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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR TREATING POLIOVIRUS

FIG. 5A

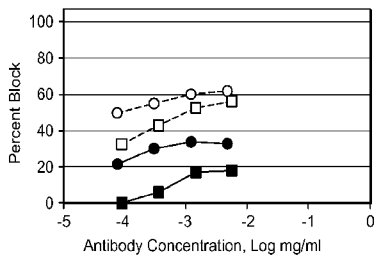


FIG. 5B

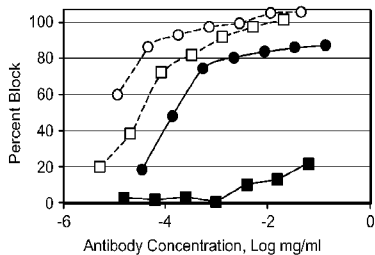
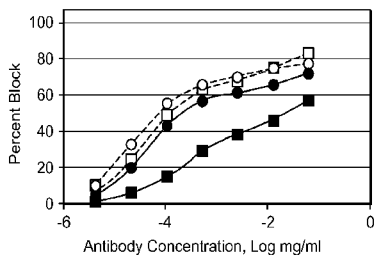


FIG. 5C



(57) Abstract: Chimeric chimpanzee/human antibodies and antigen-binding fragments thereof that are capable of neutralizing poliovirus are provided. In addition, methods of using the antibodies and antigen-binding fragments thereof to prevent or treat poliovirus infection are also provided.



MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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COMPOSITIONS AND METHODS FOR TREATING POLIOVIRUS

RELATED APPLICATIONS

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This application claims the benefit of U.S. Provisional Application Ser. No. 61/443,915, filed February 17, 2011 the entire contents of which are hereby incorporated herein by reference.

10 STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

This work was supported by the National Institutes of Health. The government has certain rights in the invention.

15

BACKGROUND OF THE INVENTION

Poliomyelitis is an infectious neurological disease that is caused by polioviruses of three distinct serological types. Two highly effective vaccines, one prepared from formalin-inactivated virulent virus and another from live attenuated strains administered orally, were developed in the 1950s. Their worldwide use resulted in almost complete eradication of the disease, with only a few remaining endemic countries and a few thousand paralytic cases annually. This dramatic success diminished an interest in development of new protective measures, as complete eradication of poliomyelitis was perceived to be very close. However, the original eradication target date of 2000 was missed by at least 10 years due to a variety of scientific, logistical, and political obstacles. Therefore in recent years the setbacks in the global efforts to eradicate poliomyelitis, newly recognized challenges of the final phases of the WHO-coordinated campaign, and the need to prevent re-emergence of poliomyelitis in the post-eradication period led to renewed efforts to develop more efficient vaccines, new strategies for their use, and other tools to protect against poliomyelitis. They include licensure of more potent monovalent (mOPV) and bivalent (bOPV) oral vaccines, development of a new generation of inactivated poliovirus vaccines for use in the post-eradication period, and development of drugs effective against poliovirus.

Use of OPV is associated with a small risk of vaccine-associated paralytic poliomyelitis (VAPP) in vaccine recipients and their immediate contacts. It has also led to emergence of circulating vaccine-derived polioviruses (cVDPV) and immunodeficiency-associated vaccine-derived polioviruses (iVDPV). The VDPVs of the first type cause outbreaks of paralytic poliomyelitis in inadequately-immunized communities and are indistinguishable from wild polioviruses in their pathogenic properties. The iVDPV emerge in OPV-vaccinated individuals with primary B-cell immunodeficiencies and can establish chronic infection and be excreted into the environment for several years. Besides the immediate danger to the chronic carriers (some of the patients eventually become paralyzed by the continuously evolving poliovirus), the presence of chronic excretors poses a serious challenge to the polio eradication campaign, providing an ample source of virulent polioviruses in the environment, making it impossible to stop immunization against poliomyelitis. Therefore finding an effective treatment for these patients is an important public health objective. In 2006 the US National Research Council recommended development of at least two polio antiviral drugs to treat chronically infected individuals and to assist in management of outbreaks in the post-eradication period.

Early work suggested the effectiveness of gamma globulin for prevention of poliomyelitis. Therefore passive immunotherapy could be another way to treat chronic excretors. Even though prior attempts to use intravenous immunoglobulin (IVIG) and breast milk were unsuccessful, there is a reason to think that higher doses of anti-poliovirus antibodies could result in complete clearance of poliovirus from chronically infected individuals. Accordingly, improved agents for treating or preventing poliovirus infection are needed.

SUMMARY OF THE INVENTION

As described below, the present invention features chimpanzee/human chimeric anti-poliovirus immunoglobulins and methods for the use of such antibodies in the treatment of chronic poliovirus excretors as well as for post-exposure emergency prophylaxis and disease prevention if there is a re-emergence of poliomyelitis in the post-eradication period. Preferred embodiments include chimpanzee/human chimeric anti-poliovirus antibodies and methods of their use. The chimpanzee immunoglobulins of the invention are virtually identical to human

immunoglobulins, therefore, the chimpanzee immunoglobulins described herein can be used in humans without further modifications such as “humanizing.”

In one aspect, the invention generally features an isolated chimpanzee/human chimeric antibody or antigen-binding portion thereof that is capable of neutralizing poliovirus.

In another aspect, the invention features an isolated polypeptide having the sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, and SEQ ID NO: 24.

In yet another aspect, the invention features a method of treating a subject that has been infected with poliovirus, the method involving administering to the subject a therapeutically effective amount of a chimeric chimpanzee/human antibody that neutralizes poliovirus, thereby treating the subject.

In a further aspect, the invention features a pharmaceutical composition for the treatment or prevention of poliovirus infection containing a therapeutically effective amount of an isolated polypeptide comprising a sequence selected from the group consisting of 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, and SEQ ID NO: 24.

In other aspects, the invention features a pharmaceutical composition for the treatment or prevention of poliovirus infection containing a therapeutically effective amount of an isolated polypeptide comprising a sequence selected from the group consisting of 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, and SEQ ID NO: 24.

In yet another aspect, the invention features a kit for the treatment or prevention of poliovirus infection, the kit containing a therapeutically effective amount of a chimeric chimpanzee/human chimeric antibody capable of neutralizing poliovirus or an isolated polypeptide comprising a sequence selected from the group consisting of 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, and SEQ ID NO: 24, and directions for using the kit in any of the methods provided herein.

In various embodiments of any of the above aspects or any other aspects of the invention delineated herein, the antibody or antigen-binding portion thereof is capable of neutralizing more than one serotype of poliovirus. In another embodiment the antibody is isolated from a culture of prokaryotic or eukaryotic cells. In further
5 embodiments the chimpanzee portion of the antibody has at least 85% identity to a sequence selected from the group consisting of 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, and SEQ ID NO: 24. In yet another embodiment the chimpanzee portion of the antibody comprises SEQ ID
10 NO: 13 and SEQ ID NO: 14. In another embodiment the chimpanzee portion of the antibody comprises SEQ ID NO: 15 and SEQ ID NO: 16. In a further embodiment the chimpanzee portion of the antibody comprises SEQ ID NO: 17 and SEQ ID NO: 18. In other embodiments the chimpanzee portion of the antibody comprises SEQ ID NO: 19 and SEQ ID NO: 20. In yet another embodiment the chimpanzee portion of the
15 antibody comprises SEQ ID NO: 21 and SEQ ID NO: 22. In other embodiments the chimpanzee portion of the antibody comprises SEQ ID NO: 23 and SEQ ID NO: 24.

In various embodiments of any of the above aspects or any other aspect of the invention delineated herein, the antibody neutralizes both type 1 and type 2 polioviruses. In further embodiments the antibody or a fragment thereof is capable of
20 preventing poliovirus infection. In yet other embodiments the invention includes an isolated polynucleotide encoding a chimeric chimpanzee/human antibody capable of neutralizing poliovirus. In additional embodiments the polynucleotide has a 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
25 ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12. In yet another embodiment includes an expression vector having a polynucleotide encoding a chimeric chimpanzee/human antibody capable of neutralizing poliovirus. Another embodiment includes a cell containing the expression vector. In further embodiments the cell is a prokaryotic or eukaryotic cell. In further embodiments, cells containing
30 expression vectors are cultured under conditions suitable for expression of a chimeric chimpanzee/human antibody. In other embodiments, the antibody is isolated from the cultured cell medium.

In further aspects, the invention features a method of producing a chimeric chimpanzee/human antibody capable of neutralizing poliovirus, the method involving:

a) immunizing a chimpanzee with all three serotypes of polio vaccine; b) aspirating bone marrow from the immunized chimpanzee; c) isolating RNA from bone marrow derived lymphocytes; d) constructing a Fab phage display library from the isolated RNA; e) selecting for poliovirus-specific Fabs by panning the phage library; and f) converting the poliovirus-specific Fabs into full-length IgGs. In an additional embodiment the method further involves the step of selecting for poliovirus-specific Fabs capable of neutralizing more than one serotype of poliovirus. In yet another embodiment the method further involves the step of selecting for poliovirus-specific Fabs capable of neutralizing both type 1 and type 2 polioviruses.

In yet another aspect, the invention features a method of ameliorating the symptoms of poliovirus infection in a subject, the method involves administering to the subject a therapeutically effective amount of a chimeric chimpanzee/human antibody that neutralizes poliovirus, thereby ameliorating the symptoms of poliovirus infection in the subject. In further aspects, the invention features a method of treating or preventing polio in a subject, the method involves administering to the subject a therapeutically effective amount of a chimeric chimpanzee/human antibody that neutralizes poliovirus, thereby treating or preventing polio in the subject. In additional embodiments the method further involves administering the antibody systemically or locally. In further embodiments the antibody is covalently linked to a functional moiety. In particular embodiments the functional moiety is horseradish peroxidase (HRP) or alkaline phosphatase (ALP). In further embodiments the method of treatment includes administering to a subject a therapeutically effective amount of one or more antiviral agents. In particular embodiments the antiviral agents include V-037, rupintrivir, pirodavir, enviroxime, ribavirin, and derivatives thereof. In yet another embodiment, the method includes administering to a subject a poliovirus vaccine.

Other aspects of the invention are disclosed infra.

Definitions

By "A12" is meant an isolated anti-poliovirus antibody where the heavy chain is encoded by **SEQ ID NO: 1**

```
CTCGAGTGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCAGTGTCTCTAGTGGC
TTCATCAATCTATATTCGTGGAATTGGATCCGCCAGGCACCAGGGACGGGACTGGAGTGGATTGGGTCC
ATCCGTTATGGTGAGACCGCTATTACAACCCATCCCTCAGGGGTCGAGTCTTCATATCACTAGACACG
```

TCCAAGAACCAGTTCTACCTGAAAAATGAGCTCTCTGACCGCCGACAGACACGGCCGTTTATTATTGTGCG
 AGAGACTACAGTAAGAGTGGCTACGATCGCTCGGGGCGGCTCGACCCCTGGGGCCAGGGAACCCTAGTC
 ATCGTCTCC;

and the light chain is encoded by **SEQ ID NO: 2**

5 GAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCC
 AGTCAGGACGTTAGTACTTATTTAGCCTGGTATCACCAAAAACCAGGAAAAGCCCCGAGGGTCTTCATC
 TATTATGCATCCAATTTACAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAGTTC
 ACTCTACAATCACCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAACAGCTTGATAATTTT
 CCGCCGACTTTTCGGCCAAGGGACCAAGCTGGAAATCAAACGA;

10 where the heavy chain has the following amino acid sequence, **SEQ ID NO:**

13

LEWGPGLVKPSETLSLTCVSSGFINLYSWNWIRQAPGTGLEWIGSIRYGETAYYNPSLR
 GRVFIISLDTSKNQFYLKMSLTAADTAVYYCARDYSKSGYDRSGRLDPWGQTLVIVS;

and the light chain has the following amino acid sequence, **SEQ ID NO:14**

15 ELTQSPSSLSASVGDRTVITCRASQDVSTYLAWYHQKPGKAPRVLIYYASNLQSGVPSRF
 SSGSGTEFTLTIITSLQPEDFATYYCQQLDNFPPTFGQGTKLEIKR.

By "H2" is meant an isolated anti-poliovirus antibody where the heavy chain
 is encoded by **SEQ ID NO: 3**

20 CTCGAGTCTGGGGGAGGCGTGGTCCAGCCCGGAGGTCCTTGAGACTCTCCTGTGCAGCCTCTGGATT
 ACCTTCACTAATTTTGGCATGAATTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAACA
 CTTTCGTTTGATCCGAATGATAAATATATATGCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAGAC
 GATTCCACACACACGTTGTATCTTCAGATGGACAGCCTGCGAGTTGAGGACACGGCTGTGTATTTCTGT
 GCGAGGAGGGATTTTGATTACATTTTCGGGACTTCTCGTTCATACTTCTTTGACTATTGGGGCCAGGGC
 25 GCCCAGGTCACCGTCTCC;

and the light chain is encoded by **SEQ ID NO: 4**

GAGCTCACGCAGTCTCCAGACTTTCAGTCTGTGCCCTCAAAGGAGAAAAGTCACCATCACCTGCCGGGCC
 AGTCAGAGCATTGGCAGTAGCTTACACTGGTACCAGCAGAAAACCAGGTCAGTCTCCAAAACCTCTCATC
 AAGTATGCTTCCCAGTCCATCTCAGGGTCCCTCGAGTTCAGTGGCAGTGGCTCTGGGACAGACTTC
 30 GCCCTCACCATCAATGGCCTGGAAGCTGAAGATGCCGCAACGTATTACTGTCTGCAGAGTAGTAGTTTA
 CCTCAGACGTTTCGGCCAAGGGACCAAGCTGGAAATCAAACGA;

where the heavy chain has the following amino acid sequence, **SEQ ID NO:**

15

LESGGGVVQPGRSLRLSCAASGFTFTNFGMNVWRQAPGKLEWVATLSFDPNDKYYADSV
 35 KGRFTISRDDSTHTLYLQMDSLRVEDTAVYFCARRDFDYISGTSRSYFFDYWGQGAQVTV
 S;

and the light chain has the following amino acid sequence, **SEQ ID NO:16**

ELTQSPDFQSVPPKEKVTIITCRASQSIGSSLHWYQQKPGQSPKLLIKYASQISGVPSRF
 SSGSGTDFALTINGLEAEADAATYYCLQSSSLPQTFGQGTKLEIKR.

By “A6” is meant an isolated anti-poliovirus antibody where the heavy chain is encoded by **SEQ ID NO: 5**

5 CTGAGTCTGGGGGAGGCTTGGTAAAGCCTGGGGGTTCCTGAGACTCTCGTGTGCGGCCTCTGGATTT
 ATCTTCAATTCCTATGATATGCACTGGGTCCGCCAGGCTCCAGAGAAGGGGCTGGAGTGGGTCTCAACT
 GTCAGTCGTGGAGGTAGTAACATGTGGTACCGAGACTCTGTCCAGGGCCGATTACCATCTCCAGAGAC
 AATTCCAAGAATACATTGTATCTCCAAATGAATGGCCTCAGAGCCGAGGACAGGGCCGTGTATTACTGT
 GCGATTGGCGACTGGGGTTTTGACTACTGGGGCCAGGGAACCCCTGGTCACCGTCTCC;

and the light chain is encoded by **SEQ ID NO: 6**

10 GAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGACTCACCATCACTTGCCGGGCA
 AGTCAGGGCATTACCAATAGATTAAATTGGTATCAGCAGAAACCAGGGAAAGCTCCTAAGCTCCTGATC
 TATGATGCCTCCAGTTTTGGAAAAGTGGGGTCCCTTCAAGGTTACAGCGGCAGTGGATCTGGGACAGATTC
 ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAACAGTTTAATAGTTAC
 CCGCTCACTTTTCGGTGGAGGGACCAAGGTGGAGATCAAACGA;

15 where the heavy chain has the following amino acid sequence, **SEQ ID NO:**

17

LESGGGLVKPGGSLRLSCAASGFI FNSYDMHWVRQAPEKGLEWVSTVSRGGSNMWYRDSV
 QGRFTISRDN SKNTLYLQMNGLRAEDRAVYYCAIGDWGFDYWGQGLVTVS;

and the light chain has the following amino acid sequence, **SEQ ID NO:18**

20 ELTQSPSSLSASVGDRLTITCRASQGITNRLNWIYQQKPKAPKLLIYDASSLESGVPSRF
 SGSGSGTDFLTITISSLPEDFATYYCQQFNSYPLTFGGGTKVEIKR.

By “B2” is meant an isolated anti-poliovirus antibody where the heavy chain is encoded by **SEQ ID NO: 7**

25 CTCGAGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCTTGAGACTCTCCTGTGCAGCCTCTGGA
 TTCACCTTCGTTAATTACCACATAAATTGGGTCCGCCAGGCTCCAGGGAAGGGACTGGAGTGGGTGGCA
 CTTATAACAACATGATGGAACCTCGTGAATTTTATGCTGACTCCGTGAAGGGCCGATTACCATCTCCAAA
 GACAATTCCAAGAGCATGCTGTATCTTCAAATGGACAGCCTGAGAACTGAAGACACGGCTATGTATTAT
 TGTGCCAGAGAGATGTATAGTACCTACGATGATGGTTTTGATCTTTGGGGCCAAGGGACAATGGTCACC
 30 GTCTCT;

and the light chain is encoded by **SEQ ID NO: 8**

GAGCTCGTGCTGACTCAGCCACCCTCAGTGTCTGCGACCCCCGGGCGGAGGGTCACCATCTCTTGTCT
 GGAAGCAGCTCCAACATCGGAAGTAATGCTGTAACCTGGTACCAGCAGCTGCCAGGAACGGCCCCCAA
 CTCCTCATCTATACTAATGATCGGCGGCCCTCAGGGTCCCTGACCGATTCTCTGGCTCCAAGTCTGGC
 35 ACCTCAGCCTCCCTGGCCATCAGTGGGCTCCGGTCTGAGGATGAGGCTGATTATTACTGTGCAGCATGG
 GATGACAGCCTGAATGGCGTGGTATTTCGGCGGAGGGACCAAGCTGACCGTCCCTAGGTCAG;

where the heavy chain has the following amino acid sequence, **SEQ ID NO:**

19

LEESGGGVVQPGRSLRLSCAASGFTFVNYHINWVRQAPGKGLEWVALIQHDGTREFYADS
VKGRFTISKDNSKSMYLYLQMDSLRTEdTAMYCAREMYSTYDDGFDLWGQGMVTVS ;

and the light chain has the following amino acid sequence, **SEQ ID NO:**

20

5 ELVLTQPPSVSATPGRRVITISCSGSSSNIGSNAVWYQQLPGTAPKLLIYTNDRRPSGVP
DRFSGSKSGTSASLAI SGLRSEDEADYCAAWDDSLNGVVFGGGTKLTVLGQ .

By “B10” is meant an isolated anti-poliovirus antibody where the heavy chain is encoded by **SEQ ID NO: 9**

10 CTCGAGTCTGGGGGAGACTTGGTCCAGCCGGGGGAGTCCCTGACACTCTCCTGTGTAGCCTCTGGATT
AGAGTCGGCAATTATTGGATGAGCTGGGTCCGCCAGGCTCCGGGAAGGGGCCGAGTGGGTCGCCAAC
ATAAATAAAGATGGAAGTGCACATATTATGCGGACTCCGTGAAGGGCCGATTACCATTTCCAGAGAC
AACGCCCAGAATTCAGTATATGCAAATGAACAGCCTGAGAGTCGAGGACATGGCTGTGTATTACTGT
15 GCGAGAGAGACTGATCAGGTTGTTTGGACTGGTAATTACGCCTCTTCTATTATTACATGGACGTCTGG
GGCCAAGGGACCACGGTCACCGTCTCC ;

and the light chain is encoded by **SEQ ID NO: 10**

gagctcaccagtcctccgtccaccctgtctgcatctgtaggtgacagagtcaccatcacttgtcgggca
agtcagggcattagtaggtatttacattggtatcagcaaaaaccagggaaagcccctaaggtcctgatc
tatgctgctccaccttggaaagtgggggtcccatcaaggttcagcggcagtggtctgggacagaattc
20 actctcaccattagcagcctgcagcccgatgattttgcaacttattactgtcaggagatatttcactgac
gctcgaactttcggccctgggaccaaagtggatatccaacgaactgtg ;

where the heavy chain has the following amino acid sequence, **SEQ ID NO:**

21

LESGGDLVQPGESLTLSCVASGFRVGNYSWVRQAPGKGPWVANINKDGSATYYADSV
25 KGRFTISRDNALQNSLYMQMNSLRVEDMAVYYCARETDQVWVTGNYASSYYYMDVWGQGT
VTVS ;

and the light chain has the following amino acid sequence, **SEQ ID NO: 22**

ELTQSPSTLSASVGDRTITCRASQGISRYLHWYQQKPGKAPKVLIIYAASSTLESVPSRF
SGSGSGTEFTLTITSSLPDDFATYYCQYFTDARTFGPGTKVDIQRV .

30

By “C10” is meant an isolated anti-poliovirus antibody where the heavy chain is encoded by **SEQ ID NO: 11**

CTCGAGTCGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGACACTCTCCTGTGCAGCCTCTGGATT
GCGTTCGGTAGTTATTGGATGAGTTGGGTCCGCCAGGCTCCGGGAAGCGGCCGAGTGGGTGGCCAAC
35 ATGAATAAAAAAGATGGAAGTGAAGAAATACTATGCGGACTCTGTGAAGGGCCGATTACCATCTCCAGA
GACAACACCAAGAACTCACTGTTTCTGAAATGAACAGCCTGAGAGGCGAGGACTCGGCTGTATATTAT
TGTGCGAGAGAGACTTCTCAGGTTGTTTGGACTGGTTCTTACGGCGCTTCTACTACTACATGGACGTC
TGGGGCATGGGGACCACGGTCACCGTCTCC ;

and the light chain is encoded by **SEQ ID NO: 12**

gagctccagatgacccagtcctccgtccaccctgtctgcatctgttaggtgacagagtcaccatcacttgt
 cgggcaagt cagggcattagtaggtatttacattggtatcagcaaaaaccagggaaagcccctaaggtc
 ctgatctatgctgcgctccaccttggaaagtgggggtcccatcaagggttcagcggcagtggtatctgggaca
 5 gaattcactctcaccattagcagcctgcagcccgatgattttgcaacttattactgtcaggagatatttc
 actgacgctcgaactttcggccctgggaccaaagtggatatccaacgaactgtg;

where the heavy chain has the following amino acid sequence, **SEQ ID NO:**

23

LESGGGLVQPGGSLTLSAASGFAGFSYWMSWVRQAPGKRPEWVANMNNKKGSEKYYADS
 10 VKGRFTISRDN TKNSLFLFLEMNSLRGEDSAVYYCARETSQVVTGSGYASYYMDVWGMGT
 TVTVS;

and the light chain has the following amino acid sequence, **SEQ ID NO: 24**

ELQMTQSPSTLSASVGDRTITCRASQGISRYLHWYQQKPGKAPKVLIIYAASLTLESGVPS
 15 RFSGSGSGTEFTLTITSSLPDDFATYYCQEFYFDARTFGPGTKVDIQRTV.

By "agent" is meant any small molecule chemical compound, antibody, nucleic acid molecule, or polypeptide, or fragments thereof.

By "ameliorate" is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

20 By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression levels, preferably a 25% change, more preferably a 40% change, and most preferably a 50% or greater change in expression levels.

25 By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for
 30 example, ligand binding. An analog may include an unnatural amino acid.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean "includes," "including," and the like; "consisting essentially of" or "consists
 35 essentially" likewise has the meaning ascribed in U.S. Patent law and the term is

open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

“Detect” refers to identifying the presence, absence or amount of the analyte
5 to be detected.

By "detectable label" is meant a composition that when linked to a molecule of interest renders the latter detectable, via spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include radioactive isotopes, magnetic beads, metallic beads, colloidal particles, fluorescent dyes,
10 electron-dense reagents, enzymes (for example, as commonly used in an ELISA), biotin, digoxigenin, or haptens.

By “disease” is meant any condition or disorder that damages or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include
[insert]

By "effective amount" is meant the amount of a required to ameliorate the symptoms of a disease relative to an untreated patient. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian
15 will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.
20

The invention provides a number of targets that are useful for the development of highly specific drugs to treat or a disorder characterized by the methods delineated herein. In addition, the methods of the invention provide a facile means to identify
25 therapies that are safe for use in subjects. In addition, the methods of the invention provide a route for analyzing virtually any number of compounds for effects on a disease described herein with high-volume throughput, high sensitivity, and low complexity.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule.
30 This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

5 By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote
10 or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

15 By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most
20 preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel
25 electrophoresis, or by HPLC analysis.

By "marker" is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

As used herein, "obtaining" as in "obtaining an agent" includes synthesizing, purchasing, or otherwise acquiring the agent.

30 "Primer set" means a set of oligonucleotides that may be used, for example, for PCR. A primer set would consist of at least 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 30, 40, 50, 60, 80, 100, 200, 250, 300, 400, 500, 600, or more primers.

By "reduces" is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By "reference" is meant a standard or control condition.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the
5 complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will
10 generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

By "specifically binds" is meant a compound or antibody that recognizes and binds a polypeptide of the invention, but which does not substantially recognize and
15 bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous
20 nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such
25 nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary
30 polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) *Methods Enzymol.* 152:399; Kimmel, A. R. (1987) *Methods Enzymol.* 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and

50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 .mu.g/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art. Hybridization techniques are well known to those skilled in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness

(Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 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, or 50.

As used herein, the terms "treat," "treating," "treatment," and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not

require that the disorder, condition or symptoms associated therewith be completely eliminated.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

20

SEQUENCE LISTING

>A12-1H

SEQ ID NO: 1

CTCGAGTGGGGCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCTGCAGTGTCTCTAGTGGC
 TTCATCAATCTATATTCGTGGAATTGGATCCGCCAGGCACCAGGGACGGGACTGGAGTGGATTGGGTCC
 ATCCGTTATGGTGAGACCGCTATTACAACCCATCCCTCAGGGGTCGAGTCTTCATATCACTAGACACG
 TCCAAGAACCAGTTCTACCTGAAAATGAGCTCTCTGACCCGCCGACAGACACGGCCGTTTATTATTGTGCG
 AGAGACTACAGTAAGAGTGGCTACGATCGCTCGGGGCGGCTCGACCCCTGGGGCCAGGGAACCCTAGTC
 ATCGTCTCC

30

>A12-1L

SEQ ID NO: 2

GAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCC
 AGTCAGGACGTTAGTACTTATTTAGCCTGGTATCACCAAAAACCAGGAAAAGCCCCGAGGGTCTCATC
 TATTATGCATCCAATTTACAAAGTGGGGTCCCATCAAGGTTTCAGCGGCAGTGGATCTGGGACAGAGTTC
 ACTCTCACAATCACCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAACAGCTTGATAATTTT
 CCGCCGACTTTTCGGCCAAGGGACCAAGCTGGAAATCAAACGA

35

>H2-1H

SEQ ID NO: 3

CTCGAGTCTGGGGGAGGCGTGGTCCAGCCCGGGAGGTCCTTGAGACTCTCCTGTGCAGCCTCTGGATT
5 ACCTTCACTAATTTTGGCATGAATTGGGTCCGCCAGGCTCCAGGCAAGGGGCTGGAGTGGGTGGCAACA
CTTTTCGTTTGTATCCGAATGATAAAATATTATGCAGACTCCGTGAAGGGCCGATTACCATCTCCAGAGAC
GATTCCACACACACGTTGTATCTTCAGATGGACAGCCTGCGAGTTGAGGACACGGCTGTGTATTTCTGT
GCGAGGAGGGATTTTGTATTACATTTCTGGGGACTTCTCGTTCATACTTCTTTGACTATTGGGGCCAGGGC
GCCAGGTCACCGTCTCC

10

>H2-1L

SEQ ID NO: 4

GAGCTCACGCAGTCTCCAGACTTTTCAGTCTGTGCCGCCAAAGGAGAAAGTCACCATCACCTGCCGGGCC
AGTCAGAGCATTGGCAGTAGCTTACACTGGTACCAGCAGAAAACCAGGTCAGTCTCCAAAACCTCTCATC
15 AAGTATGCTTCCCAGTCCATCTCAGGGGTCCCTCGAGGTTTCAGTGGCAGTGGCTCTGGGACAGACTTC
GCCCTCACCATCAATGGCCTGGAAGCTGAAGATGCCGCAACGTATTACTGTCTGCAGAGTAGTAGTTTA
CCTCAGACGTTTCGGCCAAGGGACCAAGCTGGAAATCAAACGA

>A6-2H

SEQ ID NO: 5

CTCGAGTCTGGGGGAGGCTTGGTAAAAGCCTGGGGGTTCCTTGAGACTCTCGTGTGCGGCCCTCTGGATT
ATCTTCAATTCCATGATATGCACTGGGTCCGCCAGGCTCCAGAGAAGGGGCTGGAGTGGGTCTCAACT
GTCAGTCTGGAGGTAGTAACATGTGGTACCGAGACTCTGTCCAGGGCCGATTACCATCTCCAGAGAC
AATTCCAAGAATACATTGTATCTCCAAATGAATGGCTCAGAGCCGAGGACAGGGCCGTGTATTACTGT
25 GCGATTGGCGACTGGGGTTTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCC

25

>A6-2L

SEQ ID NO: 6

GAGCTCACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGACTCACCATCACTTGCCGGGCA
30 AGTCAGGGCATTACCAATAGATTAATTTGGTATCAGCAGAAAACCAGGGAAAGCTCCTAAGCTCCTGATC
TATGATGCCTCCAGTTTGGAAAAGTGGGGTCCCTTCAAGGTTTCAGCGGCAGTGGATCTGGGACAGATTC
ACTCTCACCATCAGCAGCCTGCAGCCTGAAGATTTTGCAACTTATTACTGTCAACAGTTTAATAGTTAC
CCGCTCACTTTCCGGTGGAGGGACCAAGGTGGAGATCAAACGA

35

>B2-2H

SEQ ID NO: 7

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CTTATAACAACATGATGGAACCTCGTGAATTTTATGCTGACTCCGTGAAGGGCCGATTACCATCTCCAAA
40 GACAATTCGAAGAGCATGCTGTATCTTCAAATGGACAGCCTGAGAACTGAAGACACGGCTATGTATTAT

40

TGTGCCAGAGAGATGTATAGTACCTACGATGATGGTTTTGATCTTTGGGGCCAAGGGACAATGGTCACC
GTCTCT

>B2-2L

5 **SEQ ID NO: 8**

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CTCCTCATCTATACTAATGATCGGCGGCCCTCAGGGGTCCTGACCGATTCTCTGGCTCCAAGTCTGGC
ACCTCAGCCTCCCTGGCCATCAGTGGGCTCCGGTCTGAGGATGAGGCTGATTATTACTGTGCAGCATGG
10 GATGACAGCCTGAATGGCGTGGTATTTCGGCGGAGGGACCAAGCTGACCGTCCTAGGTCAG

>B10-3H

SEQ ID NO: 9

15 CTCGAGTCTGGGGGAGACTTGGTCCAGCCGGGGGAGTCCCTGACACTCTCCTGTGTAGCCTCTGGATTC
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ATAAATAAAGATGGAAGTGCACATATTATGCGGACTCCGTGAAGGGCCGATTACCATTTCCAGAGAC
AACGCCCAGAATTCACTGTATATGCAAATGAACAGCCTGAGAGTCGAGGACATGGCTGTGTATTACTGT
GCGAGAGAGACTGATCAGGTGTGTTGGACTGGTAATTACGCCCTCTTCCTATTATTACATGGACGTCTGG
20 GGCCAAGGGACCACGGTCACCGTCTCC

>B10-3L

SEQ ID NO: 10

gagctcaccagctctccgtccaccctgtctgcatctgtaggtgacagagtcaccatcacttgtcgggca
25 agtcagggcattagtaggtatctacattggtatcagcaaaaaccagggaaagcccctaaggtcctgatc
tatgtctgctccacccttggaaagtggggtcccatcaagggtcagcggcagtggtatctgggacagaattc
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30 >C10-3H

SEQ ID NO: 11

CTCGAGTCGGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGACACTCTCCTGTGCAGCCTCTGGATTC
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ATGAATAAAAAAGATGGAAGTGAGAAATACTATGCGGACTCTGTGAAGGGCCGATTACCATCTCCAGA
35 GACAACACCAAGAACTCACTGTTTCTGGAAAATGAACAGCCTGAGAGGCGAGGACTCGGCTGTATATTAT
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>C10-3L

40 **SEQ ID NO: 12**

Gagctccagatgaccagtcctccgtccaccctgtctgcatctgttaggtgacagagtcaccatcacttgt
cgggcaagtcagggcattagtaggtatttacattggatcagcaaaaaccagggaaagcccctaaggtc
ctgatctatgctgctccaccttggaaagtgggggtcccatcaagggtcagcggcagtgatctgggaca
gaattcactctcaccattagcagcctgcagcccgatgattttgcaacttattactgtcaggagatatttc
5 actgacgctcgaactttcggccctgggaccaaagtggatatccaacgaactgtg

