Most commercially available immunoglobulins are derived from human plasma collected, processed, and distributed for sale by the blood and plasma products industry. The first purified human immunoglobulin G (IgG) preparation used clinically was immune serum globulin which was produced in the 1940s (Cohn, E. J., et al "J. Am Chem. Soc., 68:459-475 (1946)) and Oncely, J. L. et al., J. Am Chem Soc. 71:541-550 (1949). The gammaglobulin produced by this method show a molecular distribution having a high molecular weight, when analyzed by way of high resolution size exclusion chromatography.

Standard immune globulin (IVIG) has been shown to have lot to lot variability for opsonic activity to a very common commensal organism that is ubiquitous on human skin, *S. epidermidis* (L. A. Clark and C. S. F. Easmon, J. Clin. Pathol. 39:856 (1986)). For example, in the study by Clark and Easmon, one third of the IVIG lots tested had poor opsonic activity with complement, and only two of fourteen were opsonic without complement. Thus, despite the fact that the IVIG lots were made from large plasma donor pools, good opsonic antibody to *S. epidermidis* was not uniformly present.

IVIG has generally been successful to prevent severe lower respiratory tract infections in immune compromised patients. However, despite the fact the immune compromised patients receiving IVIG appear to have acceptable levels of total immunoglobulin as well as sufficient levels of anti *S. pneumonia* antibody to prevent serious bacterial infections caused by *S. pneumonia,* there exists a significant percentage of the patients who experience upper respiratory tract infections and non-respiratory infections that is debilitating, lowers their quality of life, leads to increased use of antibiotics which are not effective and also leads to enhanced medical expenditures (See, e.g., Favre et al., Allergy 2005 60:385-390).

SUMMARY OF THE INVENTION

The present disclosure relates to compositions and methods for the treatment of infection caused by *Streptococcus pneumonia*. In particular, the disclosure provides human immune globulin and compositions thereof for preventing or treating pneumococcal infection. The disclosure provides methods of producing immune globulin containing high titers of anti-pneumococcal antibodies with opsonic activity (e.g., to a multitude of *S. pneumonia* serotypes), compositions containing same, and methods of using the compositions for the prevention and treatment of pneumococcal infection. The disclosure further provides methods of preventing or treating pneumococcal infection (e.g., upper respiratory infections (e.g., pharyngitis, otitis media, sinusitis, etc.)) in immunocompromised subjects (e.g., via administration of immune globulin compositions of the disclosure (e.g., containing a high titer

of opsonic anti-pneumococcal antibodies) to immunocompromised subjects who have not been adequately protected by regular infusions of standard IVIG).

In one aspect, the present disclosure refers to a method for preparing an immune globulin having an opsonophagocytic antibody titer of at least 1:256 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F comprising the steps of: (a) pooling plasma from vaccinated plasma donors in order to obtain a pooled plasma containing an opsonophagocytic antibody titer of at least 1:256 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; wherein the plasma was harvested from the plasma donors subsequent to a booster immunization, wherein the plasma donors are healthy adult human plasma donors between the ages of 18-60 who were immunized with a primary immunization with a multivalent *S. pneumonia* vaccine followed by a booster immunization with a multivalent *S. pneumonia* vaccine that is different than the primary vaccine; and (b) preparing an immune globulin from the plasma pooled according to step (a).In another aspect, the present disclosure refers to an immune globulin prepared according to the method as disclosed herein.

In another aspect, the present disclosure refers to the use of a therapeutically effective amount of an immune globulin prepared according to the method as disclosed herein in the manufacture of a medicament for treating *S. pneumonia* infection in a subject. In another aspect, the present disclosure refers to effective amount of an immune globulin prepared according to the method of claim 1 for use in immunotherapy.

In another aspect, there is also provided a method of preparing immune globulin having enhanced opsonophagocytic bactericidal activity against at least seven serotypes of *Streptococcus pneumonia* for the prevention or treatment of *S. pneumonia* infection comprising the steps of: (a) preparing immune globulin from pooled plasma, wherein the pooled plasma was harvested and pooled from immunized plasma of healthy adult human plasma donors, wherein said healthy adult human plasma donors were immunized with a primary immunization with a multivalent *S. pneumonia* conjugate vaccine followed by boost immunization with a multivalent polysaccharide *S. pneumonia* vaccine;
wherein the immune globulin contains an opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* that is three-fold or higher than the opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* present in immune globulin prepared from plasma pooled from 1000 or more

random non-vaccinated human plasma donors. In another aspect, the present disclosure refers to an immune globulin prepared according to the method as disclosed herein.

In yet another aspect, the present disclosure refers to a pooled plasma composition having an opsonophagocytic antibody titer specific for at least 55% of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F that is at least 3-fold higher than the opsonophagocytic antibody titer present in immune globulin prepared from a mixture of plasma samples obtained from 500 or more random human plasma donors that have not been vaccinated with an anti-pneumococcal vaccine.

