METHOD FOR INHIBITING HIV-1 REPLICAION UTILIZING ANTI-GBV-C E2 ANTIBODIES

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Field of Classification Search
None
See application file for complete search history.

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ABSTRACT
GB virus C (GBV-C or hepatitis G virus) is a flavivirus that frequently leads to chronic viremia in humans. The invention provides compositions and methods involving an anti-GBV-C antibody or other GBV-C binding agent, or a GBV-C antigen, for inhibiting and treating HIV infections.

8 Claims, 14 Drawing Sheets
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* cited by examiner
FIG. 1
FIG. 2
FIG. 5
FIG. 6
R5-1 = no antibody control;
R5-IC = IC/no Ab;
BD/IC and VS/IC.
All values = $1 - \frac{\text{sample/IC}}{100} = \%$ inhibition

FIG. 7
S1000-Co-elution of P24 antigen and CPM of 48 Hr.

FIG. 13
METHOD FOR INHIBITING HIV-1 REPLICATION UTILIZING ANTI-GBV-C E2 ANTIBODIES

This application is a divisional of U.S. patent application Ser. No. 10/862,061 filed on Jun. 4, 2004, now abandoned which in turn claims benefit of the filing date of U.S. Provisional Patent Application Ser. No. 60/475,987, filed on Jun. 5, 2003, both of which are incorporated herein by reference in their entirety.

The U.S. Government owns rights in this invention pursuant to grant number RO1AA12671 from the National Institutes of Health and a merit grant awarded to Jack Stapleton from the Veterans Administration.

BACKGROUND OF THE INVENTION

I. Field of the Invention

The present invention relates generally to the fields of molecular biology and virology. More particularly, it concerns methods and compositions to treat, inhibit or prevent HIV infection.

II. Description of Related Art

A. GB Virus Type C

GB virus type C (GBV-C), also known as hepatitis G virus (HGV), is a virus whose genomic organization and nucleotide sequence places it in the Flavivirus family (Robertson et al., 1998). It is the most closely related human viral virus to hepatitis C virus (HCV) (Leary et al., 1996; Linnen et al., 1996; Simons et al., 1995). It has been suggested that these viruses should be classified together with non-human GB-hepatitis agents as the hepacivirus genus. Although GBV-C was originally associated with post-transfusion hepatitis in humans (Linnen et al., 1996), subsequent epidemiological studies indicated that it does not cause acute or chronic hepatitis (Alter et al., 1997a; Alter et al., 1997b). In addition, experimental GBV-C infection of chimpanzees was not associated with acute hepatitis (Bukh et al., 1998).

Persistent GBV-C viremia (as detected by RT-PCR) is common, with 0.9% to 3% of healthy U.S. blood donors and approximately 20%-30% of patients with HCV infection persistently infected with GBV-C (Dawson et al., 1996; Feucht et al., 1997; Simons et al., 1995a; Simons et al., 1995b; Tacke et al., 1997). Following infection, about 80% of people clear their viremia, concomitantly developing antibody to the GBV-C E2 protein (Feucht et al., 1997; Thomas et al., 1998). Thus, it is estimated that approximately 20% of infected people remain viremic for long periods of time. GBV-C appears to be transmitted primarily by parenteral exposure (Simons et al., 1995), although there are data suggesting that sexual and/or household transmission of GBV-C infection may occur (Akiyoshi et al., 1999; de Martino et al., 1998; Nenrkar et al., 1998; Tanaka et al., 1997; Wu et al., 1997).

B. GBV-C and HIV

GBV-C has been investigated in the context of HIV infection. The course of HIV-1 infection is extremely variable among infected individuals, although the reasons for this observation are not fully understood. Individuals whose HIV disease progresses slowly are often called long-term non-progressors (LTNPs). The prevalence of LTNPs varies from 1% to 25% of infected people, depending upon the definition used (reviewed in Easterbrook, 1999). There are no specific clinical criteria for LTNP. However, non-progression generally implies the absence of HIV-related clinical disease 10 or more years following infection and an absolute CD4 count of ≥500 cells/µL (Easterbrook, 1999). Evaluation of LTNPs has identified HIV isolates with deletions in key replicative genes (Deacon et al., 1995) and host genetic factors, including specific HLA haplotypes (reviewed in reference Rowland-Jones, 1999). In some individuals, polymorphisms that result in absent or reduced expression of HIV co-receptors have been identified (Huang et al., 1996). However, these findings are uncommon and thought to account for no more than one-third of LTNPs (Rowland-Jones, 1999).

Persistent GBV-C infection is common in humans, with infection rates of approximately 0.9% to 3% in healthy blood donors, 20-30% in HCV-positive people (Dawson et al., 1996), and 35%-40% in HIV-positive individuals. GBV-C infection can persist for decades in the absence of any clinical morbidity or mortality. Among immune-competent individuals, it is estimated that 60% to 75% of GBV-C-infected people clear the infection, concomitantly developing antibodies to the envelope glycoprotein E2 (Thomas et al., 1998). It is also known that GBV-C can be propagated in cultures of peripheral blood mononuclear cells (PBMCs) (Fogeda et al., 1999).

In 1998, Toyoda et al. found that hemophiliacs co-infected with HIV and GBV-C had a lower plasma HIV RNA concentration and a lower incidence of AIDS diagnoses compared to those infected with HIV alone (Toyoda et al., 1998), although the differences were not statistically significant. In contrast, Sabin and colleagues found an increased rate of AIDS and death in hemophiliacs "exposed" to GBV-C (Sabin et al., 1998) compared to non-exposed individuals. This study included HIV-positive subjects who were either GBV-C viremic as determined by detection of GBV-C RNA in plasma, or HIV-infected people who were not viremic but were anti-GBV-C E2 antibody-positive. Although the mortality rate was higher among the GBV-C "exposed" individuals, the results were not statistically significant. Looking at HIV-infected persons, Lefèvre and colleagues reported a significant delay in the rate of CD4+ T cell decline, development of AIDS, and death in 23 HIV-positive individuals with GBV-C viremia compared to 72 HIV-infected people without GBV-C viremia (Lefèvre et al., 1999). In this study, HIV-infected individuals who were also GBV-C-positive were compared to HIV-infected individuals who were GBV-C-negative. When these subjects were matched by age, sex, baseline HIV RNA load, and baseline CD4+ T cell count, HIV disease progression appeared to be worse in GBV-C-negative subjects.

During progressive human immunodeficiency virus type 1 (HIV-1) infection, the virus-specific immune responses of an infected subject gradually deteriorate, leading to the development of acquired immunodeficiency syndrome (AIDS). Most infected patients do not exhibit overt clinical manifestations of the disease for six to ten years following initial infection, however, most individuals infected with HIV eventually die from conditions or infections; that the individual's immune system is no longer equipped to fight. While treatment for AIDS has been forthcoming, no effective cure has been reported. Thus, preventative and treatment options against HIV infection and the development of AIDS remain highly desirable.

SUMMARY OF THE INVENTION

Embodiments of the invention provide improved methods and compositions for therapeutic and/or prophylactic treatment of HIV infection. Aspects of the invention include compositions and methods related to antigens and/or polypeptides or peptides derived from GBV-C proteins or envelope proteins, in particular GBV-C envelope protein E2 (E2). In other aspects, the invention includes compositions and methods related to antibodies and other binding agents that bind antigens derived from GBV-C proteins. In other aspects, the
invention includes compositions and methods related to the use of compositions comprising one or more GBV-C polypeptides or peptides for therapeutic or prophylactic administration. Compositions and methods comprising GBV-C polypeptides, GBV-C binding agents, or polynucleotides expressing the same can be used to stimulate or provide anti-HIV activity, including, but not limited to inhibition of HIV replication, inhibition of HIV processing, HIV neutralization, inhibition of HIV infection, or a decreased or delayed mortality in infected persons.

Embellishments of the invention include a therapeutic composition comprising a GBV-C polypeptide or peptide, or a GBV-C polypeptide or peptide binding agent, wherein the composition attenuates HIV infectivity. The binding agent may be an antibody, an aptamer, or any other known binding agent that can be selected or screened for binding to GBV-C polypeptides or fragments thereof, including, but not limited to GBV-C E2 polypeptides or peptides. In certain embodiments, the binding antibody may be a polyclonal antibody, a monovalent antibody or a fragment or mimetic thereof. An antibody of the invention may be a humanized antibody, human antibody, or a human mouse, or human library derived monovalent antibody. In certain embodiments, a GBV-C polypeptide can be derived from a GBV-C envelope protein. In particular embodiments, the GBV-C envelope protein is an E2 protein.

Embellishments of the invention include methods for preventing or treating HIV infection comprising administering to a subject a composition comprising a GBV-C polypeptide or peptide-binding agent. The binding agent may attenuate HIV infectivity. The binding agent may be an antibody, an anti-GBV-C antibody, an antibody-like molecule, or another known binding agent that binds to a GBV-C polypeptide or peptide. In certain embodiments, the anti-GBV-C binding agent binds to a GBV-C E2 polypeptide or peptide. An anti-GBV-C antibody can be an anti-GBV-C E2 antibody. An antibody of the invention may be a polyclonal, monoclonal or a fragment or mimetic thereof. An antibody of the invention may be a humanized antibody, human antibody, or a human mouse, or human library derived monoclonal antibody.

In certain embodiments, methods may include administration of at least a second anti-HIV therapy. A second anti-HIV therapy may be an administration of an infectious GBV-C virus, HAART therapy, AZT therapy, or other known HIV therapies. The second therapy may be administered before, after or during a therapy comprising a GBV-C binding agent or GBV-C polypeptide or peptide. In a particular embodiment, a method may include administering the GBV-C virus before a therapeutic composition of the invention. A therapeutic composition of the invention may be administered at least 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times over minutes, hours, days, weeks, months and/or years.

Embellishments of the invention include methods of preparing an antibody or other GBV-C binding agent by immunizing a non-human animal with a GBV-C polypeptide or fragment thereof, or a GBV-C E2 polypeptide or fragment thereof, or screening recombinant human antibody libraries with the above. In certain aspects, an antigen may be a GBV-C E2 derived peptide. In particular embodiments, the peptide may include, but is not limited to, LGGGFYFLVRRC (SEQ ID NO:6), GGAAGLGAGGFYFLVRRC (SEQ ID NO:7), or FYFLVRRC (SEQ ID NO:8).

Methods of preparing a therapeutic composition may comprise contacting a cell with a polynucleotide encoding a GBV-C polypeptide or peptide binding agent under conditions effective to allow expression of all or part of a GBV-C polypeptide or peptide binding agent; collecting the expressed GBV-C polypeptide or peptide binding agent; and constituting the GBV-C polypeptide or peptide binding agent in a pharmaceutically acceptable solution. The binding agent may attenuate, inhibit, and/or modify HIV. A GBV-C polypeptide or peptide binding agent may be an aptamer, an antibody, or a related molecule. An antibody or related molecule may be humanized.

Certain embodiments include vaccines comprising an antigen derived from a GBV-C polypeptide. The antigen may be all or part of a GBV-C polypeptide including, but not limited to a GBV-C E2 polypeptide. In certain embodiments, the antigen may be a GBV-C E2-derived peptide. In particular embodiments, the peptide may include, but is not limited to, LGGGFYFLVRRC (SEQ ID NO:6), or GGAAGLGAGGFYFLVRRC (SEQ ID NO:7), or FYFLVRRC (SEQ ID NO:8).

Embellishments of the invention include methods of immunizing a subject comprising contacting said subject with a composition comprising a GBV-C polypeptide or fragment thereof. The composition may further comprise an adjuvant. In certain embodiments, the GBV-C polypeptide is an E2 polypeptide.

In still further embodiments, polypeptides and/or peptides of the invention may be used as competitors for HIV binding to or association with various components of the human body. The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.” Furthermore, where multiple steps of a method of process are cited, it is understood that the steps are not required to be performed in the particular order recited unless one of skill in the art is not be able to practice the method in a different order.

Other objects, features, and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating 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

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Exemplary effect of E2 antibody positive serum on HIV replication.

FIG. 2. Example of inhibition of HIV by two E2 antibody positive sera in a dose-dependent fashion; whereas, E2 antibody negative sera does not inhibit HIV.

FIG. 3. IgG purified from E2 antibody positive sera inhibits HIV replication in PBMC cultures.

FIG. 4. IgG purified from E2 antibody positive sera inhibits HIV replication in PBMC cultures (same data shown in FIG. 3, but represents the data as percent inhibition in HIV p24 Ag in culture supernatant fluids).

FIG. 5. GBV-C E2 antibody positive IgG inhibition of clinical HIV strain.

FIG. 6. The Roche M6 monoclonal antibody inhibits HIV-1 (R5 strain)
FIG. 7. The Biodesign (Saco, Me.) and Virostat (Portland, Me.) antibodies also inhibited HIV-1, although not as efficiently as M6.

FIG. 8. A map of the epitopes identified by the Roche mAbs as described in Schmolke et al. (1998).

FIG. 9. A map of exemplary GBV-C epitopes (Peptide GAGLITGFFPY EPLVRRC (SEQ ID NO:6)).

FIG. 10. Illustrates inhibition of HIV-R5 by 17-mer Rabbit serum day 3.

FIG. 11. Illustrates inhibition of HIV (X4) by Rabbit anti-peptide lgG day 2.

FIG. 12. Illustrates inhibition of HIV (X4) by 17-mer Rabbit serum day 3.

FIG. 13. Illustrates the elution profile of P24 antigen and CPMs.

FIG. 14. Metabolically 35S-labeled HIV particles were partially purified by size-exclusion chromatography and incubated with either isotype control (IC) or anti-E2 McAb (concentrations indicated on X-axis). HIV-lgG complexes were immuno-precipitated using staph protein A (PANSORBIND®). Data represent HIV cpm precipitated by Mc6 (over background isotype control cpm).

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

Anti-retroviral medications suppress viral replication in HIV disease, yet they have failed to eradicate the virus from the body due to the multi-faceted nature of HIV infection, as well as the complexities of the immune system. Methods are being developed that both prevent infection and boost the immune system to keep it functioning at a level where it can assist in fighting HIV infection.

Thus, embodiments of the invention provide additional methods and compositions for therapeutic and/or prophylactic treatment of HIV infection. Aspects of the invention include compositions and methods related to antibodies derived from GBV-C envelope proteins, in particular GBV-C envelope protein E2 (E2). In other aspects, the invention includes compositions and methods related to antibodies and other binding agents that bind antibodies derived from GBV-C proteins. In particular, binding agents, such as aptamers and anti-GBV-C antibodies that bind GBV-C E2 proteins, are contemplated. In particular embodiments, a therapeutic GBV-C binding agent is contemplated for the treatment of HIV infection. Certain embodiments of the invention include combination treatments for HIV infection using compositions of the invention in combination with other anti-retroviral or HIV therapies.