>A12-1H

SEQ ID NO: 13

LEWGPGLVKPSETLSLTCSVSSGFINLYSWNWIRQAPGTGLEWIGSIRYGETAYYNPSLR
10 GRVFI SLDTSKNQFYLKMS SLTAADTAVYYCARDYSKSGYDRSGRLDPWGQGLVIVS

>A12-1L

SEQ ID NO:14

ELTQSPSSLSASVGDRTTITCRASQDVSTYLAWYHQKPGKAPRVLIYYASNLSQSGVPSRF
15 SGSGSGTEFTLTITISLQPEDFATYYCQQLDNFPPTFGQGTKLEIKR

>H2-1H

SEQ ID NO: 15

LESGGGVVQPGRSLRLSCAASGFTFTNFGMNWVRQAPGKLEWVATLSFDPNDKYYADSV
20 KGRFTISRDDSTHTLYLQMDSLRVEDTAVYFCARRDFDYISGTSRSYFFDYWGQGAQVTV
S

>H2-1L

SEQ ID NO: 16

ELTQSPDFQSVPPKEKVTITCRASQSIGSSLHWYQQKPGQSPKLLIKYASQSIGVPSRF
25 SGSGSGTDFALTINGLEAEADAATYYCLQSSSLPQTFGQGTKLEIKR

>A6-2H

SEQ ID NO: 17

LESGGGLVKPGGSLRLSCAASGFI FNSYDMHWVRQAPEKLEWVSTVSRGGSNMWYRDSV
30 QGRFTISRDN SKNTLYLQMNGLRAEDRAVYYCAIGDWGFDYWGQGLVTVS

>A6-2L

SEQ ID NO: 18

ELTQSPSSLSASVGDRLTITCRASQGITNRLNHWYQQKPGKAPKLLIYDASSLESGVPSRF
35 SGSGSGTDFLTITISSLQPEDFATYYCQFNSYPLTFGGGKVEIKR

>B2-2H

SEQ ID NO: 19

LEESGGGVVQPGRSLRLSCAASGFTFVNYHINWVRQAPGKLEWVALIQHDGTREFYADS
40

VKGRFTISKDNSKSMYLYLQMDSLRTEDTAMYYCAREMYSTYDDGFDLWGQGTMTVTS

>B2-2L

SEQ ID NO: 20

5 ELVLTQPPSVSATPGRRVTIISCSGSSSNIGSNAVITWYQQLPGTAPKLLIYTNDRRPSGVP
DRFSGSKSGTSASLAIISGLRSEDEADYYCAAWDDSLNGVVFGGGTKLTVLGQ

>B10-3H

SEQ ID NO: 21

10 LESGGDLVQPGESLTLSCVASGFRVGNYSWVRQAPGKGPPEWVANINKDGSATYYADSV
KGRFTISRDNQNSLYMQMNSLRVEDMAVYYCARETDQVVWTGNYASSYYYMDVWGQGT
TVTS

>B10-3L

SEQ ID NO: 22

15 ELTQSPSTLSASVGDRTIITCRASQGISRYLHWYQQKPGKAPKVLIIAASTLESGVPSRF
SGSGSGTEFTLTITISSLPDDFATYYCQYFTDARTFGPGTKVDIQRTV

>C10-3H

SEQ ID NO: 23

20 LESGGGLVQPGSLTLSCAASGFAGSYWMSWVRQAPGKRPEWVANMNKKGSEKYYADS
VKGRFTISRDNKNSLFLFLEMNSLRGEDSAVYYCARETSQVVWTGSGYASYYYMDVWGMGT
TVTTS

25 >C10-3L

SEQ ID NO: 24

ELQMTQSPSTLSASVGDRTIITCRASQGISRYLHWYQQKPGKAPKVLIIAASTLESGVPS
RFSGSGSGTEFTLTITISSLPDDFATYYCQYFTDARTFGPGTKVDIQRTV

30

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a table showing the specific binding and neutralization of selected
35 Fabs for the various polio-virus serotypes.

Figure 2 is a table showing the assignment of poliovirus-neutralizing Fab
clones to their closest human germ line counterparts, based on nucleotide sequence
homology.

Figures 3A-3C show the binding specificity of anti-poliovirus MAbs. Binding in response to different concentrations of anti-poliovirus MAbs on serotype 1 (Figure 3A), 2 (Figure 3B) and 3 (Figure 3C) of Sabin poliovirus was measured by ELISA with virions directly attached to the solid phase. Type 1 MAbs: H2, □; A12, ■; Type 2 MAbs: A6, ▲; B2, △; Type 3 MAbs: B10, ○; C10, ●.

Figure 4 is a table showing the results of the block-ELISA reaction between human monoclonal antibodies and polioviruses.

Figures 5A-5C show the block-ELISA reaction of primate MAbs with wild and attenuated Sabin polioviruses. Figures 5A, 5B, and 5C show reactions with type 1, type 2, and type 3 polioviruses, respectively. Figure 5A: Sabin 1 / H2, ○; Mahoney / H2, ●; Sabin 1 / A12, □; Mahoney / A12, ■. Figure 5B: Sabin 2 / B2, ○; MEF-1 / B2, ●; Sabin 2 / A6, □; MEF-1 / A6, ■. Figure 5C: Sabin 3 / B10, ○; Saukett / B10, ●; Sabin 3 / C10, □; Saukett / C10, ■.

Figure 6 is a table showing the cross-neutralization of Sabin and wild polioviruses with humanized MAbs.

Figure 7 is a table showing neutralization of polioviruses with rabbit hyperimmune sera.

Figure 8 is a schematic poliovirus structure showing the location of the epitope to antibody H2 on the surface of type 1 poliovirus. Capsid proteins VP1, VP2, and VP3 are shown in pink, green, and light blue, respectively. Antigenic sites 1, 2, 3, and 4 are shown in red, orange, gold, and yellow, respectively. Amino acids that are replaced in escape mutants are shown in black.

Figures 9A and 9B show the location of the epitope to antibody A12 on the surface of poliovirus. Figure 9A: Scheme of poliovirus capsid showing location of a single capsomer relative to the 5, 3 and 2-fold axes of symmetry. Canyon surrounds the 5-fold axis. VP1, VP2, and VP3 proteins are shown in pink, green, and light blue, respectively; Figure 9B: 3-D structure of poliovirus capsomer. Color codes for proteins VP1, VP2, and VP3 are the same as on Figure 9A. Antigenic sites 1 and 2 are shown in red and orange, respectively, and amino acids replaced in escape mutants are shown in black.

Figure 10 is a table showing that H2 MAb protects transgenic mice from a challenge with ten paralytic doses of wild poliovirus (Mahoney strain).

Figure 11 is a table showing that H2 MAb protects transgenic mice even when administered after challenge with five paralytic doses of Mahoney virus.

Figure 12 is a table showing protection of PVRTg-mice from poliovirus infection by monoclonal antibody A12.

Figure 13 is an image of the A12 Fab complex bound to Type 1 poliovirus.

Figure 14 is an image of the A12 Fab complex bound to Type 2 poliovirus.

5 Figure 15 is a schematic image of the A12 epitope.

DETAILED DESCRIPTION OF THE INVENTION

The invention features compositions and methods that are useful for the treatment or prevention of poliovirus infection.

10 Chimeric chimpanzee/human anti-poliovirus antibodies were isolated by combining hyper-immunization of chimpanzees with phage Fab display library technology. Compared to other methods based on human hybridoma technology (Dessain, S. K. et al., (2004) J Immunol Methods 291:109-22) and B-cell immortalization with EBV (Steinitz, M. et al., (1977) Nature 269:420-2; Traggiai, E. et al., (2004) Nat Med 10:871-5), the method described herein has an advantage of
15 producing cloned immunoglobulin genes that can be used directly for production of antibodies expressed *in vitro*, as well as for genetic manipulations that improve their therapeutic properties. Furthermore, the chimpanzee immunoglobulins described herein are virtually identical to humans, therefore, they can be used in humans
20 without further modifications such as “humanizing.”

The antigenic structure of polioviruses has been extensively studied in the past (Minor, P. D. (1986) Microbiol Sci 3:141-4). There are three distinct serotypes of poliovirus that are defined by their inability to be cross-neutralized by sera raised against two other serotypes (Pallansch, M. A., and R. P. Roos. (2001) p. 723-775. *In*
25 *Fields Virology*, Fourth ed.). Elucidation of the molecular structure of poliovirus and the introduction of MAb technology allowed epitopes to mouse MAbs to be mapped on the surface of polio virions. Mutations rendering poliovirus resistant to MAbs identify epitopes with which the antibodies interact. Epitopes to different MAbs are clustered into several structural regions (antigenic sites) on the surface of polio virions
30 (Humphrey, D. et al., (1982) Infection and Immunity 36:841-843; Uhlig, H. et al., (1983) Journal of General Virology 64:2809-2812). Studies with three serotypes of poliovirus identified three or four such antigenic sites. Antigenic site 1 is composed of the so-called B-C loop and the adjacent regions within VP1, while three other antigenic sites are composed of a combination of different capsid proteins, in some

cases belonging to adjacent capsomers. Most poliovirus epitopes are believed to be conformational and are very sensitive to disruption of the virion structure.

The methods described herein resulted in the generation of antibodies that are capable of binding to more than one serotype of poliovirus. One illustrative example described herein is the dual specificity of antibody A12. It binds and neutralizes both type 1 and type 2 polioviruses. Cross-neutralization experiments with hyper-immune rabbit antisera indicate that such dual-specific antibodies may not exist in the rabbit immunoglobulin repertoire. In contrast, the methods described herein is well suited for targeted screening for cross-neutralizing antibodies by phage display panning Fab fragments induced by one serotype against antigens of another serotype.

Dual type 1/type 2-specific antibody A12 appears to bind to both antigenic sites 1 and 2, and thus fills the canyon on the virion surface where the receptor-binding site is located (Belnap, D. M. et al., (2000) *Proc Natl Acad Sci U S A* 97:73-8; Colston, E., and V. R. Racaniello, (1994) *EMBO J* 13:5855-62; Wien, M. W. et al., (1997) *Nat Struct Biol* 4:666-74). Therefore it is reasonable to suggest that antibody A12 prevents virus from binding to its cellular receptor.

Comparison of amino acid sequences and three-dimensional structures of relevant parts of Sabin 1 and Sabin 2 capsid proteins shows that they are similar, and amino acid differences in this region involve chemically similar amino acids. This provides an explanation of dual reactivity of A12 antibody. Another antibody, H2, binds to the space between antigenic sites 2 and 3. Therefore parts of the virion that are immunologically important in mice and chimpanzees appear to differ.

Previous attempts to use intravenous immunoglobulin and breast milk to treat poliovirus were unsuccessful, even though a significant temporary decrease in shedding was observed when the patient was treated with breast milk (MacLennan, C., (2004) *Lancet* 363:1509-13). The failure to completely stop shedding may be due to insufficient levels of anti-poliovirus antibodies in IVIG and breast milk.

Availability of highly potent human-like antibodies to poliovirus described herein opens the possibility of curing chronically infected individuals of their poliovirus infection, thereby stopping shedding of virus into the environment. Addition of the recombinant chimpanzee/human chimeric antibodies described herein could boost neutralizing titers to extremely high levels and thereby resolve chronic infection.

The transgenic mouse data presented herein indicate that the antibodies can protect against poliomyelitis not only when given prior to infection, but also after

exposure to poliovirus. This means that the antibodies can stop development of the infectious process even after it has started. This demonstrates their utility in emergency prophylaxis of contacts of a paralytic polio case, or to prevent spread of poliovirus, should it re-emerge after apparent eradication. Under these circumstances
5 emergency vaccinations will be performed to stop the outbreak. Adding treatment with anti-poliovirus antibodies to this intervention will result in immediate protection of the involved individuals, while allowing the longer-term effect of a vaccine to develop.

The present invention provides methods of treating or preventing polio or
10 symptoms thereof which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a compound of the formulae herein to a subject (e.g., a mammal such as a human). Thus, one embodiment is a method of treating a subject suffering from or susceptible to poliovirus infection. The method includes the step of administering to the mammal a therapeutic amount of an amount
15 of a compound herein sufficient to treat the disease or disorder or symptom thereof, under conditions such that the disease or disorder is treated.

The methods herein include administering to the subject (including a subject identified as in need of such treatment) an effective amount of a compound described herein, or a composition described herein to produce such effect. Identifying a
20 subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g. opinion) or objective (e.g. measurable by a test or diagnostic method).

As used herein, the terms “treat,” “treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be
25 appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

As used herein, the terms “prevent,” “preventing,” “prevention,” “prophylactic treatment” and the like refer to reducing the probability of developing a disorder or
30 condition in a subject, who does not have, but is at risk of or susceptible to developing a disorder or condition.

The therapeutic methods of the invention (which include prophylactic treatment) in general comprise administration of a therapeutically effective amount of the compounds herein, such as a compound of the formulae herein to a subject (e.g.,

animal, human) in need thereof, including a mammal, particularly a human. Such treatment will be suitably administered to subjects, particularly humans, suffering from, having, susceptible to, or at risk for a disease, disorder, or symptom thereof. Determination of those subjects "at risk" can be made by any objective or subjective
5 determination by a diagnostic test or opinion of a subject or health care provider (e.g., genetic test, enzyme or protein marker, Marker (as defined herein), family history, and the like). The compounds herein may be also used in the treatment of any other disorders in which poliovirus may be implicated.

10 In one embodiment, the invention provides a method of monitoring treatment progress. The method includes the step of determining a level of diagnostic marker (Marker) (e.g., any target delineated herein modulated by a compound herein, a protein or indicator thereof, etc.) or diagnostic measurement (e.g., screen, assay) in a subject suffering from or susceptible to a disorder or symptoms thereof associated
15 with poliovirus, in which the subject has been administered a therapeutic amount of a compound herein sufficient to treat the disease or symptoms thereof. The level of Marker determined in the method can be compared to known levels of Marker in either healthy normal controls or in other afflicted patients to establish the subject's disease status. In preferred embodiments, a second level of Marker in the subject is
20 determined at a time point later than the determination of the first level, and the two levels are compared to monitor the course of disease or the efficacy of the therapy. In certain preferred embodiments, a pre-treatment level of Marker in the subject is determined prior to beginning treatment according to this invention; this pre-treatment level of Marker can then be compared to the level of Marker in the subject after the
25 treatment commences, to determine the efficacy of the treatment.

Antibodies

30 Antibodies (e.g., chimpanzee/human chimeric antibodies) that selectively bind poliovirus are useful in the methods of the invention. Such antibodies are particularly useful for neutralizing poliovirus and/or treating and preventing poliovirus infection. As described herein below, binding to poliovirus neutralizes poliovirus as determined by a plaque reduction neutralization test. In some embodiments the antibodies were used to prevent paralytic disease in transgenic mice treated with poliovirus. In some

embodiments, the protection was seen when the antibodies were administered before the mice were treated with poliovirus. In certain embodiments, the protection occurred when the antibodies were administered after the mice were treated with poliovirus.

5 Methods of preparing antibodies are well known to those of ordinary skill in the science of immunology. As used herein, the term “antibody” means not only intact antibody molecules, but also fragments of antibody molecules that retain immunogen-binding ability. Such fragments are also well known in the art and are regularly employed both *in vitro* and *in vivo*. Accordingly, as used herein, the term
10 “antibody” means not only intact immunoglobulin molecules, but also the well-known active fragments $F(ab')_2$, and Fab. $F(ab')_2$, and Fab fragments that lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding of an intact antibody (Wahl et al., *J. Nucl. Med.* 24:316-325 (1983). Preferably, the antibodies of the invention comprise chimeric
15 antibodies, humanized antibodies, fusion polypeptides, and unconventional antibodies. In preferred embodiments, the invention provides hybrid antibodies, in which one portion of the antibody is obtained from a first antibody (e.g., a chimpanzee/human chimeric antibody), while the other portion is obtained from a different second antibody (e.g., human antibody). Such antibodies are often referred
20 to as “chimeric” antibodies. Such hybrids may also be formed using humanized antibodies.

In general, intact antibodies are said to contain “Fc” and “Fab” regions. The Fc regions are involved in complement activation and are not involved in antigen binding. An antibody from which the Fc’ region has been enzymatically cleaved, or
25 which has been produced without the Fc’ region, designated an “ $F(ab)_2$ ” fragment, retains both of the antigen binding sites of the intact antibody. Similarly, an antibody from which the Fc region has been enzymatically cleaved, or which has been produced without the Fc region, designated an “Fab” fragment, retains one of the antigen binding sites of the intact antibody. Fab fragments consist of a covalently
30 bound antibody light chain and a portion of the antibody heavy chain, denoted “Fd.” The Fd fragments are the major determinants of antibody specificity (a single Fd fragment may be associated with up to ten different light chains without altering antibody specificity).