In yet another aspect, there is also provided an immune globulin having an opsonophagocytic antibody titer of at least 1:256 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

In one example, it is an object of the disclosure to provide a novel immune globulin composition prepared from plasma donors according to methods of the disclosure (e.g., human plasma donors (e.g., healthy human plasma donors) that have been vaccinated with one or more pneumococcal vaccines according to methods described herein) that contains an elevated titer of anti-pneumococcal-specific, antibodies with opsonic activity when compared to a control sample.

The invention is not limited by the type of control sample utilized. For example, in one example, a control sample is immune globulin prepared from a healthy human plasma donor(s) pre-vaccination (e.g., such that the pre-immunization levels of anti-pneumococcal-specific, opsonic antibody titers of the human plasma donors are used as a baseline to measure the increase in the anti-pneumococcal-specific, opsonic antibody titers post immunization). In another example, a control sample is immune globulin prepared from a mixture of plasma samples obtained from random human plasma donors (e.g., 100, 300, 500, 1000 or more random human plasma donors) that have not been vaccinated with an anti-pneumococcal vaccine (e.g., such that the levels of anti-pneumococcal-specific, opsonic antibody titers of the non-vaccinated human plasma donors are used as a baseline to measure the increase in the anti-pneumococcal-specific, opsonic antibody titers present in human plasma donors vaccinated according to methods of the disclosure). Those of skill in the art will appreciate that other control samples may be utilized. The invention is not limited by the method or assay used to measure the opsonic (e.g., opsonophagocytic) titer of antibody present. Indeed, a variety of assays may be used including, but not limited to, those described herein (e.g., in Example 1).

In one example, it is an object of the disclosure to provide a novel immune globulin composition containing a high titer of opsonic, anti-pneumococcal antibodies. It is a further object of the disclosure to provide a novel intravenous immune globulin composition containing a high titer of opsonic, anti- pneumococcal antibodies (e.g., “anti-pneumococcal hyper-immune globulin”) and methods of producing and utilizing the same (e.g., for preventing and/or treating pneumococcal infection (e.g., in immune compromised patients)). The term "high titer" in this context means the presence of opsonic anti-pneumococcal antibody in an amount which is 2-fold or greater, e.g., 3-, 5-, 7-, 10-, 15-, 20- or more times higher than that found in a control sample.

Accordingly, a novel immune globulin composition (e.g., anti-pneumococcal hyper-immune globulin) containing a high titer of opsonic, anti-pneumococcal antibodies of the disclosure is different than standard/conventional immune globulin preparations (e.g., standard, conventional IVIG) and is different than other IVIG preparations (e.g., other hyper-immune IVIG preparations) in that it has a high titer of opsonic, human anti-pneumococcal antibodies that are functionally and broadly reactive against a multitude of *S. pneumonia* serotypes and enhance phagocytosis and killing of *S. pneumonia* (e.g., in vitro and/or in vivo (e.g., the antibodies are opsonophagocytic)), independent of the total amount of binding anti-pneumococcal antibodies (e.g., as measured by ELISA) that are present in the composition.

This is surprising since data generated and disclosed herein indicated that there was no consistent or predictable correlation between total anti-pneumococcal IgG antibody titer and the titer of opsonic anti-pneumococcal antibodies present in plasma or immune globulin prepared from vaccinated donors. Due to the surprising identification of the great discordance between the total amount of binding anti-pneumococcal IgG antibody titer and the titer of opsonic anti-pneumococcal antibodies present in plasma, or immune globulin prepared from same, from vaccinated donor plasma disclosed herein, it is a further object of the disclosure to provide a plasma and/or immune globulin composition (e.g., anti-pneumococcal hyper-immune globulin) containing significantly elevated functional, opsonic anti-pneumococcal antibody titers (e.g., regardless of the total amount of binding anti-pneumococcal antibody titer (e.g., by pooling plasma and/or immune globulin harvested from vaccinated donor plasma, or, by pooling vaccinated donor plasma together with immune globulin obtained from unvaccinated donors that may not have a high titer of opsonic anti-pneumococcal antibodies that when pooled with the significantly elevated titer opsonic anti-pneumococcal antibody donor plasma and/or immune globulin do not dilute the total opsonic titer to a non-protective level)) compared to a control sample. Encompassed by the disclosure is sera/plasma as well as immune globulin (e.g., hyperimmune globulin) prepared from same containing significantly elevated functional, and broadly reactive opsonic anti-pneumococcal antibody titers. In one example, the disclosure provides plasma/sera and/or immune globulin prepared from same containing an elevated anti-pneumococcal-specific, opsonic antibody titer that is at least 2-fold, 3-fold, 4-fold, 5-fold, 7-fold, 10-fold, or more greater for at least about 55% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85% or more) of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) compared to the anti-pneumococcal-specific, opsonic antibody titers for the same serotypes present in a control sample (immune globulin prepared from a mixture of plasma samples obtained from random

human plasma donors (e.g., 100, 300, 500, 1000 or more random human plasma donors) that have not been vaccinated with an anti-pneumococcal vaccine).