The inventors have shown that HIV-infected subjects that are co-infected with GB virus C (GBV-C) typically have reduced mortality and slower progression to AIDS as compared to HIV-infected subjects without GBV-C co-infection. Infection of peripheral blood mononuclear cells (PBMCs) with GBV-C and HIV results in inhibition of HIV-1 replication. GBV-C infection typically inhibits HIV by inducing β-chemokines and reducing expression of the HIV co-receptor CXCR5, explaining part of the beneficial clinical findings of GBV-C on HIV disease progression. Antibodies directed to the GBV-C virus have been noted and are typically used as a diagnostic agents, with no therapeutic use having been ascribed to them. The inventors now describe a therapeutic use for antibodies and/or binding agents that bind GBV-C proteins (e.g., envelope proteins), and antigens used for producing these antibodies or binding agents. In certain embodiments, a vaccine composition includes peptides derived from GBV-C polypeptides. In further aspects, the peptides themselves may bind to or associate with binding sites within an organism that also bind to HIV, thus the peptides themselves may be used as competitive inhibitors of HIV binding or localization in a host organism.

Embodiments of the invention include anti-GBV-C antibodies that also attenuate the infectivity of HIV. Antibodies against the GBV-C envelope glycoprotein E2 (GBV-C-E2), derived form either passive or active vaccination, are of particular interest for attenuation of HIV. The invention concerns the observation that antibodies against GBV-C peptides and polypeptides may react with and attenuate HIV. These antibodies may be induced, administered, in a pharmaceutical composition for the therapeutic or prophylactic treatment of HIV infection. Infectious GBV-C can be used in combination with the present invention for preventative or therapeutic treatments for HIV infection and related conditions such as AIDS.

I. GBV-C Virus

Like other members of the Flaviviridae, GBV-C is a positive-strand RNA virus that encodes a single long open reading frame (Leary et al., 1996). GBV-C does not cause acute or chronic hepatitis, yet it is the family member most closely related to HCV, the cause of hepatitis C. Sequences of GBV-C have been previously reported, for example in U.S. Pat. No. 5,874,563, which is specifically incorporated by reference. In particular, an infectious GBV-C clone has been described in the PCT application WO 01/77157, which is incorporated herein by reference.

The GBV-C polyprotein is predicted to be cleaved into two envelope proteins (E1 and E2, referred to collectively as GBV-C envelope protein), an RNA helicase, a trypsin-like serine protease, and an RNA-dependent RNA polymerase. A major difference between GBV-C and HCV is in the amino terminus of the polyprotein. In many isolates, this region is truncated, and no core (or nucleocapsid) protein is present (Simons et al., 1995; Xiang et al., 1999). In vitro translation experiments suggest that the AUG immediately upstream of the putative E1 protein is preferentially used to initiate translation, although there may be as many as four AUG’s in frame with the polyprotein upstream of this AUG (Simons et al., 1996).

The site of GBV-C replication has not been clearly identified, but it appears that replication in the hepatocyte, if it occurs, is not the primary source of virus in infected individuals (Lankus et al., 1998; Pessoa et al., 1998; Seipp et al., 1999). Recently, there were reports that human peripheral blood mononuclear cells (PBMCs) and interferon-resistant Daudi cells are permissive for GBV-C replication (Foged et al., 1999; Shinizu, 1999). In addition, transient replication of GBV-C was described in MT-2 cells (a human T-cell line), and PBMC (a human hepatocyte line immortalized with simian virus 40 large T antigen) (Seipp et al., 1999).

II. GBV-C Polypeptides

In certain aspects, the invention is directed to the function, activity, or antigenicity of various components of an infectious GBV-C virus or a polypeptide derived therefrom, in particular the E2 protein. The expression or isolation of certain GBV-C polypeptides can be used to stimulate an anti-HIV activity, including inhibition of replication, processing, neutralization, and infection. SEQ ID NO:2 and 4 represent the translated product of SEQ ID NO:1 (GBV-C polyprotein) and 3 (GBV-C E2 protein), respectively. It is contemplated that the compositions and methods disclosed herein may be utilized to express all or part of SEQ ID NO:2 or 4 and derivatives thereof. In certain embodiments, compositions of the invention may include the nucleic acids encoding the peptides as set forth in SEQ ID Nos. 5, 6, 7 or 8. Determination of which molecules possess or stimulate an anti-HIV
response may be achieved using functional assays measuring HIV infectivity, which are familiar to those of skill in the art. In other embodiments of the invention, heterologous polypeptides may be encoded by a sequence that also contains GBV-C sequences. “Heterologous” polypeptide indicates the polypeptide is not a GBV-C polypeptide. An endogenous GBV-C polypeptide refers to a polypeptide encoded by GBV-C viral RNA. Such a polypeptide would possess the same or similar sequence as SEQ ID NO:2, 4, 5, 6, 7 or 8.

In certain embodiments, an antigen comprising a 9 amino acid sequence FYEPLVRRC (SEQ ID NO:8) or derivative thereof is contemplated. In certain embodiments, an antigen comprising a 13 amino acid sequence (LI6GIFY6PVLRRC, SEQ ID NO:6) or a derivative thereof is contemplated. In still further embodiments, an antigen comprising a 17 amino acid sequence (GGAGL6GIFY6PVLRRC, SEQ ID NO:7) or derivative thereof is contemplated. The structure of the various peptides can be modeled or resolved by computer modeling, NMR, or x-ray crystallography. Peptide structures may be used to engineer derivatives of the various E2 protein sequences or to engineer other molecules to interact with the peptides, such as antibodies or other affinity reagents. Amino acids or peptides of the invention may be used as an HIV disease-modifying immunogen (vaccine). Peptides may be used to inhibit, produce, or design inhibitors of HIV (as a prototype drug), as well as being used to induce anti-HIV antibodies (as a vaccine).

A. Variants of GBV-C Polypeptides

Embodiments of the invention include various GBV-C polypeptides, peptides, and derivatives thereof. Amino acid sequence variants of a polypeptide may be substitutonal, insertional or deletion variants. Deletion variants lack one or more residues of the native protein that are not essential for function or immunogenic activity. Insertional mutants typically involve the addition of material at a non-terminal point in the polypeptide. This may include the insertion of an immunoreactive epitope or simply a single residue. Terminal additions, called fusion proteins, are discussed below.

Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, without the loss of other functions or properties. Substitutions of this kind preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to glutamic acid; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

The term “biologically functional equivalent” is well understood in the art and is further defined in detail herein. Accordingly, sequences that have between about 70% and about 80%; or more preferably, between about 81% and about 90%; or even more preferably, between about 91% and about 99% of amino acids that are identical or functionally equivalent to the amino acids of GBV-C polypeptides, for example SEQ ID NO:2, 4, 5, 6, 7, or 8, provided the biological activity, e.g., immunogenicity, of the protein or peptide is maintained.

The term “functionally equivalent codon” is used herein to refer to codons that encode the same amino acid, such as the six codons for arginine or serine, and also refers to codons that encode biologically equivalent amino acids (see Table 1 below).

Certain embodiments of the invention include various peptides and/or fusion proteins of GBV-C polypeptides, in particular GBV-C E2 protein. For example, all or part of a GBV-C and/or a GBV-C E2 protein as set forth in SEQ ID NO:2, 4, 5, 6, 7 and/or 8 may be used in various embodiments of the invention. In certain embodiments, a fragment of the E2 or other GBV-C protein may comprise, but is not limited to about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 51, about 52, about 53, about 54, about 55, about 56, about 57, about 58, about 59, about 60, about 61, about 62, about 63, about 64, about 65, about 66, about 67, about 68, about 69, about 70, about 71, about 72, about 73, about 74, about 75, about 76, about 77, about 78, about 79, about 80, about 81, about 82, about 83, about 84, about 85, about 86, about 87, about 88, about 89, about 90, about 91, about 92, about 93, about 94, about 95, about 96, about 97, about 98, about 99, about 100, about 110, about 120, about 130, about 140, about 150, about 160, about 170, about 180, about 190, about 200, about 210, about 220, about 230, about 240, about 250, about 275, about 300, about 325, about 350, about 375, about 400, about 425, about 450, about 475, about 500, about 525, about 550, about 575, about 600, about 625, about 650, about 675, about 700, about 725, about 750, about 775, about 800, about 825, about 850, about 875, about 900, about 925, about 950, about 975, about 1000, about 1100, about 1200, about 1300, about 1400, about 1500, about 1750, about 2000, about 2250, about 2500, or greater amino acid molecule residues, and any range derivable therein.

It also will be understood that amino acid and nucleic acid sequences may include additional residues, such as additional N- or C-terminal amino acids or 5′ or 3′ sequences, and yet still be essentially as set forth in one of the sequences disclosed herein, so long as the sequence meets the criteria set forth above, including the maintenance of biological activity (e.g., immunogenicity) where protein expression is concerned. The addition of terminal sequences particularly applies to nucleic acid sequences that may, for example, include various non-coding sequences flanking either of the 5′ or 3′ portions of the coding region.

The following is a discussion based upon changing of the amino acids of a GBV-C polypeptide or peptide to create an equivalent, or even an improved, second-generation molecule. For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein’s biological functional activity, certain amino acid substitutions can be made in a protein sequence, and in its underlying DNA or RNA coding sequence, and nevertheless produce a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the DNA or RNA sequences of genes or coding regions without appreciable loss of their biological utility or activity, as discussed herein. Table 1 shows the codons that encode particular amino acids.
TABLE 1

<table>
<thead>
<tr>
<th>Amino Acids</th>
<th>Codon Table</th>
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In making such changes, the hydrophobic index of amino acids may be considered. The importance of the hydrophobic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982). It is accepted that the relative hydrophobic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like.

It also is understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U.S. Pat. No. 4,554,101, incorporated herein by reference, states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

It is understood that an amino acid substituted for another having a similar hydrophilicity value still produces a biologically equivalent and immunologically equivalent protein.

In certain embodiments, a GBV-C polypeptide may be a fusion protein. Fusion proteins may alter the characteristics of a given polypeptide, such antigenicity or purification characteristics. A fusion protein is a specialized type of insertional variant. This molecule generally has all or a substantial portion of the native molecule, linked at the N- or C-terminus, to all or a portion of a second polypeptide. For example, fusions typically employ leader sequences from other species to permit the recombinant expression of a protein in a heterologous host. Another useful fusion includes the addition of an immunologically active domain, such as an antibody epitope, to facilitate purification of the fusion protein. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other useful fusions include linking of functional domains, such as active sites from enzymes such as a hydrolase, glycosylation domains, cellular targeting signals, or transmembrane regions.

B. In vitro Production of GBV-C or Anti-GBV-C Polypeptides or Peptides

Various types of expression vectors are known in the art that can be used for the production of protein products. Following transfection with an expression vector, a cell in culture, e.g., a primary mammalian cell, a recombinant product may be prepared in various ways. A host cell strain may be chosen that modulates the expression of the inserted sequences, or that modifies and processes the gene product in the manner desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins. Appropriate cell lines or host systems can be chosen to insure the correct modification and processing of the foreign protein expressed. In order for the cells to be kept viable while in vitro and in contact with the expression construct, it is necessary to ensure that the cells maintain contact with the correct ratio of oxygen and carbon dioxide and nutrients but are protected from microbial contamination. Cell culture techniques are well documented (for exemplary methods see Freshney, 1992).

Animal cells can be propagated in vitro in two modes: as non-anchorage-dependent cells growing in suspension throughout the bulk of the culture or as anchorage-dependent cells requiring attachment to a solid substrate for propagation (i.e., a monolayer type of cell growth).

Non-anchorage dependent or suspension cultures from continuous cell line are the most widely used means of large-scale production of cells and cell products. However, suspension cultured cells have limitations, such as tumorigenic potential and lower protein production than adherent cells.

In further aspects of the invention, other protein production methods known in the art may be used, including but not limited to prokaryotic, yeast, and other eukaryotic hosts such as insect cells and the like.

C. Protein Purification

It may be desirable to purify anti-GBV-C and/or GBV-C polypeptides and peptides, or variants and derivatives thereof. Protein purification techniques are well known to those of skill in the art. These techniques involve, at one level, the crude fractionation of the cellular milieu to polypeptide and non-polypeptide fractions. Having separated the polypeptide from other proteins, the polypeptide of interest may be further purified using chromatographic and electrophoretic techniques to achieve partial or complete purification (or purification to homogeneity). Analytical methods particularly suited to the preparation of a pure peptide are ion-exchange chromatography, hydrophobic interaction chromatography, exclusion chromatography; polyacrylamide gel electrophoresis; isoelectric focusing. A particularly efficient method of purifying peptides is fast protein liquid chromatography or even FPLC.

Certain aspects of the present invention concern the purification, and in particular embodiments, the substantial purification, of an encoded protein or peptide. The term "purified protein or peptide" as used herein, is intended to refer to a composition, isolatable from other components, wherein the protein or peptide is purified to any degree relative to its naturally obtainable state. A purified protein or peptide therefore also refers to a protein or peptide, free from the environment in which it may naturally occur.

Generally, "purified" will refer to a protein or peptide composition that has been subjected to fractionation to remove various other components, and which composition substantially retains its expressed biological activity. Where the term "substantially purified" is used, this designation will refer to a composition in which the protein or peptide forms the major component of the composition, such as constituting about 50%, about 60%, about 70%, about 80%, about 90%, about 95% or more of the proteins in the composition.
Various methods for quantifying the degree of purification of the protein or peptide will be known to those of skill in the art in light of the present disclosure. These include, for example, determining the specific activity of an active fraction, or assessing the amount of polypeptides within a fraction by SDS/PAGE analysis. A preferred method for assessing the purity of a fraction is to calculate the specific activity of the fraction, to compare it to the specific activity of the initial extract, and to thus calculate the degree of purity, herein assessed by a “fold purification number.” The actual units used to represent the amount of activity will, of course, be dependent upon the particular assay technique chosen to follow the purification and whether or not the expressed protein or peptide exhibits a detectable activity.

There is no general requirement that the protein or peptide always be provided in their most purified state. Indeed, it is contemplated that less substantially purified products will have utility in certain embodiments. Partial purification may be accomplished by using fewer purification steps in combination, or by utilizing different forms of the same general purification scheme.

III. GBV-C Polynucleotides

Certain embodiments of the invention include GBV-C polynucleotides or nucleic acid molecules and fragments thereof. The polynucleotides of the invention may be isolated and purified from GBV-C virus or cells infected or transfected with GBV-C polynucleotides. The term isolated indicating they are free or substantially free from total viral or cellular genomic RNA or DNA, and proteins. It is contemplated that an isolated and purified GBV-C nucleic acid molecule may take the form of RNA or DNA. A GBV-C nucleic acid molecule refers to an RNA or DNA molecule that is capable of yielding all or part of a GBV-C polypeptide from a transfected cell.

As used in this application, the term “polynucleotide” refers to a nucleic acid molecule, RNA, or DNA that has been isolated free of total genomic nucleic acid. Therefore, a “polynucleotide encoding all or part of GBV-C” refers to a nucleic acid segment that contains GBV-C coding sequences, yet is isolated away from, or purified and free of, total viral genomic RNA and proteins; similarly, a “polynucleotide encoding full-length GBV-C” refers to a nucleic acid segment that contains full-length GBV-C coding sequences yet is isolated away from, or purified and free of, total viral genomic RNA and protein. Therefore, when the present application refers to the function or activity of an infectious GBV-C that is encoded by a GBV-C polynucleotide, it is meant that the polynucleotide encodes a molecule that has the ability to propagate an infectious GBV-C virus particle from a cell. It is contemplated that a GBV-C polynucleotide may refer to a GBV-C RNA transcript that is able to propagate an infectious GBV-C virus particle after introduction to a cell or to a GBV-C expression construct, clone, or vector composed of double-stranded DNA or DNA/RNA hybrid that is similarly capable.

The term “cDNA” is intended to refer to DNA prepared using RNA as a template. The advantage of using a cDNA, as opposed to genomic RNA or an RNA transcript is stability and the ability to manipulate the sequence using recombinant DNA technology (See Maniatis, 1989; Ausubel, 1994). There may be times when the full or partial genomic sequence is preferred. Alternatively, cDNAs may be advantageous because it represents coding regions of a polypeptide and eliminates introns and other regulatory regions.

It also is contemplated that a given GBV-C may be represented by natural variants or strains that have slightly different nucleic acid sequences but, nonetheless, encode the same viral polypeptides (see Table I above). Consequently, the present invention also encompasses derivatives of GBV-C with minimal amino acid changes in its viral proteins, but that possesses the same activities.

The term “gene” is used for simplicity to refer to the nucleic acid giving rise to a functional protein, polypeptide, or peptide-encoding unit. As will be understood by those in the art, this functional term includes genomic sequences, cDNA sequences, and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, domains, peptides, fusion proteins, and mutants. The nucleic acid molecule encoding GBV-C may contain a contiguous nucleic acid sequence encoding one or more GBV-C genes and regulatory regions and be of the following lengths: about 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1010, 1020, 1030, 1040, 1050, 1060, 1070, 1080, 1090, 1100, 1120, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000, 10100, 10200, 10300, 10400, 10500, 10600, 10700, 10800, 10900, 11000, 11100, 11200, 11300, 11400, 11500, 11600, 11700, 11800, 11900, 12000 or more nucleotides, nucleosides, or base pairs. Such sequences may be identical or complementary to all or part of SEQ ID NO:1, 3 or Genbank Accession numbers AF196904 or AF1070476 or segments thereof, e.g., those segments related to peptides of SEQ ID NO:5, 6, 7 or 8.

“Isolated substantially away from other coding sequences” means that the gene of interest forms part of the coding region of the nucleic acid segment, and that the segment does not contain large portions of naturally-occurring coding nucleic acid, such as large chromosomal fragments or other functional genes or cDNA coding regions. Of course, this refers to the nucleic acid segment as originally isolated, and does not exclude genes or coding regions later added to the segment by human manipulation.

In particular embodiments, the invention concerns isolated nucleic acid segments and recombinant vectors incorporating DNA sequences that encode GBV-C polypeptides or peptides that include within its amino acid sequence a contiguous amino acid sequence in accordance with, or essentially corresponding to GBV-C polypeptides.

Certain embodiments include nucleic acids segments and recombinant vectors encoding polypeptides and peptides to induce or enhance immune responses in both subjects having HIV, suspected of having HIV, at risk of being exposed to HIV and/or animals or cells for the production of anti-GBV-C antibodies.

The nucleic acid segments used in the present invention, regardless of the length of the coding sequence itself, may be combined with other DNA or RNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their overall length may vary consid-
erably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol.

In a non-limiting example, one or more nucleic acid constructs may be prepared that include a contiguous stretch of nucleotides identical to or complementary to GBV-C. A nucleic acid construct may be about 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 2,000, 3,000, 4,000, 5,000, 6,000, 7,000, 8,000, 9,000, 10,000, 11,000, 12,000, 13,000, 14,000, 15,000, 20,000, 30,000, 50,000, 100,000, 250,000, about 500,000, 750,000, to about 1,000,000 nucleotides in length, as well as constructs of greater size, up to and including chromosomal sizes (including all intermediate lengths and intermediate ranges), given the advent of nucleic acids constructs such as a yeast artificial chromosome known to those of ordinary skill in the art. It will be readily understood that "intermediate lengths" and "intermediate ranges," as used herein, means any length or range including or between the quoted values (i.e., all integers including and between such values). Non-limiting examples of intermediate lengths include about 11, about 12, about 13, about 16, about 17, about 18, about 19, etc.; about 21, about 22, about 23, etc.; about 31, about 32, etc.; about 51, about 52, about 53, etc.; about 101, about 102, about 103, etc.; about 151, about 152, about 153, etc.

The nucleic acid segments used in the present invention encompass biologically functional and/or immunogenically equivalent GBV-C proteins and peptides. Such sequences may arise as a consequence of codon redundancy and functional equivalency that are known to occur naturally within nucleic acid sequences and the proteins thus encoded. Alternatively, functionally and immunologically equivalent proteins or peptides may be created via the application of recombinant DNA technology, in which changes in the protein structure may be engineered, based on considerations of the properties of the amino acids being exchanged. Changes designed by human may be introduced through the application of site-directed mutagenesis techniques, e.g., to introduce improvements to the antigenicity of the protein.

A. Vectors Encoding GBV-C

The present invention encompasses the use of vectors to encode for all or part of one or more GBV-C polypeptides, including an infectious GBV-C. The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). In particular embodiments, gene therapy or immunization vectors are contemplated. One of skill in the art would be well equipped to construct a vector through standard recombinant techniques, which are described in Maniatis et al., 1988 and Ausubel et al., 1994, both incorporated herein by reference.

The term "expression vector" or "expression construct" refers to a vector containing a nucleic acid sequence coding for at least part of a gene product capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules or ribozymes. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host organism. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described infra. It is contemplated that an infectious GBV-C particle of the present invention may arise from a vector containing GBV-C sequence or RNA encoding GBV-C sequence into a cell. Either of these, or any other nucleic acid molecules of the present invention may be constructed with any of the following nucleic acid control sequences. Thus, the full-length RNA transcript may contain the benefit of recombinant DNA technology such that it contains exogenous control sequences or genes.

1. Promoters and Enhancers

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

A promoter may be one naturally associated with a gene or sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as "endogenous." Similarly, an enhancer may be one naturally associated with a nucleic acid sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other prokaryotic, viral, or eukaryotic cell, and promoters or enhancers not "naturally occurring," i.e., containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. In addition to producing nucleic acid sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR™, in connection with the compositions disclosed herein (see U.S. Pat. Nos. 4,683,202 and 5,928,906, each incorporated herein by reference). Furthermore, it is contemplated the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the nucleic acid segment in the cell type, organelle, and organism chosen.
for expression. Those of skill in the art of molecular biology generally know the use of promoters, enhancers, and cell type combinations for protein expression, for example, see Sambrook et al. (1989), incorporated herein by reference. The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or eukaryotic, i.e., from a different source than GBV-C sequence. In some examples, a prokaryotic promoter is employed for use with in vitro transcription of a desired sequence. Prokaryotic promoters for use with many commercially available systems include T7, T3, and Sp6.

Table 2 lists several elements/promoters that may be employed, in the context of the present invention, to regulate the expression of a gene. This list is not intended to be exhaustive of all the possible elements involved in the promotion of expression but, merely, to be exemplary thereof. Table 3 provides examples of inducible elements, which are regions of a nucleic acid sequence that can be activated in response to a specific stimulus.

**TABLE 2**

<table>
<thead>
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<th>Promoter/Enhancer</th>
<th>References</th>
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<tr>
<td>Immunoglobulin Heavy Chain</td>
<td>Banerji et al., 1983; Gilles et al., 1983; Groussard et al., 1985; Archimone et al., 1986, 1987; Linzler et al., 1987; Weisberger et al., 1984; Kiehl et al., 1988; Porten et al., 1990</td>
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<tr>
<td>Immunoglobulin Light Chain</td>
<td>Queen et al., 1983; Picard et al., 1984</td>
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<td>T-Cell Receptor</td>
<td>Luria et al., 1987; Winoto et al., 1989; Redondo et al., 1990</td>
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<td>HLA-DQA a and/or DQB</td>
<td>Sullivan et al., 1987</td>
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<td>β-Interferon</td>
<td>Goldboim et al., 1986; Fujita et al., 1987; Goldboim et al., 1988</td>
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<td>Interleukin-2</td>
<td>Grete et al., 1989</td>
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<td>Interleukin-2 Receptor</td>
<td>Oreone et al., 1989; Lin et al., 1990</td>
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<td>MHC Class II HLA-DRα</td>
<td>Sherman et al., 1989</td>
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<td>β-Actin</td>
<td>Kawamoto et al., 1988; Ng et al., 1989; Johnson et al., 1989</td>
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<td>Muscle Creatine Kinase (MCK)</td>
<td>Jaynes et al., 1988; Horlick et al., 1989;</td>
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<td>Prealbumin (Transferrin)</td>
<td>Costa et al., 1988</td>
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<td>Metallothionein (MTII)</td>
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<td>Insulin</td>
<td>Edlund et al., 1985</td>
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<td>Neural Cell Adhesion Molecule (NCAM)</td>
<td>Hirsh et al., 1990</td>
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<td>α1-Antitrypsin</td>
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<td>H2B (H2B2) Histone</td>
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<td>Mouse and/or Type I Collagen</td>
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<td>Glucose-Regulated Proteins (GRP94 and GRP78)</td>
<td>Chang et al., 1989</td>
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<td>Rat Growth Hormone</td>
<td>Lams et al., 1986</td>
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<td>Human Serum Amyloid A (SAA)</td>
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<td>Troponin I (TN1)</td>
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<td>SV40</td>
<td>Banerji et al., 1981; Moreau et al., 1981; Sleigh et al., 1985; Finak et al., 1986; Herr et al., 1986; Imber et al., 1986; Kadash et al., 1986; Wang et al., 1986; Oudek et al., 1987; Kuhl et al., 1987; Schaffner et al., 1988</td>
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**Polyoma**

Swaartgrodber et al., 1975; Vaseur et al., 1980; Kataoka et al., 1980, 1981; Tyndall et al., 1981; Dundol et al., 1983; de Villiers et al., 1984; Hen et al., 1986; Satake et al., 1988; Campbell and/or Villareal, 1988

**Retroviruses**

Kriegler et al., 1982, 1983; Levinson et al., 1982; Kriegler et al., 1983, 1984a, b, 1988; Boze et al., 1986; Miksicek et al., 1986; Celander et al., 1987; Thiesen et al., 1988; Celander et al., 1988; Chol et al., 1988; Reisman et al., 1989
TABLE 2-continued

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<td>Papilloma Virus</td>
<td>Campo et al., 1983; Lusky et al., 1983; Spandios and/or Wilkie, 1983; Spahlholz et al., 1985; Lusky et al., 1986; Cripe et al., 1987; Goss et al., 1987; Hirochika et al., 1987; Stephens et al., 1987; Glae et al., 1988</td>
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<tr>
<td>Hepatitis B Virus</td>
<td>Buja et al., 1986; Jameel et al., 1986; Shaul et al., 1987; Spandau et al., 1988; Vannice et al., 1988</td>
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<tr>
<td>Human Immunodeficiency Virus</td>
<td>Muir et al., 1987; Hauber et al., 1988; Jakubovits et al., 1988; Feng et al., 1988; Takebo et al., 1988; Rosén et al., 1988; Berkhou et al., 1989; Lampia et al., 1989; Sharp et al., 1989; Braddock et al., 1989</td>
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<tr>
<td>Cytomegalovirus (CMV)</td>
<td>Weber et al., 1984; Boshart et al., 1985</td>
<td></td>
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<tr>
<td>Gibbon Ape Leukemia Virus</td>
<td>Hoibrook et al., 1987; Quinn et al., 1989</td>
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TABLE 3

<table>
<thead>
<tr>
<th>Element</th>
<th>Inducer</th>
<th>References</th>
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<tbody>
<tr>
<td>MT II</td>
<td>Phorbol Ester (TFA)</td>
<td>Palmer et al., 1982; Haslinger et al., 1985; Searle et al., 1985; Stuart et al., 1985; Imamura et al., 1987; Kazin et al., 1987; Angel et al., 1987b; McNeall et al., 1989</td>
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<td></td>
<td>Heavy metals</td>
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<td>MMTV (mouse mammary tumor virus)</td>
<td>Glucocorticoids</td>
<td>Huang et al., 1981; Lee et al., 1981; Majors et al., 1983; Chaudier et al., 1983; Lee et al., 1984; Pouza et al., 1985; Sakaizawa et al., 1988</td>
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<tr>
<td>β-Interferon</td>
<td>poly (rX)</td>
<td>Tavernier et al., 1983</td>
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<td></td>
<td>poly (rC)</td>
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<td>Adenovirus 5 E2</td>
<td>EIA</td>
<td>Imperiale et al., 1984</td>
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<td>Collagenase</td>
<td>Phorbol Ester (TFA)</td>
<td>Angel and et al., 1987a</td>
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<td>Angel et al., 1987b</td>
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<td>SV40</td>
<td>Phorbol Ester (TFA)</td>
<td>Angel et al., 1987b</td>
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<td>Murine MX Gene</td>
<td>Interferon, Newcastle Disease Virus</td>
<td>Hagiwara et al., 1988</td>
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<tr>
<td>GRP78 Gene</td>
<td>A23187</td>
<td>Resendez et al., 1988</td>
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<td>α-2-Macroglobulin</td>
<td>IL-6</td>
<td>Kurz et al., 1989</td>
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<td>Vimentin</td>
<td>Serum</td>
<td>Ritting et al., 1989</td>
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<td>MHC Class I Gene H-2kb</td>
<td>Interferon</td>
<td>Blau et al., 1989</td>
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<td>HSP70</td>
<td>EIA, SV40 Large T</td>
<td>Taylor et al., 1989, 1990a, 1990b</td>
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<td>Proliferin</td>
<td>Phorbol Ester-TPA</td>
<td>Mora-Cot, et al., 1989</td>
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<tr>
<td>Thyroid Stimulating Hormone</td>
<td>PMA</td>
<td>Hensel et al., 1989</td>
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<tr>
<td>Hormone α Gene</td>
<td>Thyroid Hormone</td>
<td>Chatterjee et al., 1989</td>
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The identity of tissue-specific promoters or elements, as well as assays to characterize their activity, is well known to those of skill in the art. Examples of such regions include the human LIMK2 gene (Nomoto et al., 1999), the somatostatin receptor 2 gene (Kraus et al., 1998), murine epididymal retinoic acid-binding gene (Lareyre et al., 1999), human CD4 (Zhao-Emonet et al., 1998), mouse alpha2 (XI) collagen (Tsumaki, et al., 1998), D1A dopamine receptor gene (Lee et al., 1997), insulin-like growth factor II (Wu et al., 1997), human platelet endothelial cell adhesion molecule-1 (Almendro et al., 1996).  

2. Initiation Signals and Internal Ribosome Binding Sites
A specific initiation signal also may be required for efficient translation of coding sequences. These signals include the ATG initiation codon or adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals. It is well known that the initiation codon must be “in-frame” with the reading frame of the desired coding sequence to ensure translation of the entire insert. The exogenous translational control signals and initiation codons can be either natural or synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements.

In certain embodiments of the invention, the use of internal ribosome entry sites (IRES) elements are used to create multigene, or polycistronic, messages. IRES elements are able to bypass the ribosome-scanning model of S' methylated Cap dependent translation and begin translation at internal sites.
(Pelletier and Sonenberg, 1988). IRES elements from two members of the picornavirus family (polio and encephalomyocarditis) have been described (Pelletier and Sonenberg, 1988), as well as an IRES from a mammalian message (Macejak and Sarnow, 1991). IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message (see U.S. Pat. Nos. 5,925,565 and 5,935,819, herein incorporated by reference).

3. Multiple Cloning Sites

Vectors can include a multiple cloning site (MCS), which is a nucleic acid region that contains multiple restriction enzyme sites, any of which can be used in conjunction with standard recombinant technology to digest the vector. (See Carbonelli et al., 1999, Levenson et al., 1998, and Cocen, 1997, incorporated herein by reference.) "Restriction enzyme digestion" refers to catalytic cleavage of a nucleic acid molecule with an enzyme that functions only at specific locations in a nucleic acid molecule. Many of these restriction enzymes are commercially available. Use of such enzymes is widely understood by those of skill in the art. Frequently, a vector is linearized or fragmented using a restriction enzyme that cuts within the MCS to enable exogenous sequences to be ligated to the vector. "Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid fragments, which may or may not be contiguous with each other. Techniques involving restriction enzymes and ligation reactions are well known to those of skill in the art of recombinant technology.

4. Splicing Sites

Most transcribed eukaryotic RNA molecules will undergo RNA splicing to remove introns from the primary transcripts. Vectors containing genomic eukaryotic sequences may require donor and/or acceptor splicing sites to ensure proper processing of the transcript for protein expression. (See Chandler et al., 1997, herein incorporated by reference.)

5. Termination Signals

The vectors or constructs of the present invention will generally comprise at least one termination signal. A "termination signal" or "terminator" is comprised of the DNA sequences involved in specific termination of an RNA transcript by an RNA polymerase. Thus, in certain embodiments a termination signal that ends the production of an RNA transcript is contemplated. A terminator may be necessary in vivo to achieve desirable message levels.

In eukaryotic systems, the terminator region may also comprise specific DNA sequences that prevent site-specific cleavage of the new transcript to expose a polyadenylation site. This signals a specialized endogenous polyadenylase to add a stretch of about 200 A residues (polyA) to the 3' end of the transcript. RNA molecules modified with this polyA tail appear to be more stable and are translated more efficiently. Thus, in other embodiments involving eukaryotes, it is preferable that a terminator comprises a signal for the cleavage of the RNA, and it is more preferred that the terminator signal promotes polyadenylation of the message. The terminator and/or polyadenylation site elements can serve to enhance message levels and/or to minimize read through from the cassette into other sequences.

Terminators contemplated for use in the invention include any known terminator of transcription described herein or known to one of ordinary skill in the art, including but not limited to, for example, the termination sequences of genes, such as for example the bovine growth hormone terminator or viral termination sequences, such as for example the SV40 terminator. In certain embodiments, the termination signal may be a lack of transcribable or translatable sequence, such as due to a sequence truncation.

6. Polyadenylation Signals

For expression, particularly eukaryotic expression, one will typically include a polyadenylation signal to effect proper polyadenylation of the transcript. The nature of the polyadenylation signal is not believed to be crucial to the successful practice of the invention, and/or any such sequence may be employed. Preferred embodiments include the SV40 polyadenylation signal and/or the bovine growth hormone polyadenylation signal, convenient and/or known to function well in various target cells. Polyadenylation may increase the stability of the transcript or may facilitate cytoplasmic transport.

7. Origins of Replication

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

8. Selectable and Screenable Markers

In certain embodiments of the invention, the cells containing a nucleic acid construct of the present invention may be identified in vitro or in vivo by including a marker in the expression vector. Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the expression vector. Generally, a selectable marker is one that confers a property that allows for selection. A positive selectable marker is one in which the presence of the marker allows for its selection, while a negative selectable marker is one in which its presence prevents its selection. An example of a positive selectable marker is a drug resistance marker.

Usually the inclusion of a drug selection marker aids in the cloning and identification of transformants, for example, genes that confer resistance to neomycin, puromycin, hygromycin, DHFR, GPT, zeocin and histidinol are useful selectable markers. In addition to markers conferring a phenotype that allows for the discrimination of transformants based on the implementation of conditions, other types of markers including screenable markers such as GFP, whose basis is colorimetric analysis, are also contemplated. Alternatively, screenable enzymes such as herpes simplex virus thymidine kinase (tk) or chloramphenicol acetyltransferase (CAT) may be utilized. One of skill in the art would also know how to employ immunologic markers, possibly in conjunction with FACS analysis. The marker used is not believed to be important, so long as it is capable of being expressed simultaneously with the nucleic acid encoding a gene product. Further examples of selectable and screenable markers are well known to one of skill in the art.

B. Host Cells

As used herein, the terms "cell," "cell line," and "cell culture" may be used interchangeably. All of these terms also include their progeny, which refers to any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a prokaryotic or eukaryotic cell, and it includes any transformable organisms that is capable of replicating a vector and/or expressing a heterologous gene encoded by a vector. A host cell can, and has been, used as a recipient for vectors. A host cell may be "transfected" or "transformed," which refers to a process by which exogenous
nucleic acid is transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny.

Host cells may be derived from prokaryotes or eukaryotes, depending upon whether the desired result is replication of the vector, expression of part or all of the vector-encoded nucleic acid sequences, or production of infectious viral particles. Numerous cell lines and cultures are available for use as a host cell, and they can be obtained through the American Type Culture Collection (ATCC), which is an organization that serves as an archive for living cultures and genetic materials. An appropriate host can be determined by one of skill in the art based on the vector backbone and the desired result. A plasmid or cosmid, for example, can be introduced into a prokaryote host cell for replication of many vectors. Bacterial cells used as host cells for vector replication and/or expression include DH5α, JM109, and KC8, as well as a number of commercially available bacterial hosts such as SURE® Competent Cells and SOLARCK® Gold Cells (Stratagene®, La Jolla). Alternatively, bacterial cells such as E. coli LE392 could be used as host cells for phase viruses.

Examples of eukaryotic host cells for replication and/or expression of a vector include HeLa, NIH3T3, Jurkat, 293, Cos, CHO, Saos, and PC12. Many host cells from various cell types and organisms are available and would be known to one of skill in the art. Similarly, a viral vector may be used in conjunction with either an eukaryotic or prokaryotic host cell, particularly one that is permissive for replication or expression of the vector.

C. Expression Systems

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

The insect cell/baculovirus system can produce a high level of protein expression of a heterologous nucleic acid segment, such as described in U.S. Pat. Nos. 5,871,986 and 4,879,236, both herein incorporated by reference, and which can be bought, for example, under the name MaxiBAC® 2.0 from INVITROGEN® and BacPACK™ Baculovirus Expression System from CLONTECH®.

Other examples of expression systems include STRATAGENE®'s COMPLETE CONTROL™ Inducible Mammalian Expression System, which involves a synthetic ecdysone-inducible receptor, or its pET Expression System, an E. coli expression system. Another example of an inducible expression system is available from INVITROGEN®, which carries the T-REX™ (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. The Tet-On™ and Tet-Off™ systems from CLONTECH® can be used to regulate expression in a mammalian host using tetracycline or its derivatives. The implementation of these systems is described in Gossen et al., 1992 and Gossen et al., 1995, and U.S. Pat. No. 5,650,298, all of which are incorporated by reference.

INVITROGEN® also provides a yeast expression system called the Pichia methanolica Expression System, which is designed for high-level production of recombinant proteins in the methylotrophic yeast Pichia methanolica. One of skill in the art would know how to express a vector, such as an expression construct, to produce a nucleic acid sequence or its cognate polypeptide, protein, or peptide.

Introduction of Nucleic Acids into Cells

In certain embodiments, a nucleic acid may be introduced into a cell in vitro for production of polypeptides or in vivo for immunization purposes. There are a number of ways in which nucleic acid molecules such as expression vectors may be introduced into cells. In certain embodiments of the invention, the expression vector comprises a GBV-C infectious particle or engineered vector derived from a GBV-C genome. In other embodiments, an expression vector known to one of skill in the art may be used to express a segment of a GBV-C nucleic acid, which may be translated into a GBV-C polypeptide or peptide. The ability of certain viruses to enter cells via receptor-mediated endocytosis, to integrate into host cell genome and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign genes into mammalian cells (Ridgeway, 1988; Nicolas and Rubenstein, 1988; Baichwal and Sugden, 1986; Temin, 1986).

"Viral expression vector" is meant to include those vectors containing sequences of that virus sufficient to (a) support packaging of the vector and (b) to express a polynucleotide that has been cloned therein. In this context, expression may require that the gene product be synthesized. A number of such viral vectors have already been thoroughly researched, including adenovirus, adeno-associated viruses, retroviruses, herpesviruses, and vaccinia viruses.

Delivery may be accomplished in vitro, as in laboratory procedures for transforming cells lines, or in vivo or ex vivo, as in the treatment of certain disease states. One mechanism for delivery is via viral infection where the expression vector is encapsulated in an infectious viral particle. Several non-viral methods for the transfer of expression vectors into cultured mammalian cells also are contemplated by the present invention. These include calcium phosphate precipitation (Graham and Van Der Eb, 1973; Chen and Okayama, 1987; Rippe et al., 1990) DEAE-dextran (Gopal, 1985), electroporation (Tur-Kaspa et al., 1986; Potter et al., 1984), direct microinjection (Harland and Weintraub, 1985), DNA-loaded liposomes (Nicolaou and Sene, 1982; Fraley et al., 1979) and lipofectamine-DNA complexes, cell sonication (Fechheimer et al., 1987), gene bombardment using high velocity microprojectiles (Yang et al., 1990), liposome (Ghosh and Bachmann, 1991; Kaneda et al., 1989) and receptor-mediated transfection (Wu and Wu, 1987; Wu and Wu, 1988). Some of these techniques may be successfully adapted for in vivo or ex vivo use.

In certain embodiments, the nucleic acid encoding a gene or genes may be stably integrated into the genome of the cell. This integration may be in the cognate location and orientation via homologous recombination (gene replacement) or may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronisation with the host cell cycle. How the expression vector is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression vector employed.

Transfer of a nucleic acid molecule may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer in vitro, but it may be applied to in vivo use as well.

IV. GBV-C Related Immunotherapy

Embodiments of the invention include various compositions and methods for stimulating, supplementing or enhancing the immune system of a subject that has or may be exposed to HIV. Immunotherapy in general is a treatment to stimulate, enhance, or restore the ability of the immune sys-
system to fight infection and disease. Immunotherapy is thus any form of treatment that uses the immune system to fight infection and disease or to protect the body from some of the side effects of treatment. Examples include active immunization, passive immunization, and adoptive immunotherapies.

Immunoglobulins typically mediate humoral immunity by attaching to foreign antigens and activating effector modalities (e.g., complement, granulocytes, cytotoxic T-cells, etc.) to destroy and clear the antigens and also by passive inactivation, exclusion or immobilization of pathogens. Each of the five Ig isotypes possesses its own spectrum of effector systems with which it interacts via its Fc domain. The constant region isotype of the antibody is determined following T-cell mediated, Fc class-switching which endows a given antibody with the specific effector modalities of the new isotype. Administration and/or elicitation of antibodies to GBV-C derived peptides, in particular GBV-C E2, may be used as a therapeutic in various immunotherapies.

A. Passive Immunotherapy Related to Anti-GBV-C Antibodies or Binding Agents

Purified or partially purified anti-GBV-C antibodies or binding agents may be administered to a subject for prophylactic or therapeutic treatment of HIV. Passive immunization has been administered for several bacterial infections including pneumococcal pneumonia and H. influenzae pneumonia. In pneumococcal disease it was essential to identify the infecting serotype and obtain the appropriate type specific antisera. The problems that arose from using horse serum and the difficulty in precisely defining the serotype led to the abandonment of this procedure as soon as antibiotic therapy was introduced into clinical medicine.

In recent years, passive immunotherapy has been used for several viral diseases such as hepatitis A, hepatitis B, polio, etc., and the use of intravenous Ig-globulin has grown as its applications have expanded. There have been several clinical trials with human monoclonal antibodies in various infectious diseases that document not only efficacy but also safety. It is contemplated therefore, that antibodies to GBV-C derived epitopes that have similar structural attributes to an infective pathogen, such as HIV, may be effective in either preventing infections or in actual therapy.

Although the bulk of contemporary opinion in virology and immunology supports the prevailing paradigm that immunity to the human immunodeficiency virus is largely cellular in nature, a significant body of evidence in vaccine studies in animals suggest a pivotal role for the humoral immune system (Sawyer et al., 1990, Moore et al., 1991). In chronic viral infections, antibodies may be critical at certain stages. As such, antibodies may play a crucial role in the control of HIV-1 infections. In particular, through the use of the present invention HIV may be inhibited in its ability to infect the body, or at least the reduce the level of infection or replication.

B. Active Immunotherapy Related to GBV-C Antigens

Certain embodiments of the invention include the vaccination of a subject with an antigen derived from a GBV-C protein, in particular a GBV-C envelope protein, for the therapeutic or prophylactic treatment of HIV. In certain embodiments, the antigen can be all or part of the GBV-C E2 polypeptide or mimes thereof. Appropriate mimetics may be designed base on secondary or tertiary structure of a protein or peptide. This vaccination elicits the production of antibodies, i.e. GBV-C and HIV binding agents.

In particular aspects, anti-HIV properties may be elicited by expression or over-expression of a GBV-C antigen by an attenuated GBV-C viral vector. Anti-HIV properties will typically result in the modification of an HIV infection or the sensitivity to such an infection. Anti-HIV properties include, but are not limited to, delaying or slowing propagation of HIV; reducing viral load; reducing viral spread; reducing or limiting the severity of secondary pathologies, such as opportunistic infections and the like; preventing or reducing the probability of infection; neutralizing HIV particles; or competing with HIV binding sites on cells and in tissues and organs of a person exposed to HIV.

Active immunotherapy involves immunization of a subject to enhance existing or to elicit novel pathogen-specific immune responses, i.e., an HIV immune response, and, for example, provide systemic anti-pathogen immunity. Immunotherapeutic vaccination is the concept of inducing or enhancing immune responses of the subject to antigenic determinants that are uniquely expressed or expressed at increased levels on pathogens or cells infected by pathogens. Antigen determinants may be in the form of peptides, polypeptides, attenuated pathogens, and the like.

The immune response is the way the body defends itself against microorganisms, viruses, and other potentially harmful substances or organisms. Antigens are typically molecules (usually proteins) on the surface of cells, viruses, fungi, bacteria, and some non-living substances such as toxins, chemicals, drugs, and foreign particles. The immune system recognizes and destroys substances containing these antigens. The immune response may be an active immune response. Active immunity develops when the body is exposed to various antigens (antigenic epitopes), such as those described herein. It involves lymphocytes, of which there are 2 main groups, B-lymphocytes, and T lymphocytes. B lymphocytes (also called B cells) produce antibodies. Antibodies attach to a specific antigen and make it easier for the phagocytes to destroy the antigen. T lymphocytes (T cells) attack antigens directly, and some T lymphocytes provide control of the immune response. B cells and T cells develop that are specific for an antigen type. When a subject is exposed to a different antigen, different B cells and T cells are formed.

1. B Cells

B cells are a type of lymphocyte. The B cell produces antibodies that bind antigens. Each B cell is programmed to make a specific antibody. When a B cell encounters its antigen (along with collaborating T cells and accessory cells), it gives rise to many large plasma cells. Every plasma cell is a factory for producing antibody. Each of the plasma cells descended from a given B cell (which are all members of the same family, or clone) manufactures millions of identical antibody molecules and pours them into the bloodstream.

A given antibody has an affinity for a particular antigen. The antibody-antigen interaction marks the antigen or the cell displaying the antigen for destruction. After the human body has recovered from a disease, B-cells produce memory cells that attack the disease-causing organism if it invades again. This second response is much quicker than the first, thus preventing symptoms of the disease from occurring. The second phase involves the formation of the memory B-cell pool and seeding of long-lived plasma cells to the bone marrow. Plasma cells are terminally differentiated and do not give rise to memory cells.

Development of memory T cells (CD4 and CD8) may occur after activation, cells differentiate into effector T cells. Memory T cells may be generated from effector T cells. There may be two subsets of memory cells: quiescent, central memory cells that recirculate from blood to secondary lymphoid organs, and effector memory cells that migrate through tissues and deliver a very rapid response on reactivation with antigen.
2. Cytotoxic T Lymphocytes

In certain embodiments, T-lymphocytes are activated by contact with an antigen-presenting cell that is in contact with an antigen of the invention.

T cells express a unique antigen binding receptor on their membrane (T-cell receptor), which can only recognize antigen in association with major histocompatibility complex (MHC) molecules on the surface of other cells. There are several populations of T cells, such as T helper cells and T cytotoxic cells. T helper cells and T cytotoxic cells are primarily distinguished by their display of the membrane bound glycoproteins CD4 and CD8, respectively. T helper cells secrete various lymphokines that are crucial for the activation of B cells, T cytotoxic cells, macrophages, and other cells of the immune system. In contrast, a T cytotoxic cell that recognizes an antigen-MHC complex proliferates and differentiates into an effector cell called a cytotoxic T lymphocyte (CTL). CTLs eliminate cells of the body displaying antigen, such as virus-infected cells and tumor cells, by producing substances that result in cell lysis.

CTL activity may be assessed in freshly isolated peripheral blood mononuclear cells (PBMC), in a phytohaemagglutinin-stimulated IL-2 expanded cell line derived from PBMC (Bernard et al., 1998) or by T cells isolated from a previously immunized subject and restimulated for 6 days with dendritic cells infected with an adenovirus vector containing antigen using standard 4 hr 51Cr release microtiter assays. One type of assay uses cloned T-cells. Cloned T-cells have been tested for their ability to mediate both perforin and Fas ligand-dependent killing in redirected cytotoxicity assays (Simpson et al., 1998). The cloned cytotoxic T lymphocytes displayed both Fas- and perforin-dependent killing. An in vitro dehydrogenase release assay has been developed that takes advantage of a fluorescent amplification system (Page et al., 1998).

This approach is sensitive, rapid, and reproducible and may be used advantageously for mixed lymphocyte reaction (MLR). It may easily be further automated for large scale cytotoxicity testing using cell membrane integrity, and is thus considered in the present invention. In another fluorometric assay developed for detecting cell-mediated cytotoxicity, the fluorophore used is the non-toxic molecule alamarBlue (Nociari et al., 1998). The alamarBlue is fluorescently quenched (i.e., low quantum yield) until mitochondrial reduction occurs, which then results in a dramatic increase in the alamarBlue fluorescence intensity (i.e., increase in the quantum yield). This assay is reported to be extremely sensitive, specific and requires a significantly lower number of effector cells than the standard 51Cr release assay.

In certain aspects, T helper cell responses can be measured by in vitro or in vivo assay with peptides, polyepitopes, or proteins. In vitro assays include measurement of a specific cytokine release by enzyme, radioisotope, chromophore, or fluorescent assays. In vivo assays include delayed type hypersensitivity responses called skin tests, as would be known to one of ordinary skill in the art.

3. Antigen Presenting Cells

In general, the term "antigen presenting cell" can be any cell that accomplishes the goal of the invention by aiding the enhancement of an immune response (e.g., from the T-cell or B-cell arms of the immune system) against an antigenic composition of the present invention or a heterologous antigen or an immunologically functional equivalent. Such cells can be defined by those of skill in the art, using methods disclosed herein and in the art. As is understood by one of ordinary skill in the art (see for example Kuby, 1993, incorporated herein by reference), and used in certain embodiments, a cell that displays or presents an antigen normally or preferentially with a class II major histocompatibility molecule or complex to an immune cell is an "antigen presenting cell." In certain aspects, a cell (e.g., an APC cell) may be fused with another cell, such as a recombinant cell or a tumor cell that expresses the desired antigen. Methods for preparing a fusion of two or more cells is well known in the art, such as for example, the methods disclosed in Goding, pp. 65-66, 71-74, 1986; Campbell, pp. 75-83, 1984; Kohler and Milstein (1975); Kohler and Milstein (1976), Gefer et al. (1977), such incorporated herein by reference. In some cases, the immune cell to which an antigen-presenting cell displays or presents an antigen to is a CD4+TH cell. Additional molecules expressed on the APC or other immune cells may aid or improve the enhancement of an immune response. Secreted or soluble molecules, such as for example, cytokines and adjuvants, may also aid or enhance the immune response against an antigen. Such molecules are well known to one of skill in the art, and various examples are described herein.

The dendritic cell (DC) is the cell type best suited for vaccine antigen delivery, as they are the most potent antigen presenting cells, effective in the stimulation of both primary and secondary immune responses (Steinman, 1999; Celluzzi and Falco, 1997). It is contemplated in the present invention that the exposure of dendritic cells with a GBV-C vaccine of the invention, will elicit a potent immune response specific for the vaccine or vaccine vector of the present invention. A more detailed description of vaccines is provided below.

C. Adoptive Immunotherapy Related to GBV-C Antigens

In various embodiments of the invention, it is contemplated that the antigens or anti-idiotypic antibodies may be used to stimulate autologous or heterologous immunocompetent cells for the treatment of HIV. Adoptive immunotherapy is a technique that involves either removing immunocompetent cells from the body, artificially increasing the number, and returning them to the body; or artificially altering target cells to make them more immunogenic.

Typical adoptive immunotherapy involves the administration of immunologically active cells to an individual for providing a beneficial immunological effect such as reduction or control of viral infections. The immunologically active cells are typically taken by venipuncture or leukophereses either from the individual to be treated, termed autologous treatment, or from another individual, termed an allogeneic or heterologous treatment. The lymphocytes are then cultured to increase their number and to activate their therapeutic activity, and then infused back into the patient. Thus, the majority of conventional efforts in adoptive immunotherapy are typically directed at expanding cell numbers in vitro followed by infusion back into the patient.

Immunocompetent cells that may be used in adoptive immunotherapy are T lymphocytes. A method for the activation of T lymphocytes to generate T-activated killer cells (T-ALK) has been described as taking lymphocytes by leukopheresis from peripheral blood, and stimulating said cells with a monoclonal antibody (MAb) to a T cell surface receptor such as anti-CD3 (soluble or solid phase bound). The T cells can be stimulated with or without the addition of one or more cytokines such as IL-2. Alternatively, T cells can be purified before stimulation with the MAb to a surface receptor. Experimentation with T-ALK cells has demonstrated that CD8+ cells are responsible for the non-MHC restricted cytolytic activity seen in these cultures (Anderson et al., 1989; Loeffler et al., 1991). The ability of IL-2 to expand T lymphocytes having immune reactivity and the ability to lyse fresh autologous, syngeneic, or allogeneic natural killer (NK) cell-resistant tumor cells, but not normal cells, has resulted in the development of cell transfer therapies, such as autologous
adoptive immunotherapy. Immunocompetent cells may include T lymphocytes, dendritic cells, and the like.

V. Anti-GBV-C Antibodies or Binding Agents

Embodiments of the invention may include polypeptides in the form of antibodies, single chain antibodies and the like that bind various GBV-C polypeptides, peptides, or derivatives thereof. Means for preparing and characterizing antibodies are well known in the art (see, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, 1988; and Humphreys and Glover, 2001, each of which is incorporated herein by reference).

A. Anti-GBV-C Antibody Generation

The present invention provides therapeutic uses for anti-GBV-C antibodies. In some embodiments, monoclonal antibodies as well as polyclonal antibodies against GBV-C antigens may be used effectively in preventive and therapeutic treatment of HIV. Thus, the present invention is directed to anti-GBV-C antibody/antibodies that bind a GBV-C protein, polypeptide, or peptide, and attenuate HIV virus infectivity or replication. In particular, antibodies that bind a GBV-C envelope protein, polypeptide, or peptide are contemplated. In particular embodiments, antibodies that bind a GBV-C E2 protein, polypeptide, or peptide, as described herein, are contemplated. The invention also contemplates the use of a biologically functional equivalent of an anti-GBV-C antibody or a GBV-C antigen. The term “GBV-C protein/polypeptide or polypeptide” or “GBV-C antigen” is used herein to refer to a GBV-C protein, polypeptide or peptide, irrespective of whether it occurs naturally, is purified, is partially purified, or is produced by recombinant DNA methods, fusion-protein methods, protein synthesis methods or is a biological functional equivalent thereof.

A biologically functional equivalent is molecule where modifications and/or changes may be made in the structure of the polynucleotides encoding and/or the protein molecule, while obtaining molecules having similar or improved characteristics. In context of this invention, the molecule may be either a GBV-C antigen or an anti-GBV-C antibody. The biological functional equivalent may comprise a polynucleotide that has been engineered to contain distinct sequences while at the same time retaining the capacity to encode a “wild-type” or a functional polypeptide or peptide. This can be accomplished through the degeneracy of the genetic code, i.e., the presence of multiple codons, which encode for the same amino acids. Methods for preparing such equivalents are well known in the art.

The term “antibody” is used to refer to any antibody-like molecule that has an antigen binding region, and includes antibody fragments such as Fab, F(ab')2, single domain antibodies (DABs), Fv, scFv (single chain Fv or single chain antibodies), chimeras and the like. Methods and techniques of producing the above antibody-based constructs and fragments are well known in the art (U.S. Pat. Nos. 5,889,157; 5,821,333; 5,888,773, each specifically incorporated herein by reference).

1. Polyclonal Antibodies

A polyclonal antibody typically is prepared by immunizing an animal with an immunogenic composition (comprising a GBV-C antigen, for example) and collecting antisera from that immunized animal. A wide range of animal species can be used for the production of antisera. Typically, the animal used for production of anti-antisera is a rabbit, a mouse, a rat, a hamster, a guinea pig, or a goat. Because of the relatively large blood volume of rabbits, a rabbit is a preferred choice for production of polyclonal antibodies.

As well known in the art, a given composition may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling a peptide or polypeptide immunogen to a carrier. Exemplary carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other proteins such as ovalbumin, mouse serum albumin, rabbit serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are also well known in the art. Exemplary methods of conjugation include glutaraldehyde, m-maleimidobenzoylethynyl-N-hydroxysuccinimide ester, carbodiimide, and dis-biazo-4-tized benzidine. Other bifunctional or derivatizing agent may also be used for linking, for example maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, sucineic anhydride, SOCl₂, or R'N—C═NR, where R and R' are different alkyl groups.

As is also well known in the art, the immunogenicity of a particular immunogen composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Exemplary and preferred adjuvants include complete Freund’s adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis(s), incomplete Freund’s adjuvants and aluminum hydroxide adjuvant.

The amount of immunogen composition used in the production of polyclonal antibodies varies upon the nature of the immunogen as well as the animal used for immunization. A variety of routes can be used to administer the immunogen (subcutaneous, intramuscular, intradermal, intravenous, and intraperitoneal). The production of polyclonal antibodies may be monitored by sampling blood of the immunized animal at various points following immunization.

A second, booster injection, also may be given. The process of boosting and titering is repeated until a suitable titer is achieved. When a desired level of immunogenicity is obtained, the immunized animal can be bled and the serum isolated and stored, and/or the animal can be used to generate mAbs.

2. Monoclonal Antibodies

A “monoclonal antibody” refers to homogenous populations of immunoglobulins that are capable of specifically binding to a GBV-C protein. It is understood that the GBV-C protein or peptide, as described herein, may have one or more antigenic determinants. The antibodies of the invention may be directed against one or more of these determinants.

Monoclonal antibodies (mAbs) may be readily prepared through use of well-known techniques, such as those exemplified in U.S. Pat. No. 4,196,265, incorporated herein by reference. Typically, this technique involves immunizing a suitable animal with a selected immunogen composition, e.g., a purified or partially purified GBV-C antigen protein, polypeptide, or peptide. The immunizing composition is administered in a manner effective to stimulate antibody-producing cells.

The methods for generating in mAbs generally begin along the same lines as those for preparing polyclonal antibodies. Rodents such as mice and rats are preferred animals, however, the use of rabbit, sheep, goat, monkey cells is also possible. The use of rats may provide certain advantages (Goding, 1986, pp. 60-61), but mice are preferred, with the BALB/c mouse being most preferred. The BALB/c mouse is most routinely used and generally gives a higher percentage of stable fusions.

The animals are injected with antigen, generally as described above. Following immunization, somatic cells with the potential for producing antibodies, specifically B-lymphocytes (B-cells), are selected for use in the mAb generating
protocol. These cells may be obtained from biopsied spleens or lymph nodes. Spleen cells and lymph node cells are preferred, the former because they are a rich source of antibody producing cells that are in the dividing plasmablast stage.

Often, a panel of animals will have been immunized and the spleen of animals with the highest antibody titer will be removed. The spleen lymphocytes are obtained by homogenizing the spleen with a syringe.

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

Any one of a number of myeloma cells may be used, as are known to those of skill in the art (Goding, pp. 65-66, 1986; Campbell, pp. 75-83, 1984; each incorporated herein by reference). For example, where the immunized animal is a mouse, one may use P3-X63Ag8.653, NS/1.Ag 4.1, Sp2-10A-B1, F0, NSO/B, MPC-11, MPC11-X45-GTG 17 and S194/SXO Bu1; for rats, one may use R120.RC3Y3, Y3-Ag 1.23, IRX53F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC720-6 are all useful in connection with human cell fusions.

One preferred murine myeloma cell line is the NS-1 myeloma cell line (also termed P3-NS-1-Ag4-1), which is readily available from the NIGMS Human Genetic Mutant-cell Repository by requesting cell line repository number GM3573. Another mouse myeloma cell line that may be used is the 8-aquaguanine-resistant mouse murine myeloma SP2/0 nonproducer cell line.

Methods for generating hybrids of antibody-producing spleen or lymph node cells and myeloma cells usually comprise mixing somatic cells with myeloma cells in a 2:1 proportion, though the proportion may vary from about 20:1 to about 1:1, respectively, in the presence of an agent or agents (chemical or electrical) that promote the fusion of cell membranes. Fusion methods using Sendai virus have been described by Kohler and Milstein (1975; 1976), and those using polyethylene glycol (PEG), such as 37% (v/v) PEG, by Gefter et al. (1977). The use of electrically induced fusion methods also is appropriate (Goding pp. 71-74, 1986).

Fusion procedures usually produce viable hybrids at low frequencies, about 1 x 10^-6 to 1 x 10^-8. However, this does not pose a problem, as the viable, fused hybrids are differentiated from the parental, unfused cells (particularly the fused myeloma cells that would normally continue to divide indefinitely) by culturing in a selective medium. The selective medium is generally one that contains an agent that blocks the de novo synthesis of nucleotides in the tissue culture media. Exemplary and preferred agents are aminopterin, methotrexate, and asazaserine. Aminopterin and methotrexate block de novo synthesis of both purines and pyrimidines, whereas asazaserine blocks only purine synthesis. Where aminopterin or methotrexate is used, the media is supplemented with hypoxanthine and thymidine as a source of nucleotides (hypoxanthine-aminopterin-thymidine (HAT) medium). Where asazaserine is used, the media is supplemented with hypoxanthine. One preferred selection medium is HAT. Only cells capable of operating nucleotide salvage pathways are able to survive in HAT medium.

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

The selected hybridomas would then be serially diluted and cloned into individual antibody-producing cell lines, which clones can then be propagated indefinitely to provide mAbs. The cell lines may be exploited for mAb production in two basic ways.

A sample of the hybridoma can be injected (often into the peritoneal cavity) into a histocompatible animal of the type that was used to provide the somatic and myeloma cells for the original fusion (e.g., a syngeneic mouse). Optionally, the animals are primed with a hydrocarbon, especially oils such as pristane (tetramethylpentadecane) prior to injection. The injected animal develops tumors secreting the specific mAb produced by the fused cell hybrid. The body fluids of the animal, such as serum or ascites fluid, can then be tapped to provide mAbs in high concentration.

The individual cell lines could also be cultured in vitro, where the mAbs are naturally secreted into the culture medium from which they can be readily obtained in high concentrations.

mAbs produced by either means may be further purified, if desired, using filtration, centrifugation, and various chromatographic methods such as FPLC or affinity chromatography. Fragments of the mAbs of the invention can be obtained from the purified mAbs by methods that include digestion with enzymes, such as pepsin or papain, and/or cleavage of disulfide bonds by chemical reduction. Alternatively, mAb fragments encompassed by the present invention can be synthesized using an automated peptide synthesizer.

It also is contemplated that a molecular cloning approach may be used to generate monoclonals. For this, combinatorial immunoglobulin phagemid libraries are prepared from RNA isolated from the spleen of the immunized animal, or from human cells derived from non-immunized individuals, and phagemids expressing appropriate antibodies are selected by panning using cells expressing the antigen and control cells. The advantages of this approach over conventional hybridoma techniques are that approximately 10^6 times as many antibodies can be produced and screened in a single round, and that new specificities are generated by H and L chain combination which further increases the chance of finding appropriate antibodies. A second advantage of monoclonal antibody production by screening recombinant libraries is the lack of need for immunization and a third is the ability to produce totally human monoclonal antibodies. Two commercially available anti-GVH-C E2 monoclonal antibodies have been tested for HIV-inhibitory effects on an R5 HIV strain. The M6 (Roche) was the best, but all three inhibited HIV. This was done in duplicate, and is similar to results seen in another previous experiment. The Roche monoclonal antibody has been studied for binding to E2 protein by pepscan. The antibody recognizes a linear epitope found on two overlapping peptides representing the GBV-C E2 protein. Thus, the epitope may be contained in a 9 amino acid sequence FYE-PLVRRC (SEQ ID NO:8). This amino acid may be an HIV disease-modifying immunogen (Vaccine). The peptide may be used to inhibit HIV (as a prototype drug), and induce anti-HIV antibodies (as a vaccine).

Antibodies from Roche are described in Tacke et al. (1997) and Schmolke et al. (1998). For a description of epitope mapping studies see Schmolke et al. (1998). A "BD" and "WS" antibodies are commercially available monoclonal
antibodies against GBV-C E2 sold by Biodesign and Virostat, respectively. Other Roche antibodies may also demonstrate these same effects, M13, and M30.

3. Humanized Anti-GBV-C Antibodies

In certain embodiments of the invention, anti-GBV-C antibodies may be humanized for therapeutic purposes. Humanized mAbs are antibodies of animal origin that have been modified using genetic engineering techniques to replace constant regions and/or variable region framework sequences with human sequences, while retaining the original antigen specificity. Such antibodies can also include a humanized heavy chain associated with a donor or acceptor unmodified light chain or a chimeric light chain, or vice versa. Such antibodies are commonly derived from rodent antibodies, for example, the murine Ab of the present invention. Rodent-derived antibodies may demonstrate a specificity against human antigens and are generally useful for in vivo therapeutic applications. This strategy reduces the host response to the foreign antibody and allows selection of the human effector functions.

The techniques for producing humanized immunoglobulins are well known to those of skill in the art. For example, U.S. Pat. No. 5,693,762 discloses methods for producing, and compositions of, humanized immunoglobulins having one or more complementarity determining regions (CDR’s). “CDRs” are defined as the complementarity determining region amino acid sequences of an antibody. CDRs are contained within the hypervariable regions of immunoglobulin heavy and light chains. CDRs provide the majority of contact residues for the binding of the antibody to the antigen or epitope. CDRs of interest in this invention are derived from donor antibody variable heavy and light chain sequences, and include functional fragments and analogs of the naturally occurring CDRs, which fragments and analogs also share or retain the same antigen binding specificity and/or neutralizing ability as the donor antibody from which they were derived. When combined into an intact antibody, the humanized immunoglobulins are substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen, such as a protein or other compound containing an epitope.

Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. A humanized antibody is one in which only the antigen-recognized sites, or complementarity-determining hypervariable regions (CDRs) are of non-human origin, whereas all framework regions (FR) of variable domains are products of human genes.

Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., 1986; Reichmann et al., 1988; Verhoeven et al., 1988), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such “humanized” antibodies are chimeric antibodies, wherein less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some framework region (FR) residues are substituted by residues from analogous sites in rodent antibodies.

Other U.S. patents, each incorporated herein by reference, that teach the production of antibodies useful in the present invention include U.S. Pat. No. 5,565,332, which describes the production of chimeric antibodies using a combinatorial approach; U.S. Pat. No. 4,816,567 which describes recombinant immunoglobin preparations and U.S. Pat. No. 4,867,973 which describes antibody-therapeutic agent conjugates.

U.S. Pat. No. 5,565,332, which incorporated herein by reference, describes methods for the production of antibodies, or antibody fragments, which have the same binding specificity as a parent antibody, but have increased human characteristics. Humanized antibodies may be obtained by chain shuffling, perhaps using phage display technology. Human antibodies may also be produced by transforming B-cells with EBV and subsequent cloning of secretors as described by Hoon et al., (1993).

4. Human Anti-GBV-C Antibodies

Embodiments of the invention may use human monoclonal antibodies in compositions and methods described herein. Human mAbs can be made using a hybridoma method. Human myeloma and mouse-human heteromyeloma cell lines for the production of HuMAbs have been described, for example, by Kozbor (1984), and Brodeur et al. (1987).

It is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy chain joining region (J_{H}) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge (Jackovits et al., 1993).

Alternatively, phage display technology can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors (Mccafferty et al., 1990). According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle.

Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats (Johnson et al., 1993). Several sources of V-gene segments can be used for phage display. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al. (1991), or Griffith et al. (1993). In this method, the affinity of “primary” human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This techniques allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse et al. (1993), and the isolation
of a high affinity human antibody directly from such large phage library is reported by Griffith et al. (1993).

5. Anti-GBV-C Antibody Conjugates

Antibody conjugates comprising a GBV-C antibody linked to another agent, such as but not limited to a therapeutic agent, an anti-viral agent, a detectable label, a cytotoxic agent, a chemical, a toxin, an enzyme inhibitor, a pharmaceutical agent, etc. form further aspects of the invention. Antibody conjugates may be used both in vitro diagnostics and in a variety of immunocassettes.

Certain antibody conjugates include may be for use in vitro, where the antibody is linked to a secondary binding ligand or to an enzyme (an enzyme tag) that will generate a colored product upon contact with a chromogenic substrate. Examples of suitable enzymes include urease, alkaline phosphatase, (horseradish) hydrogen peroxidase and glucose oxidase. Preferred secondary binding ligands are biotin and avidin or streptavidin compounds. The use of such labels is well known to those of skill in the art and is described, for example, in U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, each incorporated herein by reference.

6. Single Chain Antibodies

The Fv portion of an antibody is a 26 kDa heterodimer consisting of the amino-terminal variable domains of the heavy and light chains, and is the smallest fragment to bear the antigen binding site. Genetically engineered single chain Fv (scFv) peptides have been synthesized by attaching the carboxyl terminus of one variable domain to the amino terminus of the other with a peptide linker. These Fv fragments have been shown to bind specific antigens, like the transferrin receptor, have been used to localize fusion proteins to targeted cells.

VI. Anti-HIV GBV-C Vaccines

The present invention includes methods for preventing the development or treating AIDS in both infected and uninfected persons, as well as the elicitation or enhancement of an immune response. As such, the invention contemplates vaccines for use in active, passive, and adoptive immunization embodiments. Immunogenic compositions, proposed to be suitable for use as active vaccines, may be prepared from an infectious, conditionally replicative, or replication defective GBV-C nucleic acid. Immunogenic compositions may also be prepared from a recombinant expression construct or synthesized in a manner disclosed herein or is known in the art. Preferably the antigenic material is extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle.

The present invention, in certain embodiments, involves the use of a GBV-C antigen, for example, and E2 antigen. The antigen may also be a fragment of a GBV-C virus protein, such as a peptide (discussed above). In a particular embodiment, the antigen is contemplated that contains the 9 amino acid sequence FYEPLVLR (SEQ ID NO:8). In preferred embodiments, the antigen is contemplated to comprise the 13 amino acid LGTFYEGYEPYLRV (SEQ ID NO:6). In more preferred embodiments, the antigen is contemplated to comprise the 17 amino acid sequence GGAGLGGFYEPYLRV (SEQ ID NO:7). This amino acid may be an HIV disease-modifying immunogen (vaccine) and/or induce anti-HIV antibodies (as a vaccine).

A. Carrier Molecules for Vaccination Against GBV-C Antigens

As is well known in the art, a given composition may vary in its immunogenicity. It is often necessary therefore to boost the host immune system, as may be achieved by coupling the heterologous polypeptide immunogen to a carrier. EXEMPLARY carriers are keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA). Other albumins such as ovalbumin, mouse serum albumin, or rabbit serum albumin can also be used as carriers. Means for conjugating a polypeptide to a carrier protein are well known in the art and include glutaraldehyde, m-maleimidobencyl-N-hydroxysuccinimide ester, carbodiimide, and bis-biotinized benzidine.

B. Adjuvants

As is also well known in the art, the immunogenicity of a polypeptide or peptide composition can be enhanced by the use of non-specific stimulators of the immune response, known as adjuvants. Suitable adjuvants include all acceptable immunostimulatory compounds, such as cytokines, toxins, or synthetic compositions.

Adjuvants that may be used include IL-1, IL-2, IL-4, IL-7, IL-12, γ-interferon, GMCSF, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CPG (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). Ribi, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion. MHC antigens may even be used. Exemplary, often preferred adjuvants include complete Freund’s adjuvant (a non-specific stimulator of the immune response containing killed Mycobacterium tuberculosis), incomplete Freund’s adjuvant and aluminum hydroxide adjuvant.

In addition to adjuvants, it may be desirable to coadminister biologic response modifiers (BRM), which have been shown to upregulate T cell immunity or downregulate suppressor cell activity. Such BRMs include, but are not limited to, Cimetidine (CIM; 1200 mg/d) (Smith/Kline, PA); or low-dose Cyclophosphamide (CYP, 300 mg/m²) (Johnson/Mead, NJ) and cytokines such as γ-interferon, IL-2, or IL-12 or genes encoding proteins involved in immune helper functions, such as B-7.

VII. Anti-HIV Therapies

In certain embodiments, therapeutic methods will include administering to a patient or subject a composition comprising an antigen or an antibody derived from a GBV-C polypeptide. In various embodiments, the treatment methods of the invention may be used in combination with other anti-HIV treatments, such as GBV-C infection as a therapeutic or preventative treatment for AIDS. For exemplary compositions and methods see PCT application WO 01/77157, which is incorporated herein by reference.

As a therapeutic measure, a binding agent that binds a GBV-C derived amino acid molecule can be used to reduce the severity of progression of AIDS, including the prevention of AIDS in HIV-infected individuals. A reduction in severity or progression of AIDS includes, but is not limited to, prevention or a reduction in the severity, duration, or discomfort associated with the following conditions: prolonged and unexplained fatigue; swollen glands; prolonged fever; chills; excessive sweating; swollen gums and mouth lesions; sore throat; cough; shortness of breath; constipation; diarrhea; symptoms of well-known opportunistic infections; Kaposi sarcomas; skin rashes or lesions; loss of appetite or weight loss; malaise; headaches; speech impairment; muscle atrophy; memory loss; reduced cognitive functioning; swelling of the joints; joint stiffness or pain; cold intolerance; pain or tenderness in bones; energy level; anxiety, stress, and tension; groin lump; priapism; genital sores; blurred or decreased vision; diplopia; light sensitivity; pain in chest, sides, back, muscle or stomach; and seizures.

As a preventative measure, a patient may be administered a pharmaceutically acceptable composition comprising a HIV
neutralizing or attenuating binding agent derived from a GBV-C polypeptide. The anti-HIV GBV-C binding agent may be used in conjunction with infection of CD4+ T cells with GBV-C or a recombinant version of GBV-C to inhibit infection of these cells by HIV. Alternatively, treatment with the GBV-C compositions of the present invention may effect a combination of preventative and therapeutic treatments insofar as infection of other cells in an HIV-infected subject’s body is prevented or attenuated.

Inhibition of AIDS progression may be demonstrated by reduction of detectable HIV in the infected subject; maintaining a CD4 count above 200 for a longer than average period of time; maintaining a normal T cell count; or maintaining normal p24 antigen. The term “therapeutic benefit” or “therapeutic effect” used throughout this application refers to anything that promotes or enhances the well-being of the subject with respect to the median of his/her condition, which includes treatment of HIV-infection (before the onset of AIDS), AIDS, as well as treatment of Hepatitis C. A list of nonexclusive examples of this includes extension of the subject’s life by any period of time; decrease or delay in the progression of AIDS (HIV, as described above) or Hepatitis C; decrease in viral load of HIV or HCV; decrease in HIV replication; clearance of HIV or HCV viremia reduced transmission of HCV or HIV; decrease in liver damage or complications; and a decrease in pain to the subject that can be attributed to the subject’s condition.

A. Combination Therapies

Of course it is understood that the method of the present invention, particularly administration of agents that bind a GBV-C amino acid molecule as treatment for an HIV-infected subject, may also be used in combination with the administration of traditional therapies. Alternatively, the compositions of the present invention may be given in combination with treatment or prevention of hepatitis C, such as α-interferon. Some such therapies are described below.

In many clinical situations, it is advisable to use a combination of distinct therapies. Thus, it is envisioned that, in addition to the therapies described herein, one would also wish to provide to the patient more “standard” pharmaceutical anti-retroviral therapies. Examples of standard therapies are provided below.

Combinations may be achieved by administering to a patient a single composition or pharmaceutical formulation that includes both agents, or by administering to a patient two distinct compositions or formulations, at the same time, wherein one composition may include a GBV-C binding agent, GBV-C antigen, or expression construct encoding a binding agent or antigen, and the other includes the standard anti-retroviral therapy. Alternatively, a GBV-C based therapeutic may precede or follow the other treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and GBV-C based therapeutic are administered separately to the patient, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and GBV-C based therapeutic would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one would administer to the patient both modalities within about 12-24 hours of each other and, more preferably, within about 6-12 hours of each other, with a delay time of only about 12 hours being most preferred. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several days (2, 3, 4, 5, or 6) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between the respective administrations.
ric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropyamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The preparation of binding agent that bind GBV-C sequences as active ingredients is generally well understood in the art by analogy, as exemplified by U.S. Pat. Nos. 6,479,243, 6,399,763, 5,714,153, 5,582,981, and 4,833,077, all incorporated herein by reference. The preparation of vaccines that contain GBV-C sequences as active ingredients is generally well understood in the art by analogy, as exemplified by U.S. Pat. Nos. 5,958,895, 6,004,799, and 5,626,896, all incorporated herein by reference.

VIII. Pharmaceutical Compositions and Routes of Administration

Pharmaceutical compositions, including the immunoglobulins for passive immunotherapy or antigens for active immunotherapy, are typically used for prophylaxis of susceptible individuals and for the treatment of infections. A discussion of passive and active immunity and immunizing agents may be found in Remington’s Pharmaceutical Sciences, 1990. The immunity provided by passive immunization is typically not long lasting and the immunoglobulins provided leave the body tissues and fluids of the host within a comparatively short period of time, usually after one to two weeks, either by utilization by binding to the pathogen or by metabolism by the host’s body. Thus, the administration of an antibody for passive immunity may be during the critical period immediately after or just prior to the predicted exposure to the pathogen or toxin such that the immunoglobulins are present when immunity is most urgently required.

The percentage of active compound in any pharmaceutical preparation is dependent upon both the activity of the compound, in this case binding of an antibody(ies) or other binding agent, and its concentration in the preparation. Typically, such compositions should contain at least 0.1% active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of the unit. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy injection is possible. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, phenylmercuric nitrate, m-cresol, and the like. In many cases, it will be preferable to use isotonic solutions, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate, and gelatin.

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

The present invention contemplates GBV-C antigens, anti-GBV-C antibodies, and/or infectious GBV-C nucleic acid molecules as well as infectious nucleic acid molecules encoding, in some embodiments, a heterologous sequence, collectively "therapeutic GBV-C compositions". In some embodiments, pharmaceutical compositions are administered to a subject. Different aspects of the present invention involve administering an effective amount of an aqueous composition. In another embodiment of the present invention, therapeutic GBV-C compositions are administered to a subject to either prevent the infection by HIV or prevent the progression of HIV infection to development of AIDS. Additionally, such compounds can be administered in combination with treatment by HAART or by administration of AZT and/or other anti-HIV drugs or drug regimens. Though typically, anti-GBV-C agent or GBV-C antigens will be administered separately from medication. Such compositions will generally be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Those of skill in the art are well aware of how to apply antibodies or other binding agents, as well as genetic delivery to in vivo and ex vivo situations.

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

In addition to the compounds formulated for parenteral administration, such as those for intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g., tablets or other solids for oral administration; time release capsules; and any other form currently used, including cremes, lotions, mouthwashes, inhalants and the like.

The active compounds of the present invention can be formulated for parenteral administration, e.g., formulated for injection via the intravenous, intramuscular, intrathoracic, sub-cutaneous, or even intraperitoneal routes. Administration by i.v. or i.m. are specifically contemplated.

The preparation of an aqueous composition that contains a compound or compounds that increase the expression of an MHC class I molecule will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions
or suspensions; solid forms suitable for use to prepare solutions or suspensions upon the addition of a liquid prior to injection can also be prepared, and the preparations can also be emulsified.

The antibodies, binding agents, or other active compositions may be formulated as neutral or salt forms. Pharmaceutically acceptable salts, include the acid salts and those which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The course of the treatment may be followed by assays for antibodies against antigens. The assays may be performed by labeling with conventional labels, such as radionuclides, enzymes, fluorochromes, and the like. Samples for assaying may be serum samples, or they may be obtained from any mucosal surface, or body fluid, such as saliva, sputum, vaginal wash, or expectoration. These assay techniques are well known and may be found in a wide variety of patents, such as U.S. Pat. Nos. 3,791,932; 4,174,384 and 3,949,064, as illustrative of these types of assays.

In certain embodiments, it may be desirable to provide a continuous supply of therapeutic compositions to the patient. For intravenous or intraarterial routes, this is accomplished by drip system. For various approaches, delayed release formulations could be used that provided limited but constant amounts of the therapeutic agent over and extended period of time. For internal application, continuous perfusion, for example with an anti GBV-C antibody, binding agent, antigen and/or a GBV-C viral vector which may or may not carry a heterologous nucleic acid segment may be preferred. This could be accomplished by catheterization followed by continuous administration of the therapeutic agent. The time period for perfusion would be selected by the clinician for the particular patient and situation, but times could range from about 1-2 hours, to 2-6 hours, to about 6-10 hours, to about 24-10 hours, to about 1-2 days, to about 1-2 weeks or longer. Generally, the dose of the therapeutic composition via continuous perfusion will be equivalent to that given by single or multiple injections, adjusted for the period of time over which the injections are administered. It is believed that higher doses may be achieved via perfusion, however.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluted first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraarterial administration. In this connection, sterile aqueous media that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion. (see for example, Remington's Pharmaceutical Sciences, 1990). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

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

Antibodies or other binding agents may be administered in a dose that can vary from 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 mg/kg of weight of 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200 mg/kg of weight in one or more daily, weekly, monthly, or yearly administrations during one or various days, weeks, months, or years. The antibodies can be administered by intravenous injection (intravenous, intraperitoneal, intramuscular, subcutaneous, intracervical, or intradermically). For viral vectors, one generally will prepare a viral vector stock. Depending on the kind of virus and the tier attainable, one will deliver 1 to 100, 10 to 50, 100-1000, or up to 1x10^3, 1x10^5, 1x10^6, 1x10^7, 1x10^8, 1x10^9, 1x10^10, 1x10^11, or 1x10^12 infectious particles to the patient. Similar figures may be extrapolated for liposomal or other non-viral formulations by comparing relative uptake efficiencies. Formulation as a pharmaceutically acceptable composition is discussed below.

In many instances, it will be desirable to have multiple administrations of the antibodies or other compositions of the invention. The compositions of the invention may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more times. The administrations will normally be at from one to twelve week intervals, more usually from one to four week intervals. Periodic re-administration will be desirable with recurrent exposure to the pathogen (e.g., HIV). For example, an HIV positive mother would be re-inoculated prior to parturition from a second pregnancy.

Doses commonly used for formulations that provide passive immunity are in the range of from 0.5 ml to 10 ml per dose, preferably in the range of 2 to 5 ml per dose. Repeated doses to deliver the appropriate amount of active compound are common. Both the size and size by weight of the recipient must be considered when determining the appropriate dosage of active ingredient and volume to administer.

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

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the form of injectable solutions described above, but drug release capsules and the like can also be employed.

As used herein, the term in vitro administration refers to manipulations performed on cells removed from an animal, including, but not limited to, cells in culture. The term ex vivo administration refers to cells that have been manipulated in vitro, and are subsequently administered to a living animal. The term in vivo administration includes all manipulations performed on cells within an animal.

In certain aspects of the present invention, the compositions may be administered either in vitro, ex vivo, or in vivo. In certain in vitro embodiments, transcribed RNA from a GBV-C clone is transfected into PBMC using DEAE-dextran. The transformed cells can then be used for in vitro analysis, or alternatively for in vivo administration.

U.S. Pat. Nos. 4,690,915 and 5,199,942, both incorporated herein by reference, disclose methods for ex vivo manipula-
tion of blood mononuclear cells and bone marrow cells for use in therapeutic applications.

EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

HIV Neutralization Assay

E2 antibody serum was studied for interactions with HIV in a virus neutralization assay. In one study (Fig. 1), a clinical R5 strain of HIV was incubated with a GBV-C RNA-negative E2 antibody negative serum (mock), with GBV-C E2 antibody positive-RNA negative serum (E2), GBV-C RNA positive-E2 negative serum (GBV-C), or a mixture of E2 and GBV-C sera for 1 hour at 37°C, prior to adding the mixture to PHA-IL-2 stimulated peripheral blood mononuclear cells (PBMCs). After infection, cells were washed, and media was collected daily for 3 days for testing for HIV p24 antigen (p24 Ag) in culture supernatant by ELISA. HIV p24 Ag was measured, and the percent inhibition determined by dividing the concentration present in the test sample by the HIV-mock infected control sample. The baseline HIV p24 Ag was determined using the “mock”—HIV mixture, and significant inhibition of HIV replication (as measured by p24 Ag production into culture supernatant fluids) was observed when E2-positive sera was mixed with HIV, although this inhibition declined each day. Similarly, when GBV-C RNA-positive sera (and GBV-C replication) was mixed with HIV, the HIV replication was inhibited to a greater extent than with E2 antisera alone, and the mixture of E2 and GBV-C RNA positive sera gave the greatest extent of HIV inhibition.

Reproducibility was determined by performing additional studies. Two additional GBV-C E2 antibody positive (RNA negative) sera (isolates 55 and 9 respectively) and two E2 antibody negative (and RNA negative) sera (negative control sera 1 and 2) were studied for their ability to decrease replication of the R5 HIV strain. A clinical isolate was used for this study. In addition, diluted sera at 1:10, 1:100, and 1:1,000 were used to determine if there was a dose-response relationship between the concentration of serum and the extent of HIV inhibition. After washing the PBMCs, sera was maintained in the culture media throughout the experiment, and infections were monitored on day 3 for HIV p24 Ag production in culture supernatant. Fig. 2 illustrates that HIV is inhibited by two E2 antibody positive sera in a dose-dependent fashion; whereas, E2 antibody negative sera do not inhibit HIV.

Example 2

HIV Neutralization Assay with Purified Antibody

To determine if the inhibitory substance in the GBV-C E2 antibody-positive sera was antibody, IgG from E2 antibody-positive sera and two E2 antibody-negative sera were purified by protein G column chromatography. HIV was mixed with a “no antibody” control (No Ab), or with 5 μg/ml of an E2 antibody negative control (NC-2) or E2 positive controls. The mixtures were applied to PBMCs, and after washing the HIV inocula, the cognate IgGs were maintained in the culture media. The raw p24 Ag results are shown in Fig. 3, and the percent HIV p24 Ag inhibition is shown in Fig. 4.

Example 3

HIV Neutralization Assay on HIV Strain X4

Studies were performed to determine if E2 antibody-positive serum inhibited X4 strains of HIV. Using the same experimental design as for FIGS. 3 and 4, IgG preparations from E2-negative and -positive IgG preparations were studied for their ability to inhibit a clinical X4 HIV strain in PHA-IL-2 PBMC cultures. Similar to the R5 strain, the X4 strain was inhibited by GBV-C E2-positive IgG, but not GBV-C E2-negative IgG (Fig. 5).

Since R5 viruses utilize CCR5 as their co-receptor, and X4 viruses utilize CXCR4 as their co-receptor, GBV-C E2 antibody inhibition indicates that they are cross-reacting with a conserved epitope on HIV that inhibits HIV replication, and that this epitope is on both co-receptor usage types of HIV. Since the epidemiological data indicates that E2 antibody is associated with prolonged survival in Germany, France, and the United States, this interaction has promise for HIV strains widely distributed worldwide.

Example 4

HIV Neutralization with Monoclonal Antibodies

Commercially available anti-GBV-C E2 monoclonal antibodies from Biodesign and Virostat, and one supplied by Roche were tested for HIV-inhibitory effects. Inhibition of an R5 HIV strain was detected when using all three antibodies, with M6 (Roche) being the best. Studies were performed in duplicate, and are similar to results seen in another previous study. The Roche monoclonal antibody binding to E2 protein has been studied using pepscan. M6 recognizes a linear epitope found on two overlapping peptides representing the GBV-C E2 protein. Thus, an epitope is contained in a 9 amino acid sequence of FYEPLVRRC (SEQ ID NO:8) or in an 17 amino acid sequence of GGAGLGIGFYEPLVRRC (SEQ ID NO:6). This amino acid sequence may be an HIV disease-modifying immunogen (vaccine).

Fig. 6 demonstrates that the Roche M6 monoclonal antibody inhibits HIV-1 (R5 strain). HIV was mixed with M6 antibody or an isotype control antibody (range of concentrations, as shown) for 1 hr at 37°C, then added to PBMC cultures. Four hrs later, cells were washed, and media was added to cells (media containing either M6 or IC). Culture supernatants were collected on day 3 post-infection and HIV p24 antigen determined. Percent inhibition was determined by dividing the p24 antigen concentration in the M6 culture supernatant by the Isotype control p24 antigen concentration. This value was subtracted from 1, and the result was multiplied x100.

Fig. 7 demonstrates that the biodesign and virostat antibodies also inhibited HIV-1, although not as efficiently as M6. Fig. 8 is a predicted map of the epitopes identified by the Roche mAbs as described in Schmollke et al. (1998). M5 inhibits all of the groups of antibodies. M6 was the only
antibody to react with a linear peptide in a PEPSCAN analysis. Antibodies against this M6 epitope were found to not appear to be elicited during GBV-C infection.

Example 5

HIV Neutralization with Rabbit Sera

To determine if the peptide antigen shown to react with the anti-GBV-C E2 (M6) monoclonal antibody was antigenic and if it exhibited anti-HIV activity, the inventors conjugated the 17 amino acid peptide to KLH (keyhole limpet) and immunized 2 New Zealand White rabbits (commercially done by InVitrogen). IgG was purified from serum collected post-immunization and at 8 weeks (following immunization and 2 boosts). Pre-immune and post-immune anti-GBV-C E2 peptide rabbit IgG was incubated with R5 and X4 HIV for 1 hour, and then added to primary PBMCs for 3 hours. Cells were then washed, and maintained in media containing either pre-immune or post-immune IgG. HIV production into culture supernatant was measured by p24 antigen, and the post-immune IgG reproducibly reduced HIV infectivity in both R5 and X4 viruses (Figs. 10, 11, and 12). All experiments were performed in triplicate, and the reduction in p24 antigen levels by post-immune IgG were all statistically significant at the P<0.05 level. These studies demonstrate that anti-GBV-C E2 peptide antibody inhibits HIV.

Example 6

HIV Particle Rip Precipitation

To determine if the anti-E2 antibodies cross-react with HIV, the inventors infected GHOST CD4+cells (Cecilia et al., 1998) with an R5 HIV isolate, and then grew the cells in methionine free media supplemented with 35S-methionine. Virus released into the culture supernatant was partially purified by size-exclusion chromatography (Fig. 13). The p24 antigen positive peak represents radiolabeled HIV particles, and SDS-PAGE demonstrated many 35S-labeled proteins, including proteins with relative molecular weights of 41 kD, 120 kD, and 160 kD consistent with HIV structural proteins (data not shown). The 35S-labeled material was incubated in normal mouse IgG overnight at 4°C, and material reacting with IgG non-specifically was removed by precipitation with staphylococcus A (PANSORBIN®). The supernatant was then incubated with either normal mouse IgG or murine anti-GBV-C E2 monoclonal antibody overnight (at various concentrations) at 4°C. Immune complexes were then precipitated using PANSORBIN®, and the pelleted IgG-HIV complexes were washed extensively. Following washing, radiolabeled material was released by adding SDS and boiling, and cpm released was counted. Fig. 14 demonstrates results for M6 antibody, showing a dose-dependent precipitation of radiolabeled HIV particles. Other anti-GBV-C E2 antibodies (including Biodesign, Virostat, M3, M5) immunoprecipitated HIV particles. For a positive control, a human anti-HIV monoclonal antibody and human HIV-negative antibodies were also tested, and confirmed that anti-HIV antibodies precipitated the radiolabeled HIV particles (data not shown).

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

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference:

U.S. Pat. No. 5,922,574
U.S. Pat. No. 4,277,437
U.S. Pat. No. 4,366,241
U.S. Pat. No. 4,544,101
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U.S. Pat. No. 4,683,195
U.S. Pat. No. 4,683,202
U.S. Pat. No. 4,690,915
U.S. Pat. No. 4,800,159
U.S. Pat. No. 4,816,567
U.S. Pat. No. 4,833,077
U.S. Pat. No. 4,867,973
U.S. Pat. No. 4,879,236
U.S. Pat. No. 4,883,750
U.S. Pat. No. 4,946,773
U.S. Pat. No. 5,199,942
U.S. Pat. No. 5,279,721
U.S. Pat. No. 5,354,855
U.S. Pat. No. 5,359,046
U.S. Pat. No. 5,565,332
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U.S. Pat. No. 5,693,762
U.S. Pat. No. 5,714,153
U.S. Pat. No. 5,840,873
U.S. Pat. No. 5,843,640
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U.S. Pat. No. 5,843,663
U.S. Pat. No. 5,846,708
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FEATURE: 221: NAME/KEY: MOD_RES
LOCATION: (30)...(283)
OTHER INFORMATION: xaa = anything

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Trp Glu Trp Val Met Arg Gln Val Arg Met Val Met Ser Arg Leu Arg 1875 1880 1885
Ala Leu Cys Pro Val Val Ser Leu Pro Leu Trp His Cys Gly Glu Gly 1890 1895 1900
Trp Ser Gly Glu Trp Leu Leu Asp Gly His Val Glu Ser Arg Cys Leu 1905 1910 1915 1920
Cys Gly Cys Val Ile Thr Gly Asp Val Leu Asn Gly Glu Leu Lys Asp 1925 1930 1935
Pro Val Tyr Ser Thr Lys Leu Cys Arg His Tyr Trp Met Gly Thr Val 1940 1945 1950
Pro Val Asn Met Leu Gly Tyr Gly Thr Ser Pro Leu Leu Ala Ser 1955 1960 1965
Asp Thr Pro Lys Val Val Pro Phe Gly Thr Ser Gly Trp Ala Glu Val 1970 1975 1980
Val Val Thr Pro Thr His Val Val Ile Arg Arg Thr Ser Cys Tyr Lys 1985 1990 1995 2000
Leu Leu Arg Gln Gln Ile Leu Ser Ala Ala Val Ala Glu Pro Tyr Tyr 2005 2010 2015 2020
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Ser Ser Glu Val Ser Ile Glu Ile Gly Thr Glu Thr Glu Asp Ser Glu 2065 2070 2075 2080
Leu Thr Glu Ala Asp Leu Pro Pro Ala Ala Ala Ala Leu Gln Ala Ile 2085 2090 2095
Glu Asn Ala Ala Arg Ile Leu Glu Pro His Ile Asp Val Xaa Met Glu 2100 2105 2110
Asp Cys Ser Thr Pro Ser Leu Cys Gly Ser Ser Arg Glu Met Pro Val 2115 2120 2125
Trp Gly Glu Asp Ile Pro Arg Thr Pro Ser Pro Ala Leu Ile Ser Val 2130 2135 2140
Thr Glu Ser Ser Ser Asp Lys Thr Leu Ser Val Thr Ser Ser Gln 2145 2150 2155 2160
Glu Asp Thr Pro Ser Ser Asp Ser Phe Glu Val Ile Gln Glu Ser Asp 2165 2170 2175
Thr Ala Glu Ser Glu Glu Ser Val Phe Asn Val Ala Leu Ser Val Leu 2180 2185 2190
Lys Ala Leu Phe Pro Gln Ser Asp Ala Thr Arg Lys Leu Thr Val Lys 2195 2200 2205
Met Ser Cys Cys Val Glu Lys Ser Val Thr Arg Phe Phe Ser Leu Gly 2210 2215 2220
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2370 2375 2380
Val Lys Asp Leu Ala Thr Pro Ala Gly Lys Met Ala Val His Asp Arg
2385 2390 2395 2400
Leu Gln Glu Ile Leu Glu Gly Thr Pro Val Pro Phe Thr Leu Thr Val
2405 2410 2415
Lys Lys Glu Val Phe Phe Lys Asp Arg Lys Glu Glu Lys Ala Pro Arg
2420 2425 2430
Leu Ile Val Phe Pro Pro Leu Asp Phe Arg Ile Ala Glu Lys Leu Ile
2435 2440 2445
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2450 2455 2460
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2465 2470 2475 2480
Trp Glu Ser Lys Thr Pro Cys Ala Ile Cys Val Asp Ala Thr Cys
2485 2490 2495
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Cys Leu Thr Cys Tyr Ile Lys Val Arg Ala Ala Cys Arg Ile Gly
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Cys Glu Arg Pro Val Cys Asp Pro Cys Glu Ala Leu Gly Arg Thr Leu
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2625 2630 2635 2640
Xaa Arg His Phe Phe Leu Thr Thr Asp Phe Arg Arg Pro Leu Ala Arg
2645 2650 2655
83

Met Ser Ser Glu Tyr Ser Asp Pro Met Ala Ser Ala Ile Gly Tyr Ile
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Leu Leu Tyr Pro Trp Xaa Pro Ile Thr Arg Trp Val Ile Ile Pro His
2675 2680 2685

Val Leu Thr Cys Ala Ser Arg Gly Gly Thr Xaa Ser Asp Pro
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Val Trp Cys Gln Val His Gly Asn Tyr Tyr Lys Phe Pro Leu Asp Lys
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Leu Pro Asn Ile Ile Val Ala Leu His Gly Pro Ala Ala Leu Arg Val
2725 2730 2735

Thr Ala Asp Thr Thr Lys Thr Lys Met Glu Ala Gly Ala Val Leu Ser
2740 2745 2750

Asp Leu Lys Leu Pro Gly Leu Ala Val His Arg Lys Ala Gly Ala
2755 2760 2765

Leu Arg Thr Arg Met Leu Arg Ser Arg Gly Trp Ala Glu Leu Ala Arg
2770 2775 2780

Gly Leu Leu Trp His Pro Gly Leu Arg Leu Pro Pro Glu Ile Ala
2785 2790 2795 2800

Gly Ile Pro Gly Gly Phe Pro Leu Ser Pro Pro Tyr Met Gly Val Val
2805 2810 2815

His Gln Leu Asp Phe Thr Xaa Gln Arg Ser Arg Trp Arg Trp Leu Gly
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18

tcc gag tgg gga att ccc tgc ggc act tgt atc ctg gac agg cgg cct
Ser Glu Trp Gly Ile Pro Cys Ala Thr Cys Ile Leu Arg Arg Pro
20 25 30

90

gcc tgt tgt ggc acc tgt tgt agg gac tgc tgg ccc gag acc ggg tgt
Ala Ser Cys Gly Thr Cys Val Arg Asp Cys Trp Pro Glu Thr Gly Ser
35 40 45

138

gta cgt ttc cca ttc cac agg tgt gcc ggc cgg cga ccc agg cct ccc gca
Val Arg Phe Pro Phe His Arg Cys Gly Ala Gly Pro Arg Leu Thr Arg
50 55 60

186

gac tgt gag gct ggc ccc ttc tgt gcc aac agg aca act ccc ccc acc ata
Asp Leu Glu Ala Val Pro Phe Val Asn Arg Thr Thr Pro Phe Thr Ile
65 70 75 80

234

agg ggg ccc ctg gcc aac cag ggg cga ggc aac ccc gtt cgg ctc ccc
Arg Gly Pro Leu Gly Asn Gly Arg Gly Asn Pro Val Arg Ser Pro
85 90 95

282

ttg cgt gtt ggg ccc tac acc aag aat cct cca gag tcc ttc ccc
Leu Gly Phe Phe Ser Tyr Thr Met Thr Lys Ile Arg Asp Ser Leu His
100 105 110

330

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Leu Val Lys Cys Pro Thr Pro Ala Ile Glu Pro Pro Thr Gly Thr Phe
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378
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<221> NAME/KEY: MOD_RES
<222> LOCATION: (253) .. (258)
<223> OTHER INFORMATION: xaa = anything

<400> SEQUENCE: 4

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Ser Glu Trp Gly Ile Pro Cys Ala Thr Cys Ile Leu Asp Arg Arg Pro 20 25 30
Ala Ser Cys Gly Thr Cys Val Arg Asp Cys Trp Pro Glu Thr Gly Ser 35 40 45
Val Arg Phe Pro Phe His Arg Cys Gly Ala Gly Pro Arg Leu Thr Arg 50 55 60
Asp Leu Glu Ala Val Pro Phe Val Asn Arg Thr Thr Pro Phe Thr Ile 65 70 75 90
Arg Gly Pro Leu Gly Asn Gly Gin Gly Arg Gly Asn Pro Val Arg Ser Pro 85 90 95

432
480
528
576
624
672
720
768
816
864
912
936
Leu Gly Phe Gly Ser Tyr Thr Met Thr Lys Ile Arg Asp Ser Leu His 100 105 110
Leu Val Lys Cys Pro Thr Pro Ala Ile Glu Pro Pro Thr Gly Thr Phe 115 120 125
Gly Phe Phe Pro Gly Val Pro Pro Leu Asn Asn Cys Met Leu Leu Gly 130 135 140
Thr Glu Val Ser Glu Val Leu Gly Gly Ala Gly Leu Thr Gly Gly Phe 145 150 155 160
Tyr Glu Pro Leu Val Arg Arg Cys Ser Glu Leu Met Gly Arg Arg Asn 165 170 175
Pro Val Cys Pro Gly Phe Ala Trp Leu Ser Ser Gly Arg Pro Asp Gly 180 185 190
Phe Ile His Val Glu Gly His Leu Glu Gly Val Asp Ala Gly Asp Phe 195 200 205
Ile Pro Pro Pro Arg Trp Leu Leu Leu Asp Phe Val Phe Val Leu Leu 210 215 220
Tyr Leu Met Lys Leu Ala Glu Ala Arg Leu Val Pro Leu Ile Leu Leu 225 230 235 240
Leu Leu Trp Trp Trp Val Asn Glu Leu Ala Val Leu Xaa Val Xaa Ala 245 250 255
Xaa Xaa Ala Val Ala Gly Val Phe Ala Gly Pro Ala Leu Ser 260 265 270
Trp Cys Leu Gly Leu Pro Phe Val Ser Met Ile Leu Gly Leu Ala Asn 275 280 285
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Val Leu Trp Lys Leu Ala Arg Gly 305 310

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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
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Cys

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<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic Peptide
What is claimed is:

1. A method for inhibiting HIV-1 replication comprising providing to a subject an anti-GBV-C E2 antibody, wherein the antibody attenuates HIV-1 infectivity.

2. The method of claim 1, wherein the antibody is a human antibody.

3. The method of claim 1, wherein the antibody is a monoclonal antibody.

4. The method of claim 3, wherein the antibody is a humanized antibody.

5. The method of claim 1, further comprising administration of at least a second anti-HIV therapy.

6. The method of claim 5, wherein the second anti-HIV therapy is HAART therapy.

7. The method of claim 5, wherein the second anti-HIV therapy is AZT therapy.

8. The method of claim 1, wherein the antibody is provided at least twice.

* * * * *

In the specification, delete the paragraph at column 1, lines 11-14 and replace with --This invention was made with Government support under R01 AA12671 awarded by the National Institutes of Health and a merit grant to Jack Stapleton awarded by the Veterans Administration. The Government has certain rights in the invention.-- therefor.

Signed and Sealed this
Twenty-seventh Day of January, 2015

[Signature]

Michelle K. Lee
Deputy Director of the United States Patent and Trademark Office