Antibodies can be made by any of the methods known in the art utilizing poliovirus vaccines, or immunogenic fragments thereof, as an immunogen. One method of obtaining antibodies is to immunize suitable host animals with an immunogen and to follow standard procedures for polyclonal or monoclonal antibody production. The immunogen will facilitate presentation of the immunogen on the cell surface. Immunization of a suitable host can be carried out in a number of ways. Nucleic acid sequences encoding a poliovirus polypeptide or immunogenic fragments thereof, can be provided to the host in a delivery vehicle that is taken up by immune cells of the host. The cells will in turn express the poliovirus polypeptide on the cell surface generating an immunogenic response in the host. Alternatively, nucleic acid sequences encoding a poliovirus polypeptide, or immunogenic fragments thereof, can be expressed in cells *in vitro*, followed by isolation of the polypeptide and administration of the polypeptide to a suitable host in which antibodies are raised.

Alternatively, antibodies against a poliovirus may, if desired, be derived from an antibody phage display library. A bacteriophage is capable of infecting and reproducing within bacteria, which can be engineered, when combined with human antibody genes, to display human antibody proteins. Phage display is the process by which the phage is made to 'display' the human antibody proteins on its surface. Genes from the human antibody gene libraries are inserted into a population of phage. Each phage carries the genes for a different antibody and thus displays a different antibody on its surface.

Antibodies made by any method known in the art can then be purified from the host. Antibody purification methods may include salt precipitation (for example, with ammonium sulfate), ion exchange chromatography (for example, on a cationic or anionic exchange column preferably run at neutral pH and eluted with step gradients of increasing ionic strength), gel filtration chromatography (including gel filtration HPLC), and chromatography on affinity resins such as protein A, protein G, hydroxyapatite, and anti-immunoglobulin.

Antibodies can be conveniently produced from hybridoma cells engineered to express the antibody. Methods of making hybridomas are well known in the art. The hybridoma cells can be cultured in a suitable medium, and spent medium can be used as an antibody source. Polynucleotides encoding the antibody of interest can in turn be obtained from the hybridoma that produces the antibody, and then the antibody may be produced synthetically or recombinantly from these DNA sequences. For the

production of large amounts of antibody, it is generally more convenient to obtain an ascites fluid. The method of raising ascites generally comprises injecting hybridoma cells into an immunologically naive histocompatible or immunotolerant mammal, especially a mouse. The mammal may be primed for ascites production by prior administration of a suitable composition (e.g., Pristane).

Antibodies produced by methods of the invention can be "humanized" by methods known in the art. "Humanized" antibodies are antibodies in which at least part of the sequence has been altered from its initial form to render it more like human immunoglobulins. Techniques to humanize antibodies are particularly useful when non-human animal (e.g., murine) antibodies are generated. Examples of methods for humanizing a murine antibody are provided in U.S. patents 4,816,567, 5,530,101, 5,225,539, 5,585,089, 5,693,762 and 5,859,205. However, chimpanzee immunoglobulins described herein are virtually identical to human immunoglobulins, therefore, chimpanzee immunoglobulins of the invention may be used in humans without further modifications such as "humanizing."

In other embodiments, the invention provides "unconventional antibodies." Unconventional antibodies include, but are not limited to, nanobodies, linear antibodies (Zapata et al., *Protein Eng.* 8(10): 1057-1062,1995), single domain antibodies, single chain antibodies, and antibodies having multiple valencies (e.g., diabodies, tribodies, tetrabodies, and pentabodies). Nanobodies are the smallest fragments of naturally occurring heavy-chain antibodies that have evolved to be fully functional in the absence of a light chain. Nanobodies have the affinity and specificity of conventional antibodies although they are only half of the size of a single chain Fv fragment. The consequence of this unique structure, combined with their extreme stability and a high degree of homology with human antibody frameworks, is that nanobodies can bind therapeutic targets not accessible to conventional antibodies. Recombinant antibody fragments with multiple valencies provide high binding avidity and unique targeting specificity to cancer cells. These multimeric scFvs (e.g., diabodies, tetrabodies) offer an improvement over the parent antibody since small molecules of ~60-100kDa in size provide faster blood clearance and rapid tissue uptake. See Power et al., (Generation of recombinant multimeric antibody fragments for tumor diagnosis and therapy. *Methods Mol Biol*, 207, 335-50, 2003); and Wu et al. (Anti-carcinoembryonic antigen (CEA) diabody for rapid tumor targeting and imaging. *Tumor Targeting*, 4, 47-58, 1999).

Various techniques for making unconventional antibodies have been described. Bispecific antibodies produced using leucine zippers are described by Kostelny et al. (J. Immunol. 148(5):1547-1553, 1992). Diabody technology is described by Hollinger et al. (Proc. Natl. Acad. Sci. USA 90:6444-6448, 1993).

5 Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers is described by Gruber et al. (J. Immunol. 152:5368, 1994). Trispecific antibodies are described by Tutt et al. (J. Immunol. 147:60, 1991). Single chain Fv polypeptide antibodies include a covalently linked VH::VL heterodimer which can be expressed from a nucleic acid including V_H- and V_L-encoding sequences
10 either joined directly or joined by a peptide-encoding linker as described by Huston, et al. (Proc. Nat. Acad. Sci. USA, 85:5879-5883, 1988). See, also, U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778; and U.S. Patent Publication Nos. 20050196754 and 20050196754.

Antibodies of the invention are particularly useful for the treatment of
15 poliovirus. Accordingly, the present invention provides methods of treating and/or preventing polio and/or disorders or symptoms thereof which comprise administering a therapeutically effective amount of a pharmaceutical composition comprising a chimeric antibody described herein to a subject (e.g., a mammal such as a human). Thus, one embodiment is a method of treating a subject suffering from or susceptible
20 to polio or disorder or symptom thereof. The method includes the step of administering to the mammal a therapeutic amount (of an amount) of a compound herein sufficient to treat the disease or disorder or symptom thereof, under conditions such that the disease or disorder is treated.

The methods herein include administering to the subject (including a subject
25 identified as in need of such treatment) a therapeutically effective amount of a compound described herein, or a composition described herein to produce such effect. Identifying a subject in need of such treatment can be in the judgment of a subject or a health care professional and can be subjective (e.g. opinion) or objective (e.g. measurable by a test or diagnostic method). For example, the antibodies of the
30 invention may be administered to a subject who had been exposed to polio virus, exposed to a person excreting poliovirus, or exposed to a person who is suspected of being infected with poliovirus.

The therapeutic methods of the invention (which include prophylactic treatment) in general comprise administration of a therapeutically effective amount of

the chimeric antibodies delineated herein to a subject (e.g., animal, human) in need thereof, including a mammal, particularly a human. Such treatment will be suitably administered to subjects, particularly humans, suffering from, having, susceptible to, or at risk for poliovirus infection. Determination of those subjects "at risk" can be
5 made by any objective or subjective determination by a diagnostic test or opinion of a subject or health care provider (e.g., genetic test, enzyme or protein marker, Marker (as defined herein), family history, and the like).

In one embodiment, the invention provides a method of monitoring treatment progress. The method includes the step of determining a level of diagnostic marker
10 (Marker) (e.g., any target delineated herein modulated by a compound herein, a protein or indicator thereof, etc.) or diagnostic measurement (e.g., screen, assay) in a subject suffering from or susceptible to a disorder or symptoms thereof associated with polio, in which the subject has been administered a therapeutic amount of a compound herein sufficient to treat the disease or symptoms thereof. The level of
15 Marker determined in the method can be compared to known levels of Marker in either healthy normal controls or in other afflicted patients to establish the subject's disease status. In preferred embodiments, a second level of Marker in the subject is determined at a time point later than the determination of the first level, and the two levels are compared to monitor the course of disease or the efficacy of the therapy. In
20 certain preferred embodiments, a pre-treatment level of Marker in the subject is determined prior to beginning treatment according to this invention; this pre-treatment level of Marker can then be compared to the level of Marker in the subject after the treatment commences, to determine the efficacy of the treatment.

25 **Recombinant Polypeptide Expression**

The invention provides antibodies (chimeric antibodies) of the invention that are useful for the treatment of poliovirus infection. In particular, the invention provides chimpanzee/human chimeric, which can be expressed recombinantly. Typically, recombinant polypeptides are produced by transformation of a suitable host
30 cell with all or part of a polypeptide-encoding nucleic acid molecule or fragment thereof in a suitable expression vehicle.

Those skilled in the field of molecular biology will understand that any of a wide variety of expression systems may be used to provide the recombinant protein. The precise host cell used is not critical to the invention. A polypeptide of the

invention may be produced in a prokaryotic host (e.g., *E. coli*) or in a eukaryotic host (e.g., *Saccharomyces cerevisiae*, insect cells, e.g., Sf21 cells, or mammalian cells, e.g., NIH 3T3, HeLa, or preferably COS cells). Such cells are available from a wide range of sources (e.g., the American Type Culture Collection, Rockland, Md.; also, see, e.g., Ausubel et al., Current Protocol in Molecular Biology, New York: John Wiley and Sons, 1997). The method of transformation or transfection and the choice of expression vehicle will depend on the host system selected. Transformation and transfection methods are described, e.g., in Ausubel et al. (supra); expression vehicles may be chosen from those provided, e.g., in Cloning Vectors: A Laboratory Manual (P. H. Pouwels et al., 1985, Supp. 1987).

A variety of expression systems exist for the production of the polypeptides of the invention. Expression vectors useful for producing such polypeptides include, without limitation, chromosomal, episomal, and virus-derived vectors, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof.

One particular bacterial expression system for polypeptide production is the *E. coli* pET expression system (e.g., pET-28) (Novagen, Inc., Madison, Wis). According to this expression system, DNA encoding a polypeptide is inserted into a pET vector in an orientation designed to allow expression. Since the gene encoding such a polypeptide is under the control of the T7 regulatory signals, expression of the polypeptide is achieved by inducing the expression of T7 RNA polymerase in the host cell. This is typically achieved using host strains that express T7 RNA polymerase in response to IPTG induction. Once produced, recombinant polypeptide is then isolated according to standard methods known in the art, for example, those described herein.

Another bacterial expression system for polypeptide production is the pGEX expression system (Pharmacia). This system employs a GST gene fusion system that is designed for high-level expression of genes or gene fragments as fusion proteins with rapid purification and recovery of functional gene products. The protein of interest is fused to the carboxyl terminus of the glutathione S-transferase protein from *Schistosoma japonicum* and is readily purified from bacterial lysates by affinity chromatography using Glutathione Sepharose 4B. Fusion proteins can be recovered

under mild conditions by elution with glutathione. Cleavage of the glutathione S-transferase domain from the fusion protein is facilitated by the presence of recognition sites for site-specific proteases upstream of this domain. For example, proteins expressed in pGEX-2T plasmids may be cleaved with thrombin; those expressed in
5 pGEX-3X may be cleaved with factor Xa.

Alternatively, recombinant polypeptides of the invention are expressed in *Pichia pastoris*, a methylotrophic yeast. *Pichia* is capable of metabolizing methanol as the sole carbon source. The first step in the metabolism of methanol is the oxidation of methanol to formaldehyde by the enzyme, alcohol oxidase. Expression
10 of this enzyme, which is coded for by the AOX1 gene is induced by methanol. The AOX1 promoter can be used for inducible polypeptide expression or the GAP promoter for constitutive expression of a gene of interest.

Once the recombinant polypeptide of the invention is expressed, it is isolated, for example, using affinity chromatography. In one example, an antibody (e.g.,
15 produced as described herein) raised against a polypeptide of the invention may be attached to a column and used to isolate the recombinant polypeptide. Lysis and fractionation of polypeptide-harboring cells prior to affinity chromatography may be performed by standard methods (see, e.g., Ausubel et al., supra). Alternatively, the polypeptide is isolated using a sequence tag, such as a hexahistidine tag, that binds to
20 nickel column.

Once isolated, the recombinant protein can, if desired, be further purified, e.g., by high performance liquid chromatography (see, e.g., Fisher, Laboratory Techniques In Biochemistry and Molecular Biology, eds., Work and Burdon, Elsevier, 1980). Polypeptides of the invention, particularly short peptide fragments, can also be
25 produced by chemical synthesis (e.g., by the methods described in Solid Phase Peptide Synthesis, 2nd ed., 1984 The Pierce Chemical Co., Rockford, Ill.). These general techniques of polypeptide expression and purification can also be used to produce and isolate useful peptide fragments or analogs (described herein).

30 **Antibodies and Analogs Thereof**

The invention further provides antibodies (e.g., chimpanzee/human chimeric antibodies) or fragments thereof that are modified in ways that enhance or do not inhibit their ability to neutralize, inhibit, or prevent poliovirus infection. In one embodiment, the invention provides methods for optimizing the amino acid sequence

of the chimeric antibody or the nucleic acid sequence encoding the chimeric antibody by producing an alteration. Such changes may include certain mutations, deletions, insertions, or post-translational modifications. In one preferred embodiment, the amino acid sequence is modified to enhance protease resistance. Accordingly, the invention further includes analogs of any naturally-occurring polypeptide of the invention. Analogs can differ from the naturally-occurring the polypeptide of the invention by amino acid sequence differences, by post-translational modifications, or by both. Analogs of the invention will generally exhibit at least 85%, more preferably 90%, and still more preferably 95% or even 99% identity with all or part of a naturally-occurring amino, acid sequence of the invention. The length of sequence comparison is at least 10, 13, 15 amino acid residues, preferably at least 25 amino acid residues, and more preferably more than 35 amino acid residues. Again, in an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence. Modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation; such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. Analogs can also differ from the naturally-occurring polypeptides of the invention by alterations in primary sequence. These include genetic variants, both natural and induced (for example, resulting from random mutagenesis by irradiation or exposure to ethanemethylsulfate or by site-specific mutagenesis as described in Sambrook, Fritsch and Maniatis, *Molecular Cloning: A Laboratory Manual* (2d ed.), CSH Press, 1989, or Ausubel et al., *supra*). Also included are cyclized peptides, molecules, and analogs which contain residues other than L-amino acids, e.g., D-amino acids or non-naturally occurring or synthetic amino acids, e.g., β or γ amino acids.

In addition to full-length polypeptides, the invention also includes fragments of any one of the polypeptides of the invention. As used herein, the term "a fragment" means at least 5, 10, 13, or 15 amino acids in length. In other embodiments a fragment is at least 20 contiguous amino acids, at least 30 contiguous amino acids, or at least 50 contiguous amino acids, and in other embodiments at least 60 to 80 or more contiguous amino acids. Fragments of the invention can be generated by methods known to those skilled in the art or may result from normal protein processing (e.g., removal of amino acids from the nascent polypeptide that are not

required for biological activity or removal of amino acids by alternative mRNA splicing or alternative protein processing events).

Antibody analogs having a chemical structure designed to mimic antibody functional activity (e.g., anti-neoplastic activity, antigen binding activity) can be administered according to methods of the invention. Methods of analog design are well known in the art, and synthesis of analogs can be carried out according to such methods by modifying the chemical structures such that the resultant analogs exhibit the poliovirus neutralizing activity of 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, or SEQ ID NO:24 or a chimeric antibody comprising SEQ ID NOs:13-24. Preferably, the antibody analogs are relatively resistant to *in vivo* degradation, resulting in a more prolonged therapeutic effect upon administration. Assays for measuring functional activity include, but are not limited to, those described in the Examples below.

15

Pharmaceutical Therapeutics

The invention comprises chimeric antibodies that are useful for the treatment of polio. In one particular embodiment, the chimeric antibodies of the invention are useful for preventing or reducing poliovirus infection. For therapeutic uses, the antibodies disclosed herein may be administered systemically, for example, formulated in a pharmaceutically-acceptable buffer such as physiological saline. Preferable routes of administration include, for example, subcutaneous, intravenous, interperitoneally, intramuscular, or intradermal injections that provide continuous, sustained levels of the drug in the patient. Treatment of human patients or other animals will be carried out using a therapeutically effective amount of a therapeutic identified herein in a physiologically-acceptable carrier. Suitable carriers and their formulation are described, for example, in Remington's Pharmaceutical Sciences by E. W. Martin. The amount of the therapeutic agent to be administered varies depending upon the manner of administration, the age and body weight of the patient, and with the clinical symptoms of the poliovirus infection. Generally, amounts will be in the range of those used for other agents used in the treatment of other diseases associated with polio, although in certain instances lower amounts will be needed because of the increased specificity of the compound.

30

Therapeutic Methods

Antibodies of the invention (e.g. chimpanzee/human chimeric antibodies described herein) are useful for preventing or ameliorating poliovirus infection. In one therapeutic approach, an agent identified or described herein is administered to the site of a potential or actual disease-affected tissue or is administered systemically. The dosage of the administered agent depends on a number of factors, including the size and health of the individual patient. For any particular subject, the specific dosage regimes should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions.

Formulation of Pharmaceutical Compositions

The administration of a compound for the treatment of polio may be by any suitable means that results in a concentration of the therapeutic that, combined with other components, is effective in ameliorating, reducing, or stabilizing a poliovirus infection. The compound may be contained in any appropriate amount in any suitable carrier substance, and is generally present in an amount of 1-95% by weight of the total weight of the composition. The composition may be provided in a dosage form that is suitable for parenteral (e.g., subcutaneously, intravenously, intramuscularly, or intraperitoneally) administration route. An advantageous method of administration is intravenous infusion. The pharmaceutical compositions may be formulated according to conventional pharmaceutical practice (see, e.g., Remington: The Science and Practice of Pharmacy (20th ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2000 and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York).

Pharmaceutical compositions according to the invention may be formulated to release the antibody substantially immediately upon administration or at any predetermined time or time period after administration. The latter types of compositions are generally known as controlled release formulations, which include (i) formulations that create a substantially constant concentration of the drug within the body over an extended period of time; (ii) formulations that after a predetermined lag time create a substantially constant concentration of the drug within the body over

an extended period of time; (iii) formulations that sustain action during a predetermined time period by maintaining a relatively, constant, effective level in the body with concomitant minimization of undesirable side effects associated with fluctuations in the plasma level of the active substance (sawtooth kinetic pattern); (iv) formulations that localize action by, e.g., spatial placement of a controlled release composition adjacent to or in contact with the thymus; (v) formulations that allow for convenient dosing, such that doses are administered, for example, once every one or two weeks; and (vi) formulations that target poliovirus by using carriers or chemical derivatives to deliver the therapeutic agent to a particular cell type (e.g., poliovirus infected cell). For some applications, controlled release formulations obviate the need for frequent dosing during the day to sustain the plasma level at a therapeutic level.

Any of a number of strategies can be pursued in order to obtain controlled release in which the rate of release outweighs the rate of metabolism of the compound in question. In one example, controlled release is obtained by appropriate selection of various formulation parameters and ingredients, including, e.g., various types of controlled release compositions and coatings. Thus, the therapeutic is formulated with appropriate excipients into a pharmaceutical composition that, upon administration, releases the therapeutic in a controlled manner. Examples include single or multiple unit tablet or capsule compositions, oil solutions, suspensions, emulsions, microcapsules, microspheres, molecular complexes, nanoparticles, patches, and liposomes.

Parenteral Compositions

The pharmaceutical composition may be administered parenterally by injection, infusion or implantation (subcutaneous, intravenous, intramuscular, intraperitoneal, or the like) in dosage forms, formulations, or via suitable delivery devices or implants containing conventional, non-toxic pharmaceutically acceptable carriers and adjuvants. The formulation and preparation of such compositions are well known to those skilled in the art of pharmaceutical formulation. Formulations can be found in Remington: The Science and Practice of Pharmacy, *supra*.

Compositions for parenteral use may be provided in unit dosage forms (e.g., in single-dose ampoules), or in vials containing several doses and in which a suitable preservative may be added (see below). The composition may be in the form of a solution, a suspension, an emulsion, an infusion device, or a delivery device for

implantation, or it may be presented as a dry powder to be reconstituted with water or another suitable vehicle before use. Apart from the active agent that reduces or ameliorates poliovirus, the composition may include suitable parenterally acceptable carriers and/or excipients. The active therapeutic agent(s) may be incorporated into
5 microspheres, microcapsules, nanoparticles, liposomes, or the like for controlled release. Furthermore, the composition may include suspending, solubilizing, stabilizing, pH-adjusting agents, tonicity adjusting agents, and/or dispersing agents.

As indicated above, the pharmaceutical compositions according to the invention may be in the form suitable for sterile injection. To prepare such a
10 composition, the suitable therapeutic(s) are dissolved or suspended in a parenterally acceptable liquid vehicle. Among acceptable vehicles and solvents that may be employed are water, water adjusted to a suitable pH by addition of an appropriate amount of hydrochloric acid, sodium hydroxide or a suitable buffer, 1,3-butanediol, Ringer's solution, and isotonic sodium chloride solution and dextrose solution. The
15 aqueous formulation may also contain one or more preservatives (e.g., methyl, ethyl or n-propyl p-hydroxybenzoate). In cases where one of the compounds is only sparingly or slightly soluble in water, a dissolution enhancing or solubilizing agent can be added, or the solvent may include 10-60% w/w of propylene glycol or the like.

20 **Controlled Release Parenteral Compositions**

Controlled release parenteral compositions may be in form of aqueous suspensions, microspheres, microcapsules, magnetic microspheres, oil solutions, oil suspensions, or emulsions. Alternatively, the antibody may be incorporated in biocompatible carriers, liposomes, nanoparticles, implants, or infusion devices.

25 Materials for use in the preparation of microspheres and/or microcapsules are, e.g., biodegradable/bioerodible polymers such as polygalactin, poly-(isobutyl cyanoacrylate), poly(2-hydroxyethyl-L-glutam- nine) and, poly(lactic acid). Biocompatible carriers that may be used when formulating a controlled release parenteral formulation are carbohydrates (e.g., dextrans), proteins (e.g., albumin),
30 lipoproteins, or antibodies. Materials for use in implants can be non-biodegradable (e.g., polydimethyl siloxane) or biodegradable (e.g., poly(caprolactone), poly(lactic acid), poly(glycolic acid) or poly(ortho esters) or combinations thereof).

Combination Therapies

Optionally, chimeric antibody therapeutics of the invention may be administered in combination with any other chemotherapeutic; such methods are known to the skilled artisan and described in Remington's Pharmaceutical Sciences by E. W. Martin. For example, a chimpanzee/human chimeric anti-polio antibody may be administered with an antiviral agent. Non-limiting examples of suitable antiviral agents include V-037, rupintrivir, pirodavir, enviroxime, ribavirin, and derivatives thereof.

Kits

The invention provides kits for the treatment or prevention of polio. In one embodiment, the kit includes a therapeutic or prophylactic composition containing a therapeutically effective amount of a chimeric antibody in unit dosage form. In some embodiments, the kit comprises a sterile container which contains a therapeutic or prophylactic cellular composition; such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

If desired a chimeric antibody of the invention is provided together with instructions for administering the chimeric antibody to a subject having or at risk of developing polio. The instructions will generally include information about the use of the composition for the treatment or prevention of polio. In other embodiments, the instructions include at least one of the following: description of the therapeutic agent; dosage schedule and administration for treatment or prevention of polio or symptoms thereof; precautions; warnings; indications; counter-indications; overdose information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney,

1987); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1996); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Current Protocols in Molecular Biology” (Ausubel, 1987); “PCR: The Polymerase Chain Reaction”, (Mullis, 1994); “Current Protocols in Immunology” (Coligan, 5 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention.

Recombinant Polypeptide Expression

10 The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, “Molecular Cloning: A Laboratory Manual”, second edition (Sambrook, 15 1989); “Oligonucleotide Synthesis” (Gait, 1984); “Animal Cell Culture” (Freshney, 1987); “Methods in Enzymology” “Handbook of Experimental Immunology” (Weir, 1996); “Gene Transfer Vectors for Mammalian Cells” (Miller and Calos, 1987); “Current Protocols in Molecular Biology” (Ausubel, 1987); “PCR: The Polymerase Chain Reaction”, (Mullis, 1994); “Current Protocols in Immunology” (Coligan, 20 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art 25 with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

30

Example 1: Generation of poliovirus-specific chimpanzee Fabs.

A phage Fab display library constructed from chimpanzees immunized with poliovirus was panned for three cycles against polioviruses of each serotype. The phage-Fab clones specific to each of three serotypes were identified by phage ELISA.

The sequencing analysis of the variable domain of heavy (V_H) and light (V_L) chains showed that nine clones specific to serotype 1, three clones specific to serotype 2, and seven clones specific to serotype 3 were recovered. These positive clones were subsequently converted to produce soluble Fabs. Each soluble Fab was confirmed for its specific binding to its panning antigen (Figure 1). Further measurement of their neutralization activity by PRNT showed that Fabs A12 and H2, Fabs A6 and B2, and Fabs B10, A2, E3 and C10 neutralized Sabin poliovirus serotypes 1, 2 and 3, respectively (Figure 1). Six Fab clones (A12, H2, A6, B2, B10 and C10) had relatively higher neutralizing activities and therefore were selected for conversion to full-length IgGs (referred to as MAbs) and further characterization. The closest human V-gene germline usage and possible somatic mutations for each of these six poliovirus-neutralizing MAbs were identified by conducting sequence similarity searches of all of the known human Ig genes compiled in the IMGT database. The analysis showed that family 3 of heavy chain gene and family 1 of kappa chain genes were over-represented. Both V_H and V_L genes were highly mutated since an identity of 85-90% for V_H and 90-96% for V_L, compared to germline V-gene was observed (Figure 2), indicating that significant affinity maturation had taken place.

Example 2: Specificity of poliovirus-neutralizing MAbs

Each of the six poliovirus-neutralizing MAbs was examined by ELISA for binding to poliovirus serotype 1, 2, and 3 virions immobilized on a 96-well plate. MAbs A12 and H2 recovered from panning against type 1 poliovirus were the only ones that reacted with Sabin 1 virus (Figure 3A). Interestingly, MAb A12 exhibited dual specificity by reacting with both Sabin 1 and Sabin 2 polioviruses (Figure 3B). MAbs A6 and B2 that were selected by panning against type 2 poliovirus reacted well with Sabin 2 poliovirus (Figure 3B). MAbs B10 and C10 that were recovered from panning against type 3 poliovirus reacted well with Sabin 3 (Figure 3C). Sabin 3 virus also reacted weakly with type 1-specific MAbs A12 and H2.

Direct ELISA may not necessarily reflect functional interactions that lead to neutralization because it can detect relatively weak binding between antibodies and antigens. Previously it had been shown that the block-ELISA procedure in which MAbs are used to compete with neutralizing polyclonal serum is much more predictive of the functional interactions, and correlates well with neutralization test results (Rezapkin, G. V. et al., (2005) *Biologicals* 33:17-27). Figure 4 shows that,

indeed, weak interactions between type 1-specific antibodies A12 and H2 and Sabin 3 poliovirus that were detected in a previous experiment did not occur in this setting. However, type 1-specific A12 antibody strongly reacted with type 2 poliovirus, indicating that it may neutralize two serotypes of poliovirus. In another experiment the block-ELISA test was performed with varying concentrations of MABs using both attenuated Sabin and wild polioviruses as antigens (Figure 5). With the exception of type-3-specific MAb B10 that reacted equally well with Sabin 3 and wild Saukett strains, all other MABs reacted better with Sabin strains than with wild strains of the same serotype. The difference was especially pronounced for type-2-specific antibody A6 that practically did not block wild MEF-1 poliovirus.

Example 3: MABs neutralize the vaccine and wild-type strains of poliovirus

The results of *in vitro* neutralization of the six MABs presented in Figure 6 showed that all MABs selected against each serotype effectively neutralized homotypic viruses. In addition, and consistent with ELISA data, A12 MAB neutralized both type 1 and type 2 polioviruses. While some MABs, such as A12 and H2, neutralized both Sabin and wild strains of poliovirus, others exhibited a significant bias for Sabin strains. The neutralizing titer of MAb A6 tested against wild MEF-1 was more than 1,000 times lower than against the Sabin 2 strain. MABs B2, B10, and C10 had about 10-100 fold lower neutralizing activity to wild type than to Sabin strains.

Cross-neutralization of different serotypes of poliovirus is a relatively unknown phenomenon. It is difficult if not impossible to study it in humans because of the maternal antibodies present in newborn children and also since routinely used vaccines are trivalent. Therefore, whether rabbit antisera raised by immunization with purified monovalent polioviruses would demonstrate any evidence of cross-neutralization was determined. Figure 7 shows that there was very little cross neutralization among serotypes, indicating that poly-specific antibodies do not represent a majority of neutralizing antibodies in rabbit polyclonal antisera.

Example 4: Location of Neutralizing Epitopes

To identify epitopes to which the primate MABs bind escape mutants were selected for by treating Sabin polioviruses with the antibodies, plaque-purifying the

resistant strains, and sequencing their RNA to identify mutations that rendered the strains resistant to neutralization. Escape mutants to antibodies specific to types 1 and 2 were successfully generated. In contrast, no resistant variants of type 3 poliovirus were obtained.

5 The H2-resistant mutants contained several mutations, most frequently D₁₂₁₉G and D₁₂₁₉E in VP1, located close to antigenic site 2. Other mutations making the virus resistant to H2 antibody were in VP3 (K₃₁₄₄R,E,T), in the vicinity of the first mutation but closer to antigenic site 3, also composed of VP3 (Figure 8). Different mutations at the same amino acid site appeared to produce different effects on block-ELISA
10 reactivity of the strains. For instance, block-activity of H2 antibody measured with D₁₂₁₉G virus was stronger than with D₁₂₁₉E (8±3% and 20±12%, respectively).

Resistant strains generated in response to dual-reactive antibody A12 also contained an unusual pattern of mutations. All clones isolated from Sabin 1 strain contained a V₁₁₆₆E mutation, while clones isolated from the Sabin 2 strain contained a
15 G₁₂₂₅D mutation (Figure 9). Some Sabin 1 escape clones also contained I₁₀₉₀M. It may not be necessary for the resistance because this mutation leads to a direct reversion to the Mahoney amino acid, and Mahoney virus is sensitive to A12. Mutation V₁₁₆₆E was not previously identified as a part of an antigenic site 1, but is located close to it on the virion surface close to the canyon surrounding the 5-fold axis
20 of symmetry. In contrast, mutation G₁₂₂₅D, which makes the Sabin 2 strain resistant to the same A12 antibody is located on the opposite side of the canyon and is a part of antigenic site 2. Thus it appears that A12 binds the virion by interacting with both sides of the canyon, at the bottom of which the binding site for cellular receptor is located (Belnap, D. M. et al., (2000) Proc Natl Acad Sci U S A 97:73-8; Colston, E.,
25 and V. R. Racaniello, (1994) EMBO J 13:5855-62; Wien, M. W. et al., (1997) Nat Struct Biol 4:666-74).

Escape mutants generated in response to type 2-specific antibodies A6 and B2 contained mutations R₁₁₀₀C,L and A₁₁₀₁D, respectively, both located in the B-C loop in VP1 protein that was previously defined as epitope 1.

30

Example 5: MAbs protect mice from infection by virulent polioviruses both pre- and post-exposure to polioviruses

To determine if the MAbs can protect against poliomyelitis *in vivo*, Tg-21PVR transgenic mice expressing human poliovirus receptor CD155 were intravenously

inoculated with varying doses of H2 MAb and challenged intramuscularly with 10
paralytic doses (PD₅₀) of wild poliovirus (Mahoney strain). Figure 10 shows that 1 µg
of MAb protected 30% of the mice and 5 µg protected 100%. The average
neutralizing titers in serum in the 5 µg and 1 µg groups were 1:32 and 1:9,
5 respectively, consistent with the common notion that a neutralization titer of 1:8
protects against polio.

The protective effect of MAbs is quite predictable, considering the high
potency of the antibodies. It is also important to determine whether the antibodies
could be used to prevent paralytic disease if administered after exposure to the virus.
10 The results shown in Figure 11 demonstrate that some mice survived challenge with a
5 PD₅₀ dose if they were treated with 5 or 25 µg of H2 antibody at either 6 or 12 hours
after infection. There was a clear protective effect, even though not all mice survived.
This observation indicates that these antibodies could be used for emergency
prophylaxis of non-immune individuals exposed to poliovirus.

15

**Example 6: Monoclonal antibody A12 provided pre- and post-exposure
protection against infection with virulent type 1 and type 2 poliovirus.**

Following the observation that A12 can cross-neutralize type 1 and type 2
polioviruses *in vitro*, whether the antibody can provide protection from infection of
20 both types *in vivo* was tested. Transgenic mice with human poliovirus receptor
(TgPVR21-mice) that are susceptible to poliovirus infection were used in the
protection experiment. Groups of 10 mice were injected intravenously (IV) with 25
µg per mouse of A12 antibody, with injection of PBS as a control. Mice were
challenged intramuscularly (IM) with 5-times the 50% paralytic dose (PD₅₀) of wild-
25 type poliovirus Mahoney strain (type 1) or MEF1 strain (type 2) 6 hour after or 3 and
6 hours before antibody administration (pre- or post-exposure protection). Mice were
observed for signs of paresis/paralysis for 14 days. The results showed that A12
antibody conferred 90-100% pre-exposure protection and 30-80% post-exposure
protection against virulent poliovirus infection of type 1 and type2 (Figure 12).
30 Although A12 can protect mice from both type 1 and type 2 infections, the protection
from type 1 infection was better than protection from type 2 infection. This is the first
report of an anti-poliovirus mAb that can cross neutralize type 1 and type2 poliovirus
in vitro and protect against lethal challenge of type1 and type2 polioviruses *in vivo*.

Example 7: A12 binds to the canyon region of the virus particle.

In addition to generation and analysis of antibody-escape mutants, cryo-electron microscopy was used to map the epitope for A12. Polio type 1 or 2 virus was incubated with A12 Fab at a molar ratio of approximately 1:1 for 30 minutes.

5 Specimens were vitrified by plunge freezing into liquid ethane and specimens were examined under cryo conditions at 300 kV with a FEG Titan Krios transmission electron microscope (FEI). Images were recorded at a nominal magnification of 47,000X and an electron dose of $\sim 15 \text{ e}^-/\text{Angstroms}^2$. Particles with a defocus range of 1-5 μm were boxed with EMAN2, and then processed using the standard single
10 particle reconstruction procedure with full contrast transfer function correction and icosahedral symmetry imposed. The available atomic model for the virus particle was docked into the cryo-EM reconstruction, and the density indicating the contacting point between capsid and antibody was localized to identify the interacting residues. The images of poliovirus particle of type 1 (Figure 13) and type 2 (Figure 14)
15 decorated with A12 Fabs (shown as spikes) clearly indicated that A12 Fab bound to type 1 and type 2 in the circular canyon region with slightly different conformations. For a better view of the location of the canyon region, the icosahedral particle of poliovirus with 2-, 3- and 5-fold axis of symmetry (A) and formation of the circular canyon region around 5-fold axis of symmetry (B) was shown in Figure 15. The
20 result from cryo-EM is in agreement with our previous results with the escape mutants as residues responsible for escaping from antibody neutralization was also located near the canyon region, as shown in Figure 15. Since poliovirus receptor, CD155 has been shown to bind to the canyon region of the virus (D.M. Belnap, B.M. McDermott, Jr., D.J. Filman, N. Cheng, B.L. Trus, H.J. Zuccola, V.R. Racaniello,
25 J.M. Hogle, A.C. Steven, Three-dimensional structure of poliovirus receptor bound to poliovirus, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 73-78), it appears that the neutralization activity of A12 is most likely achieved by blocking virus from receptor binding.

30 The results described above were obtained using the following methods and materials.

Immunization of chimpanzees with poliovirus vaccines and construction of combinatorial Fab antibody library.

Two chimpanzees (1603 and 1609) were immunized orally with all three serotypes of the oral polio vaccine (OPV) (GlaxoSmithKline) twice at a two-month interval and were boosted intramuscularly with inactivated polio vaccine (IPV) (GlaxoSmithKline) seven months and two years after second immunization. Bone marrow was aspirated from each chimpanzee at days 10, 20, 30, and 40 after last immunization. Bone marrow-derived lymphocytes were prepared by centrifugation on a Ficoll-Paque gradient and were used for construction of a combinatorial Fab phage display library as described (Chen, Z. et al., (2007) J Virol 81:8989-95; Chen, Z., (2006) Proc Natl Acad Sci U S A 103:1882-7). A Fab phage display library containing $\gamma 1$ heavy chain and K, λ light chain with a diversity of 10^8 was constructed. The chimpanzee protocols were approved by the Institutional Animal Care and Use Committee and were housed in facilities accredited by AAALAC.

Panning of phage library and selection of poliovirus-specific Fabs.

Phages were produced from the library as described (Chen, Z., (2006) Proc Natl Acad Sci U S A 103:1882-7) and panned by affinity binding separately on serotypes 1, 2, and 3 of Sabin strains of poliovirus in a 96-well ELISA plate. For each cycle of panning, 100 μ l of OPV, which are the same viruses used for immunization, at 5×10^6 PFU/ml in PBS was used to coat each well of a ELISA plate and specific phages were selected by incubating 10^{12} phages with the coated virus. The poliovirus-specific Fab clones were enriched by three cycles of panning against the three serotypes. After panning, 96 single phage-Fab clones were cultured in a 96-well plate for phage production. The resulting phages were screened for specific binding to poliovirus by phage ELISA (Chen, Z., (2006) Proc Natl Acad Sci U S A 103:1882-7). Clones that bound to the virus, but not to BSA were scored as poliovirus-specific clones.

DNA sequence analysis of specific Fabs.

The genes coding for the variable regions of the heavy (VH) and light (VL) chains of poliovirus-specific Fabs were sequenced. Fabs with distinct VH and VL sequences were regarded as separate Fab clones. The presumed immunoglobulin family usage, germline origin, and somatic mutations were identified by comparing to those deposited in the IMGT sequence database.

Production of Fabs and IgGs.

As described (Chen, Z., (2006) Proc Natl Acad Sci U S A 103:1882-7), histidine-tagged Fab was expressed in *E. coli* and was affinity-purified on a nickel column. IgG was expressed in transiently transfected 293T mammalian cells and purified on a Protein A column. Both Fab and IgG were further purified through a cation-exchange SP column (GE Healthcare). Purities of the Fab and IgG were
5 evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the protein concentrations were determined by optical density measurements at 280 nm, assuming that 1.35 A₂₈₀ corresponds to 1.0 mg/ml.

Measurement of neutralizing activity of specific Fabs.

10 Purified Fabs were used in a plaque reduction neutralization test (PRNT) to determine their neutralizing activities against each of the three polio serotypes. Typically, approximately 50 plaque-forming units (PFU) of Sabin poliovirus in 150 µl of serum-free DMEM were mixed with the same volume of serial dilutions of a Fab. The virus-Fab mixture was incubated at room temperature for 1h and added to
15 confluent HeLa cells in a 6-well plate in duplicate. After 1 h incubation at room temperature, an overlay of DMEM containing 2% FBS and 0.8% methyl cellulose was added and the plates were incubated in a 5% CO₂ incubator at 37°C for 72 h. The plaques were visualized by staining with 0.1% crystal violet for 5 min.

ELISA.

20 Wells in a 96-well ELISA plate were coated with 100 µl of poliovirus, OPV at a concentration of 5x10⁶ PFU/ml, followed by treatment with 3% milk in PBS. After washing, the plate was incubated with 3-fold serially-diluted anti-poliovirus IgG for 2 h at room temperature. After washing, the plates were incubated for 1 h with horseradish peroxidase-conjugated antibodies against human Fc. The color was
25 developed by adding tetramethylbenzidine reagent (KPL, Gaithersburg, MD) and stopped with H₂SO₄ after 10 min. The optical density (OD) at 450 nm was read in an ELISA plate reader. The data were plotted and the dose-response curves were generated with Prism software (Graphpad Software Inc, San Diego).

Block-ELISA.

30 The test is based on materials and reagents developed in a previous study (32). 0.5-1.0 D-antigen units /ml of poliovirus was captured on 96-well ELISA plates coated with purified rabbit polyclonal serotype-specific IgG (2 µg/ml). Then some wells containing captured antigen were incubated with serial dilutions of a MAbs or polyclonal serum (block reaction), while control wells were treated with normal rabbit

serum. Wells with no antigen served as a background control. Anti-poliovirus antibodies reacted with respective antigenic sites and blocked them from subsequent reaction with biotin-conjugated polyclonal anti-polio IgG (0.5 µg/ml). Next, ExtrAvidin-peroxidase conjugate (Sigma, 1:1000 dilution) was added and the reaction of bound peroxidase with tetra-methyl benzidine substrate (TMB, Sigma) resulted in development of a color reaction. After addition of a stop-reagent (Sigma), the plate was scanned at 450 nm. The OD₄₅₀ in the background wells that contained no antigen corresponded to 100% block activity, and the OD₄₅₀ for antigen-containing wells incubated with normal rabbit serum corresponded to 0% block activity. The percentage of the block activity of specific MAb or serum with a particular antigen was calculated from the difference between the average OD of blocked and non-blocked wells containing the same antigen. The final results of block reactivity were calculated from the following formula: . Block % = (Average OD for wells containing normal rabbit serum – Average OD for wells containing test serum) / (Average OD for wells with normal rabbit serum – Average OD for background wells with no antigen). The titer was defined as a serum dilution producing 50% block. Graphical linearization of S-shaped block-ELISA titration curves was performed by plotting of the logarithm of Block/(1-Block) against the logarithm of dilution.

Polyclonal rabbit antisera were obtained by immunizing female rabbits with Sabin strains of poliovirus as described elsewhere (Ivanov, A. P., and E. M. Dragunsky, (2005) *Expert Rev Vaccines* 4:167-72).

Micro-neutralization test.

Poliovirus-neutralizing antibody titers were determined in a micro-neutralization test (NT) according to WHO recommendations ((1997) *Manual for the virological investigation of polio*) with some modifications. The MAb samples were diluted to 1 µg/ml in maintenance medium (DMEM supplemented with 2% FBS and 1% of antibiotic/antimycotic solution, Invitrogen, Carlsbad, CA) and sterilized by filtration through Spin-X columns (Corning, Corning, NY). Two-fold serial dilutions of the antibodies starting at 1 µg/ml were incubated in quadruplicate for 3 hr at 36°C with 100 50% tissue culture infectious doses (TCID₅₀) of the respective poliovirus strain in an atmosphere of 5% CO₂. After the incubation, 1x10⁴ HEp-2c cells were added to the wells. The plates were incubated for 10 days at 36°C, 5% CO₂, evaluated microscopically, and neutralizing antibody titers were calculated using the Kärber formula.

Generation of escape mutants. The antibodies were diluted to approximately 200 µg/ml in maintenance medium and sterilized by filtration through a Spin-X column. Four five-fold dilutions of antibodies starting at 200 µg/ml were incubated for 1 hr at room temperature with approximately 10^8 TCID₅₀ of poliovirus, followed by
5 incubation for 2 h at 36°C to allow for neutralization of susceptible virus. Monolayers of HEp-2c cells were inoculated with virus and incubated at 36°C, in 5% CO₂ until cytopathic effect (CPE) developed. The virus-containing cell suspension was subjected to three freeze-thaw cycles to release intracellular virus, clarified by centrifugation, and viral titer was determined by microtitration assay ((1997) Manual
10 for the virological investigation of polio).

For plaque purification, HEp-2c monolayers in 6-well plates were inoculated with serial dilutions of mutant viruses (10^1 - 10^4 TCID₅₀/ml), and incubated for 1 hr at room temperature. Next, the medium was replaced with 4 ml MEM/0.5% agarose overlay containing 6% FBS (Invitrogen). Plaques were picked after 48 hr of
15 incubation at 36°C, 5% CO₂, transferred into 12-well plates with confluent HEp-2c monolayers, and incubated until CPE developed. Virus-containing cell suspensions were subjected to three freeze-thaw cycles to release intracellular virus and clarified by centrifugation.

Nucleotide sequencing.

20 Viral RNA was isolated from virus-containing media using QIAamp viral RNA mini kit (QIAGEN, Valencia, CA). The RNA was amplified by RT-PCR using QIAGEN OneStep RT-PCR Kit (QIAGEN) with type-specific primers, allowing amplification of the entire capsid region of poliovirus. Reaction mixtures were assembled according to manufacturer's recommendations. The reverse transcription
25 step was at 55°C for 90 minutes followed by 15 min incubation at 95°C and 40 cycles of PCR with 20 seconds at 94°C denaturation step, 1 min at 65°C primer annealing step and 3 min at 72°C elongation step. Results of RT-PCR reactions were confirmed by agarose gel electrophoresis and amplified DNA was purified using a QIAquick PCR purification kit (QIAGEN). Both RNA isolation and PCR purification steps were
30 performed with a QIAcube automation station (QIAGEN). Purified PCR products were sequenced by regular Sanger method with type-specific primers covering both strands of DNA and spaced about 600 bp apart. Four primers were used to sequence each strand of DNA in PCR products. Overlapping reads were assembled and aligned

with reference Sabin sequences using Macintosh MacVector® program package (MacVector Inc, Cary, NC) and used to detect mutations relative to the parental strain.

Tg mouse challenge test.

5 TgPVR21 mice were obtained from the Central Institute for Experimental Animals (Tokyo, Japan). Maintenance, containment, and transportation of mice were performed in accordance with recommendations of the WHO Memorandum on transgenic mice susceptible to human viruses ((1993) Bull World Health Organ 71:497-509). All experiments in mice were performed in accordance with the US Guide for the Care and Use of Laboratory Animals ((1985) Guide for the care and use
10 of laboratory animals).

In protection experiments, groups of 10 mice (5 males and 5 females) were injected intravenously (IV) with 1 µg, 5 µg, or 25 µg of H2 antibody in 0.1 ml PBS. For control, one group of 10 animals was injected IV with 0.1 ml of PBS. 24 hours later all animals were challenged intramuscularly (IM) with 10-times the 50%
15 paralytic doses (PD₅₀) of wild-type poliovirus Mahoney strain. Mice were observed for signs of paresis/paralysis for 14 days.

In a post-exposure treatment experiment, groups of 10 mice, equal numbers of males and females, were challenged IM with 5 PD₅₀ of Mahoney virus, and 6 or 12 hours later injected IV with 5 µg or 25 µg of H2 antibody. A control group received PBS
20 instead of H2. Animals were observed for clinical signs for 2 weeks.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following
25 claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other
30 embodiments or portions thereof.

Incorporation By Reference

The entire contents of all patents published patent applications and other references cited herein are hereby expressly incorporated herein in their entireties by reference.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

What is claimed is:

- 5 1. An isolated chimpanzee/human chimeric antibody or antigen-binding portion thereof that is capable of neutralizing poliovirus.
2. The isolated chimpanzee/human chimeric antibody or antigen-binding portion thereof of claim 1, wherein the antibody or antigen-binding portion thereof is capable of
10 neutralizing more than one serotype of poliovirus.
3. The isolated antibody of any of the preceding claims, wherein the antibody is isolated from a culture of prokaryotic or eukaryotic cells.
- 15 4. The antibody or antigen-binding portion thereof of claims 1 or 2, wherein the chimpanzee portion of the antibody has at least 85% identity to a sequence selected from the group consisting of 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, and SEQ ID NO: 24.
20
5. The antibody or antigen-binding portion thereof of claim 4, wherein the chimpanzee portion of the antibody comprises SEQ ID NO: 13 and SEQ ID NO: 14.
6. The antibody or antigen-binding portion thereof of claim 4, wherein the
25 chimpanzee portion of the antibody comprises SEQ ID NO: 15 and SEQ ID NO: 16.
7. The antibody or antigen-binding portion thereof of claim 4, wherein the chimpanzee portion of the antibody comprises SEQ ID NO: 17 and SEQ ID NO: 18.
- 30 8. The antibody or antigen-binding portion thereof of claim 4, wherein the chimpanzee portion of the antibody comprises SEQ ID NO: 19 and SEQ ID NO: 20.
9. The antibody or antigen-binding portion thereof of claim 4, wherein the chimpanzee portion of the antibody comprises SEQ ID NO: 21 and SEQ ID NO: 22.
35

10. The antibody or antigen-binding portion thereof of claim 4, wherein the chimpanzee portion of the antibody comprises SEQ ID NO: 23 and SEQ ID NO: 24.

5 11. The antibody or antigen-binding portion thereof of any of claims 1 or 2, wherein the antibody neutralizes both type 1 and type 2 polioviruses.

12. The antibody or antigen-binding portion thereof of any of claims 1 or 2, wherein the antibody or a fragment thereof is capable of preventing poliovirus infection .

10

13. An isolated polypeptide comprising the sequence selected from the group consisting of SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO:15, SEQ ID NO: 16, SEQ ID NO: 17, and SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, and SEQ ID NO: 24.

15

14. An isolated polynucleotide encoding the antibody of claims 1 or 2.

15. An isolated polynucleotide encoding the antibody of claims 1 or 2 or the isolated polypeptide of claim 13.

20

16. The isolated polynucleotide of claim 14, wherein the polynucleotide has a 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: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12.

25

17. An expression vector comprising the polynucleotide of any of claims 14-16 positioned for expression.

18. A cell comprising the expression vector of claim 17.

30

19. The cell of claim 18, wherein the cell is a prokaryotic or eukaryotic cell.

20. A method for producing the antibody of claims 1 or 2, the method comprising culturing the cell of claim 18 under conditions suitable for expression of the antibody, and isolating the antibody from the cultured cell.
- 5 21. A method of producing the antibody of claims 1 or 2, the method comprising:
- a) immunizing a chimpanzee with all three serotypes of polio vaccine;
 - b) aspirating bone marrow from the immunized chimpanzee;
 - c) isolating RNA from bone marrow derived lymphocytes;
 - d) constructing a Fab phage display library from the isolated RNA;
 - 10 e) selecting for poliovirus-specific Fabs by panning the phage library; and
 - f) converting the poliovirus-specific Fabs into full-length IgGs.
22. The method of claim 21, further comprising the step of selecting for poliovirus-specific Fabs capable of neutralizing more than one serotype of poliovirus.
- 15 23. The method of claim 21, further comprising the step of selecting for poliovirus-specific Fabs capable of neutralizing both type 1 and type 2 polioviruses.
24. A method of treating a subject that has been infected with poliovirus, the
20 method comprising administering to the subject a therapeutically effective amount of the antibody of claims 1 or 2, thereby treating the subject.
25. A method of ameliorating the symptoms of poliovirus infection in a subject, the method comprising administering to the subject a therapeutically effective amount
25 of the antibody of any of claims 1 or 2, thereby ameliorating the symptoms of poliovirus infection in the subject.
26. A method of treating or preventing polio in a subject, the method comprising administering to the subject a therapeutically effective amount of the antibody of any
30 of claims 1 or 2, thereby treating or preventing polio in the subject.
27. The method of any of claims 24-26, wherein the antibody is administered systemically or locally.

28. The method of any of claims 24-26, wherein the antibody is covalently linked to a functional moiety.
29. The method of claim 28, wherein the functional moiety is horseradish peroxidase (HRP) or alkaline phosphatase (ALP).
30. The method of any of claims 24-26, further comprising administering to a subject a therapeutically effective amount of one or more antiviral agents.
31. The method of claim 30, wherein the one or more antiviral agents is selected from the group consisting of V-037, rupintrivir, pirodavir, enviroxime, ribavirin, and derivatives thereof.
32. The method of any of claims 24-26, further comprising administering to a subject a poliovirus vaccine.
33. A pharmaceutical composition for the treatment of polio comprising a therapeutically effective amount of the antibody of any of claims 1 or 2.
34. A pharmaceutical composition for the treatment or prevention of poliovirus infection comprising a therapeutically effective amount of an isolated polypeptide comprising a sequence selected from the group consisting of 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, and SEQ ID NO: 24.
35. A kit for the treatment or prevention of poliovirus infection, the kit comprising a therapeutically effective amount of the antibody of claims 1 or 2 or an isolated polypeptide comprising a sequence selected from the group consisting of 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, and SEQ ID NO: 24, and directions for using the kit in a method of any of claims 26-33.

FIG. 1

Table 1. Binding and neutralization of selected Fabs

Fab clones ^a	Specific binding to poliovirus in ELISA ^b	In vitro neutralization ^c
A7	Type 1	-
A12	Type 1	+
B7	Type 1	-
C1	Type 1	-
D2	Type 1	-
D4	Type 1	-
E1	Type 1	-
G1	Type 1	-
H2	Type 1	+
A1	Type 2	-
A6	Type 2	+
B2	Type 2	+
G6	Type 3	-
B10	Type 3	+
A2	Type 3	+
E3	Type 3	+
C2	Type 3	-
C10	Type 3	+
F5	Type 3	-

^aFab clones were defined as clones with distinct V_H and V_L sequences.

^bBinding to specific types of Sabin polioviruses in ELISA by soluble Fabs.

^cNeutralizing activity was measured by PRNT.

Table 2. Assignment of poliovirus-neutralizing Fab clones to their closest human germ line counterparts, based on nucleotide sequence homology^a

Fab	Germline genes in:							
	Heavy chain				Light chain			
	V-gene	Identity ^b	J-gene	D-gene	V-gene	Identity	J-gene	
A12	IGHV4-59*04	86%	IGHJ5*02	IGHD5-12*01	IGKV1-9*01	92%	IGKJ1*01	
H2	IGHV3-30*03	88%	IGHJ4*02	IGHD3-16*02	IGKV6-21*01	96%	IGKJ1*01	
A6	IGHV3-48*01	85%	IGHJ4*02	IGHD4-23*01	IGKV1-13*02	96%	IGLJ4*01	
B2	IGHV3-30*03	89%	IGHJ3*01	IGHD5-12*01	IGLV1-44*01	96%	IGKJ2*01	
B10	IGHV3-7*01	90%	IGHJ6*03	IGHD3-3*01	IGKV1-9*01	90%	IGLJ3*01	
C1 0	IGHV3-7*01	93% ^c	IGHJ6*03	IGHD3-3*01	IGKV1-9*01	90%	IGLJ3*01	

^aThe closest human V-gene germ lines were identified by search of the IMG database at <http://www.imgt.org/>.

^bThe V_H and V_L genes before CDR3 region were used to calculate the percent nucleotide acid identity. The mutations in the first 20 base pairs were excluded since these mutations could be introduced by PCR primers.

^cThere was a 3 base pair insertion in CDR2 region in addition to the 93% identity.

FIG. 2

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FIG. 3A

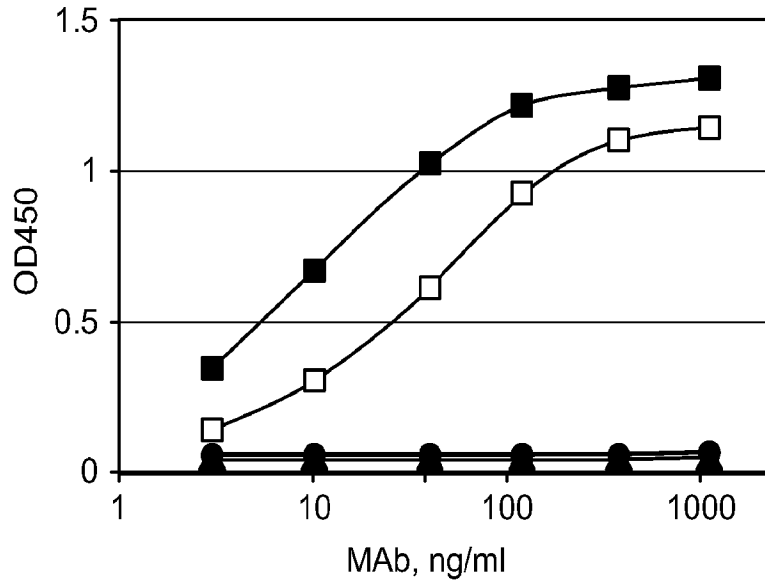


FIG. 3B

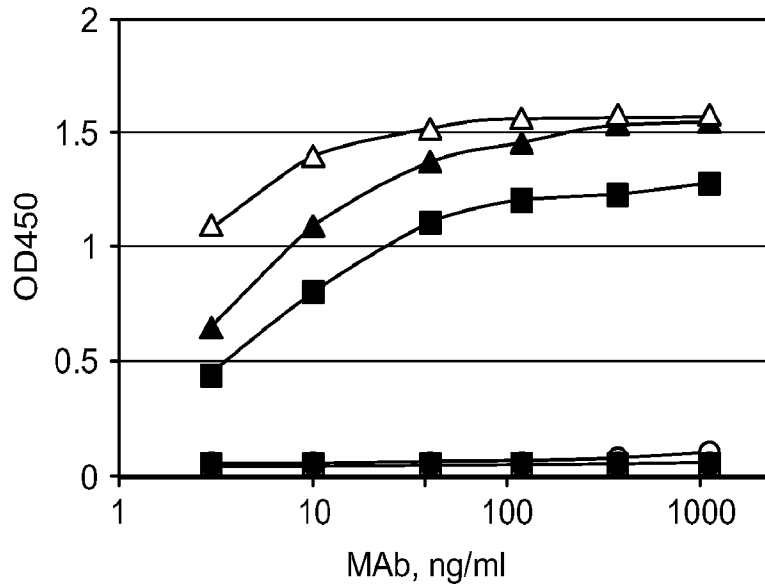


FIG. 3C

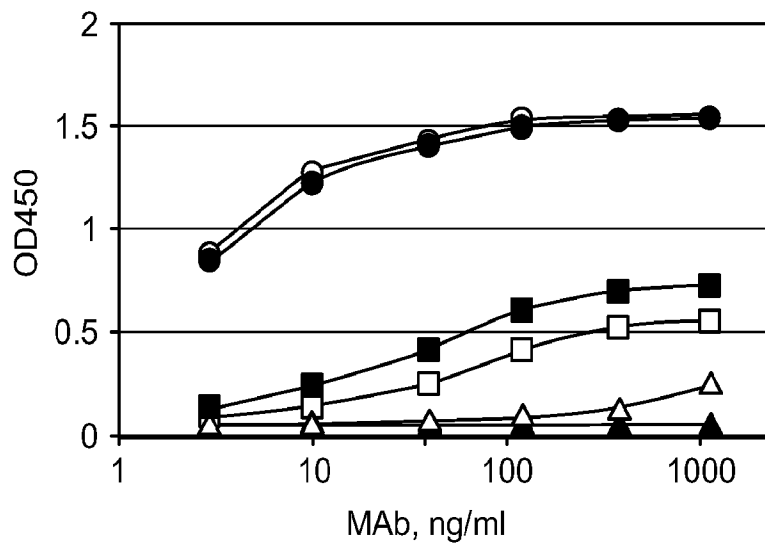


Table 3. Block-ELISA reaction between human monoclonal antibodies and polioviruses

MAb	Type 1	Type 2	Type 3
H2	0.66 ± 0.04	0 ± 0.03	0 ± 0.05
A12	0.63 ± 0.06	0.73 ± 0.04	0.01 ± 0.04
A6	0.01 ± 0.04	0.97 ± 0.05	0.01 ± 0.03
B2	-0.02 ± 0.05	0.97 ± 0.04	0.06 ± 0.04
B10	-0.02 ± 0.03	0.03 ± 0.03	0.79 ± 0.04
C10	-0.06 ± 0.04	-0.06 ± 0.03	0.73 ± 0.03

Note: The values represent relative reduction (block) of ELISA reaction between surface-captured antigen and polyclonal conjugate after treatment with monoclonal antibodies, and reflect affinities between the antibodies and the antigens.

FIG. 4

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FIG. 5A

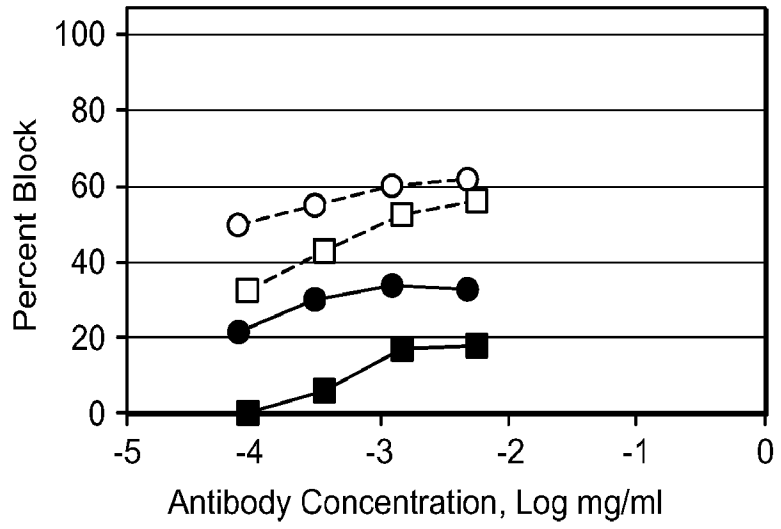


FIG. 5B

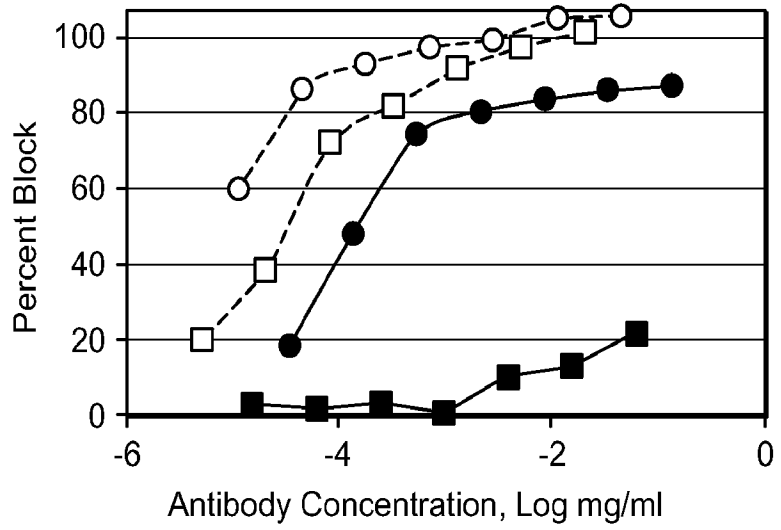
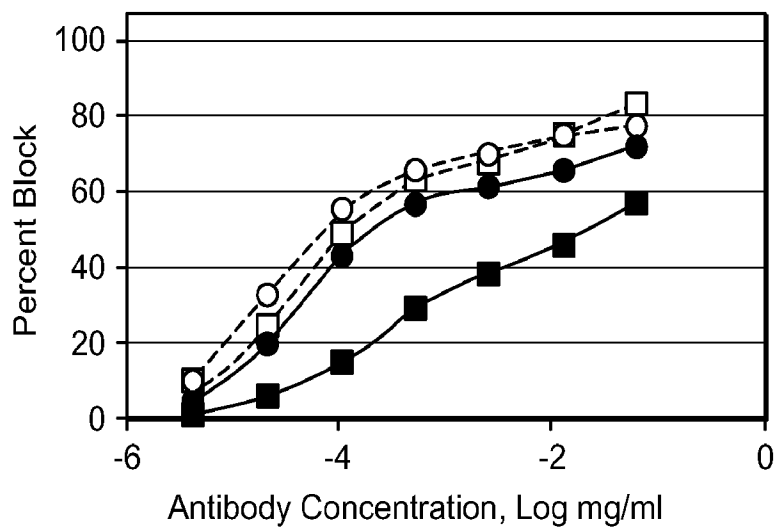


FIG. 5C



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FIG. 6

Table 4. Cross-neutralization of Sabin and wild polioviruses with humanized MAbs.

MAb, 1 mg/ml	Type 1		Type 2		Type 3	
	Sabin 1	Mahoney	Sabin 2	MEF-1	Sabin 3	Saukett
A12	102,400	121,800	121,800	172,200	n.d. ¹	n.d.
H2	43,100	18,100	n.d.	n.d.	n.d.	n.d.
A6	n.d.	n.d.	18,100	< 20	n.d.	n.d.
B2	n.d.	n.d.	289,600	21,500	n.d.	n.d.
B10	n.d.	n.d.	n.d.	n.d.	819,200	10,800
C10	n.d.	n.d.	n.d.	n.d.	1,158,500	144,800

Note: Neutralization titers per 1 mg/ml of MAb are presented.

¹n.d. - no data

FIG. 7

Table 5. Neutralization of polioviruses with rabbit hyperimmune sera

		Antiserum		
		Type 1	Type 2	Type 3
Virus	Type 1	38,968	64	<8
	Type 2	11	38,968	362
	Type 3	<11	91	28,800

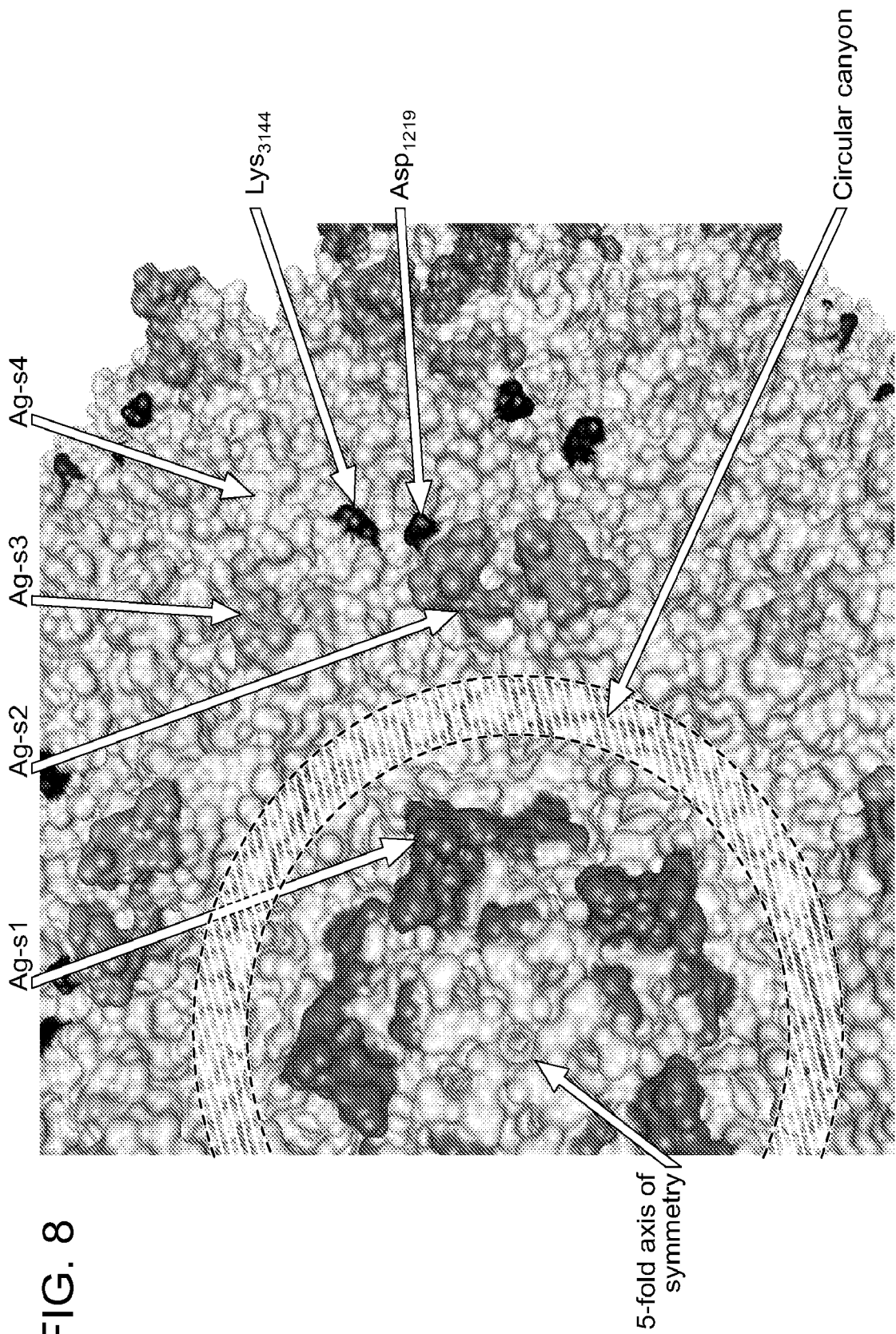


FIG. 8

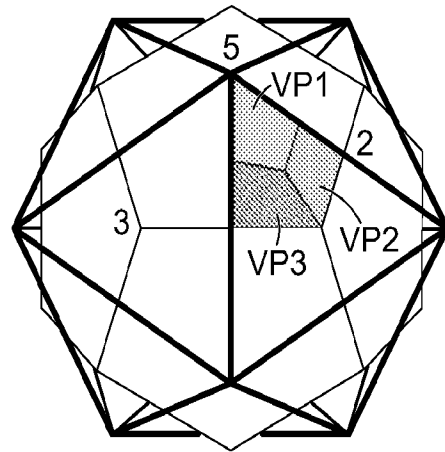


FIG. 9A

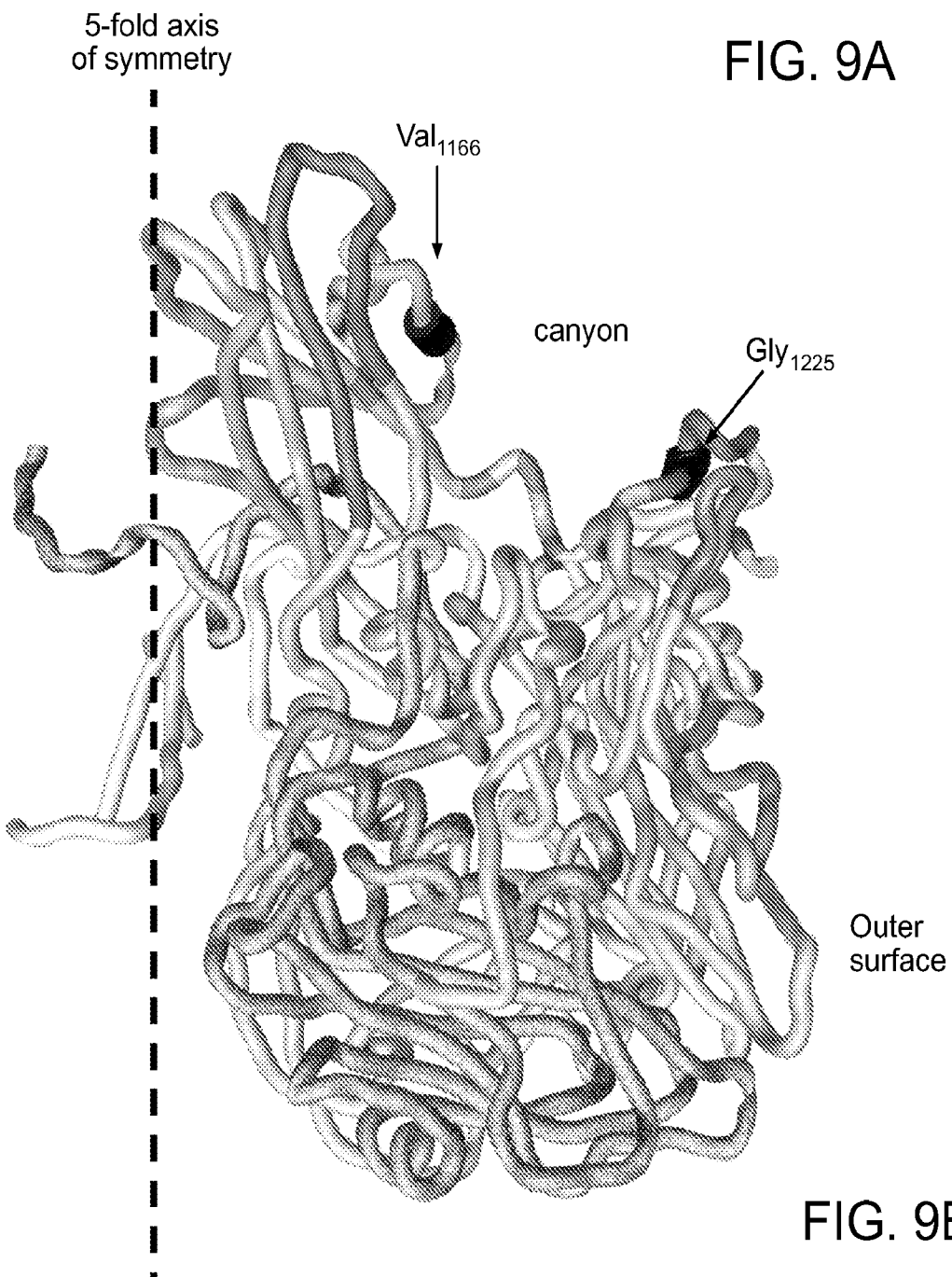


FIG. 9B

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FIG. 10

Table 6. H2 Mab protects transgenic mice from challenge with 10 PD₅₀ of Mahoney virus

MAb dose, μg / mouse	Reciprocal neutralization titer in serum	Survived / challenged mice
0	0	0 / 10
1	9	3 / 10
5	32	10 / 10
25	158	10 / 10

FIG. 11

Table 7. H2 Mab protects transgenic mice even when administered after challenge with 5 PD₅₀ of Mahoney virus

Group #	MAb dose	Time after challenge	Survived/ challenged mice	P value ¹
1	5 μg	6 h	7/10	0.000
2	25 μg	6 h	4/10	0.010
3	5 μg	12 h	4/10	0.010
4	25 μg	12 h	3/10	0.031
Control	0 μg	6 h	0/10	n/a

¹Probability of this number of mice surviving by chance at challenge dose of 5 PD₅₀ / mouse

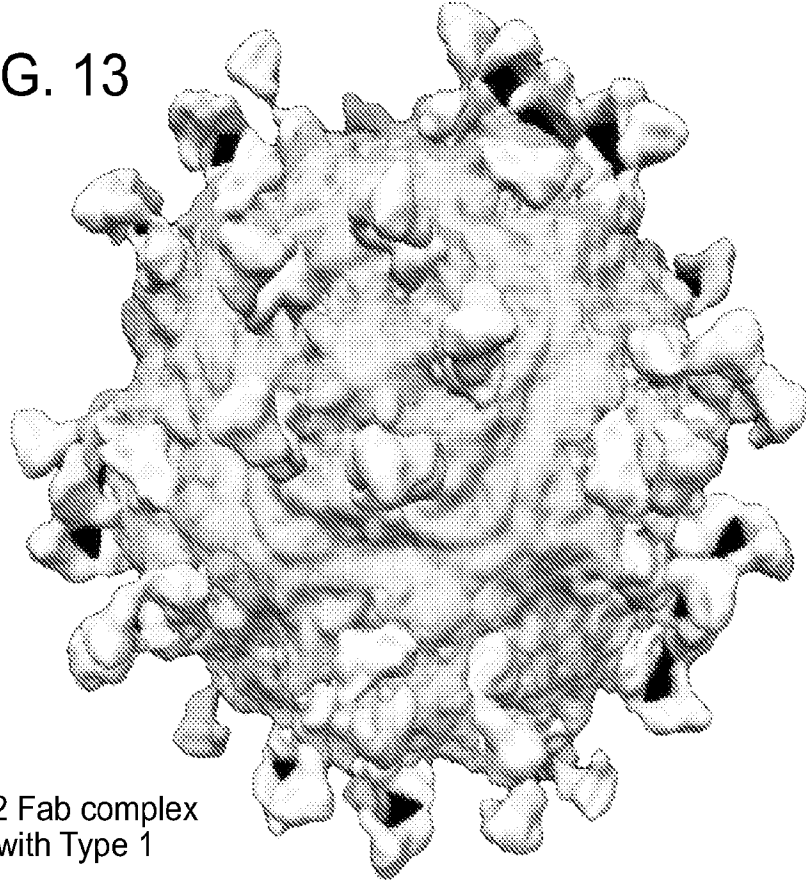
Protection of PVRTg-mice from poliovirus infection by monoclonal antibody A12

Group #	A12 IgG dose	Time before or after challenge	wt Type 1 Mahoney 5 PD ₅₀ (unaffected total)	p value*	wt Type 1 MEFI 5 PD ₅₀ (unaffected total)	p value*
1	0 µg	6h	0/9		0/9	
2	25 µg	-6h	10/10	< 0.001	9/10	< 0.001
3	25 µg	3h	8/10	< 0.001	3/10	0.010
4	25 µg	6h	4/10	0.010	3/10	0.031

* Probability of mice surviving by chance after lethal challenge

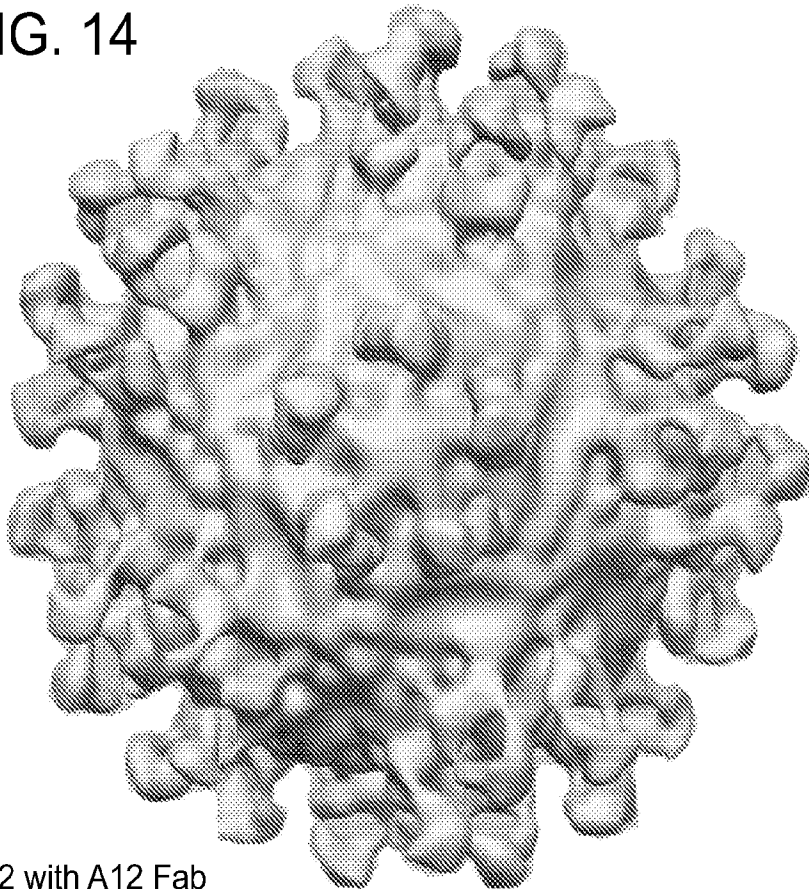
FIG. 12

FIG. 13



A12 Fab complex
with Type 1

FIG. 14



Type 2 with A12 Fab

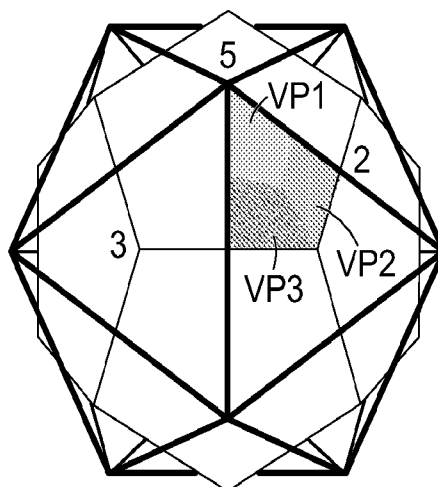


FIG. 15A

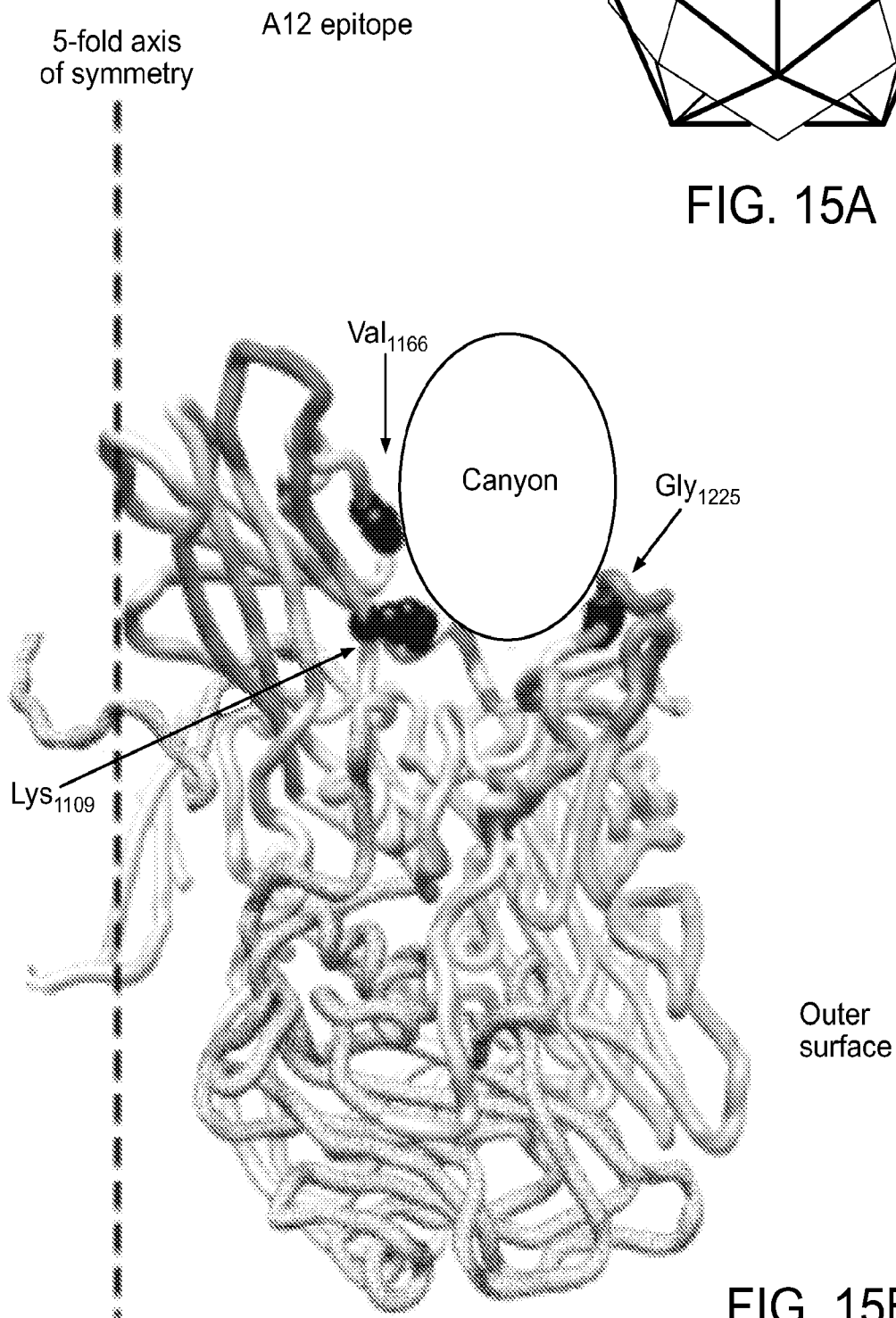


FIG. 15B