In another example, the disclosure provides a hyperimmune globulin (e.g., IVIG) composition containing a titer of broadly reactive opsonic antibodies, specific for 70% or more of the *S. pneumonia* serotypes selected from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23, that is 2 fold or greater (e.g., 3-25 fold or greater) than the titer of opsonic antibodies specific for the same *S. pneumonia* serotypes present in a control sample (e.g., immune globulin prepared from a mixture of plasma samples obtained from random human plasma donors (e.g., 100, 300, 500, 1000 or more random human plasma donors) that have not been vaccinated with an anti-pneumococcal vaccine. In another example, the disclosure provides plasma/sera and/or immune globulin prepared from same containing an anti-pneumococcal-specific, opsonic antibody titer between 1:64 and 1:8192 (e.g., for at least 50% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85% or more) of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F).

It is also an object of the invention to provide a novel immune globulin composition (e.g., anti-pneumococcal hyperimmune globulin) containing a high titer of opsonic, anti-pneumococcal antibodies that enhances immunity *in vivo* and/or preventatively and therapeutically inhibits infection (e.g., upper respiratory tract infection), in patients with compromised immune systems who do not adequately respond to regular infusions of conventional IVIG.

It is also another advantageous object of the present disclosure that while standard immunoglobulin pools of normal donors (e.g., used to generate commercially available, standard/conventional IVIG) do not have consistent, reproducible and fully protective levels of opsonic antibody for *S. pneumonia*, a hyperimmune globulin composition (e.g., anti-pneumococcal hyperimmune globulin) containing a high titer of opsonic, anti-pneumococcal antibodies of the disclosure when given intravenously immediately provides specific, functional (e.g., not merely binding) antibodies that promote phagocytosis and killing of *S. pneumonia* by phagocytes. The disclosure is not limited by the serotype or number of serotypes of *S. pneumonia* for which the functional, opsonic antibodies present within a hyperimmune globulin (e.g., IVIG) composition of the disclosure promotes the phagocytosis and/or killing. Indeed, it is a further object of the disclosure that a composition (e.g., a hyperimmune plasma composition and/or a hyperimmune globulin (e.g., IVIG) composition) of the disclosure contains broadly reactive, opsonic antibodies to at least 7 of 12, 8 of 12, 9 of 12, 10 of 12, 11 of 12 or all 12 of 12 of the following serotypes of *S. pneumonia*: 1, 3, 4, 5,

6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. That is, the disclosure provides compositions and methods of obtaining same that comprise an elevated opsonic anti-pneumococcal-specific antibody titer that is at least 2-fold, 3-fold, 4-fold, 5-fold, 7-fold, 10-fold, or more greater than the opsonic anti-pneumococcal-specific antibody titer present in a control sample (e.g., for at least about 55% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85% or more)) of the pneumococcal serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F). In one example, the disclosure provides an immune globulin (e.g., a hyperimmune globulin (e.g., IVIG)) composition containing a titer of broadly reactive opsonic antibodies, specific for 70% or more of the *S. pneumonia* serotypes selected from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23, that is 2 fold or greater (e.g., 3-25 fold or greater) than the titer of opsonic antibodies specific for the *S. pneumonia* serotypes present in a control sample (e.g., immune globulin prepared from a mixture of plasma samples obtained from random human plasma donors (e.g., 100, 300, 500, 1000 or more random human plasma donors) that have not been vaccinated with an anti-pneumococcal vaccine, or, immune globulin prepared from a healthy human plasma donor(s) pre-vaccination (e.g., such that the pre-immunization levels of serotype-specific, opsonic antibody titers of the human plasma donors are used as a baseline to measure the increase in the serotype-specific, opsonic antibody titers post immunization)). In another example, the disclosure provides an immune globulin (e.g., a hyperimmune globulin (e.g., IVIG)) composition that contains a high titer of broadly reactive, opsonic antibodies to at least 50%, to at least 55%, to at least 60%, to at least 65%, to at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, 95%, 98%, or more of each serotype present in a vaccine or a plurality of vaccines utilized to immunize one or more plasma donors from which the immune globulin composition is derived.

It is a further advantage and object of the disclosure to provide a hyperimmune globulin (e.g., IVIG) composition containing broadly reactive, opsonic antibodies specific for *S. pneumonia* to a patient (e.g., an immune compromised patient (e.g., a PIDD patient)) in order to treat or prevent pneumococcal infection in the patient (e.g., by inhibiting *S. pneumonia* growth and/or clearing *S. pneumonia* from the blood of the patient). Another advantage and object of the disclosure is to provide a hyperimmune globulin (e.g., IVIG) composition of the disclosure containing broadly reactive, opsonic antibodies specific for *S. pneumonia* to a patient (e.g., an immune compromised patient (e.g., a PIDD patient)) in order to treat pneumococcal infection in the patient (e.g., by improving or enhancing *S. pneumonia* clearance from the blood of the patient). It is a further advantage and object of the disclosure

to provide a hyperimmune globulin (e.g., IVIG) composition of the disclosure containing broadly reactive, opsonic antibodies specific for *S. pneumonia* to a patient (e.g., an immune compromised patient (e.g., a PIDD patient)) in order to prevent upper respiratory tract infections in the patient that are not preventable with conventional IVIG treatment. The disclosure is not limited by the type of upper respiratory tract infection prevented and/or treated and may include, but is not limited to, rhinosinusitis (sinusitis), otitis media, pharyngitis, epiglottitis, laryngotracheitis, and laryngotracheobronchitis. Similarly, compositions and methods of using (e.g., administering) the same find use in preventing and/or treating signs or symptoms of upper respiratory tract infection including, but not limited to, cough, sneezing, nasal discharge, nasal congestion, runny nose, fever, scratchy or sore throat, and nasal breathing.

In one example, the disclosure provides compositions and methods for obtaining a composition comprising pooled plasma samples (e.g., plasma from a plurality of donors (e.g., donors that have been vaccinated with one or more pneumococcal vaccines)) that contain high titers of opsonic anti-pneumococcal antibodies.

Thus, it is an object of the disclosure to provide methods of generating compositions (e.g., blood, plasma, and/or immune globulin (e.g., hyperimmune globulin) compositions) containing a high titer of opsonic, anti-pneumococcal antibodies. In one example, one or a plurality of healthy adult human subjects (e.g., human subjects with no known medical conditions) are administered a pneumococcal immunogen, recombinant pneumococcal protein, or a combination thereof. In some examples, a *S. pneumonia* immunogen is a *S. pneumonia* cell membrane sugar (e.g., a polysaccharide). In some examples, the *S. pneumonia* immunogen is a *S. pneumonia* vaccine containing one or a plurality of *S. pneumonia* proteins (e.g., recombinant or isolated proteins). In some examples, a *S. pneumonia* immunogen is a conjugate vaccine (e.g., conjugated to a carrier and/or adjuvant (e.g., a protein or other carrier molecule)). In some examples, a *S. pneumonia* immunogen is an unconjugated vaccine. In some examples, the conjugate vaccine or unconjugated vaccine contains 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 ,17, 18, 19, 20, 21, 22, 23 or more different immunogens (e.g., from an equal number of different serotypes of *S. pneumonia*). In some examples, the one or more different serotypes of *S. pneumonia* include, but are not limited to, serotypes 1, 2, 3, 4, 5, 6A, 6B, 7A, 7B, 7C, 7D, 7E, 7F, 8, 9A-9V, 12, 14, 18C, 19A-19F, 23A-23F, and 25. In some examples, the one or more different serotypes of *S. pneumonia* are selected from any one of the more the 90

different known *S. pneumonia* serotypes. In some examples, the one or more different serotypes of *S. pneumonia* is newly identified.

In a further example, one or a plurality of healthy human subjects (e.g., human subjects with no known medical conditions) are administered a pneumococcal immunogen, recombinant pneumococcal protein, or a combination thereof present in a commercial pneumococcal vaccine. The disclosure is not limited by the type of commercial pneumococcal vaccine. Indeed, any pneumococcal vaccine known in the art can be utilized including, but not limited to, pneumococcal conjugate vaccine (PCV13 or PREVNAR13, Wyeth Pharmaceuticals, Collegeville, PA), SYNFLORIX, and/or pneumococcal polysaccharide vaccine (PPSV23 or PNEUMOVAX23, Merck Sharp & Dohme Corp., North Wales, PA). In one example, one or a plurality of healthy human subjects receives a first or prime vaccination with a first anti-pneumococcal vaccine, and a subsequent boost vaccination with the first anti-pneumococcal vaccine or with a second, different anti-pneumococcal vaccine. For example, in one example, one or a plurality of healthy human subjects receive a first or prime vaccination/immunization with a first anti-pneumococcal vaccine (e.g., PREVNAR), and then receive a boost vaccination/immunization (e.g., at 2 weeks, 4 weeks, 6, weeks, 8 weeks, 10 weeks, 12 weeks or longer post the prime vaccination/immunization) with a second anti-pneumococcal vaccine (e.g., PNEUMOVAX23). At a time point subsequent to the sequential vaccination (e.g., at 2 weeks, 4 weeks, 6, weeks, 8 weeks, 10 weeks, 12 weeks or longer post the sequential vaccination), sera/plasma is harvested from the vaccinated, healthy human plasma donors. Plasma from the vaccinated donors may be pooled (with each other and/or with plasma from non-vaccinated donors) followed by isolation and/or purification of immune globulin from same (e.g., in order to generate an anti-pneumococcal hyper-immune globulin of the disclosure). Methods of harvesting plasma as well as extraction of immune globulin are well known by those of ordinary skill in the art.

In one example, the disclosure provides a method for preparing a hyperimmune globulin having a high titer of opsonophagocytic antibody to *Streptococcus pneumonia* (e.g., a titer that is at least 2-fold, 3-fold, 4-fold, 5-fold, 7-fold, 10-fold, or more greater for at least about 55% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85% or more) of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) compared to the anti-pneumococcal-specific, opsonophagocytic antibody titers for the same serotypes present in a control sample; or, a titer specific for 70% or more of the *S. pneumonia* serotypes selected from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23, that is 2 fold or greater (e.g., 3-25 fold or

greater) than the titer of opsonophagocytic antibodies specific for the same *S. pneumonia* serotypes present in a control sample; or, a titer between 1:64 and 1:8192 (e.g., at least 1:256 (e.g., for at least 50% or more(e.g., 60%, 65%, 70%, 75%, 80%, 85% or more)) of *S.pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) as determined by an opsonophagocytic killing assay described herein) comprising the steps of immunizing healthy adult human plasma donors between the ages of 18-60 with a primary immunization with a multivalent *S. pneumonia* vaccine followed by immunization with a boost multivalent *S. pneumonia* vaccine that is either the same or may be different than the primary vaccine; harvesting plasma from the plasma donors subsequent to the boost immunization; pooling plasma from the vaccinated donors in order to obtain a pooled plasma containing a high titer of opsonophagocytic antibody titer to *S. pneumonia*; and preparing an immune globulin from the pooled plasma. In a further example, the method comprises rendering the immune globulin obtained intravenously injectable. The immune globulin can be provided in solution and/or the pH and ionic strength of the solution can be adjusted so as to render it intravenously injectable. The disclosure is not limited by the number of individuals vaccinated according to the methods described herein. For example, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1100, 1200, 1300, 1400 or more healthy adult human plasma donors can be vaccinated and plasma harvested from the donors. In one example, the pooled plasma is made from pooling plasma from 1000 or more different vaccinated healthy adult human plasma donors. In one example, the pooled plasma contains an opsonophagocytic antibody titer that is at least 2-fold, 3-fold, 4-fold, 5-fold, 7-fold, 10-fold, or more greater for at least about 55% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85% or more) of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) compared to the opsonophagocytic anti-pneumococcal-specific antibody titers for the same serotypes present in a control sample (e.g., immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors). In another example, the pooled plasma contains an opsonophagocytic antibody titer specific for 70% or more of the *S. pneumonia* serotypes selected from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23, that is 2 fold or greater (e.g., 3-25 fold or greater) than the titer of opsonophagocytic antibodies specific for the same *S. pneumonia* serotypes present in a control sample (e.g., immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors). In yet another example, the pooled plasma contains an opsonophagocytic antibody titer between 1:64 and 1:8192(e.g.,for at least 50% or more(e.g., 60%, 65%, 70%, 75%, 80%, 85% or more)of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F,

9V, 14, 18C, 19A, 19F, and 23F) as determined by an opsonophagocytic killing assay described herein or known in the art.

The disclosure also provides an immune globulin (e.g., a hyperimmune globulin) prepared according the above described method. The immune globulin (e.g., hyperimmune globulin) so prepared can be used in various methods. For example, the immune globulin can be used in a method of treating *S. pneumonia* infection in a subject (e.g., comprising administering to the subject a therapeutically effective amount of the immune globulin). The immune globulin can also be used in a method of providing immunotherapy to a subject (e.g., comprising administering to the subject a therapeutically effective amount of the immune globulin).

The disclosure also provides, in one example, a method of preparing a hyperimmune globulin having enhanced opsonophagocytic bactericidal activity against at least seven serotypes of *Streptococcus pneumonia* for the prevention or treatment of *S. pneumonia* infection comprising the steps of immunizing healthy adult human plasma donors with a primary immunization with a multivalent *S. pneumonia* conjugate vaccine followed by boost immunization with a multivalent polysaccharide *S. pneumonia* vaccine that is different than the prime vaccine; harvesting and pooling the plasma from the immunized plasma donors; and preparing immune globulin from the pooled plasma, wherein the immune globulin contains an opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* that is two-fold or higher (e.g., 3-25 fold or more higher) than the opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* present in a control sample (e.g., immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors). The disclosure also provides a hyperimmune globulin prepared according the above described method. Hyperimmune globulin so prepared can be used in various methods. For example, the hyperimmune globulin can be used in a method of treating *S. pneumonia* infection in a subject comprising administering to the subject a therapeutically effective amount of the hyperimmune globulin. The hyperimmune globulin can also be used in a method of providing immunotherapy to a subject comprising administering to the subject a therapeutically effective amount of the hyperimmune globulin.

In some examples, the disclosure provides a method of preparing compositions (e.g., blood, plasma, and/or immune globulin (e.g., hyperimmune globulin) compositions) containing a high titer of opsonic, anti-pneumococcal antibodies involving screening plasma (e.g., pools of plasma or immunoglobulin; immunoglobulin or immunoglobulin preparations)

