



US008858946B2

(12) **United States Patent**  
**Stapleton et al.**

(10) **Patent No.:** **US 8,858,946 B2**  
(45) **Date of Patent:** **Oct. 14, 2014**

(54) **METHOD FOR INHIBITING HIV-1 REPLICATION UTILIZING ANTI-GBV-C E2 ANTIBODIES**

6,451,578 B1 9/2002 Simons et al. .... 435/235.1  
6,558,898 B1 5/2003 Simons et al. .... 435/5  
6,586,568 B1 7/2003 Simons et al. .... 530/300  
6,720,166 B2 4/2004 Simons et al. .... 435/69.1

(75) Inventors: **Jack T. Stapleton**, Iowa City, IA (US);  
**Jinhua Xiang**, Iowa City, IA (US);  
**Donna Klinzman**, Coralville, IA (US);  
**James H. McLinden**, Coralville, IA (US)

FOREIGN PATENT DOCUMENTS

EP 0 747 482 12/1996  
EP 0 832 901 4/1998  
WO WO 94/18217 8/1994  
WO WO 97/38108 10/1997  
WO WO 01/77157 10/2001  
WO WO 2004/037202 5/2004

(73) Assignees: **The University of Iowa Research Foundation**, Iowa City, IA (US); **The United States of America, as represented by the Secretary of the Department of Veterans Affairs**, Washington, DC (US)

OTHER PUBLICATIONS

George et al. Interactions between GB virus type C and HIV. *Current Infectious Disease Reports*. 2002, 4: 550-558.\*  
Jung et al. HIV entry inhibition by the envelope 2 glycoprotein of GB virus C. *AIDS*. 2007. 21(5): 645-7.\*  
Tillman et al. Infection with GB Virus C and Reduced Mortality among HIV-Infected Patients. *N Engl J Med* 2001; 345:715-724.\*  
Cohen, J. Is an Effective HIV Vaccine Feasible. *Science*. vol. 309, 2005, p. 99.\*  
Srivastava et al. Neutralizing antibody responses to HIV: role in protective immunity and challenges for vaccine design. *Expert Rev. Vaccines*. 3(4) Suppl. 33-52 (2004).\*  
Walker and Burton.\*  
Winkler, K., et al., 2000, Changing the antigen binding specificity by single point mutations of an anti-p24 (HIV-1) antibody, *J. Immunol*. 165:4505-4514.\*  
Self, C. H., and S. Thompson, 2006, How specific are therapeutic monoclonal antibodies, *Lancet* 367(960):1038-1039.\*  
West, Jr., A. P., et al., Jan. 2012, Single-chain Fv-based anti-HIV proteins: potential and limitations, *J. Virol*. 86(1):195-202.\*  
Aarons, E. J., et al., 2001, Adaptation to blockade of human immunodeficiency virus type 1 entry imposed by the anti-CCR5 monoclonal antibody 2D7, *Virol*. 297:382-390.\*  
Bansal, G. P., 2007, A summary of the workshop on passive immunization using monoclonal antibodies for HIV/AIDS, held at the National Institute of Allergy and Infectious Diseases, Bethesda, Mar. 10, 2006, *Biol*. 35:367-371.\*  
Cadogan, M., and A. G. Dalgleish, 2008, HIV immunopathogenesis and strategies for intervention, *Lancet Infect. Dis*. 8:675-684.\*  
Office Communication, issued in U.S. Appl. No. 10/862,061, dated Nov. 20, 2006.  
Office Communication, issued in U.S. Appl. No. 10/862,061, dated Mar. 9, 2007.  
Office Communication, issued in U.S. Appl. No. 10/862,061, dated Oct. 1, 2007.  
Office Communication, issued in U.S. Appl. No. 10/862,061, dated Jun. 10, 2008.  
"Infection with GBV-C linked to longer life," *AIDS Alert*. 17:7-8, 2002.  
Anderson et al., "Anit-CD3 + IL-2-stimulated murine killer cells. In vitro generation and in vivo antitumor activity," *J Immunol*, 142:1383-1394, 1989.

(\* ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1228 days.

(21) Appl. No.: **12/179,399**

(22) Filed: **Jul. 24, 2008**

(65) **Prior Publication Data**

US 2009/0010932 A1 Jan. 8, 2009

**Related U.S. Application Data**

(62) Division of application No. 10/862,061, filed on Jun. 4, 2004, now abandoned.

(60) Provisional application No. 60/475,987, filed on Jun. 5, 2003.

(51) **Int. Cl.**

**A61K 39/42** (2006.01)  
**C07K 16/10** (2006.01)  
**A61K 39/00** (2006.01)

(52) **U.S. Cl.**

CPC ..... **C07K 16/109** (2013.01); **C07K 2317/34** (2013.01); **A61K 2039/505** (2013.01)  
USPC ..... **424/147.1**; 424/159.1

(58) **Field of Classification Search**

None  
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,665,569 A \* 9/1997 Ohno ..... 435/69.6  
5,693,762 A 12/1997 Queen et al. .... 530/387.3  
5,766,840 A 6/1998 Kim et al. .... 530/388.3  
5,821,333 A 10/1998 Carter et al. .... 530/350  
5,824,507 A 10/1998 Kim et al. .... 435/69.3  
5,849,532 A 12/1998 Kim et al. .... 435/69.3  
5,856,134 A 1/1999 Kim et al. .... 435/69.3  
5,874,563 A 2/1999 Kim et al. .... 536/23.72  
5,888,773 A 3/1999 Jost et al. .... 435/69.6  
5,889,157 A 3/1999 Pastan et al. .... 530/387.1  
5,981,172 A 11/1999 Simons et al. .... 435/5  
6,020,195 A 2/2000 Schmolke et al. .... 435/339  
6,051,374 A 4/2000 Simons et al. .... 435/5  
6,156,495 A 12/2000 Pilot-Matias et al. .... 435/5  
6,387,959 B1 \* 5/2002 Nottet ..... 514/713

(Continued)

*Primary Examiner* — Jeffrey S. Parkin

(74) *Attorney, Agent, or Firm* — Parker Highlander PLLC

(57)

**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**

(56)

## References Cited

## OTHER PUBLICATIONS

- Aronoff DM, "Using live pathogens to treat infectious diseases: a historical perspective on the relationship between GB virus C and HIV," *Antivir. Ther.* 7:73-80, 2002.
- Cecilia et al., "Neutralization profiles of primary human immunodeficiency virus type 1 isolates in the context of coreceptor usage," *J. Virol.*, 72(9):6988-6996, 1998.
- Chakraborty et al. "Viral coinfections among African children infected with human immunodeficiency virus type 1," *Clin. Infect. Dis.* 36:922-924.
- Dawson et al., "Prevalence studies of GB virus-C infection using reverse transcriptase-polymerase chain reaction," *J. