demonstrated in the laboratory by detection of viral genome and antigen in fecal samples, by RT-qPCR and ELISA, respectively. In addition, the donors demonstrated a greater than four-fold rise in serum antibody levels by total antibody ELISA and by HBGA blocking activity. The protocol was reviewed and approved by the Baylor College of Medicine Institutional Review Board, and informed consent was obtained from the participants.

[0228] Generation of EBV-transformed lymphoblastoid cell lines (LCLs) secreting NV-specific human monoclonal antibodies (mAbs) – B cells were transformed by infection with Epstein Barr virus (obtained from supernatant of cultured B95.8 cotton top tamarin lymphoblastoid line) in the presence of 2.5 µg/mL TLR agonist CpG (phosphorothioate-modified oligodeoxynucleotide ZOEZOEZZZZZOEZOEZZZZT (SEQ ID NO. 62), Life Technologies), 10 µM Chk2 inhibitor [Chk2i] (Sigma), 10 µg/mL cyclosporine A (Sigma) and plated in 384-well culture plates. After 7 days of culture, cells from one 384-

well culture plate were expanded into four 96-well culture plates containing 97 CpG, Chk2i and irradiated heterologous human PBMCs to serve as feeder layers for the growth of lymphoblastoid cell line (LCL) clusters. After an additional 3 days of culture, the supernatants were screened for binding to NV GI.1 VLP or disruption of the NV VLP – glycan interaction (described below). Briefly, 5 μ L of supernatant from each well of transformed B cell cultures (in a total assay volume of 50 μ L) were added to the wells coated with 1 μ g/mL NV VLP. The bound antibodies were detected using alkaline phosphatase conjugated goat anti-human Ig (γ and α chain specific) (Southern Biotech). In blocking assays, 50 μ L of diluted supernatant as described above were mixed with NV VLP and the complexes were added to H3-PAA (Glycotect, Rockville, MD) immobilized on neutravidin-coated plates, as described below. Supernatants from LCL cultures (diluted 1:10 in assay buffer) that had been selected for rotavirus-reactive antibody were used as negative controls.

[0229] Generation of hybridomas secreting NV-specific mAbs from LCLs. – Cells from wells with desired activity were subjected to electrofusion with HMMA2.5 myeloma cells. The fused cells then were cultured in a selective medium containing 100 μ M hypoxanthine, 0.4 μ M aminopterin, 16 μ M thymidine (HAT Media Supplement, Sigma HO262), and 7 μ g/mL ouabain (Sigma O3125) and incubated for 14 – 18 days before screening hybridomas for antibody production by ELISA. Cells from the positive wells were cloned biologically by sorting single cells into 384-well plates using a FACSaria III fluorescence activated cell sorter (Becton Dickinson), cultured for about 14 days and screened for antibody production.

[0230] Sequence analysis of antibody variable region genes – Total RNA was extracted from hybridoma cells and used for amplification of genes coding for the variable domains of the antibody clones. First-strand cDNA synthesis and RT-PCR were done with gene-specific primers as previously described (Table 2) using the OneStep RT-PCR kit (Qiagen), according to the manufacturer's protocols. The thermal cycling parameters were as follows: 50 °C for 30 min, 95 °C for 15 min, 39 cycles of (94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min) followed by a final extension step for 10 min at 72 °C. PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter) and sequenced directly using an ABI3700 automated DNA sequencer without cloning. Heavy chain or light chain antibody variable region sequences were analyzed using the IMGT/V-Quest program (Brochet, *et al.*, 2008; Giudicelli, *et al.*, 2011). The analysis involved the identification of germline genes that were used for antibody production, location of

complementary determining regions (CDRs) and framework regions (FRs) as well as the number and location of somatic mutations that occurred during affinity maturation.

[0231] Table 2 Primers used in RT-PCR for amplifying heavy or light chain antibody variable genes.

Oligo Name	Sequence 5' to 3'	SEQ ID NO:
IgExp_H1	GTTTTAAAAGGTGTCCTGTGTCARRTNCAGCTGGTRCAGTC	63
IgExp_H2	GTTTTAAAAGGTGTCCTGTGTCAGRTCACCTTGARGGAGTC	64
IgExp_H3	GTTTTAAAAGGTGTCCTGTGTSARGTGCAGCTGGTGGAGTC	65
IgExp_H4	GTTTTAAAAGGTGTCCTGTGTCAGSTGCAGCTRSAGGAGTC	66
IgExp_H5	GTTTTAAAAGGTGTCCTGTGTGARGTGCAGCTGGTGCAGTC	67
IgExp_H6	GTTTTAAAAGGTGTCCTGTGTCAGGTACAGCTGCAGCAGTC	68
IgExp_H7	GTTTTAAAAGGTGTCCTGTGTCAGGTGCAGCTGGTGCAGTC	69
IgExp_Hconst	GATGGGCCCTTGAAGCTTGCTGAGGAGACGGTGACCAGGGT	70
IgExp_K1	GAATCCCAGGCATGAGATCTGMCATCCRGWTGACCCAG	71
IgExp_K2	GAATCCCAGGCATGAGATCTGAKRTTGTGATGACYCAG	72
IgExp_K3	GAATCCCAGGCATGAGATCTGAAATWGTRWTGACRCAG	73
IgExp_K4	GAATCCCAGGCATGAGATCTGACATCGTGATGACCCAG	74
IgExp_K5	GAATCCCAGGCATGAGATCTGAAACGACACTCACGCAG	75
IgExp_K6	GAATCCCAGGCATGAGATCTGAWRTTGTGMTGACWCAG	76
IgExp_Kconst	GATGGCGGGAAGATGAAGACAGATGGTGCGGCCGCGAGT	77
IgExp_L1	GAATCCCAGGCATGAGATCTCAGTCTGTSBTGACKCAG	78
IgExp_L2	GAATCCCAGGCATGAGATCTCARTCTGCCCTGACTCAG	79
IgExp_L3	GAATCCCAGGCATGAGATCTTCCTMTGDGCYRAYWCAG	80
IgExp_L4	GAATCCCAGGCATGAGATCTCWGCTGTGCTGACTCAA	81
IgExp_L5	GAATCCCAGGCATGAGATCTCAGSCTGTGCTGACTCAG	82
IgExp_L6	GAATCCCAGGCATGAGATCTAATTTTATGCTGACTCAG	83
IgExp_L7	GAATCCCAGGCATGAGATCTCAGRCTGTGGTGACTCAG	84
IgExp_L8	GAATCCCAGGCATGAGATCTCAGWCTGTGGTGACCCAG	85
IgExp_L9	GAATCCCAGGCATGAGATCTCAGCCTGTGCTGACTCAG	86
IgExp_L10	GAATCCCAGGCATGAGATCTCAGGCAGGGCTGACTCAG	87
IgExp_L11	GAATCCCAGGCATGAGATCTCGGCCCGTGCTGACTCAG	88
IgExp_Lconst	AGGGGGGAACAGAGTGACASTTGGAGCGGCCTTAGGCTG	89

[0232] Molecular engineering of antibody variable gene domains – For expression of recombinant forms of the antibody clones, the nucleotide sequences of variable domains were optimized for mammalian expression and synthesized (Genscript). The heavy chain fragments were cloned as EcoRI/HindIII fragments into 138 pML-huCG1 or pML-huCA1 vectors for expression of $\gamma 1$ or $\alpha 1$ chains, respectively (McLean, *et al.*, 2000). The light chains were cloned as BglII/NotI fragments into pML-huCk or pML-huCL vectors for κ or λ chains, respectively.

Production and purification of antibodies from hybridomas or from transfected HEK293 cells – For expression of antibodies from hybridoma clones, cells were cultured in serum-free medium, Hybridoma SFM (Life Technologies), for 21 days. Recombinant antibodies were expressed transiently in Expi293 F cells (Life Technologies), according to

the manufacturer's recommendation. Equal amounts of heavy and light chain DNA were used for transfections to generate recombinant IgG or monomeric IgA antibodies. For recombinant dimeric IgA, plasmids encoding cDNAs for the heavy chain, light chain and J chain DNA were mixed at 1:1:2 ratio as described (Aiyegbo, *et al.*, 2013). Transfection was done using ExpiFectamine 293 transfection reagent (Life Technologies) according to the manufacturer's protocols. After 7 days of culture, the supernatants were clarified by centrifugation and filtered using 0.4- μ m pore size filter devices. Antibodies were harvested from the supernatants by affinity chromatography on HiTrap KappaSelect or LambdaSelect columns (Life Technologies) as previously described (Aiyegbo, *et al.*, 2013). Antibodies eluted from affinity columns were concentrated using Amicon centrifugal filters (Millipore). Purified antibodies were resolved on polyacrylamide gels under reducing or non-reducing denaturing conditions and stained with Coomassie Blue reagent.

[0233] Antibodies used as control reagents -- Polyclonal rabbit serum raised against NoV VLPs was obtained as a positive control for detection of VLPs coated on ELISA plates. This immune sera were generated by hyperimmunization of rabbits with NV VLPs as previously described (Jiang, *et al.*, 1992). The inventors also prepared purified immunoglobulin from murine hybridoma cells secreting the mAbs 8812 or 3901. MAbs 8812 and 3901 were included in some receptor experiments as positive and negative controls for inhibition of NV VLP binding to receptor, based on previously determined activities (Hutson, *et al.*, 2003). These murine mAbs were used as controls for immunoblotting experiments to determine if mAbs bound to linear epitopes, because mAb 3901, but not mAb 8812, has been described previously to bind linear epitopes (Hardy, *et al.*, 1996). In immunoblots, mAb 3901 binds denatured VP1 but mAb 8812 does not.

[0234] Production and purification of VLPs – VLPs representing different norovirus genogroups (GI and GII) and genotypes (GI.1, NC_001959; GI.2, FJ515294; GI.4, GQ413970; GI.6, KC998959; GI.7, JN005886; GI.8, GU299761; GII.4; EU310927) were generated and purified as previously described (Kou, *et al.*, 2015). Briefly, capsid proteins (VP1 and VP2) were expressed in SF9 insect cells (2.75×10^6 cells/mL of Grace's insect cell media) from recombinant baculovirus expression vectors, and NoV VLPs were purified from culture supernatants on a cesium chloride gradient (Jiang, *et al.*, 1992). Structural integrity and purity of the VLP preparations were confirmed by electron microscopy of negatively stained VLPs (1.0% ammonium molybdenate (Sigma-Aldrich; St. Louis, MO), 180 pH 6.0)

on carbon coated grids and by Western blot, respectively. The inventors also generated a GI.1 VLP (designated CT303) in which the P domain was deleted by mutagenesis of the VP1 gene construct (Bertolotti-Ciarlet, *et al.*, 2002). A second mutated GI.1 VLP was prepared with the point mutation W375A that was previously determined to ablate HBGA binding (Choi, *et al.*, 2008).

[0235] VLP binding assay – Binding characterization of purified antibodies to NoV VLPs was carried out by ELISA. NoV VLPs were suspended in PBS at 1 µg/mL and coated in microwell plates (Nunc) for 16 h at 4 °C, and the wells were blocked with 5% skim milk and 2% goat serum in PBS-Tween. Purified antibodies were diluted serially in PBS and added to the ELISA plates. The bound antibodies were detected using alkaline phosphatase conjugated goat anti-human κ or λ chain antibodies (Southern Biotech). To compare binding between different classes of antibodies, the concentrations of antibodies were adjusted to normalize for the binding sites (Fab = 1; IgG = 2; mIgA = 2 or dIgA = 4) before use in ELISA. The genotype specificity of antibody binding was determined by direct ELISA, as described above, with the following modifications: VLPs were coated at 10 µg/mL and antibodies were used at a concentration of 20 µg/mL. Plates were developed using ultra-TMB reagent (Pierce ThermoFisher; Rockford, IL), following the manufacturer's protocol, and optical density as read at 450 nm using a SpectraMax M5 plate reader.

[0236] P domain dimer specific binding assay – The inventors prepared purified recombinant P domain dimeric protein, as previously described (Choi, *et al.*, 2008). Briefly, a NV P domain construct was expressed in *E. coli* (Novagen) and purified by affinity chromatography, followed by size exclusion chromatography. We tested binding of each of the mAbs to P domain dimer by direct antigen ELISA, using the same protocol as described above for the VLP binding assay.

[0237] Western blot – The nature of the epitopes bound by the human mAbs was determined by SDS-PAGE analysis and Western blot. NV VLPs were diluted in 5X Laemmli sample buffer and prepared for SDS-PAGE in one of the two following ways. Samples were either boiled at 100°C for 10 minutes or incubated at room temperature for 10 minutes prior to loading on separate pre-cast 14-20% polyacrylamide gels (Criterion TGX gel, BioRad; Hercules, CA) for electrophoresis. Electrophoresed proteins were transferred to nitrocellulose membrane for Western blot analysis. Human mAbs were diluted to 1 µg/mL in blocking solution (1% wt:vol, Kroger non-fat dried milk in 1X phosphate buffered saline). Two NV-reactive murine monoclonal antibodies (mAb 3901 and mAb 8812) and a Norwalk-reactive

rabbit polyclonal were used as positive controls for detection of VP1. Blots were incubated overnight at 4°C. Bound antibodies were detected using either an anti-human Ig (A, G, M)-HRP, anti-mouse-HRP, or anti-rabbit-HRP conjugate antibody (Southern Biotech; Birmingham, AL). Blots were developed by chemiluminescence using West Pico HRP substrate (Pierce ThermoFisher; Rockford, IL) following the manufacturer's instructions.

[0238] HBGA blocking assay – Disruption of interaction between VLP and HBGAs was used as a surrogate assay for measuring NoV neutralization by human monoclonal antibodies. Pre-existing titer of HBGA blocking antibodies is correlated with protection from NoV gastroenteritis (Reeck, *et al.*, 2010; Atmar, *et al.*, 2011). An HBGA blocking assay was carried out as previously described (Reeck, *et al.*, 2010). Briefly, biotin-polyacrylamide (PAA)-blood group antigen conjugates (Glycotech, Rockville, MD) were immobilized on neutravidin-coated plates (Thermo Scientific). VLPs were mixed with serial dilutions of antibodies, and the complexes were added to the glycan-coated microtiter plates. The relative amount of VLP bound to HBGAs was determined using rabbit anti-NoV antiserum followed by horseradish peroxidase-conjugated goat anti-rabbit (Southern Biotech). We tested mAbs for inhibition of binding of NoV VLPs to additional biotin-PAA-HBGA ligands, including H type 1 (H1-PAA-biotin), H type 2 (H2-PAA-biotin), H type 3 (H3-PAA-biotin), A trisaccharide (tri-A-PAA-biotin), and Lewis(y) (Le(y)-PAA-biotin) (Glycotech, Rockville, MD). Plates were developed using ultra-TMB reagent (Pierce ThermoFisher; Rockford, IL), following the manufacturer's protocol, and optical density as read at 450 nm using a SpectraMax M5 plate reader.

[0239] Hemagglutination inhibition assay – Hemagglutination inhibition assays were performed as described previously (Czakó, *et al.*, 2012). In brief, Human type O erythrocytes were collected from a healthy adult in Alsever's buffer, washed twice in Dulbecco's phosphate-buffered saline (PBS) without Ca²⁺ or Mg²⁺, and pelleted by centrifugation at 500xg for 10 min at 4 °C. Monoclonal antibodies (mAb; starting concentration 60 µg/mL for human mAb and 8.5 µg/mL for murine 245 8812) were diluted initially 1:10 in PBS with 0.85% saline (pH 5.5), and then serially 2-fold diluted. Four hemagglutination units (~2 ng) of Norwalk virus VLPs were mixed with the diluted monoclonal antibodies and incubated at room temperature for 30 min. The VLP-mAb mixture was added to an equal volume of 0.5% washed type O erythrocytes in 0.85% saline (pH 6.2) and incubated for 2 h at 250 °C. The HAI titer was determined by identifying the reciprocal of the highest dilution of mAb that inhibited hemagglutination by the VLPs.

[0240] Competition-binding ELISA analysis – Competition-binding ELISAs were carried out to determine whether the hmAbs we generated bound distinct or shared epitopes in the NV capsid protein. Briefly, each mAb was used to coat a 96-well microtiter plate (Greiner Bio-One; Monroe, NC) at a concentration of 2 µg/mL in carbonate coating buffer at 4°C overnight. Norwalk VLPs (100 ng/mL) were incubated with serial dilutions of each hMAb, ranging from 6.25 µg/mL to 250 µg/mL in assay buffer [1% non-fat dried milk (NFDM) in 1X PBS, w/v], for 2 hours at 37 °C. Each plate included an antigen-only control to which no mAb had been added. The assay plate was washed three times with PBS containing 0.05% Tween 20 (PBS-T) and blocked for 1 hour at 37 °C with 5% NFDM in PBS. The pre-incubated VLP/mAb preparations were added to the mAb-coated microtiter plate and plates were incubated for 2 hours at 37°C. Bound VLPs were detected using a rabbit anti-NV polyclonal antibody (1/10,000 in assay buffer; 2 hours at 37°C) followed by a commercial goat anti-rabbit-HRP conjugate antibody (Southern Biotech; 1/7500 in assay buffer; 45 minutes at 37°C). Plates were developed using ultra-TMB reagent (Pierce ThermoFisher; Rockford, IL), following the manufacturer's protocol, and optical density as 267 read at 450 nm using a SpectraMax M5 plate reader. Readings from duplicate wells were averaged. The percent competition for each competitor hMAb was calculated relative to the antigen-only control. MAbs were judged to compete for binding to the same site if maximum binding of the competing mAb was reduced to <25% of its un-competed binding. A level of 25-50% of its un competed binding was considered intermediate competition.

Examples of Results

[0241] Isolation of NV-specific human IgG or IgA mAbs – Currently there is not a robust method for growing NoVs, but studies suggest that blocking the interaction of VLP with glycan moieties can be used as a surrogate for neutralization activity (Reeck, *et al.*, 2010; Lindesmith, *et al.*, 2015). The inventors sought to isolate blocking mAbs to the NoV capsid protein from volunteers challenged with NV. PBMCs isolated from two NV-immune donors were transformed with EBV, and the LCL supernatants were screened for binding to NV VLPs and separately for blocking of binding to H3-PAA glycan. The transformed B cells from cell line supernatants exhibiting IgG or IgA binding to VLPs or blocking of VLPs to the glycan, or exhibiting both activities, were expanded. The supernatants from expanded LCLs then were assayed again for binding to VLPs, and bound antibodies were detected using either polyclonal anti-IgG (γ-specific) or anti-IgA (α- specific) secondary antibodies to

determine the isotype of the binding antibodies. Polyclonal secondary antibodies, instead of monoclonal antibodies, were used to minimize any differences in sensitivity of the secondary antibody to gamma or alpha chains and confirmed that the affinities of secondary antibodies did not differ measurably. About 100 wells of the 384 wells tested were positive for NV binding. Of all the binding antibodies, a higher proportion of lines contained NV-specific antibodies that were IgG than IgA. However, the proportion of NV-binding antibodies that also exhibited blocking activity was higher for IgA antibodies than for IgG, suggesting that mAbs of the IgA isotype are highly over-represented in the repertoire of antibodies that block receptor binding (FIG. 19). The cells in the positive wells were fused with a myeloma partner to generate a hybridoma clone. The inventors were able to obtain a panel of seven IgG (1A8, 2L8, 3I23, 4E7, 4I23 from Donor 1 and NV1, NV48 from Donor 2) and seven IgA (2J3, 3I3, 4B19, 4C10, 5I2 from Donor 1 and NV41, NV56 from Donor 2) clones. The proper molecular assembly of IgG and dimeric IgA was confirmed by resolving antibodies on SDS-PAGE gels and staining with Coomassie Blue reagent (FIG. 23).

[0242] IgA antibodies are more potent than IgG for receptor blocking – Interpretation of the curves for Ig binding to VLPs was conducted after normalizing for the differing molarity of binding sites of IgG and dimeric IgA. IgA antibodies as a class appeared to have a lower affinity for binding in the VLP binding assays when compared with IgG. Interestingly, however, this class distinction was not apparent in the assays to detect antibody mediated blocking of VLP binding to glycan. These data suggest that even lower affinity IgA antibodies can mediate potent blocking activity (FIG. 20). The inventors constructed average binding and blocking curves for IgG and IgA sets of antibodies using R software package and generated representative binding and blocking curves for IgG and IgA (FIG. 24). The difference between binding of average IgG and average IgA was significant ($p < 0.001$), while the blocking was not significant ($p = 0.39$). The inventors more fully characterized the antibodies obtained from Donor 1 in the experiments that follow.

[0243] Genotype-specific recognition of NoV VLPs by mAbs. – The genotype specificity of mAb binding was assessed by direct antigen ELISA. VLPs representing different human NoV genotypes were coated on an ELISA plate. Each of the mAbs isolated bound Norwalk VLPs (GI.1) and none of the mAbs detected the other GI or GII NoV genotypes tested (FIG. 21A).

[0244] MAbs bind to the P domain of the major capsid protein VP1 – Direct antigen ELISA was performed for domain mapping of the human mAbs. All 10 hmAbs bound to wild-type NV VLPs, mediated by binding to the major capsid protein VP1 (FIG. 21B). The VP1 protein has two major domains, the highly conserved shell domain and the highly variable protruding (P) domain. Each of the 10 mAbs bound to recombinant P domain preparations, suggesting that their binding epitopes are contained within the P domain. Further support for this conclusion was provided by loss of mAb binding to VLPs assembled from a mutated VP1 (designated CT303) in which the P domain had been deleted (Bertolotti-Ciarlet, *et al.*, 2002; Huang, *et al.*, 2014). The inventors also tested NV VLPs with ablated HBGA binding through introduction of a point mutation (W375A) in the HBGA binding domain. Two of the mAbs failed to bind W375A VLPs, suggesting that the residue at this position influences VP1 recognition by mAbs 3I23 IgG and 4I23 IgG (FIG. 21B). The inventors tested the specificity of three representative antibodies (2L8 IgG, 3I23 IgG and 5I2 IgA) to block VLP binding to diverse HBGA ligands, including H types 1, 2, 3 and Le(y). In every case the mAbs exhibited a strong inhibitory effect, except for 2L8 IgG, which had reduced activity to block binding to H type 3 tri-A and Le(y) (FIG. 21C).

[0245] MAbs from a NV-infected individual bind nonlinear epitopes – To characterize the epitopes recognized by mAbs derived from 335 Donor 1, we tested their ability to bind the NV major capsid protein VP1 by western blot (FIG. 22A and 22B). Murine mAbs 3901 or 8812 have been described previously to bind to linear or nonlinear epitopes, respectively, and were used as controls in this experiment (Hardy, *et al.*, 1996). Each of the 10 human mAbs and the murine mAb 8812 bound to unboiled preparations of NV VLPs, suggesting that the human mAbs recognize nonlinear epitopes in the major capsid protein VP1 (panel A). None of the human mAbs bound to denatured VP1 (panel B). Consistent with this finding, murine mAb 3901, but not murine mAb 8812, bound to denatured VP1.

[0246] MAbs recognize at least 3 overlapping epitopes in VP1 – Epitope binning was carried out by competition-binding ELISA. MAbs were assessed in a pairwise manner for their ability to inhibit binding of each other to NoV VLPs by ELISA (FIG. 22C). The observed patterns of competition-binding suggest that most of the mAbs bind to one major antigenic site. However, a few mAbs (2L8 IgG and 3I23 IgG) failed to inhibit capture of NV VLPs by other mAbs (4B19 IgA, 4C10 IgA, 4I23 IgG, 5I2 IgA). These observations suggest that members of the panel of mAbs bind to at least three distinct, but likely overlapping, epitopes on VP1.

[0247] **Isotype-switch variant IgG or IgA mAbs** – Although IgA mAbs as a group appeared more potent in receptor blocking, that comparison is complicated by the fact that the IgA or IgG antibodies compared above with respect to blocking activity do not share the same variable regions. To 356 determine if the variable domains of these antibodies contribute to the differences in activity, we analyzed the variable heavy and light chain genes of the antibodies, but did not find any unusual features in any of the antibody class in terms of gene families, mutation rate or CDR3 lengths (FIG. 27). To study the effects of antibody isotype on functional activity in a more defined manner, we prepared IgG or IgA versions of representative blocking antibodies using mammalian cell recombinant expression of isotype-switch variant Ig molecules. [0248] The inventors synthesized cDNAs coding for the variable domains after optimizing the sequence of the genes computationally for expression in mammalian cells. The heavy chain antibody variable genes were cloned in expression vectors for expression as γ or α chain. The light chain antibody variable genes were cloned in expression vectors for expression as κ or λ chains. Recombinant polymeric IgA was obtained by co-expression of joining (J) chain along with the heavy and light chains. Electrophoresis of purified proteins on SDS-PAGE gels under non-reducing conditions confirmed the correct assembly of IgG and dimeric IgA (FIG. 25). After normalizing for molarity of binding sites (IgG = 2, mIgA = 2, and dIgA = 4), each set of antibodies was tested in the binding and blocking assays. The inventors calculated half-maximal effective concentrations (EC_{50}) at which binding or blocking occurred. To enable comparison between the molecular forms of each antibody, the inventors calculated the ratio of *blocking* EC_{50} to *binding* EC_{50} for each antibody. A lower ratio of blocking to binding indicated that smaller amounts of antibodies bound to VLP were needed for blocking the VLP binding to their receptor. In the antibodies we tested, mIgA and dIgA exhibited lower blocking to-binding ratios than IgG (Table 4 and FIG. 26). Interestingly, the ratio was lower for mIgA than for IgG, despite these Igs having a similar molecular weight (150 kDa vs. 170 kDa). The activity of IgG versus IgA forms of the antibodies showed a similar trend in hemagglutination inhibition assays (Table 5). This observation suggested that the higher potency of the IgA class of antibodies for blocking stems not only from their potential to make large polymeric Ig molecules with large capacity for steric hindrance following binding, but also from structural or functional features that are found even in the monomeric form of Ig molecules of that isotype.

[0249] **Table 4 Blocking potencies of various isotypes of anti-NV mAbs** IgG (red), monomeric IgA (mIgA, blue) or dimeric IgA (dIgA, blue) forms of anti-NoV mAbs were tested for binding to VLP and for blocking VLP-glycan interaction after adjusting for the number of valencies in each moiety (IgG and mIgA = 2; dIgA =4). The concentration at which half-maximal binding (EC₅₀) or inhibition (IC₅₀) occurred was calculated from non-linear regression analysis. The ratio of IC₅₀ to EC₅₀ suggests that more IgG is needed for blocking activity compared to mIgA or dIgA for all the three antibodies compared.

Parent hybridoma	Recombinant isotype variants								
	EC ₅₀ (nM)			IC ₅₀ (nM)			IC ₅₀ / EC ₅₀		
	IgG	mIgA	dIgA	IgG	mIgA	dIgA	IgG	mIgA	dIgA
3I23 IgG	2.7	2.3	1.6	19.7	9.8	4.9	7.3	4.3	3.0
4I23 IgG	0.6	2.2	2.1	19.6	11.5	17.5	32.7	5.3	8.2
2J3 IgA	0.4	0.6	0.6	17.9	15.3	11.9	44.8	23.8	19.6
4C10 IgA	1.4	2.1	1.4	13.9	9.2	9.6	9.9	4.4	6.9
5I2 IgA	3.3	3.2	2.4	32.1	18.2	12.4	9.8	5.7	5.3

[0250] **Table 5 Hemagglutination inhibition activity for recombinant isotype switch variants.** Hemagglutination inhibition assays were performed as described previously (Czako, *et al.*, 2012). In brief, human type O erythrocytes were collected from a healthy adult in Alsever's buffer, washed twice in Dulbecco's phosphate-buffered saline (PBS) without Ca²⁺ or Mg²⁺, and pelleted by centrifugation at 500 x g for 10 min at 4 °C. Monoclonal antibodies (mAb; starting concentration 60 µg/mL) were diluted initially 1:10 in PBS with 0.85% saline (pH 5.5), and then serially 2-fold diluted. Four hemagglutination units (~2 ng) 683 of Norwalk virus VLPs were mixed with the diluted monoclonal antibodies and incubated at room temperature for 30 min. The VLP-mAb mixture was added to an equal volume of 0.5% washed type O erythrocytes in 0.85% saline (pH 6.2) and incubated for 2 h at 4 °C. The HAI titer was determined by identifying the reciprocal of the highest dilution of mAb that inhibited hemagglutination by the VLPs.

Isotype	3I23	4I23	2J3	4C10	5I2
IgG	320	20	80	640	640
mIgA	2,560	40	80	640	160
dIgA	640	40	160	1,280	640

Significance of Certain Embodiments

[0250] The molecular basis of antibody-mediated inhibition of human NoV infection is poorly understood. It has been difficult to study NoV neutralization because of the lack of a robust cell culture system for growing virus. Recent studies suggest, however, that the presence of antibodies that block NoV-HBGA interactions is associated with protection against illness (Atmar, *et al.*, 2011; Jiang, *et al.*, 1992; Choi, *et al.*, 2008). In this model, antibodies that disrupt the interaction of NoV VLPs with HBGA ligands thus act as putative virus neutralizing antibodies. The structural and functional features of these putative neutralizing antibodies are not known. Blocking activity of purified, serum derived IgA antibodies was recently described, and our group recently identified serum IgA and salivary IgA antibodies as novel correlates of protection from NoV gastroenteritis (Atmar, *et al.*, 2015; Ramani, *et al.*, 2015; Lindesmith, *et al.*, 2015). In the studies presented here, the inventors isolated a panel of hmAbs with potent NoV-HBGA blocking activity, representing IgA and IgG isotypes, from an immune individual following experimental virus challenge. The features of these antibodies reveal new aspects of antibody-mediated NoV inhibition.

[0251] An interesting finding from these detailed studies is that naturally-occurring NoV specific human antibodies of the IgA isotype exhibit enhanced potency for receptor blocking, compared to IgG antibodies isolated in a similar fashion. These data suggest that this enhanced potency stems from two principal factors. First, there appears to be some intrinsic structural or functional features of the IgA isotype that confer enhanced blocking activity, even in monomeric forms of IgA antibodies, compared to a matched IgG variant. It is known that IgG and IgA molecules differ in certain functional aspects, due to sequence polymorphisms in the constant domain (Pritsch, *et al.*, 2000; Torres, *et al.*, 2007). In fact, previous studies with antibodies to other microbial agents have suggested that polymorphisms in the constant regions even of differing IgG subclasses can mediate a profound phenotypic change in the pattern binding of antibodies (McLean, *et al.*, 2002). One can determine the molecular basis for this effect against NV virus, but the enhancement is of interest as it has relevance to both antibody and vaccine design efforts.

[0252] Second, dimeric IgAs exhibited enhanced potency for blocking compared to matched monomeric IgA or IgG counterparts. Most likely, this finding is due to the large molecular weight of dimeric IgA, which probably facilitates a more profound receptor blocking capacity.

[0253] It was interesting that many of the B cells the inventors isolated from blood that encoded NoV-specific IgA secreted polymeric IgA in the naturally-occurring form. It has been noted previously that secreted IgA proteins in the serum typically are almost exclusively monomeric. However, the inventors did not study serum antibodies here; rather they isolated NoV-specific IgA-encoding B cells from the blood, and many of these secreted dimeric IgA after recovery. It is possible that these cells are circulating in peripheral blood en route to mucosal tissues. The technique that was used predominantly isolates memory B cells, and the inventors isolated these cells during the convalescent phase from the donor. Therefore, it is not anticipated that the cells would have been secreting dimeric IgA into the serum in the donor.

[0254] Sequence analysis was performed of the antibody variable 426 genes encoding these mAbs. The data show that there is a wide diversity of antibody variable genes that encode antibodies that block receptor binding. This finding is encouraging, because it suggests that there is no genetic restriction on the ability of diverse humans to make receptor-blocking antibodies to NoV. The inventors examined the genetic features of the antibodies to see if there were any unusual characteristics. Some especially important domains in viral surface proteins that are susceptible to recognition by potent virus-neutralizing antibodies, such as HIV envelope or influenza hemagglutinin, are associated with unusual genetic and structural features such as long heavy chain CDR3 regions or a very high level of somatic mutations (McLellan, *et al.*, 2011; Pancera, *et al.*, 2010; Zhou, *et al.*, 2010). The NoV receptor-blocking antibodies did not possess any extreme genetic features. Diverse antibody variable genes were used, and the level of somatic mutation observed was typical of that found in human memory B cells (Briney, *et al.*, 2012a; Briney, *et al.*, 2012b). The length of heavy chain CDR3 regions was average, and there was no unusual occurrence of insertions or deletions.

[0255] Human NoVs cause acute gastroenteritis worldwide, and they exhibit a high degree of genetic variation in different geographical locations. Field strains of NoV evolve, and dominant novel strains emerge periodically that exhibit antigenic variation and differential glycan-binding specificities due to genetic changes that alter structure in the P domain of the NoV capsid protein (Shanker, *et al.*, 2011; Lindesmith, *et al.*, 2012). HBGAs most likely function as co-receptors or cell attachment factors to NoVs, and thus they

determine susceptibility to infection (Tan, *et al.*, 2014; Shanker, *et al.*, 2011; Ruvoën-Clouet, *et al.*, 2013).

* * *

[0256] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

[0257] All patents and publications mentioned in this specification are indicative of the level of those skilled in the art to which the invention pertains. All patents and publications herein are incorporated by reference to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference in their entirety.

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CLAIMS

We claim:

1. A conformation-specific antibody that binds a three-dimensional epitope comprising residue H381 of SEQ ID NO:61.
2. A composition comprising the antibody of claim 1.
3. The composition of claim 1 or 2, wherein the antibody comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, or a combination thereof, or a functionally active derivative thereof.
4. As a composition of matter, a binding polypeptide comprising, consisting of, or consisting essentially of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or a combination thereof, or a functionally active derivative thereof.
5. The composition of claim 4, wherein the functionally active derivative thereof is 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, respectively.

6. A method of blocking or preventing the binding of a Norovirus particle to a histo-blood group antigen (HBGA) in an individual susceptible thereto, comprising the step of providing to the individual an effective amount of a non-natural, chimeric, isolated, recombinant, and/or humanized binding polypeptide that blocks the binding of the particle to the HBGA.

7. The method of claim 6, wherein the binding polypeptide is monoclonal antibody 5I2, 1A8, 2J3, 2L8, 3I3, 3I23, 4B19, 4C10, 4E7, 4I23, 5I12, NORO 105, NORO 115, NORO 118, or NORO 123.

8. The method of claim 6 or 7, wherein the binding polypeptide comprises part or all of SEQ ID NO:1 and/or SEQ ID NO:2 or a functionally active (binding) derivative thereof.

9. The method of claim 8, wherein the functionally active derivative thereof is at least or is 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to SEQ ID NO:1 or SEQ ID NO:2, respectively.

10. The method of claim 6 or 7, wherein the binding polypeptide comprises part or all of SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, , SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53 or a functionally active (binding) derivative thereof.

11. The method of claim 10, wherein the functionally active derivative thereof is at least or is 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, , SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53 respectively.

12. The method of any one of claims 6-11, wherein the Norovirus is the GI.1 genotype.

13. A method of treating an individual for Norovirus infection or preventing Norovirus infection in an individual, comprising the step of providing to the individual a therapeutically effective amount of monoclonal antibody 5I2, 1A8, 2J3, 2L8, 3I3, 3I23, 4B19, 4C10, 4E7, 4I23, or 5I12.

14. A method of treating an individual for Norovirus infection or preventing Norovirus infection in an individual, comprising the step of providing to the individual a therapeutically effective amount of a binding polypeptide or antibody comprising one or more of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53 or a functionally active (binding) derivative thereof.

15. The method of claim 14, wherein the functionally active derivative thereof is at least or is 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, , SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, respectively.

16. A method of making an antibody, comprising the steps of harvesting from a host cell heterogeneous sequence expressing SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID

NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or a functionally active (binding) derivative thereof.

17. A pharmaceutical composition comprising an antibody of claim 1 and/or a composition of claims 2, 3, 4, and/or 5.

18. A method of making a HBGA-blocking human monoclonal antibody comprising:

- a) generating a monoclonal antibody using as an antigen part or all of a Norovirus;
- b) screening monoclonal antibodies for specific binding to the Norovirus virus but that block binding of HBGA to Norovirus virus particles;
- c) humanizing one or more monoclonal antibodies screened for specific binding to the Norovirus virus; and
- d) screening the one or more humanized monoclonal antibodies for an ability to block binding of HBGA to Norovirus virus particles or virus-like particles.

19. A method of detecting Norovirus virus infection in an individual or detecting Norovirus from an environment, comprising the step of exposing a sample from the respective individual or environment to an antibody of claim 1, 3, and/or 7 and/or a composition of claim 4, 5, 8, and/or 9.

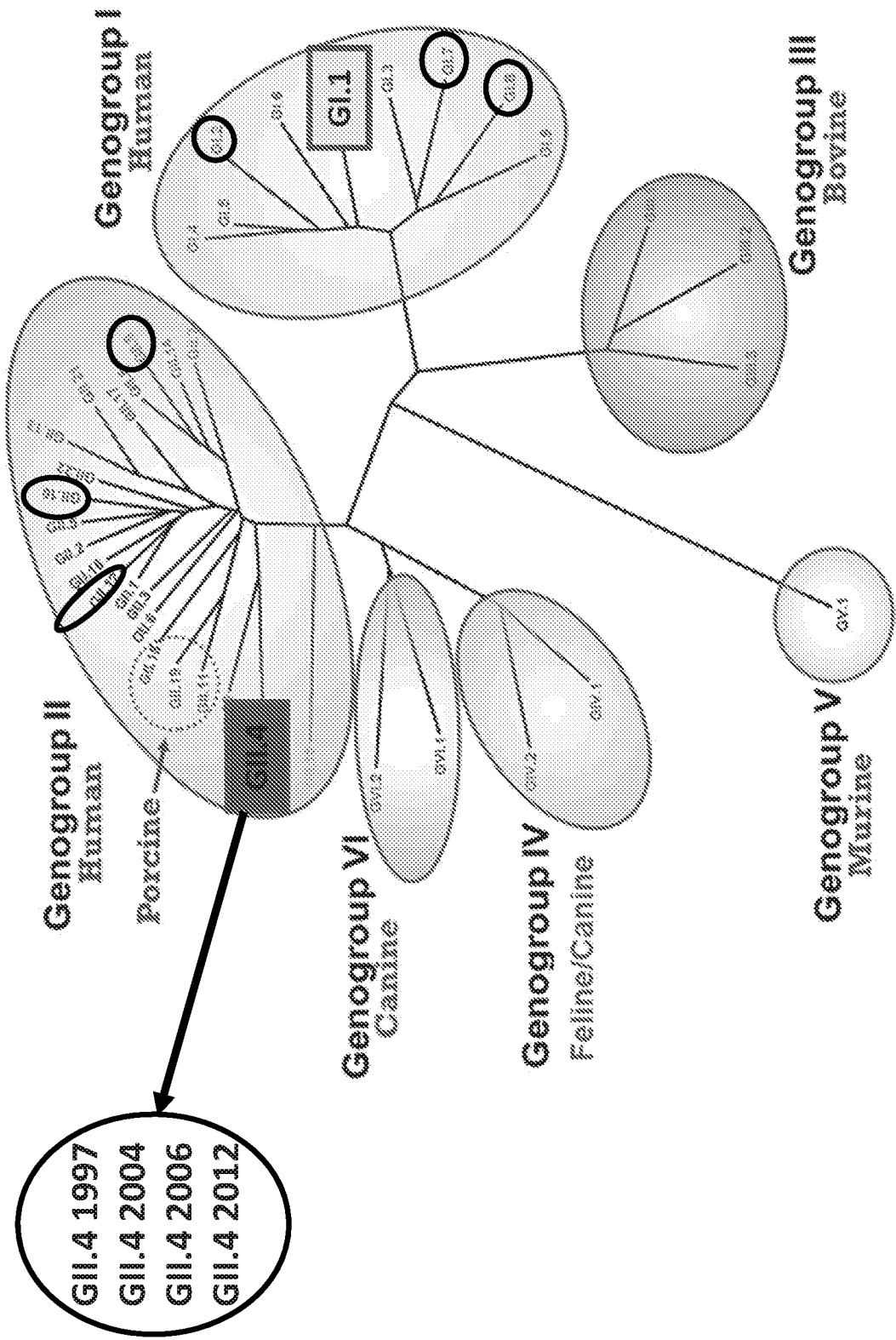
20. As a composition of matter, a nucleic acid sequence selected from the group consisting of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, or functional derivatives thereof that are at least 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25,

SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, or SEQ ID NO:60, respectively.

21. As a composition of matter, a polypeptide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53, or that is 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent identical to a polypeptide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEQ ID NO:49, SEQ ID NO:50, SEQ ID NO:51, SEQ ID NO:52, or SEQ ID NO:53.

22. A method of immunization against Norovirus infection comprising administering to an individual in need thereof a therapeutically effective amount of an antibody of claim 1 and/or a composition of claim 2, 3, 4, and/or 5.

Noroviruses Classification



Structural basis of HBGA binding to NoVs well characterized.
Structural basis of antigenic variations/neutralization is not known.

FIG. 2

Isolation and purification of anti NoVs hMAbs

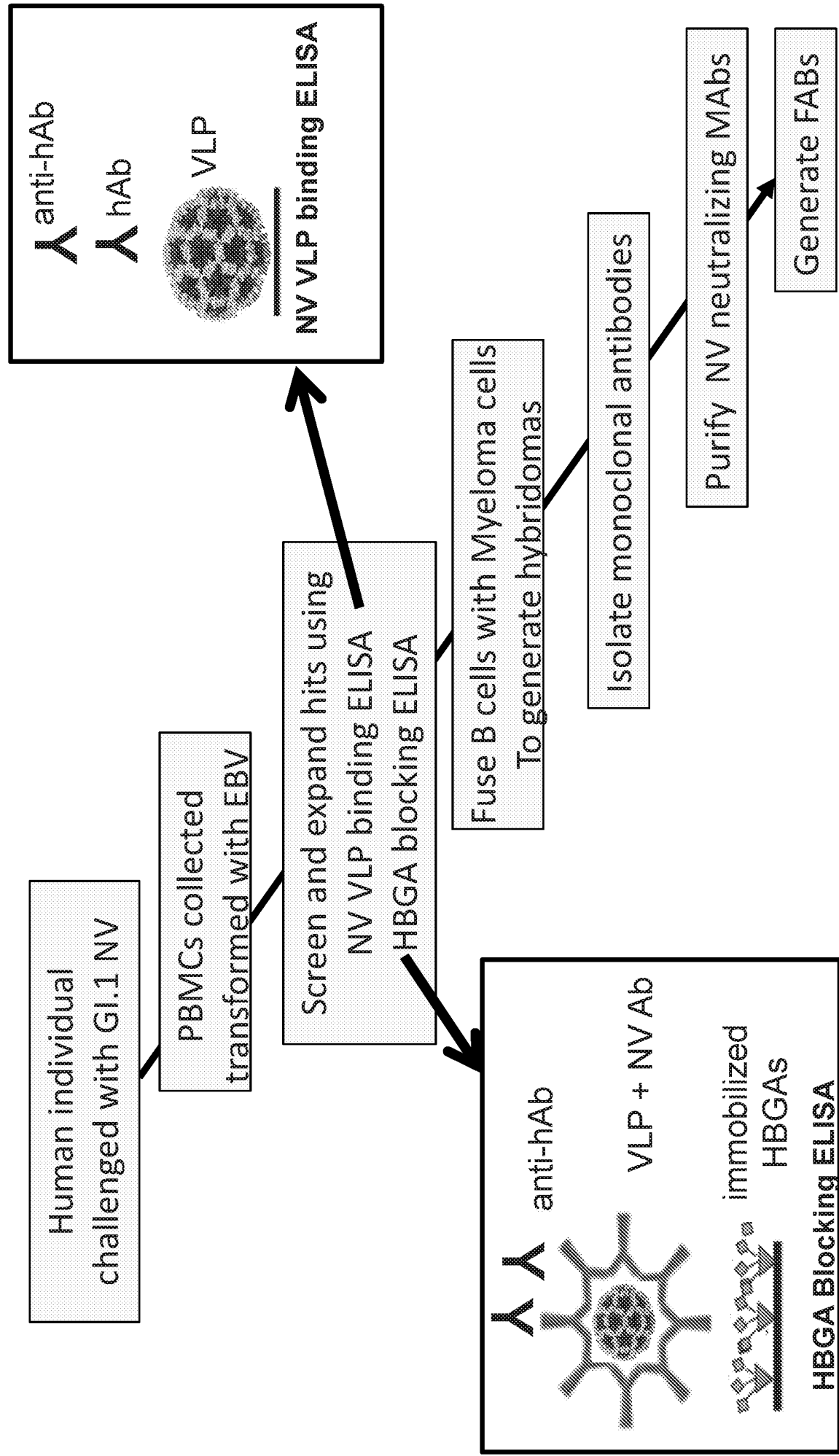
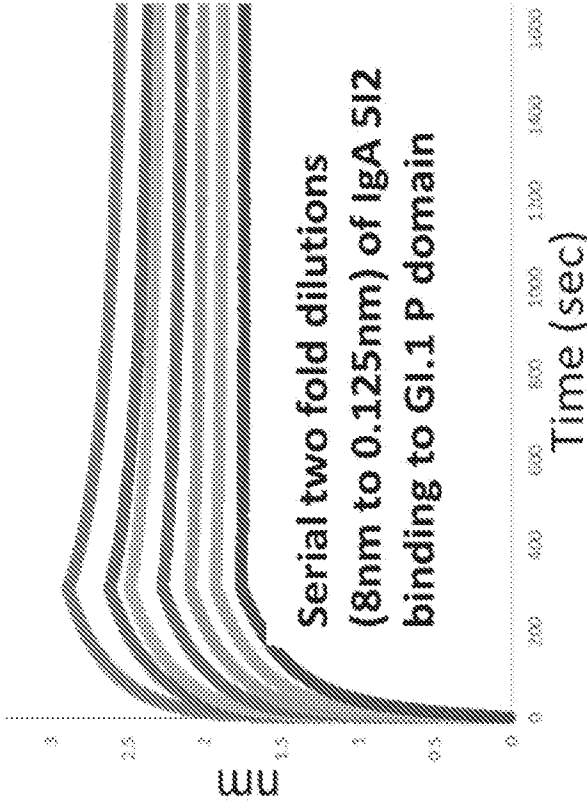
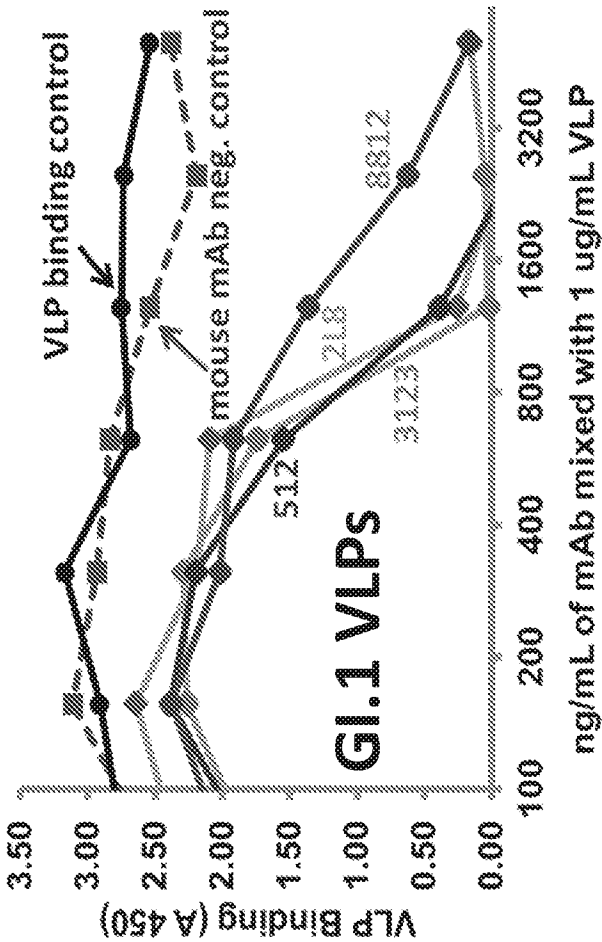
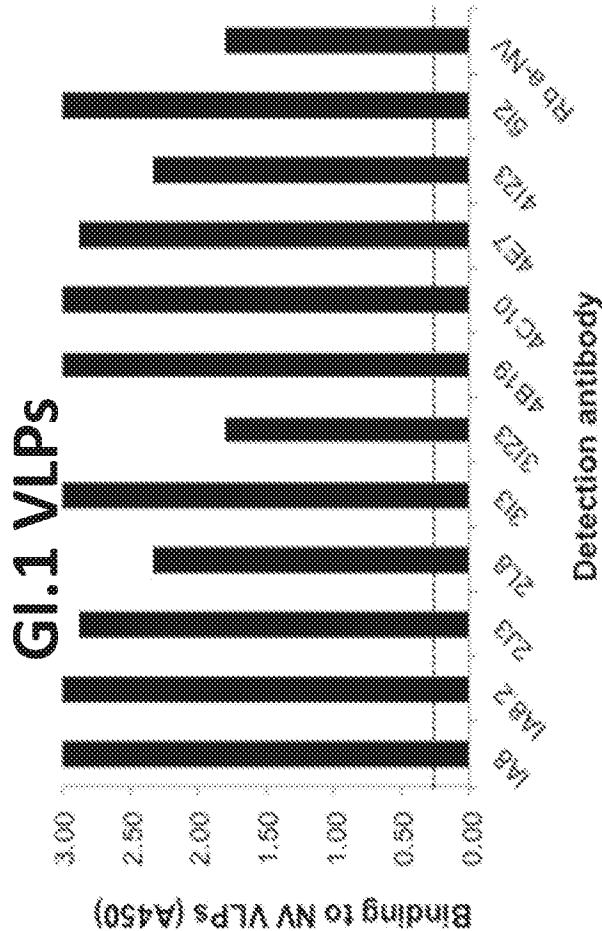


FIG. 3

Abs are specific to Gl.1 and exhibit HBGA blockade

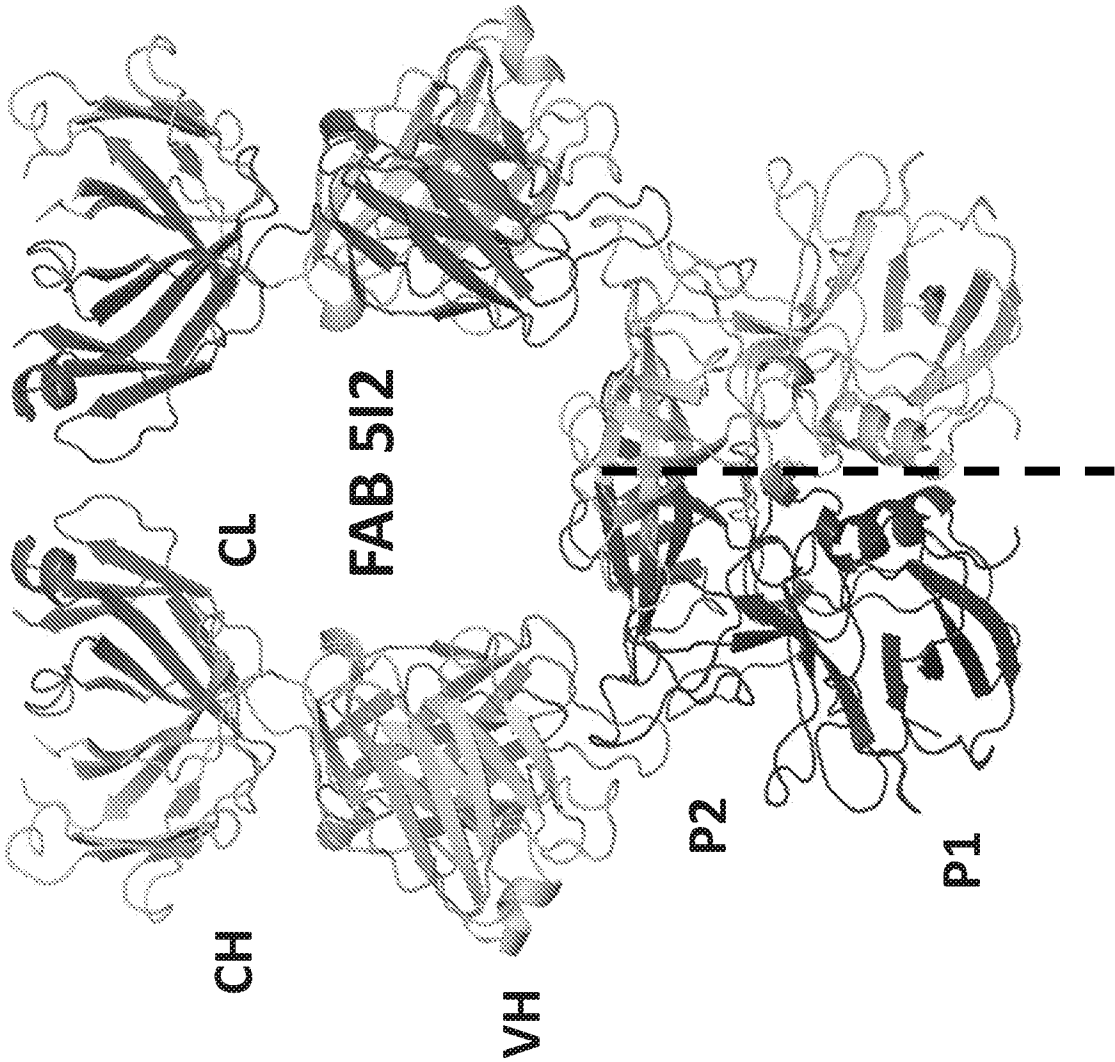


	Mab	FAB	MAB	MAB	MAB
	512	512	2L8	3123	4B19
KD (nM)	0.26	18	0.06	0.15	0.03
Chi ² /DoF	0.0017	0.016	0.0018	0.0038	0.0053
R ²	0.96	0.89	0.94	0.951	0.925

Bio Layer Interferometry studies show these NABs bind P domain with sub nanomolar affinities.

FIG. 4

Structure of NV P domain complexed with human IgA 5I2



Data collection statistics

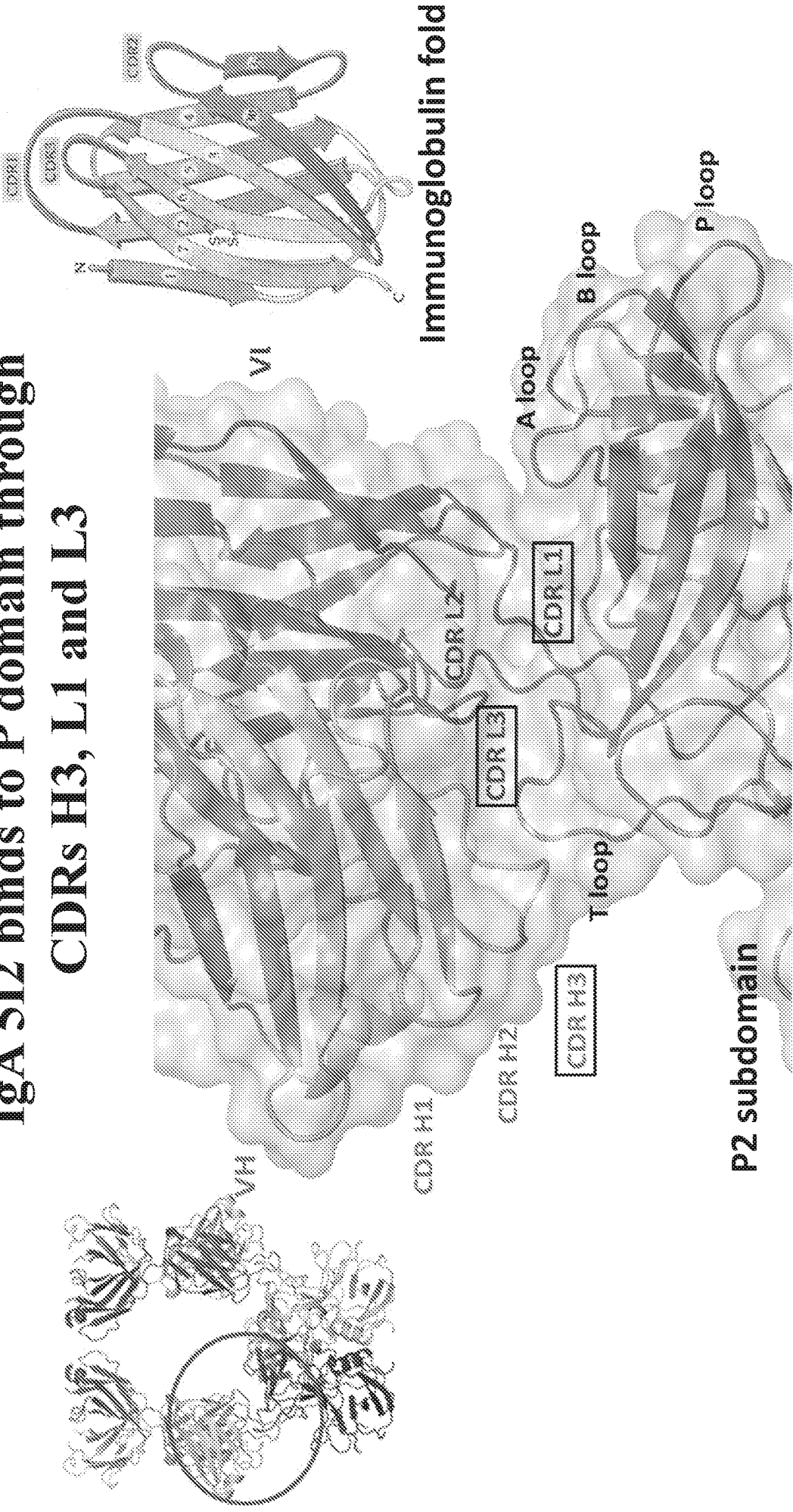
Space group : P6₅22
Resolution (Å): 2.3
Rmerge (%) : 10.9 (88)
Mean I/σ : 16.8 (2.8)
nMol/ Asym : 1

Refinement statistics

R_{fac}/R_{free}(%) : 20/24
RMSD
Bonds : 0.009
Angles : 1.30
Ramachandran
Favored : 98.7%

FIG. 5

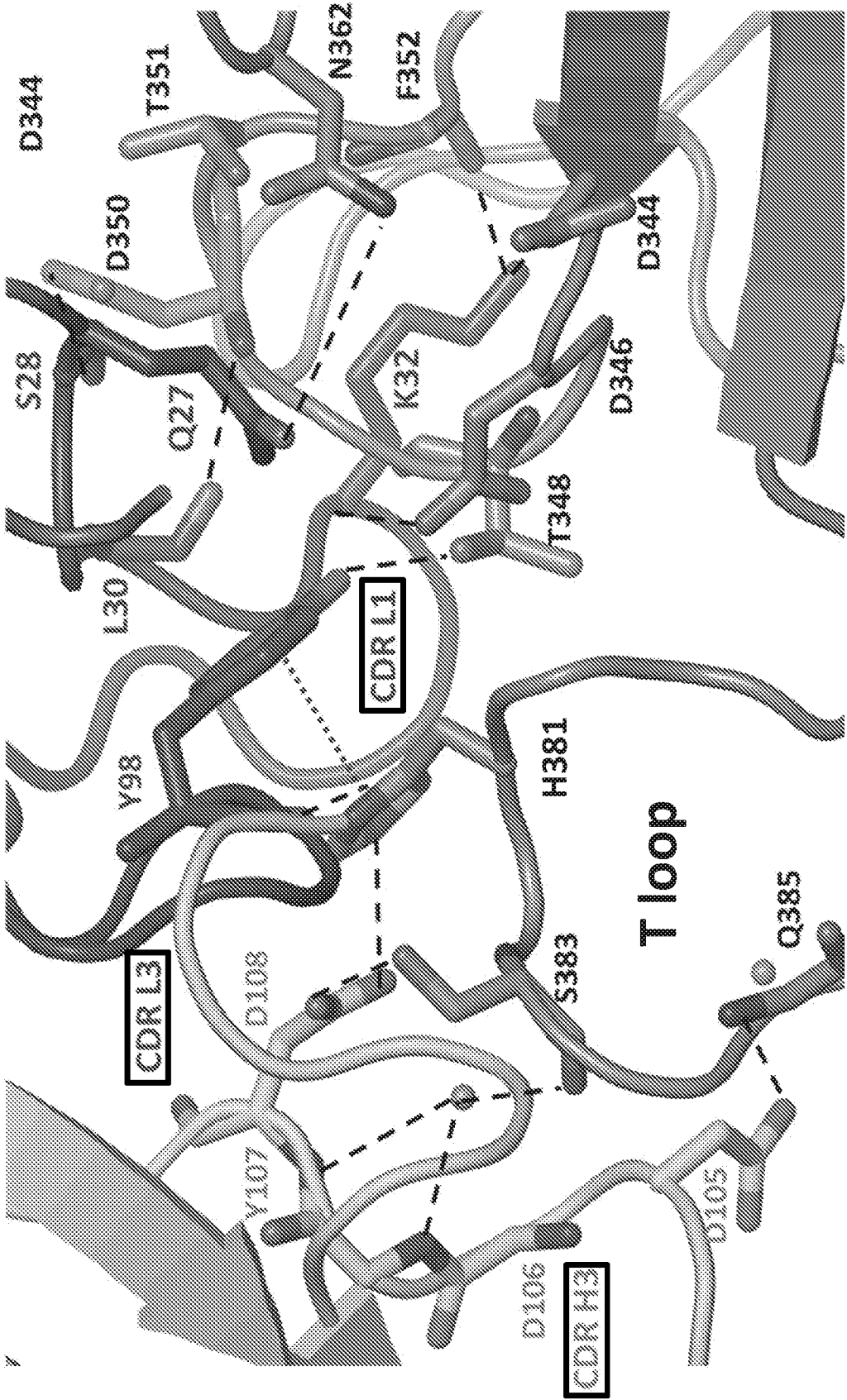
**IgA 5I2 binds to P domain through
CDRs H3, L1 and L3**



Total buried interface area of 955 Å² indicates strong binding

FIG. 6

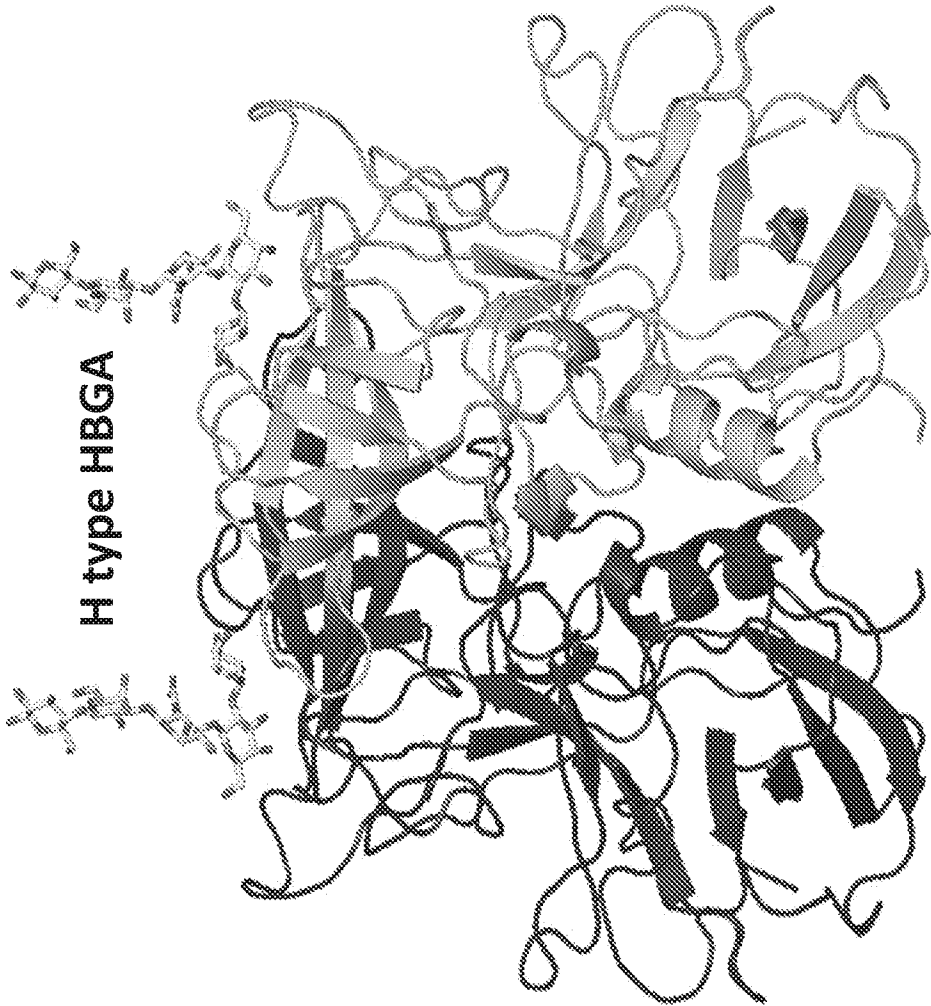
Close-up of P domain- 5I2 interactions



Hydrogen bonding interactions play a key role in FAB-P domain binding.

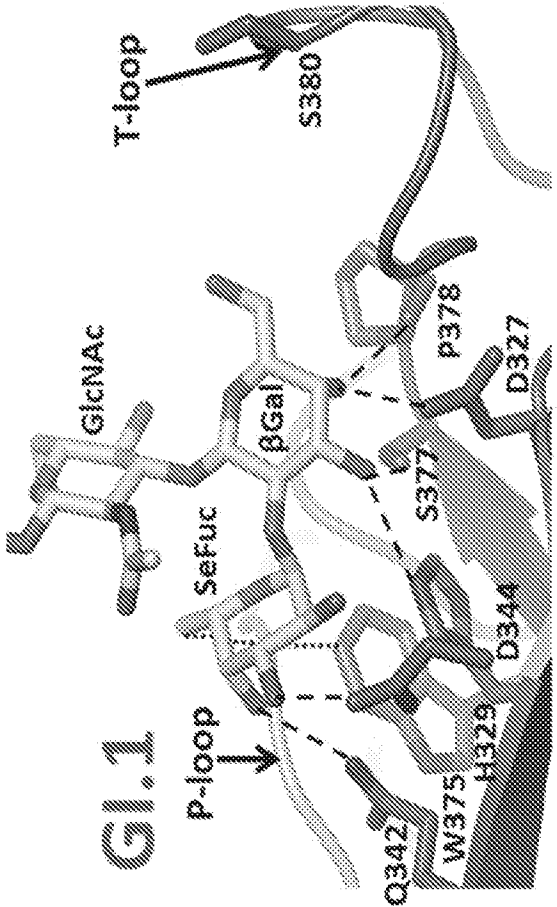
FIG. 7

Structure of NV P domain bound to H type HBGA



H type HBGA

P domain dimer



Gl.1

P-loop

T-loop

Choi et. al. PNAS, 2008

FIG. 8

IgA 512 blocks HBGA binding through steric hindrance

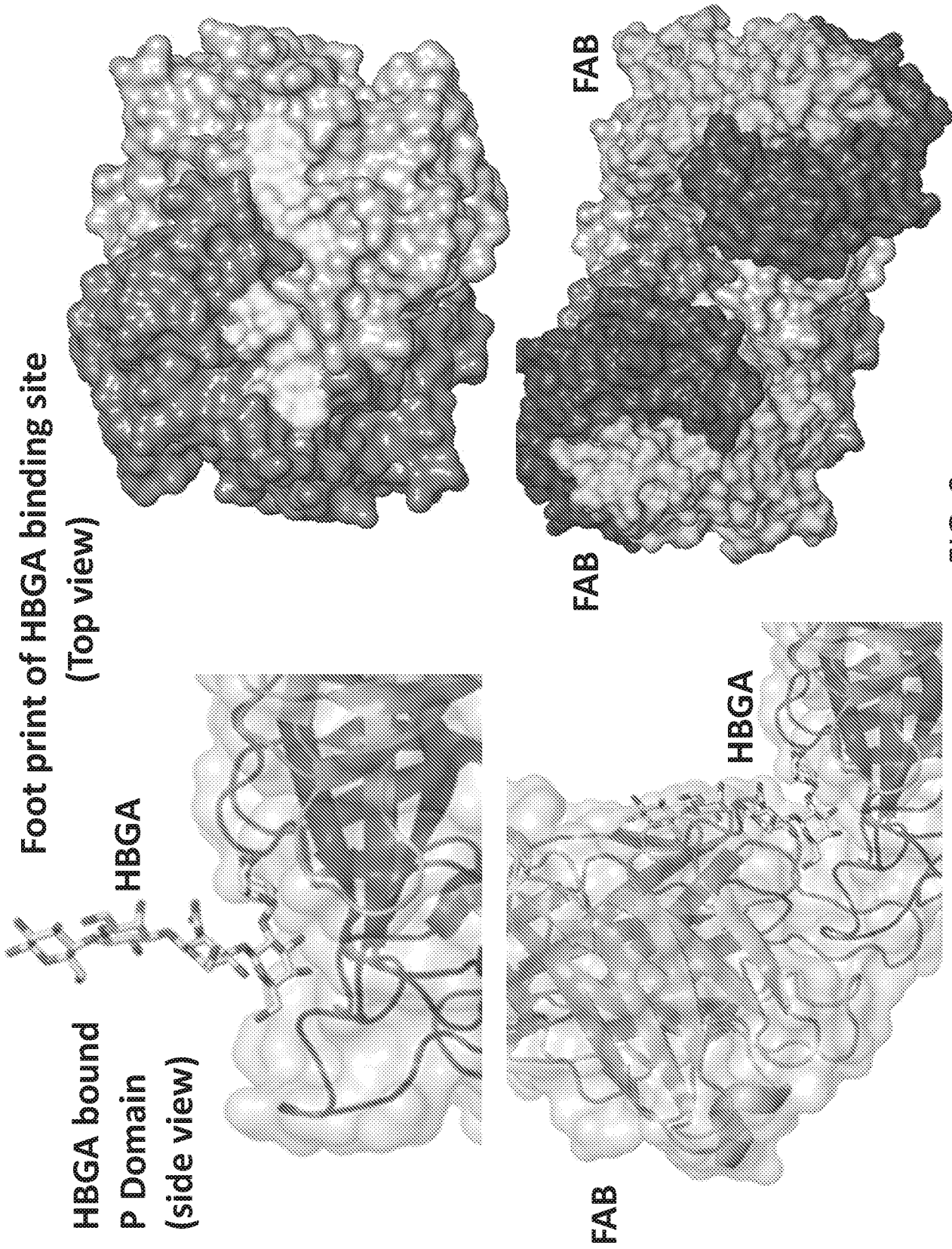
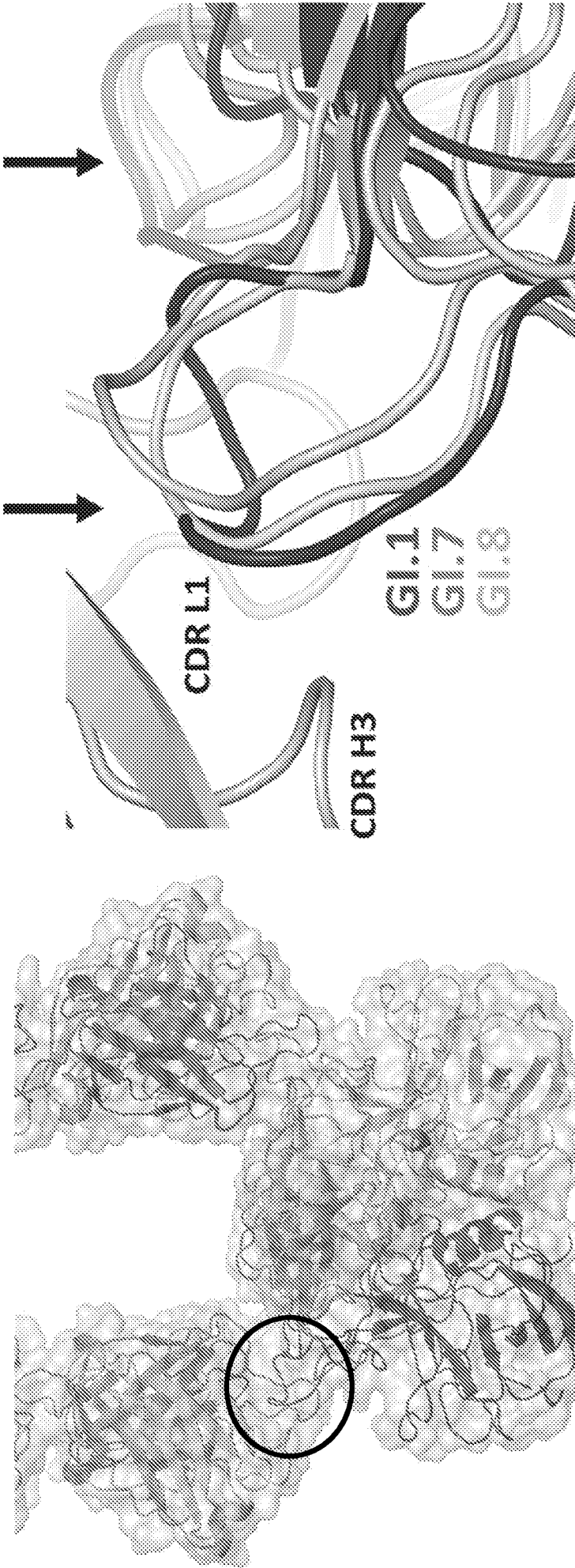


FIG. 9

Sequence and structural and alterations mediate
IgA 5L2 genotype specificity



Residue #	GI.1	GI.2	GI.3	GI.4	GI.6	GI.7	GI.8	GI.9
344	D	S	Q	R	S	R	K	V
346	D	S	N	D	T	D	V	N
348	T	N	Q	Q	N	Q	Y	Y
351	T	G	A	S	S	P	T	Q
381	H	T	D	D	D	S	G	N
383	S	P	H	T	-	A	A	N
385	Q	D	D	H	Q	-	-	-

Choi et. al PNAS, 2008
Shanker et. al JVI, 2014
Hao et. al JVI, 2015

FIG. 10

Sequence ID	V-GENE and allele	J-GENE and allele	D-GENE and allele	CDR-IMGT lengths	AA JUNCTION
NV2L8_heavy	VH1-46*01	JH4*02	D6-13*01	8.8.14	CARGISWVVTGFDYW
NV5I2_heavy	VH1-69*01	JH4*02	D5-12*01	8.8.17	CASNRRANRADDYDYFDYW
NV4C10_heavy	VH3-20*01	JH5*01	D5-12*01	8.8.17	CARDNRGQRGSSFGWFDSW
NV3I23_heavy	VH3-23*01	JH4*02	D5-24*01	8.8.16	CAKGVGSDFPTSRLDSW
NV4I23_heavy	VH3-23*01	JH4*02	D4-17*01	8.8.14	CAKNAGDYAPSPADYW
NV4B19_heavy	VH3-30-3*01	JH4*02	D4-11*01	8.8.10	CARDLSASFDYW
NV2I3_heavy	VH4-38-2*02	JH6*03	D2-2*01	9.7.18	CARDRSVVVPAAPLYYMDVW
NV3I3_heavy	VH4-39*01	JH5*02	D3-3*01	10.7.13	CARHPSWDRSWFDPW
NV4E7_heavy	VH4-4*02	JH1*01	D6-25*01	9.7.12	CAIGGSASVPTKYW
NV1A8_heavy	VH4-4*07	JH4*02	D3-10*01	10.7.13	CAREFYGGRGVVDSW
NV4C10_light	VK1-39*01	JK1*01	-	6.3.9	CQQDYITPRTF
NV4E7_light	VK1-39*01	JK2*01	-	6.3.9	CQQSYSSPYTF
NV4B19_light	VK2-30*01	JK5*01	-	11.3.10	CMQGTHWPMFTF
NV3I23_light	VK3-20*01	JK3*01	-	7.3.8	CHQYGTSFTF
NV5I2_light	VK4-1*01	JK1*01	-	12.3.9	CQQYYSIPRTF
NV4I23_light	VK6-21*01	JK1*01	-	6.3.9	CHQSSTLPRTF
NV2L8_light	VL2-14*01	IL3*02	-	9.3.10	CSSYTRSSSTWVF
NV2I3_light	VL2-23*02	IL2*01	-	9.3.10	CCSYATSTNLLF
NV3I3_light	VL2-8*01	IL2*01	-	9.3.10	CSSYVGNNNFAF

FIG. 11

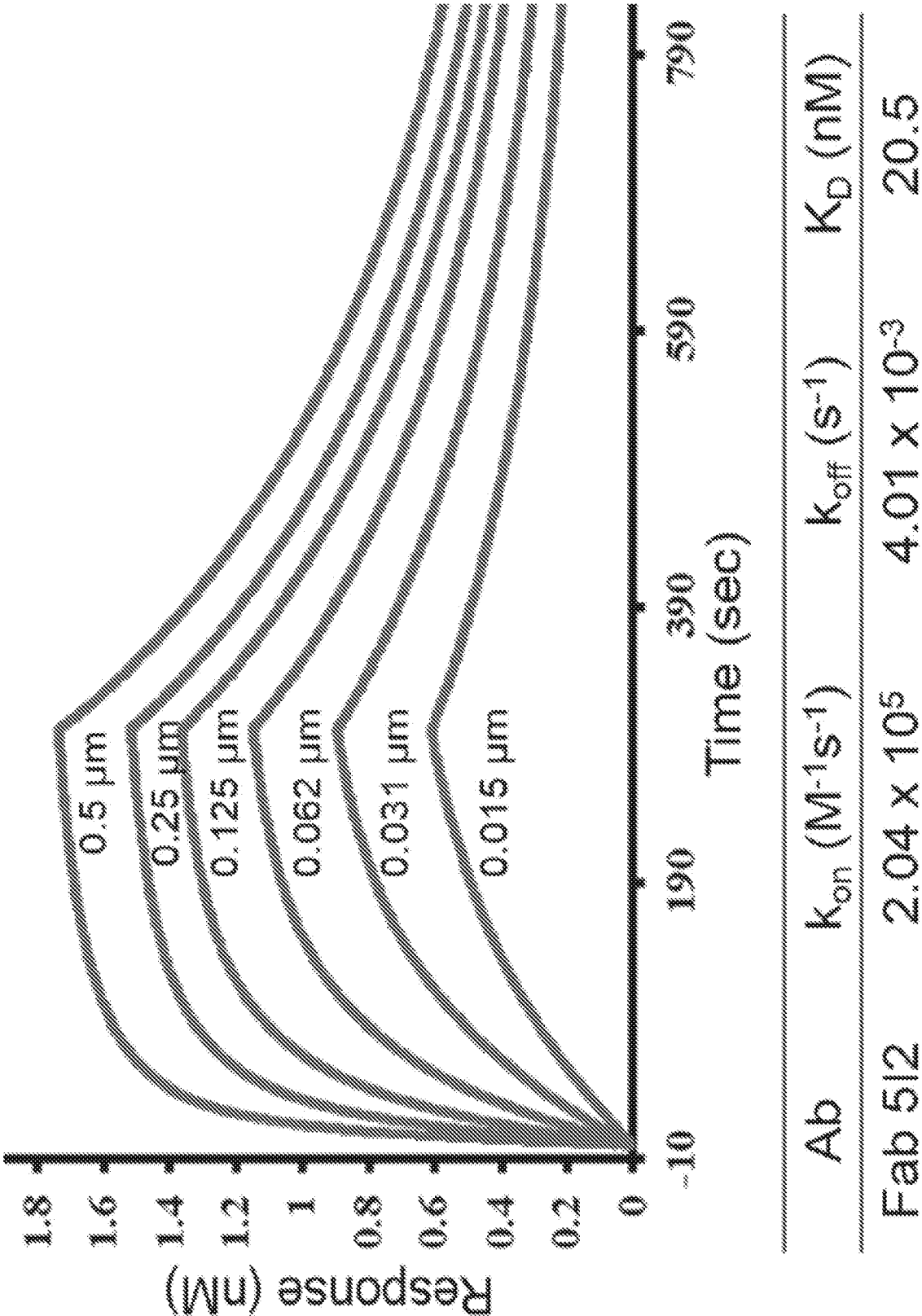


FIG. 12

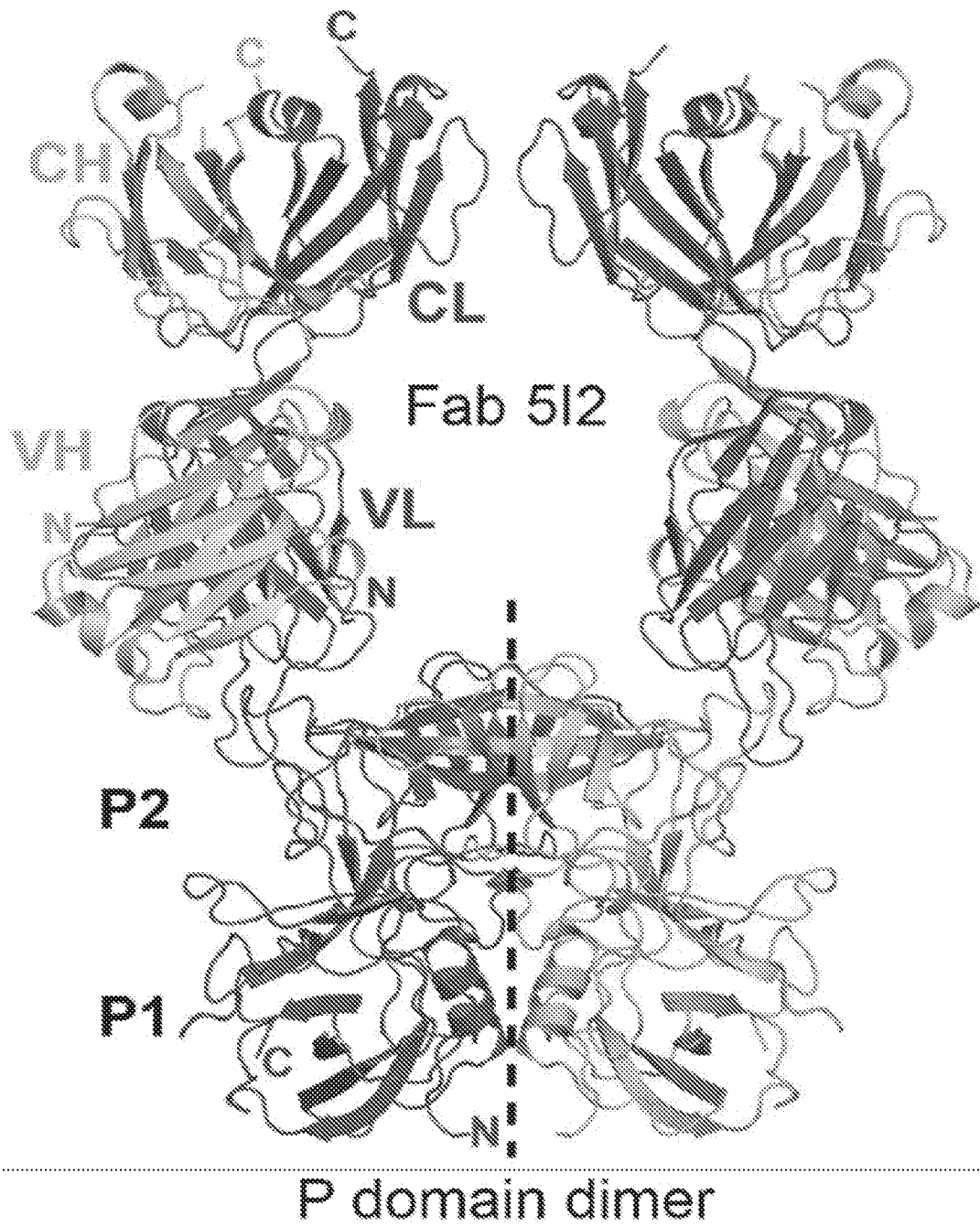


FIG. 13

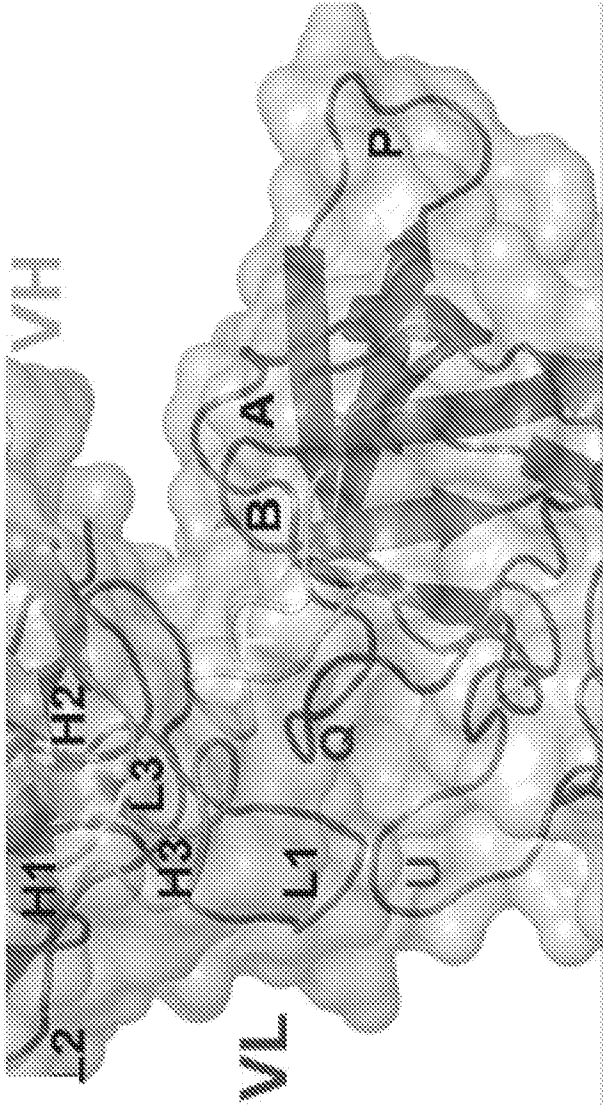


FIG. 14A

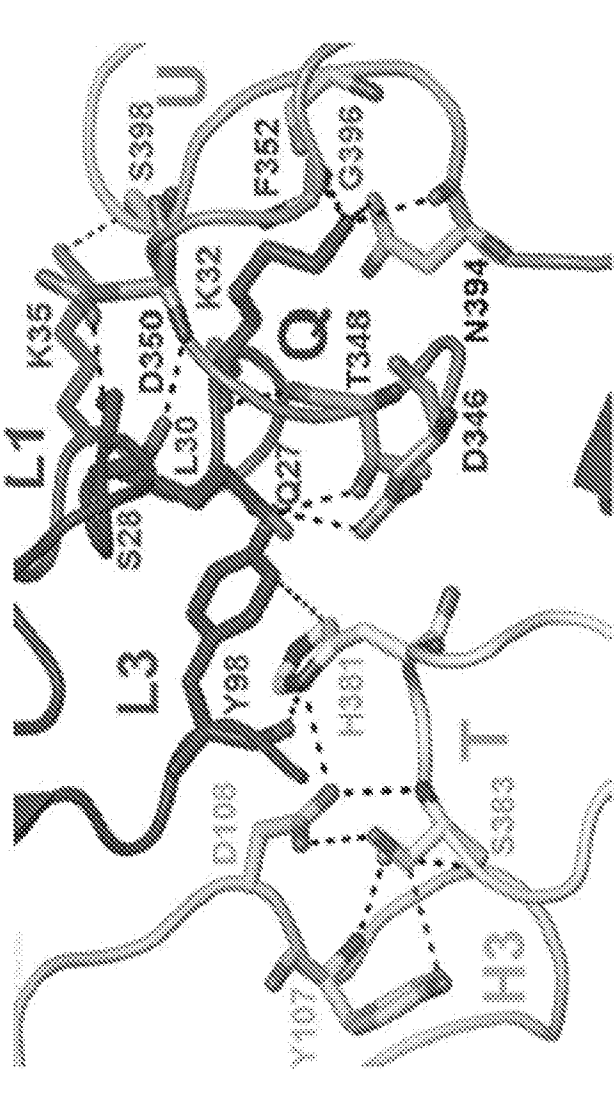


FIG. 14B

FIG. 15A

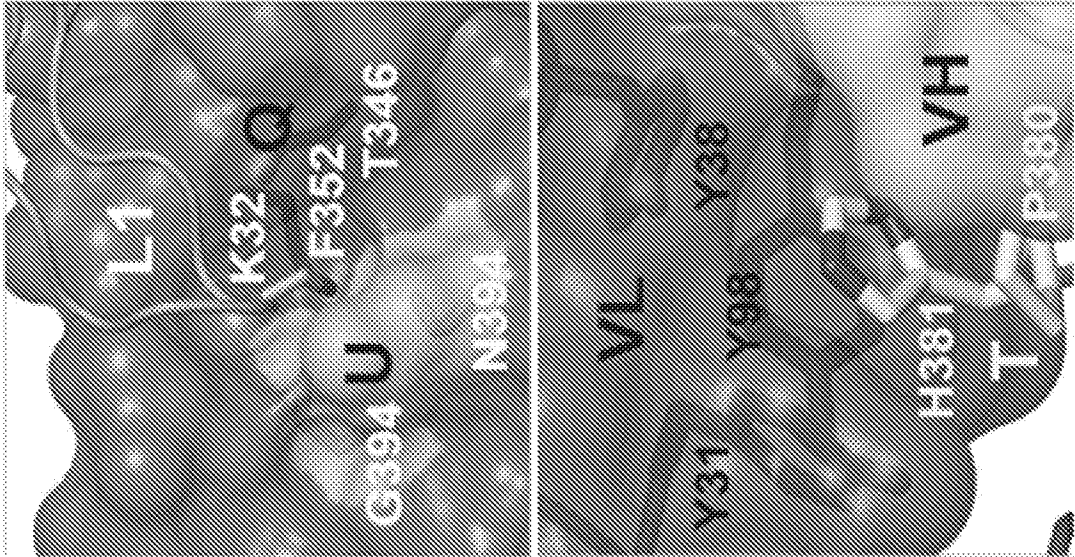


FIG. 15B

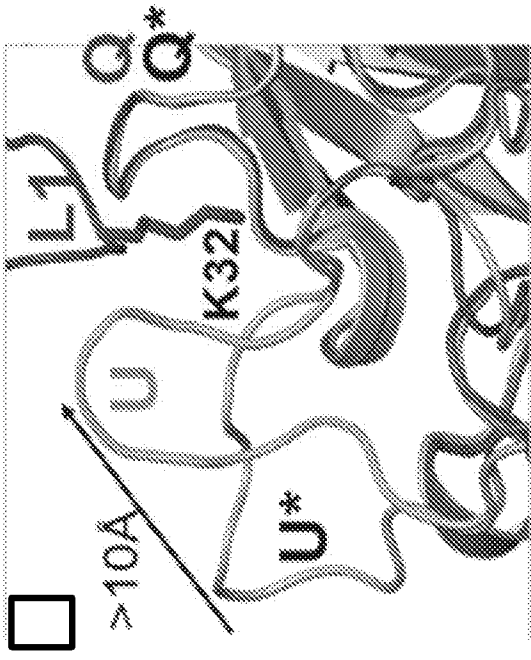


FIG. 15C

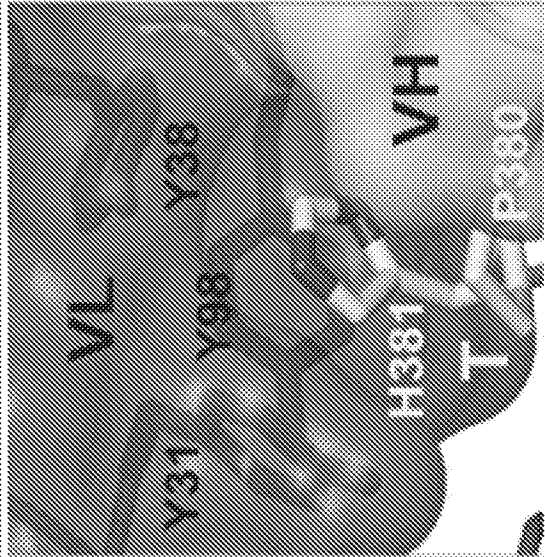


FIG. 15D

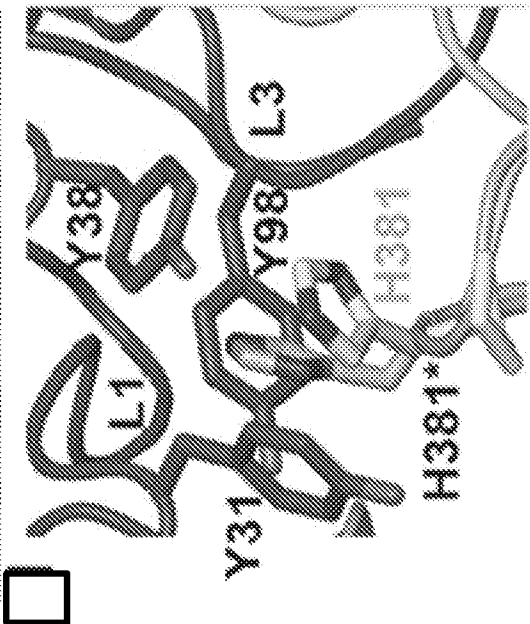
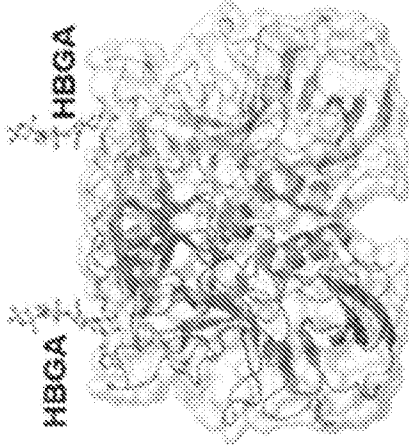


FIG. 16A



P domain dimer

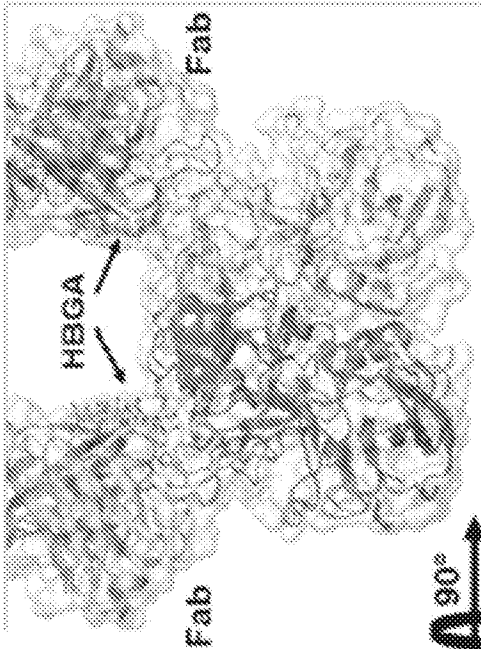
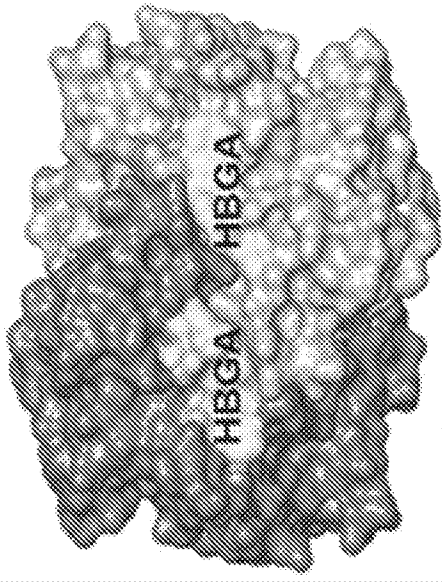


FIG. 16B



FIG. 16C



P domain dimer

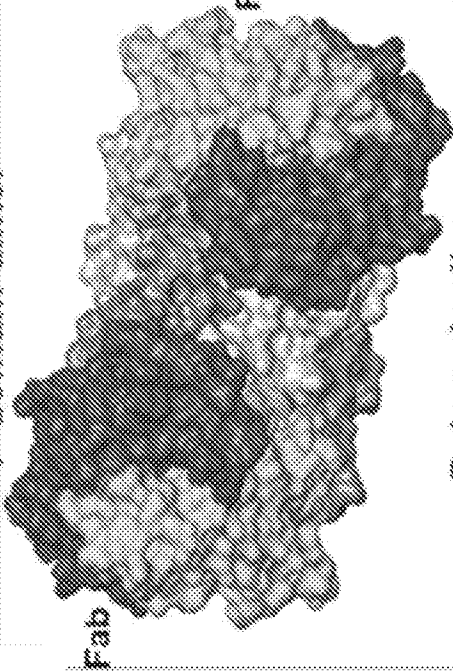


FIG. 16D

P domain dimer

FIG. 17A

NV residue#	Loop Q					Loop T			Loop U		
	346	348	350	351	352	380	381	383	394	396	398
G1.1	D	T	D	T	F	S	H	S	N	G	S
G1.2	S	N	Q	Q	F	S	T	P	D	G	S
G1.3	L	Q	S	A	F	S	D	H	R	G	T
G1.4	D	Q	A	S	F	S	D	T	S	G	T
G1.6	T	N	A	S	F	S	D	.	K	G	S
G1.7	D	Q	N	S	F	N	N	S	S	G	T
G1.8	V	Y	A	T	F	E	G	A	N	G	N
G1.9	N	Y	L	Q	F	T	N	N	N	G	T

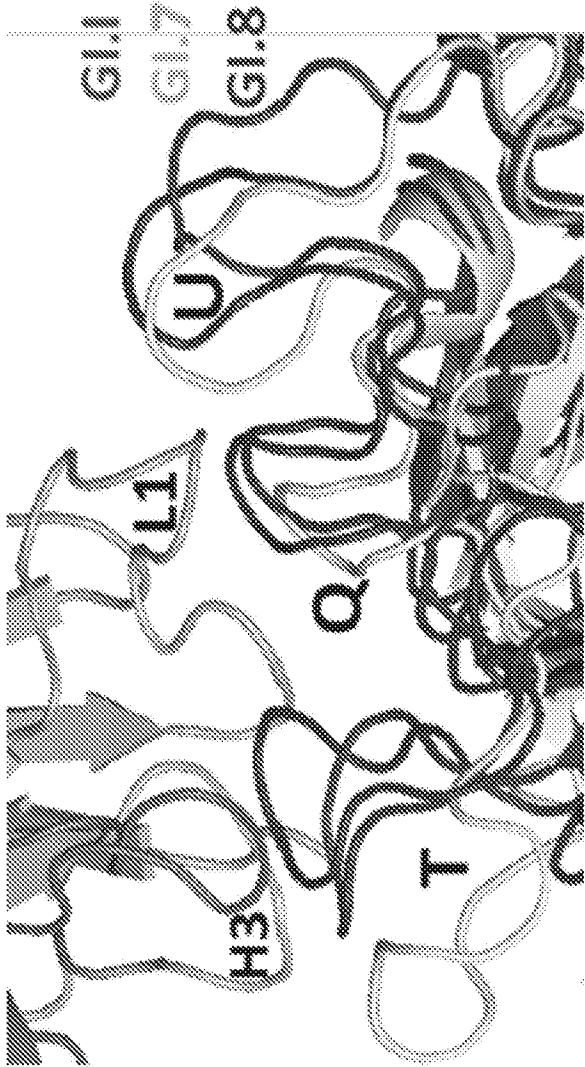


FIG. 17B

FIG. 18A

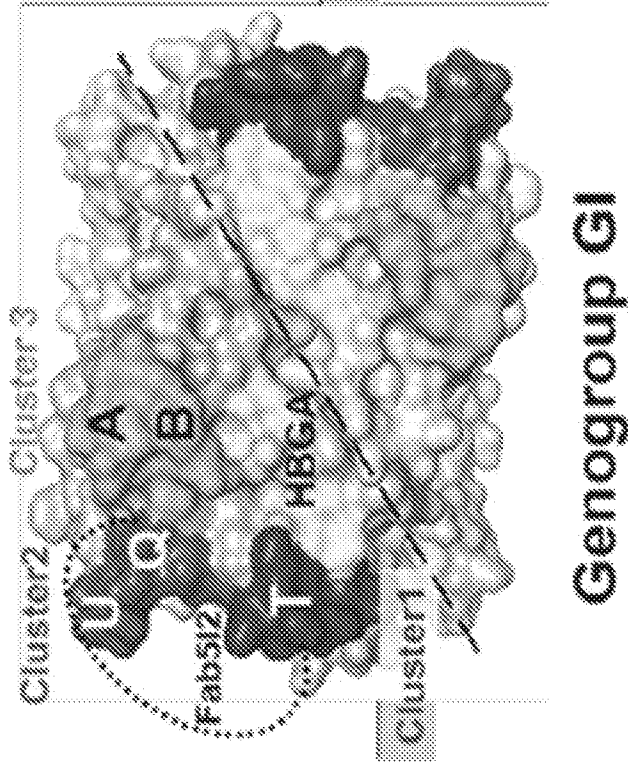


FIG. 18B

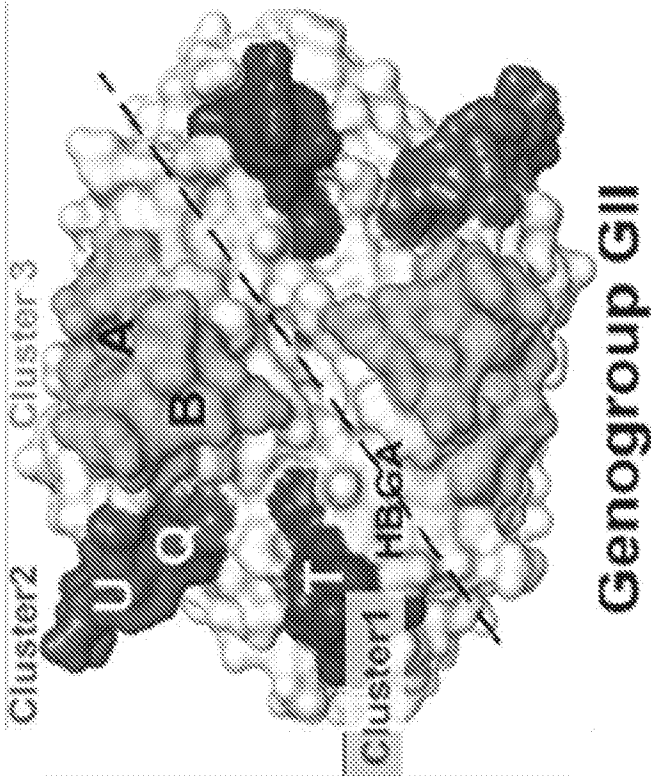


FIG. 19A

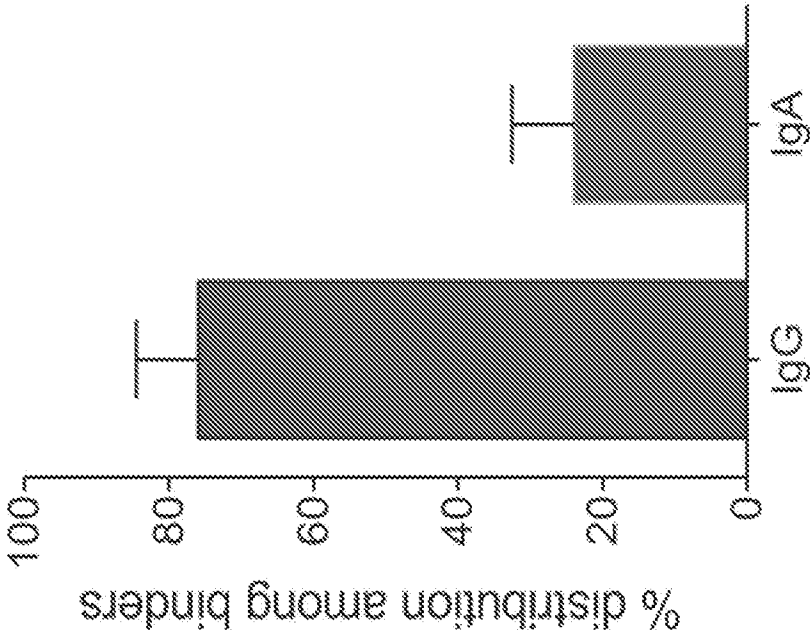


FIG. 19B

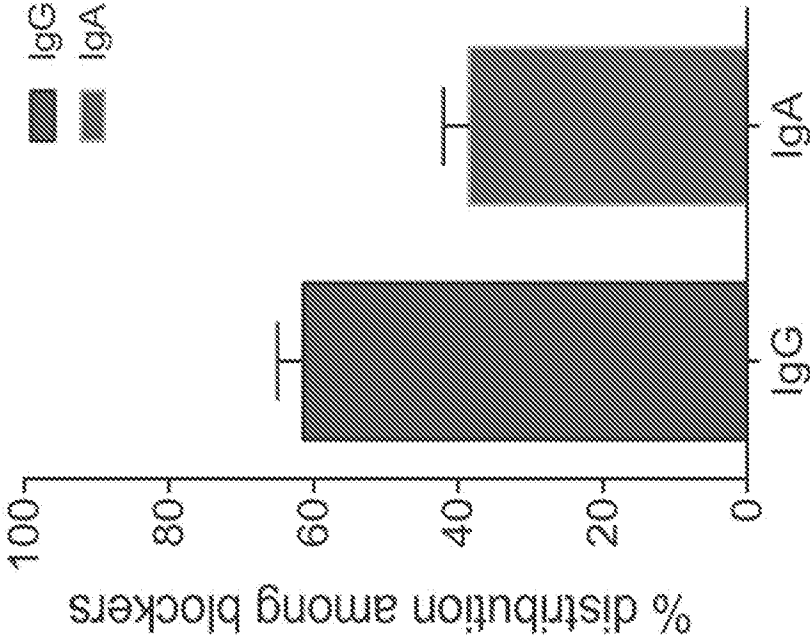


FIG. 20B

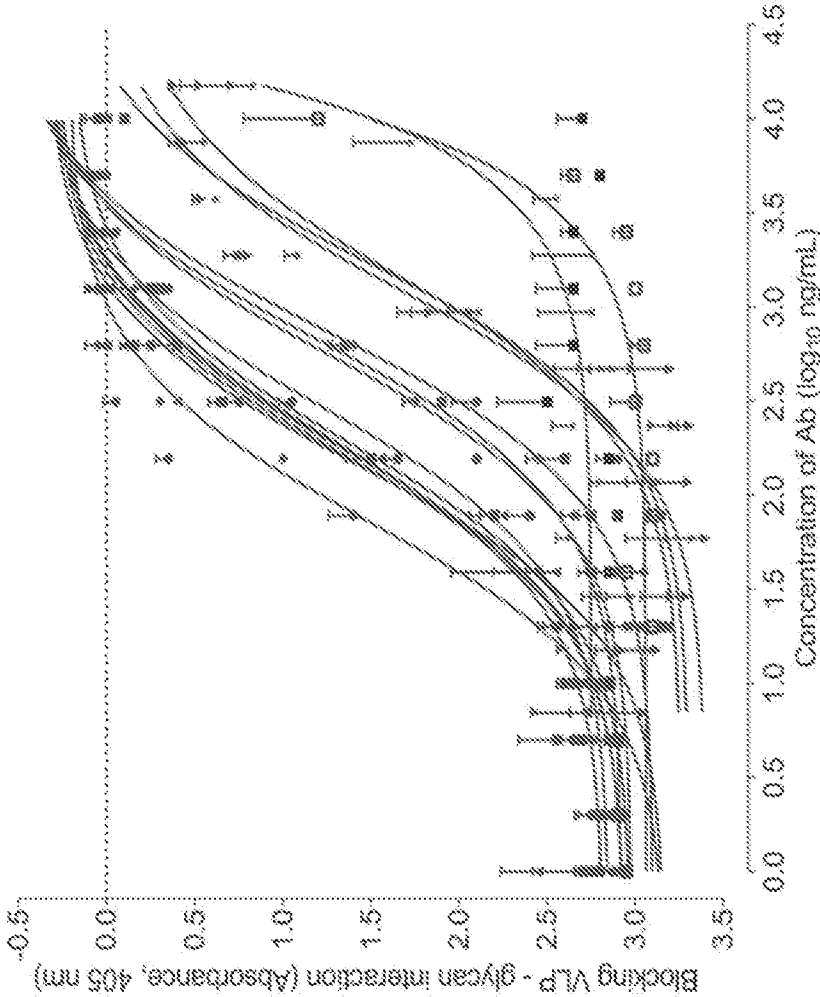


FIG. 20A

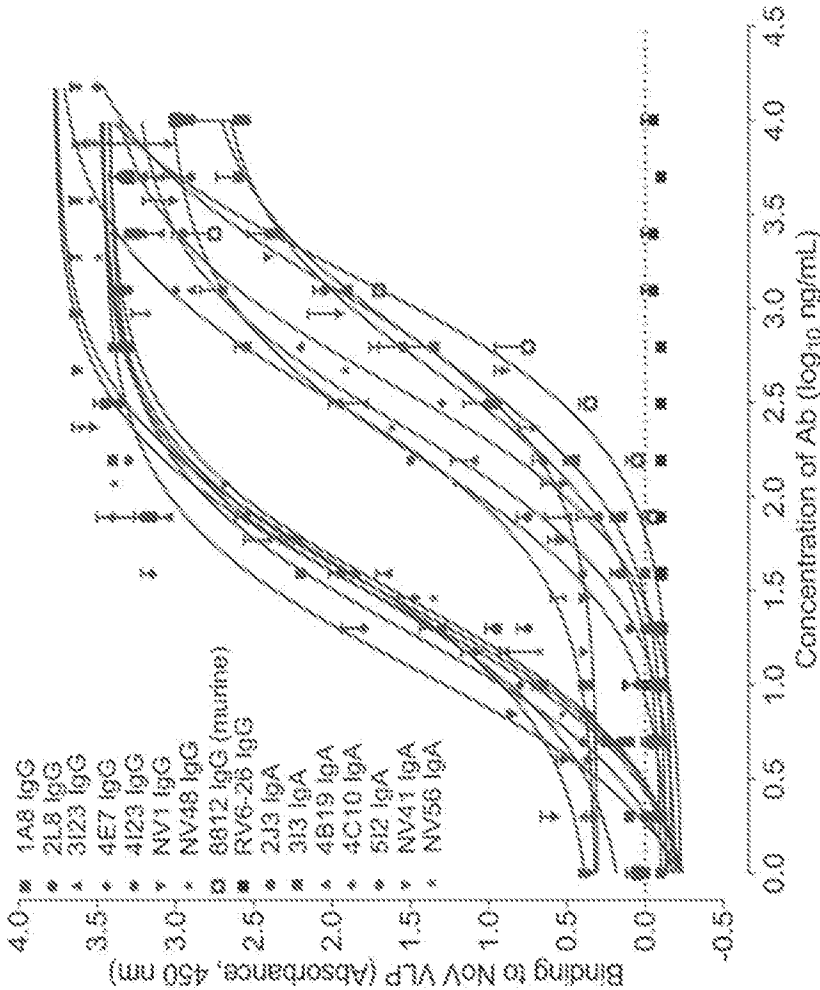


FIG. 21C

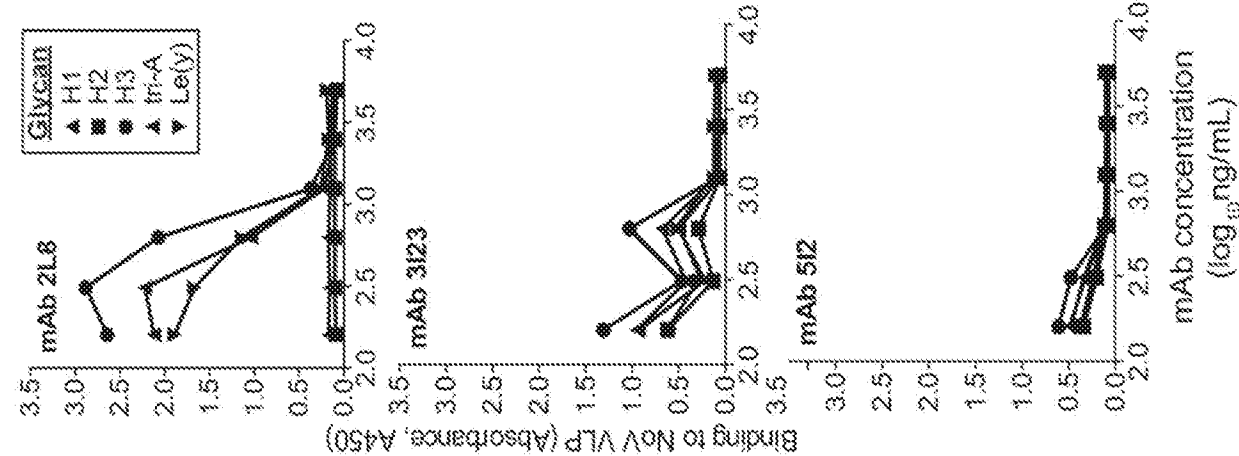


FIG. 21A

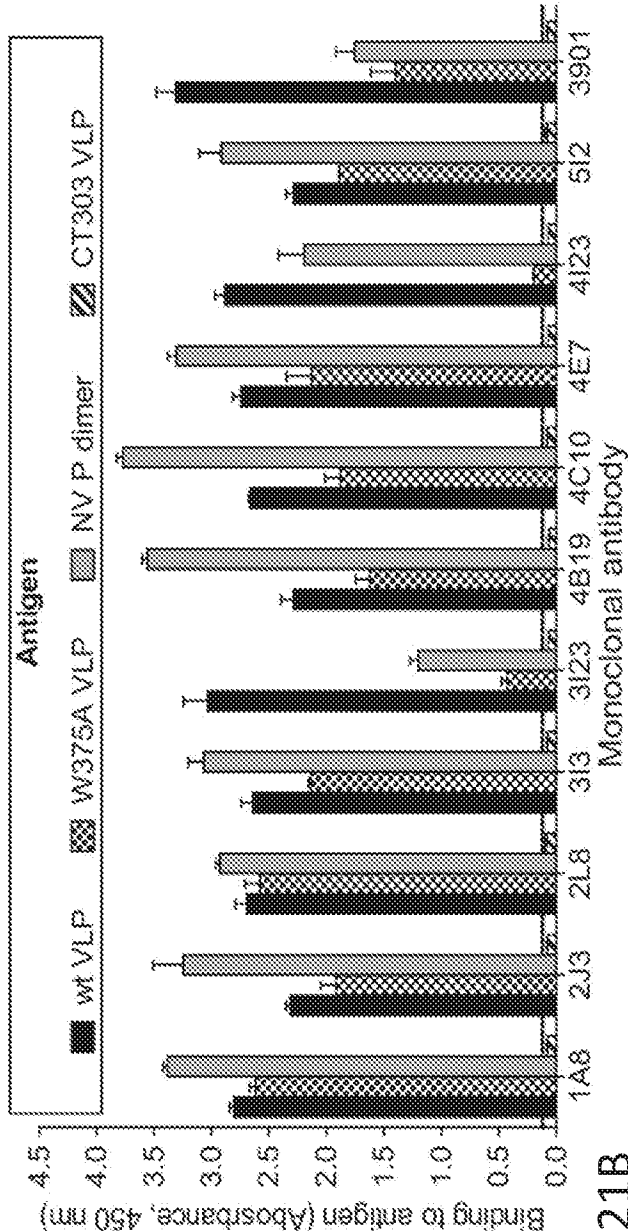
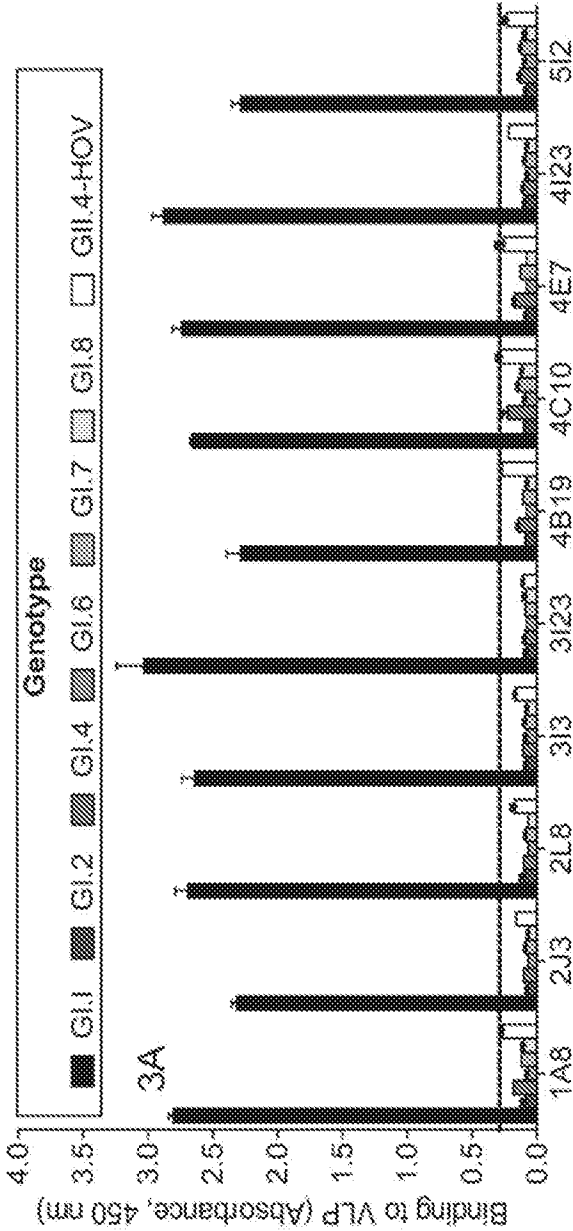