for total anti-pneumococcal binding antibodies using an in vitro antigen-binding assay (e.g., using an ELISA) together with an opsonophagocytic killing assay (e.g., an opsonophagocytic killing assay described herein or known in the art). In some examples, the disclosure provides a method of preparing compositions (e.g., blood, plasma, and/or immune globulin compositions) containing a high titer of opsonic, anti-pneumococcal antibodies involving screening plasma (e.g., pools of plasma or immunoglobulin; immunoglobulin or immunoglobulin preparations) for total anti-pneumococcal opsonic antibody titer using an opsonophagocytic bactericidal assay (e.g., an assay described in Example 1). In another example, the disclosure provides a method of preparing compositions (e.g., blood, plasma, and/or immune globulin compositions) containing a high titer of opsonic, anti-pneumococcal antibodies involving screening plasma (e.g., pools of plasma or immunoglobulin; immunoglobulin or immunoglobulin preparations) for total anti-pneumococcal binding antibodies using an in vitro antigen-binding assay (e.g., using an ELISA) followed by screening the plasma (e.g., pools of plasma or immunoglobulin; immunoglobulin or immunoglobulin preparations) for total anti-pneumococcal opsonic antibody titer using an opsonophagocytic bactericidal assay (e.g., an assay described in Example 1). In a further example, protective efficacy can be documented in vivo by analyzing protective activity of the compositions (e.g., blood, plasma, and/or immune globulin compositions) containing a high titer of opsonic, anti-pneumococcal antibodies using an animal model of *S. pneumonia* infection (e.g., to determine morbidity, mortality, and/or bacterial clearance). The disclosure is not limited by the method or assay used to measure the opsonophagocytic titer of antibody present. Indeed, a variety of assays may be used including, but not limited to, those described herein (e.g., in Example 1).

The disclosure provides a method, in one example, of generating a composition comprising obtaining plasma samples from donors (e.g., 50, 100, 300, 500, 1000 or more donors (e.g., healthy adult human plasma donors)) vaccinated with one or more anti-pneumococcal vaccines and pooling the plasma samples (e.g., with each other and/or with plasma from non-vaccinated donors) to generate a pooled plasma composition. In a further example, immune globulin is prepared from the pooled plasma samples (e.g., immune globulin is fractionated, purified, and/or isolated from plasma via any method known in the art including those described herein). In a further example, immune globulin of the disclosure is rendered intravenously injectable (e.g., via providing the immune globulin in solution, adjusting the pH, adjusting the ionic strength, etc.). As described herein, in one example, a pooled plasma composition of the disclosure contains an opsonophagocytic

antibody titer that is at least 2-fold, 3-fold, 4-fold, 5-fold, 7-fold, 10-fold, or more greater for at least about 55% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85% or more) of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) compared to the anti-pneumococcal-specific, opsonic antibody titers for the same serotypes present in a control sample (e.g., immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors). In another example, the pooled plasma composition contains an opsonophagocytic antibody titer specific for 70% or more of the *S. pneumonia* serotypes selected from serotypes 4, 6B, 9V, 14, 18C, 19F, and 23, that is 2 fold or greater (e.g., 3-25 fold or greater) than the titer of opsonic antibodies specific for the same *S. pneumonia* serotypes present in a control sample (e.g., immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors). In yet another example, the pooled plasma composition contains an opsonophagocytic antibody titer between 1:64 and 1:8192 (e.g., for at least 50% or more (e.g., 60%, 65%, 70%, 75%, 80%, 85% or more) of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) as determined by an opsonophagocytic killing assay described herein.

In one example, plasma samples (e.g., from vaccinated and/or non-vaccinated donors) are screened in order to confirm the absence of blood-borne pathogens (e.g., before or after pooling). In some examples, plasma and/or antibody samples are obtained from donor subjects in the form of donated or purchased biological material (e.g., blood or plasma). In some examples, plasma and/or antibody samples (e.g., blood, plasma, isolated antibodies, etc.) are obtained from a commercial source. In some examples, a plasma and/or antibody sample, blood donation, or plasma donation is screened for pathogens, and either cleaned or discarded if particular pathogens are present. In some examples, screening occurs prior to pooling a donor sample with other donor samples. In other examples, screening occurs after pooling of samples. Antibodies, blood, and/or plasma may be obtained from any suitable subjects. In some examples, antibodies, blood, and/or plasma are obtained from a subject who has recently (e.g., within 1 year, within 6 months, within 2 months, within 1 month, within 2 weeks, within 1 week, within 3 days, within 2 days, within 1 day) been vaccinated against or been exposed to one or more pneumococcal vaccines.

In some examples, blood, plasma and/or immune globulin samples identified (e.g., according to methods described herein) as containing a high titer of opsonic, anti-pneumococcal antibodies are combined (e.g., pooled) to produce a composition of the

disclosure (e.g., hyperimmune globulin) comprising a high titer of opsonic, anti-pneumococcal antibodies.

Any suitable method for obtaining plasma, antibody samples, pooled plasma compositions and/or immunoglobulin from same are within the scope of the present disclosure. Further, any suitable method for producing, manufacturing, purifying, fractionating, enriching, etc. antibody samples and/or plasma pools is within the bounds of the present disclosure. Exemplary techniques and procedures for collecting antibody samples and producing plasma pools are provide, for example, in: U.S. Patent No. 4,174,388; U.S. Patent No. 4,346,073; U.S. Patent No. 4,482,483; U.S. Patent No. 4,587,121; U.S. Patent No. 4,617,379; U.S. Patent No. 4,659,563; U.S. Patent No. 4,665,159; U.S. Patent No. 4,717,564; U.S. Patent No. 4,717,766; U.S. Patent No. 4,801,450; U.S. Patent No. 4,863,730; U.S. Patent No. 5,505,945; U.S. Patent No. 5,582,827; U.S. Patent No. 6,692,739; U.S. Patent No. 6,962,700; U.S. Patent No. 6,984,492; U.S. Patent No. 7,045,131; U.S. Patent No. 7,488,486; U.S. Patent No. 7,597,891; U.S. Patent No. 6,372,216; U.S. Patent App. No. 2003/0118591; U.S. Patent App. No. 2003/0133929 U.S. Patent App. No. 2005/0053605; U.S. Patent App. No. 2005/0287146; U.S. Patent App. No. 2006/0110407; U.S. Patent App. No. 2006/0198848; U.S. Patent App. No. 2006/0222651; U.S. Patent App. No. 2007/0037170; U.S. Patent App. No. 2007/0249550; U.S. Patent App. No. 2009/0232798; U.S. Patent App. No. 2009/0269359; U.S. Patent App. No. 2010/0040601; U.S. Patent App. No. 2011/0059085; and U.S. Patent App. No. 2012/0121578; herein incorporated by reference in their entireties. Examples of the present disclosure may utilize any suitable combination of techniques, methods, or compositions from the above listed references.

Isolated immune globulin of the disclosure may be of the IgG fraction or isotype, but isolated immune globulin is not restricted to any particular fraction or isotype and may be IgG, IgM, IgA, IgD, IgE, or any combination thereof. It is also preferable that the isolated immune globulin be purely or antigenically human immune globulin.

In one example, a composition comprising high titer of opsonic, anti-pneumococcal antibodies of the disclosure is a sterile solution with a pH of about 6.0-7.8 (e.g., 5.0-6.0, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6. 7.7, 7.8, or higher). In another example, a composition comprising high titer of opsonic, anti-pneumococcal antibodies of the disclosure is prepared according US FDA standards for immune globulin preparation (see e.g., 37 CFR §§640.100; 640.101; 640.102; 640.103; 640.104, April 1, 2013). In one example, a composition comprising high titer of opsonic, anti-pneumococcal antibodies of the disclosure (e.g., hyperimmune globulin described herein,

in particular, in the Examples) possesses at least the minimum level of antibody titers to Corynebacterium diphtheria, measles virus, and polio virus recommended by the FDA (e.g., see 37 CFR § 640.104) for treating patients with immune deficiency disease.

In one example, the pooled plasma composition lacks detectable levels (e.g., detected using any method known in the art (e.g., recommended by the U.S. Food and Drug Administration)) of human immunodeficiency virus (HIV) 1 (HIV-1), HIV-2, Treponema pallidum, Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax, Plasmodium knowlesi, hepatitis B virus(HBV), hepatitis C virus (HCV), prions, West Nile virus, parvovirus, Typanosoma cruzi, SARS coronavirus, and/or vaccinia virus. In one example, each individual plasma sample used in a process or composition of the disclosure is collected only at an FDA approved blood establishments and is tested by serological tests (e.g., FDA approved serological tests) for human immunodeficiency virus (HIV) 1 (HIV-1), HIV-2, Treponema pallidum, Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax, Plasmodium knowlesi, hepatitis B virus(HBV), hepatitis C virus (HCV), prions, West Nile virus, parvovirus, Typanosoma cruzi, SARS coronavirus, and/or vaccinia virus. In another example, an individual plasma sample and/or a pooled plasma composition of the disclosure is tested for the presence of HIV-1, HIV-2, HBV, HCV, or other infectious agent (e.g., pathogen) using Nucleic Acid Testing (NAT) and used in a process or composition of the disclosure only when the absence of the pathogens is confirmed.

The disclosure is not limited by the type of subject (e.g., mammal, non-human primate, human, etc.) administered or treated with a composition of the disclosure (e.g., pooled plasma samples and/or immunotherapeutic composition comprising same). For example, the disclosure is not limited by the type of patient receiving a compositions (e.g., blood, plasma, and/or immune globulin (e.g., hyperimmune globulin) compositions) containing a high titer of opsonic, anti-pneumococcal antibodies. It is an object of the disclosure to provide and/or administer a composition of the disclosure to immunocompromised patients described herein.

In one example, the pooled plasma comprises plasma samples obtained from 1000-3000 or more (e.g., more than 1000, 1250, 1500, 1750, 2000, 2500, 3000, 3500, 4000 or more) human subjects. In one example, the composition comprising pooled plasma samples further comprises a pharmaceutically acceptable carrier (e.g., natural and/or non-naturally occurring carriers). In one example, the pooled plasma composition is utilized to prepare immune globulin (e.g., for intravenous administration to a subject). In one example, the pooled plasma composition and/or immune globulin provides a therapeutic

benefit to a subject administered the composition that is not achievable via administration of a mixture of plasma samples obtained from 1000 or more random human subjects and/or immunoglobulin prepared from same (e.g., prevents or treats upper respiratory infection in a subject not prevented or treated with conventional IVIG). The disclosure is not limited by the type of therapeutic benefit provided. Indeed, a variety of therapeutic benefits may be attained including those described herein.

In one example, the pooled plasma and/or immunoglobulin prepared from same of the disclosure reduces the incidence of infection (e.g., upper respiratory tract infection) in a subject administered the composition. In another example, a pooled plasma and/or immunoglobulin prepared from same of the disclosure reduces the number of days a subject administered the pooled plasma and/or immunoglobulin of the disclosure is required to be administered antibiotics (e.g., to treat infection (e.g., upper respiratory tract infection)). In yet another example, a pooled plasma and/or immunoglobulin prepared from same of the disclosure increases the trough level of circulating opsonic anti-pneumococcal antibodies in a subject (e.g., increases the level of opsonic antibody titer specific for *S. pneumonia* (e.g., thereby providing protective levels of anti-pneumococcal specific antibodies between scheduled dates of administration of the pooled plasma and/or immune globulin prepared from same of the disclosure (e.g., that are not maintained in a subject administered conventional IVIG))).

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

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the pneumococcal serotype specific IgG antibody binding titers in individual vaccinated donor sera drawn at the following time points: immediately prior to primary PREVNAR vaccination (Pre), immediately prior to secondary PNEUMOVAX23 vaccination at week 4 (Week 4), at week 8, at week 10, at week 12 and at week 16. The average pneumococcal serotype specific IgG antibody binding titers are shown for pre and weeks 4, 8 , 10, and 12, and for pooled pre and week 12. The fold increase in pneumococcal serotype specific IgG antibody binding titers between pre and week 12 are also shown.

CLAIMS

What Is Claimed Is:

1. A method for preparing an immune globulin having an opsonophagocytic antibody titer of at least 1:256 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F comprising the steps of:
(a) pooling plasma from vaccinated plasma donors in order to obtain a pooled plasma containing an opsonophagocytic antibody titer of at least 1:256 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F; wherein the plasma was harvested from the plasma donors subsequent to a booster immunization, wherein the plasma donors are healthy adult human plasma donors between the ages of 18-60 who were immunized with a primary immunization with a multivalent *S. pneumonia* vaccine followed by a booster immunization with a multivalent *S. pneumonia* vaccine that is different than the primary vaccine; and
(b) preparing an immune globulin from the plasma pooled according to step (a).

2. The method of claim 1, further comprising the step (c) rendering the immune globulin obtained according to step (b) intravenously injectable.

3. The method of claim 2, wherein the immune globulin is provided in solution and the pH and ionic strength of the solution is adjusted so as to render it intravenously injectable.

4. The method of claim 1, wherein the pooled plasma is made via pooling plasma from 1000 or more different immunized human plasma donors.

5. An immune globulin prepared according to the method of claim 1.

6. Use of a therapeutically effective amount of an immune globulin prepared according to the method of claim 1 in the manufacture of a medicament for treating *S. pneumonia* infection in a subject.

7. An effective amount of an immune globulin prepared according to the method of claim 1 for use in immunotherapy.

8. A method of preparing immune globulin having enhanced opsonophagocytic bactericidal activity against at least seven serotypes of *Streptococcus pneumonia* for the prevention or treatment of *S. pneumonia* infection comprising the steps of:
(a) preparing immune globulin from pooled plasma, wherein the pooled plasma was harvested and pooled from immunized plasma of healthy adult human plasma donors, wherein said healthy adult human plasma donors were immunized with a primary immunization with a multivalent *S. pneumonia* conjugate vaccine followed by boost immunization with a multivalent polysaccharide *S. pneumonia* vaccine;
wherein the immune globulin contains an opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* that is three-fold or higher than the opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* present in immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors.

9. The method of claim 8, wherein the opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* that is five-fold or higher than the opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* present in immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors.

10. The method of claim 8, wherein the opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* that is ten-fold or higher than the opsonophagocytic antibody titer specific for each of the at least seven serotypes of *S. pneumonia* present in immune globulin prepared from plasma pooled from 1000 or more random non-vaccinated human plasma donors.

11. An immune globulin prepared according to the method of claim 8.

12. A pooled plasma composition having an opsonophagocytic antibody titer specific for at least 55% of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F that is at least 3-fold higher than the opsonophagocytic antibody titer present in immune globulin prepared from a mixture of plasma samples obtained from 500 or more random human plasma donors that have not been vaccinated with an anti-pneumococcal vaccine.

13. The pooled plasma composition of claim 12, wherein the opsonophagocytic antibody titer specific for at least 55% of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F is at least 5-fold higher than the opsonophagocytic antibody titer present in immune globulin prepared from a mixture of plasma samples obtained from 500 or more random human plasma donors that have not been vaccinated with an anti-pneumococcal vaccine.

14. The pooled plasma composition of claim 12, wherein the opsonophagocytic antibody titer specific for at least 55% of *S. pneumonia* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F is at least 10-fold higher than the opsonophagocytic antibody titer present in immune globulin prepared from a mixture of plasma samples obtained from 500 or more random human plasma donors that have not been vaccinated with an anti-pneumococcal vaccine.

15. An immune globulin having an opsonophagocytic antibody titer of at least 1:256 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

16. The immune globulin of claim 15, wherein the opsonophagocytic antibody titer is at least 1:512 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

17. The immune globulin of claim 15, wherein the opsonophagocytic antibody titer is at least 1:1024 for 50% or more *Streptococcus pneumonia* serotypes selected from serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.