FIG. 21B

FIG. 22A

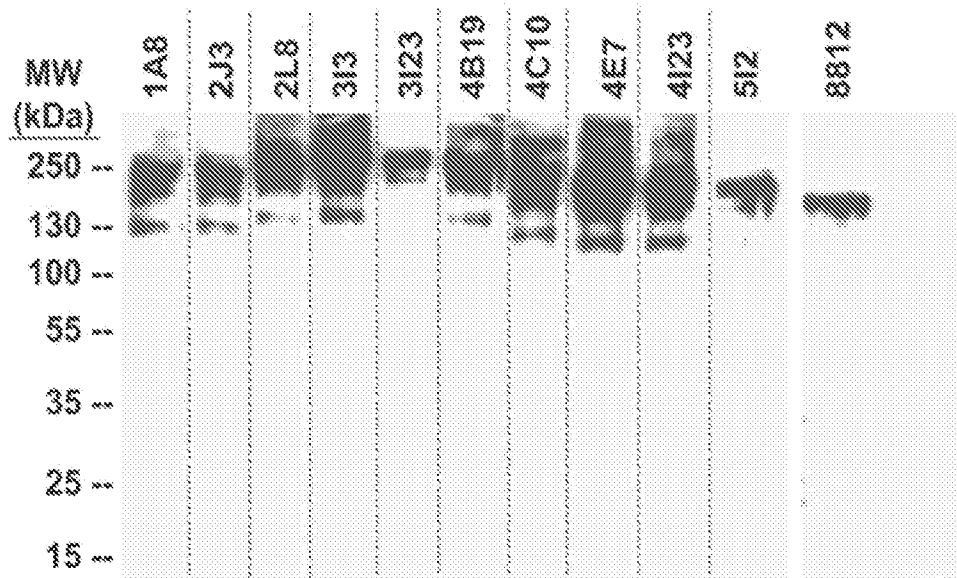


FIG. 22B

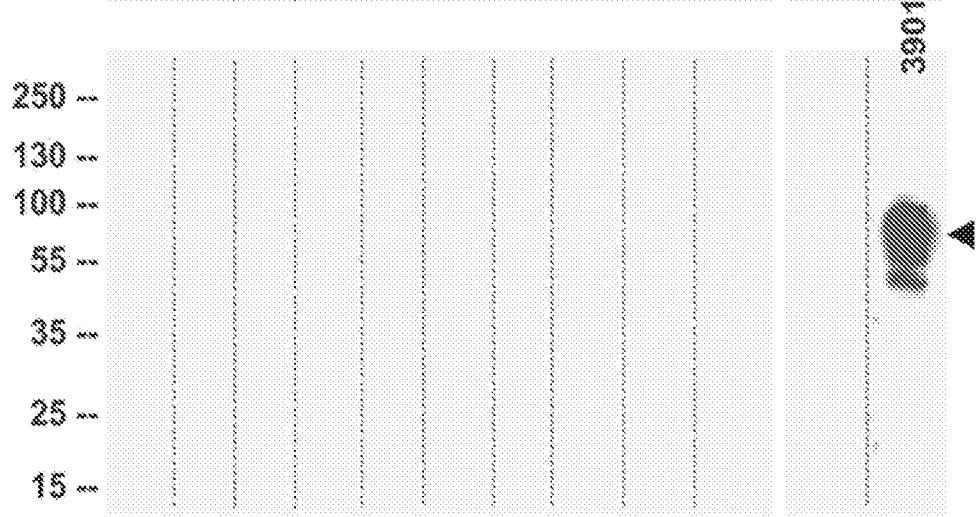


FIG. 22C

		Secondary antibody									
		1A8	2L8	3I23	4E7	4I23	2J3	3I3	4B19	4C10	5I2
Capture antibody	1A8	86	89	82	108	106	81	84	100	102	113
	2L8	102	100	100	123	101	63	104	106	102	103
	3I23	95	93	92	98	101	92	94	98	100	99
	4E7	100	84	88	90	104	95	95	101	87	93
	4I23	79	80	43	97	85	79	78	90	98	78
	2J3	99	98	98	107	100	93	102	100	100	100
	3I3	100	98	97	87	97	88	98	97	96	95
	4B19	102	53	68	97	103	104	103	101	101	102
	4C10	94	67	14	88	74	72	89	108	100	95
	5I2	96	5	3	106	103	98	94	105	106	106



>75% competition
50 - 75% competition
<50% competition

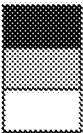


FIG. 23B

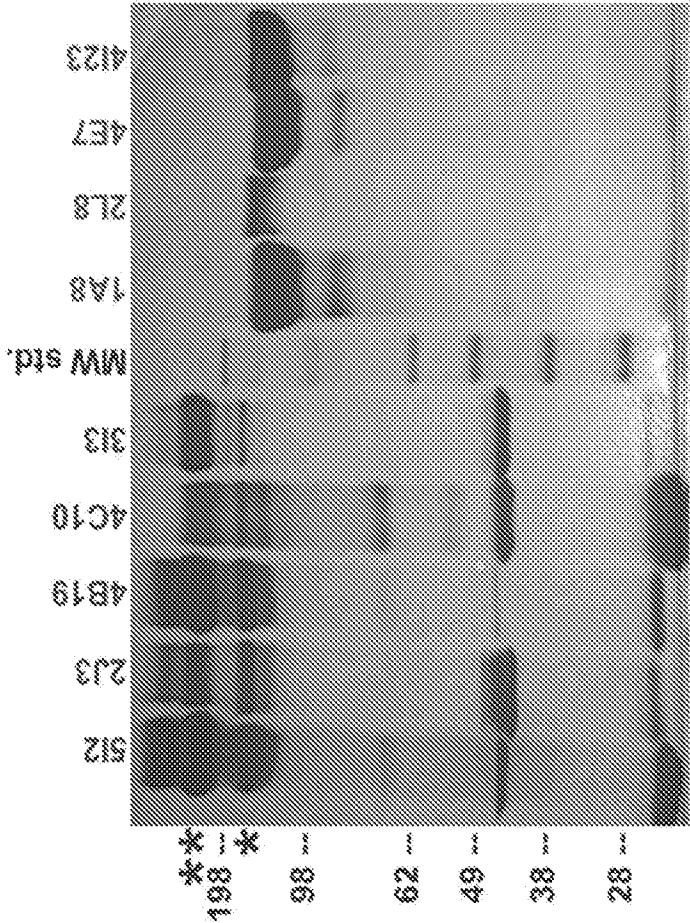


FIG. 23A

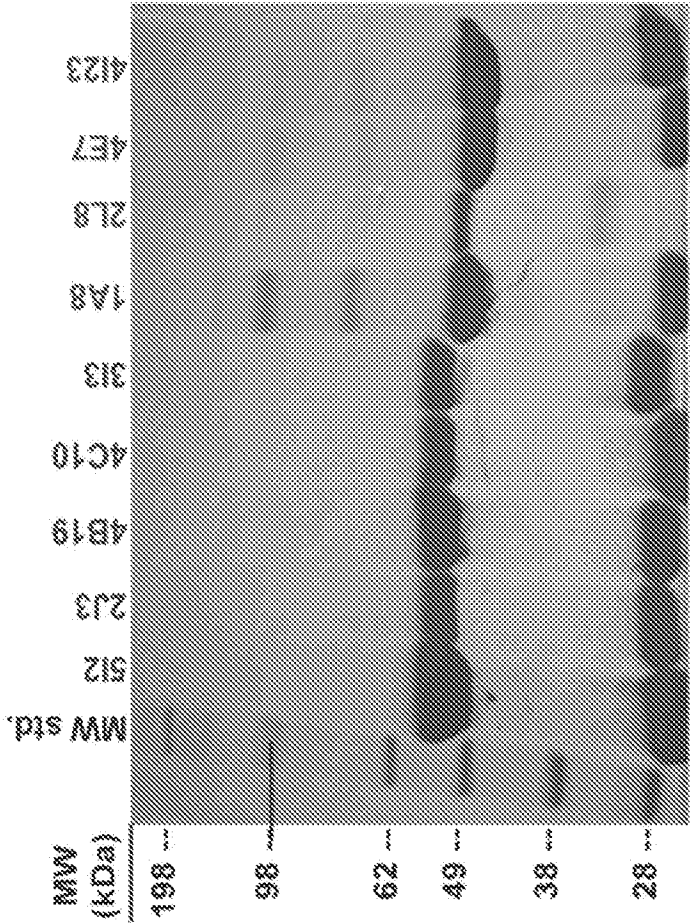


FIG. 24B

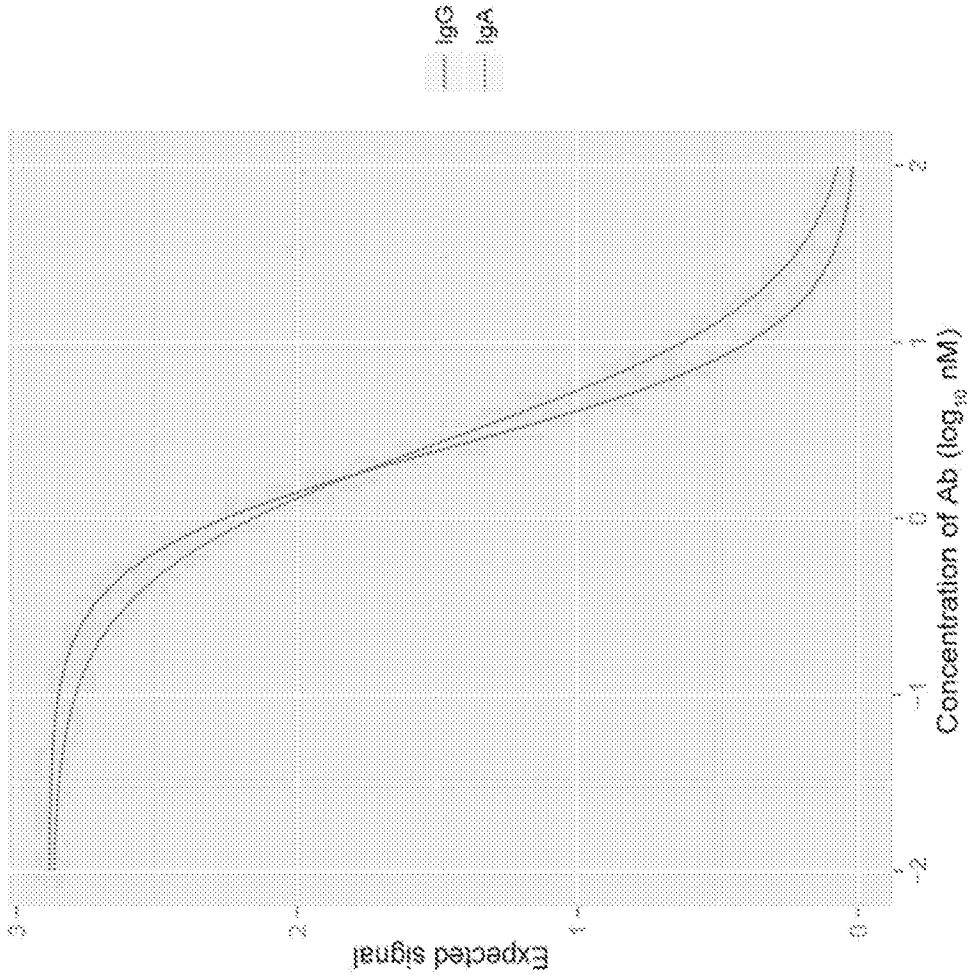
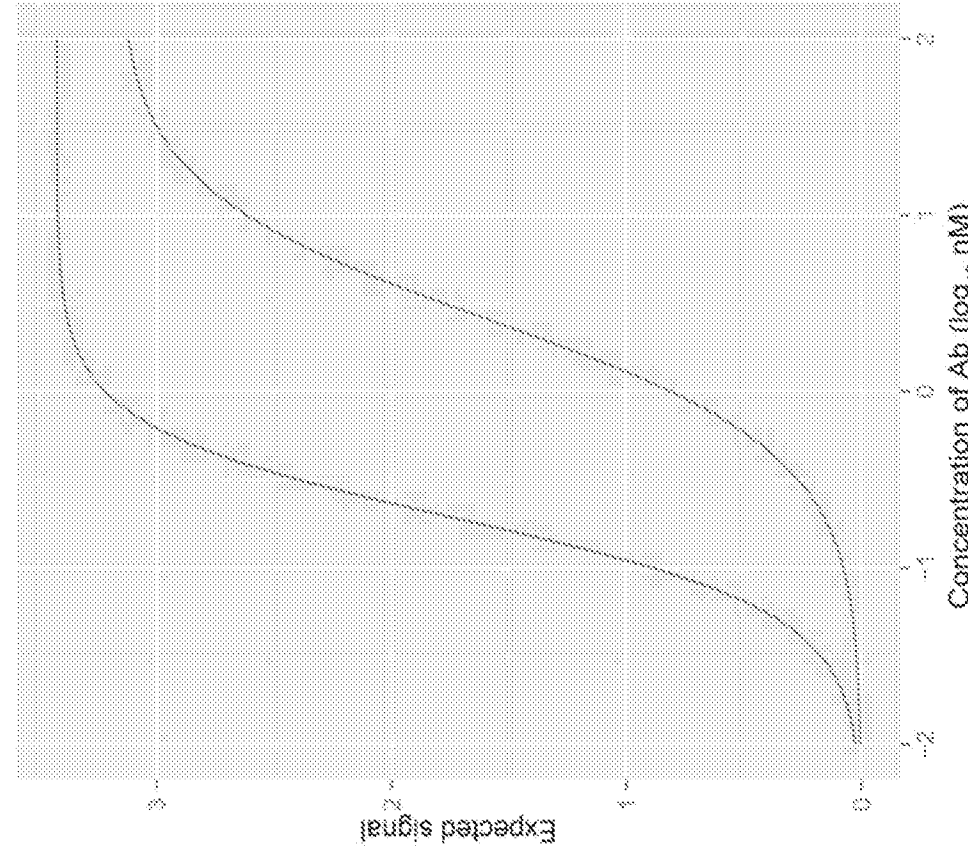


FIG. 24A



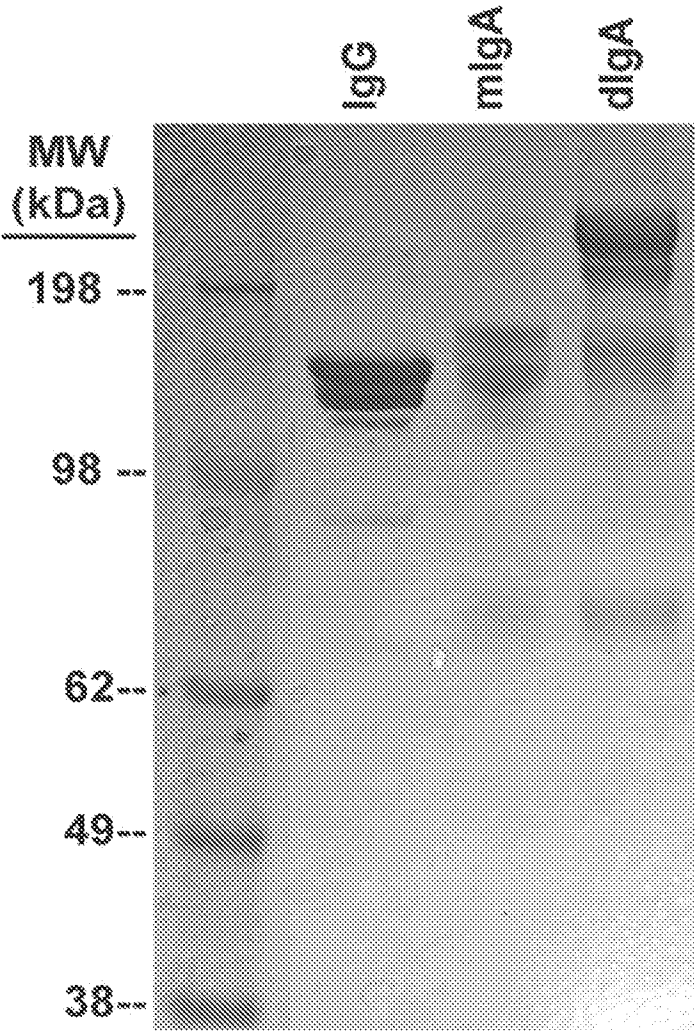


FIG. 25

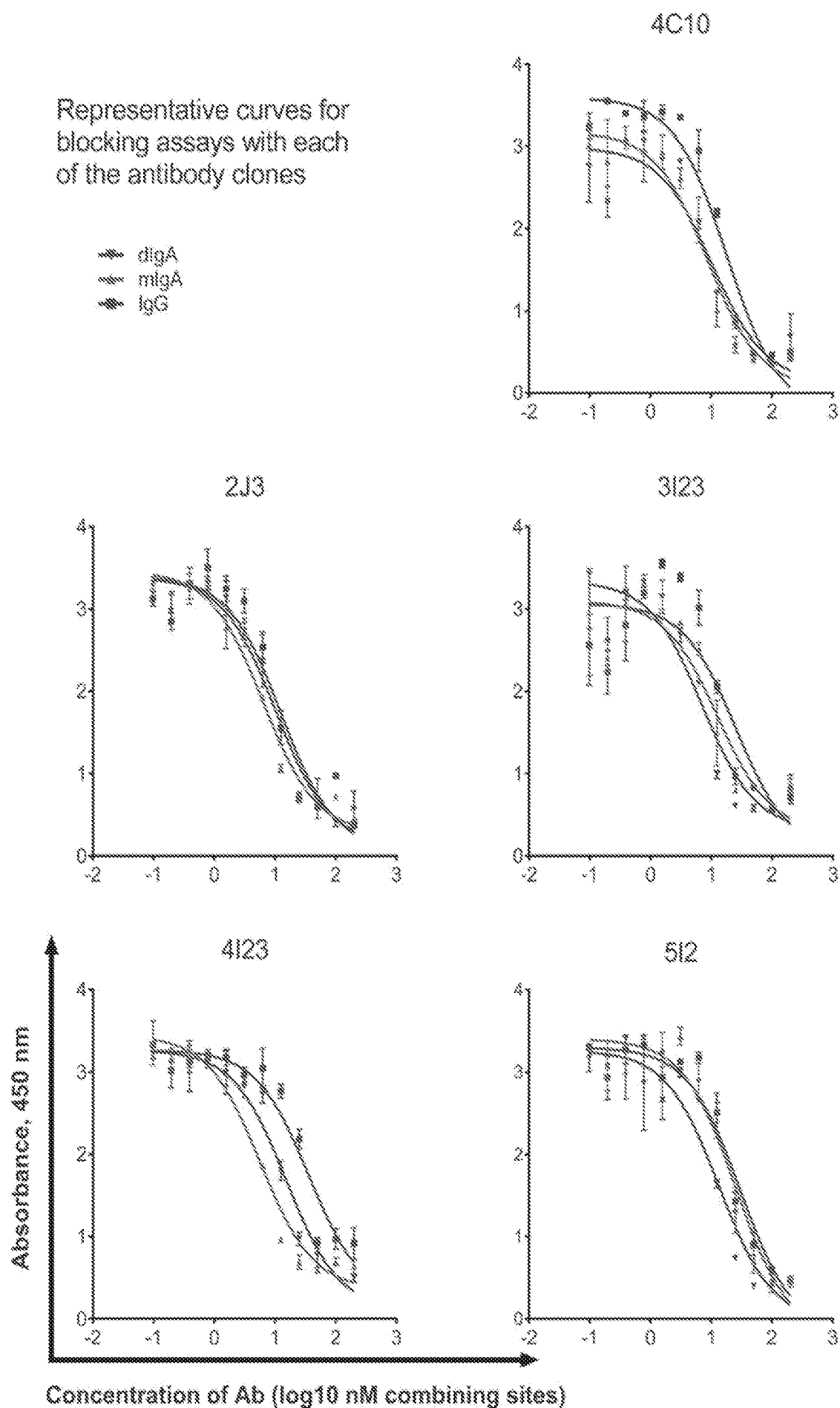


FIG. 26

Class mAb and isotype	EC ₅₀ (ng/ml.)	Heavy chain						
		V gene	J gene	D gene	Number of AA in		HCDR3 AA sequence	Nt mutations
					CDR1	CDR2		
1A8 IgG1, κ	23	4-4*07	4*02	3-10*01	10	7	13 CAREFYGGRGVDSW	34
2L8 IgG1, λ	25	1-46*01	4*02	6-13*01	8	8	14 CARGGLSWYTGDIW	12
3I23 IgG1, κ	25	3-23*01	4*02	5-24*01	8	8	16 CAKGVGSDFTSRILDSW	24
4E7 IgG1, κ	15	4-4*02	1*01	6-25*01	9	7	12 CAIGGSASVEIKYW	14
4I23 IgG1, κ	23	3-23*01	4*02	4-17*01	8	8	14 CANNAGDYAPSPADIW	8
2I3 IgA1, λ	342	4-38-2*02	6*03	2-2*01	9	7	18 CARDSSVVPEAAFLYMDNW	12
3I3 IgA1, λ	387	4-39*01	5*02	3-3*01	10	7	13 CARHESWDRSNFDEW	24
4B19 IgA1, κ	177	3-30-3*01	4*02	4-11*01	8	8	10 CARDLSASFDIW	25
4C10 IgA1, κ	459	3-20*01	5*01	5-12*01	8	8	17 CARDNRGQRGSSFGWFDW	24
5I2 IgA1, κ	120	1-69*01	4*02	5-12*01	8	8	17 CASNRANRADDIYYEYDIW	21

Class mAb and isotype	EC ₅₀ (ng/ml.)	Light chain						
		V gene	J gene	Number of AA in	LCDR3 AA sequence	Nt mutations	AA mutations	
				CDR1	CDR2			
1A8 IgG1, κ	23	nd						
2L8 IgG1, λ	25	L2-14*01	L3*02	9	3	10	CSSYTRSSSTWVF	10
3I23 IgG1, κ	25	K3-20*01	K3*01	7	3	8	CHQYGTSFTF	14
4E7 IgG1, κ	15	K1-39*01	K2*01	6	3	9	CQQSYSSPYTF	4
4I23 IgG1, κ	23	K6-21*01	K1*01	6	3	9	CHQSSILPGTF	4
2I3 IgA1, λ	342	L2-23*02	L2*01	9	3	10	CCSYAISTNLLF	11
3I3 IgA1, λ	387	L2-8*01	L2*01	9	3	10	CSSYVGNNTAF	13
4B19 IgA1, κ	177	K2-30*01	K5*01	11	3	10	CHQGHNEMFTF	9
4C10 IgA1, κ	459	K1-39*01	K1*01	6	3	9	CQQYITPRTF	12
5I2 IgA1, κ	120	K4-1*01	K1*01	12	3	9	CQQYSIERTF	8

FIG. 27

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/41763

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61P 31/14, C07K 16/08, C12P 21/08, A61K 39/42 (2016.01)

CPC - A61K 39/42, A61K 2039/505, C07K 16/10

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - A61P 31/14, C07K 16/08, C12P 21/08, A61K 39/42 (2016.01)

CPC - A61K 39/42, A61K 2039/505, C07K 16/10

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

CPC - A61K 39/395, C07K 14/005

(keyword limited; terms below)

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

PatBase, Google Patents, Google Scholar

Search terms: norovirus, Norwalk, antibody, antibodies, monoclonal, humanized, histoblood group antigen, histo-blood group antigen, HBGA, treat, therapeutic, prevent, capsid, AAB50466, encode

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y — A	US 2006/0115846 A1 (JIANG) 1 June 2006 (01.06.2006) para [0078], [0096], [0098]	6 ----- 1-2 ----- 3-5, (8-11)/6, 14-15
X — A	WO 2014/126921 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES) 21 August 2014 (21.08.2014) p 17, ln 10-13; p 17, ln 29-30; p 24, ln 26-31; p 32, ln 12-15; p 32, ln 27-29; p 61, ln 22-31; p 61, ln 26-30; p 61, ln 32 to p 62, ln 4; p 66, ln 30-32	18 ----- 16, 21
Y — A	AAB50466, GenBank Accession No. AAB50466, 58 kd capsid protein [Norwalk virus], 1 June 2006 [online]. [Retrieved on 9 September 2016]. Retrieved from the internet <URL: http://www.ncbi.nlm.nih.gov/protein/AAB50466 >	1-2 ----- 3
A	US 2008/0069822 A1 (JENSEN et al.) 20 March 2008 (20.03.2008) SEQ ID NO: 179	3-5, (8-11)/6, 14- 16, 21
A	US 2011/0158984 A1 (JENSEN et al.) 30 June 2011 (30.06.2011) SEQ ID NO: 179	3-5, (8-11)/6, 14- 16, 21
A	US 2010/0021477 A1 (TSUI et al.) 28 January 2010 (28.01.2010) SEQ ID NO: 69	3-5, (8-11)/6, 14- 16, 21

☒ Further documents are listed in the continuation of Box C.

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

Date of the actual completion of the international search 16 November 2016	Date of mailing of the international search report 07 DEC 2016
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/41763

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010/0111957 A1 (MAKLER et al.) 6 May 2010 (06.05.2010) SEQ ID NO: 16	3-5, (8-11)/6, 14- 16, 21
A	US 2014/0377281 A1 (HELDWEIN et al.) 25 December 2014 (25.12.2014) SEQ ID NO: 11	3-5, (8-11)/6, 14- 16, 21
A	U6LNH2, UniProtKB/TrEMBL submission no. U6LNH2_9EIME, 22 January 2014 [online]. [Retrieved on 16 November 2016]. Retrieved from the internet <URL: http://www.uniprot.org/uniprot/U6LNH2.txt?version=1> entire document	3-5, (8-11)/6, 14- 16, 21
A	W3XIY0, UniProtKB/TrEMBL submission no. W3XIY0_9PEZI, 19 March 2014 [online]. [Retrieved on 16 November 2016]. Retrieved from the internet <URL: http://www.uniprot.org/uniprot/W3XIY0.txt?version=1> entire document	3-5, (8-11)/6, 14- 16, 21
A	N6ZMG6, UniProtKB/TrEMBL submission no. N6ZMG6_9RHOO, 26 June 2013 [online]. [Retrieved on 16 November 2016]. Retrieved from the internet <URL: http://www.uniprot.org/uniprot/N6ZMG6.txt?version=1> entire document	3-5, (8-11)/6, 14- 16, 21
A	D2VYN6, UniProtKB/TrEMBL submission no. D2VYN6_NAEGR, 2 March 2010 [online]. [Retrieved on 16 November 2016]. Retrieved from the internet <URL: http://www.uniprot.org/uniprot/D2VYN6.txt?version=1> entire document	3-5, (8-11)/6, 14- 16, 21
A	V4ALY0, UniProtKB/TrEMBL submission no. V4ALY0_LOTGI, 22 January 2014 [online]. [Retrieved on 16 November 2016]. Retrieved from the internet <URL: http://www.uniprot.org/uniprot/V4ALY0.txt?version=1> entire document	3-5, (8-11)/6, 14- 16, 21
A	US 2012/0131696 A1 (AAYAL et al.) 24 May 2012 (24.05.2012) SEQ ID NO: 6242	3-5, (8-11)/6, 14- 16, 21

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/41763

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 12, 17, 19, 22
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

—please see extra sheet—

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-6, 8-11 (in part), 14-16, 18, 21, limited to SEQ ID NOs: 1 and 3-5

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/41763

Continuation of: Box No. III Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I+: Claims 1-11, 13-16, 18, 21, drawn to a compositions and methods comprising a binding polypeptide such as an antibody or fragment thereof. The compositions and methods will be searched to the extent that the binding polypeptide sequence encompasses NORO-512 Fab Heavy chain sequence SEQ ID NO: 1 (which comprises NORO-512 Fab Heavy chain CDR sequences of SEQ ID NOs: 3-5). It is believed that claims 1-5, 6, 8-11 (in part), 14-16, 18, 21, limited to SEQ ID NOs: 1 and 3-5, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass compositions or methods comprising a binding polypeptide NORO-512 Fab Heavy chain sequence SEQ ID NO: 1 (which comprises NORO-512 Fab Heavy chain CDR sequences of SEQ ID NOs: 3-5). [Note that claims 7 and 13 require a full monoclonal antibody (heavy chain plus light chain) and will be searched only if both the heavy chain and light chain sequences of an antibody are elected for search, for example electing for search both SEQ ID NO: 1 (NORO-512 heavy chain) and SEQ ID NO: 2 (NORO-512 light chain)]. Additional binding polypeptide sequences will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected binding polypeptide sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be the binding polypeptide sequence of NORO-512 Fab Light chain sequence SEQ ID NO: 2 (which comprises NORO-512 Fab Light chain CDR sequences of SEQ ID NOs: 6-8), i.e. claims 1-11, 13-16, 18, 21, limited to SEQ ID NO: 2 and 6-8.

Group II+: Claim 20, drawn to a composition of matter comprising a nucleic acid sequence. Group II+ will be searched upon payment of additional fees. The composition of matter may be searched for an additional fee and election as such, for example, to the extent that nucleic acid sequence encompasses SEQ ID NO: 9, i.e. claim 20 limited to SEQ ID NO: 9. Additional nucleic acid sequences will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected nucleic acid sequence(s). Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. Another exemplary election would be SEQ ID NO: 10, i.e. claim 20 limited to SEQ ID NO: 10.

The inventions listed as Groups I+ and II+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The technical feature of each of the inventions listed as Group I+ is the specific binding polypeptide sequences recited therein. Each invention requires a binding polypeptide sequence not required by any of the other inventions. Further, Group I+ requires binding polypeptide sequences, not required by Group II+.

The technical feature of each of the inventions listed as Group I+ is the specific nucleic acid sequences recited therein. Each invention requires a nucleic acid sequence not required by any of the other inventions. Further, Group II+ requires nucleic acid sequences, not required by Group I+.

Common Technical Features

The feature shared by Groups I+ and II+ is a composition of matter.

The feature shared by the inventions listed as Group I+ is the composition of matter of claims 4 and 21.

Another feature shared by the inventions listed as Group I+ is the method of blocking or preventing the binding of a Norovirus particle to a histoblood group antigen (HBGA) of claim 6.

Another feature shared by the inventions listed as Group I+ is the method of treating or preventing Norovirus infection of claims 13 and 14.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by US 2006/0115846 A1 to (Jiang).

Jiang discloses a composition of matter (para [0096] "The invention also includes a medicament and a pharmaceutical composition comprising an active compound").

Jiang discloses [claims 4, 21] a composition of matter comprising a binding polypeptide (para [0096] "The invention also includes a medicament and a pharmaceutical composition comprising an active compound ... A typical composition comprises at least one, and typically two or more, first test compounds that can prevent the host blood antigen from binding with any norovirus in vivo, thereby inhibiting an infection, or treating an infection, of the host by the norovirus"; para [0078] "The first test compound is typically selected from the group consisting of ... a poly- and monoclonal antibody. ... A monoclonal antibody to the determinant binding site of the norovirus can be prepared, and isolated by procedures that are well known to those skilled in the art").

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Continuation of: Box No. III Observations where unity of invention is lacking

Jiang discloses [claim 6] a method of blocking or preventing the binding of a Norovirus particle to a histoblood group antigen (HBGA) in an individual susceptible thereto (para [0098]), comprising the step of providing to the individual an effective amount of an isolated binding polypeptide that blocks the binding of the particle to the HBGA (para [0098] "a method for preventing an infection of a host by a norovirus, by administering to the host an effective preventative amount of a prevention compound that inhibits binding of the norovirus in vivo to a histo blood group antigen of the host"; para [0096] "a medicament and a pharmaceutical composition comprising an active compound ... A typical composition comprises at least one, and typically two or more, first test compounds that can prevent the host blood antigen from binding with any norovirus in vivo, thereby inhibiting an infection, or treating an infection, of the host by the norovirus"; para [0078] "The first test compound is typically selected from the group consisting of ... a poly- and monoclonal antibody. ... A monoclonal antibody to the determinant binding site of the norovirus can be prepared, and isolated by procedures that are well known to those skilled in the art").

Jiang discloses [claims 13 and 14] a method of treating an individual for Norovirus infection or preventing Norovirus infection in an individual, comprising the step of providing to the individual a therapeutically effective amount of a binding polypeptide or antibody (para [0098] "a method for preventing an infection of a host by a norovirus, by administering to the host an effective preventative amount of a prevention compound that inhibits binding of the norovirus in vivo to a histo blood group antigen of the host. The invention can also relate to a method for treating an active infection of a host by a norovirus, by administering to the host an effective treatment amount of a treatment compound that inhibits binding of the infecting norovirus in vivo to a histo blood group antigen of the host"; para [0096] "a medicament and a pharmaceutical composition comprising an active compound ... A typical composition comprises at least one, and typically two or more, first test compounds that can prevent the host blood antigen from binding with any norovirus in vivo, thereby inhibiting an infection, or treating an infection, of the host by the norovirus"; para [0078] "The first test compound is typically selected from the group consisting of ... a poly- and monoclonal antibody. ... A monoclonal antibody to the determinant binding site of the norovirus can be prepared, and isolated by procedures that are well known to those skilled in the art").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

The feature shared by the inventions listed as Group I+ is the composition of matter of claims 1 and 2.

I however, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by Jiang in view of GenBank Accession No. AAB50466 (58 kd capsid protein [Norwalk virus], 1 June 2006 [online]. [Retrieved on 9 September 2016]. Retrieved from the internet <URL: <http://www.ncbi.nlm.nih.gov/protein/AAB50466>>) (hereinafter "AAB50466").

Jiang discloses [claim 1] a conformation-specific antibody that binds a three-dimensional epitope of a Norovirus (para [0078] "A monoclonal antibody to the determinant binding site of the norovirus can be prepared, and isolated by procedures that are well known to those skilled in the art"). Jiang does not specifically teach that the epitope comprises residue H381 of SEQ ID NO:61. However, AAB50466 discloses the sequence of SEQ ID NO:61 as the sequence of the Norwalk virus capsid protein (sequence of AAB50466 exhibits 100% identity to claimed SEQ ID NO: 61; description of AAB50466 as "58 kd capsid protein [Norwalk virus]"). Further, Jiang teaches methods of producing antibodies that target a specific binding determinant site of a Norovirus (para [0071]-[0074]) and the use of the three-dimensional structure of the capsid protein of known Noroviruses for producing antibodies (para [0082] "The nucleotide and amino acid sequences of the norovirus capsid genes of several norovirus strains, and the three-dimensional structure of the prototype NV, are known, and could be used to model ligand-receptor (binding site-epitope) interaction for the engineering of such compounds."; para [0083] "A first test compound can also be selected from a compound that has the binding specificity of the antigenic determinant epitope of a blood antigen, and functionally equivalent molecules thereof. Such compounds can include monoclonal antibodies. A typical antibody is an anti-antibody to the antigenic determinant epitope. An example of such an antibody is the 9C3 Mab that can bind at the determinant binding site of the NV with the binding specificity of the antigenic determinant epitope of the H-type blood antigen"). Given that AAB50466 teaches the capsid protein sequence of SEQ ID NO: 61, and Jiang teaches a method of producing antibodies that bind to specific antigenic determinant epitope of a Norovirus capsid protein, one of ordinary skill in the art would have found it obvious to use an antigenic determinant epitope of the sequence of AAB50466 in the method of Jiang, to thereby obtain a conformation-specific antibody that binds a three-dimensional epitope comprising residue H381 of SEQ ID NO:61

Jiang further discloses [claim 2] a composition comprising the antibody of claim 1 (para [0096] "a medicament and a pharmaceutical composition comprising an active compound ... A typical composition comprises at least one, and typically two or more, first test compounds that can prevent the host blood antigen from binding with any norovirus in vivo, thereby inhibiting an infection, or treating an infection, of the host by the norovirus"; para [0078] "The first test compound is typically selected from the group consisting of ... a poly- and monoclonal antibody. ... A monoclonal antibody to the determinant binding site of the norovirus can be prepared, and isolated by procedures that are well known to those skilled in the art")

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

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Continuation of: Box No. III Observations where unity of invention is lacking

Another feature shared by the inventions listed as Group I+ is the method of making an antibody of claims 16 and 18.

The feature shared by the inventions listed as Group II+ is a composition of matter comprising a nucleic acid sequence encoding a binding polypeptide of claim 21.

However, these shared technical features do not represent a contribution over prior art, because the shared technical features are taught by WO 2014/126921 A1 to (US Health).

US Health discloses [claim 16] a method of making an antibody, comprising the steps of harvesting from a host cell heterogeneous sequence expressing a binding polypeptide sequence (p 41, ln 32 to p 42, ln 1 "In a further group of embodiments, the antibodies are Fv antibodies ... To produce these antibodies, the VH and the VL can be expressed from two individual nucleic acid constructs in a host cell"; p 47, ln 21-28 "Once the expression vector is transferred to a host cell by conventional techniques, the transfected cells are then cultured by conventional techniques, such as to produce an antibody. Thus, host cells are provided containing a polynucleotide encoding an antibody or fragments thereof, or a heavy or light chain thereof, or portion thereof, or a single-chain antibody of the invention, operably linked to a heterologous promoter. In certain embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule").

US Health discloses [claim 18] a method of making a HBGA-blocking human monoclonal antibody (p 17, ln 10-12 "Monoclonal antibodies are produced by methods known to those of skill in the art, for instance by making hybrid antibody-forming cells from a fusion of myeloma cells with immune spleen cells. These fused cells and their progeny are termed "hybridomas.""; p 32, ln 12-15 "In some embodiments, the antibody is neutralizing. ... In other embodiments, the antibody inhibits the binding of NoV [Norovirus] VLPs to HI HBGA carbohydrate in a dose dependent manner") comprising:

- a) generating a monoclonal antibody using as an antigen part or all of a Norovirus (p 61, ln 22-31 "Isolation of NV [Norovirus]-specific antibodies by immortalization of memory B cells: Peripheral blood (50 ml) was collected from the chimpanzee immunized with NV rVLPs. ... memory B-cells were isolated by FACS sorting ... The FACS sorted cells were used immediately for the B cell immortalization");
- b) screening monoclonal antibodies for specific binding to the Norovirus virus but that block binding of HBGA to Norovirus virus particles (p 61, ln 32 to p 62, ln 4 "The memory B-cells were cultured in U-bottom 96-well plates ... The supernatant fluid of each well was screened by ELISA for binding to NV rVLPs as described above"; p 24, ln 26-31 "In some examples, an antibody specific for NoV VP1 neutralizes the infectious titer of the virus. In vitro assays for neutralization are known in the art. Thus, in some non-limiting examples, an assay for neutralization activity is blocking the binding of NoV-like particles (VLPs) to HI HBGA carbohydrate in a dose dependent manner");
- c) humanizing one or more monoclonal antibodies screened for specific binding to the Norovirus virus (p 17, ln 12-13 "In some embodiments, monoclonal antibodies can be humanized monoclonal antibodies"; p 17, ln 29-30 "Humanized immunoglobulins can be construed by means of genetic engineering"; p 32, ln 27-29 "The monoclonal antibodies disclosed herein can be chimpanzee antibodies, and can include a chimpanzee framework region. In some embodiments, the antibodies are humanized, and thus include one or more human framework regions"); and
- d) screening the one or more humanized monoclonal antibodies for an ability to block binding of HBGA to Norovirus virus particles or virus-like particles (p 61, ln 26-30 "As disclosed herein, five NV-specific antibodies and four anti-No V-specific antibodies were isolated from immunized chimpanzees. It was demonstrated that these antibodies were able to block the binding of rVLP of NoV to HBGA carbohydrates, a known binding ligand for NoV in the intestinal mucosa"; p 66, ln 30-32 "HBGA Binding Blocking Assay: The ability of antibodies to block the binding of NV rVLPs to synthetic HBGA H type 1 (HI) carbohydrate was determined as described (Bok, 2011, Proc Natl Acad Sci U S A 108:325-30)").

US Health discloses [claim 21] a composition of matter comprising a nucleic acid sequence encoding a binding polypeptide (p 45, ln 22-24 "Nucleic acid molecules (also referred to as polynucleotides) encoding the polypeptides provided herein (including, but not limited to antibodies) can readily be produced by one of skill in the art").

As the technical features were known in the art at the time of the invention, they cannot be considered special technical features that would otherwise unify the groups.

Groups I+ and II+ therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.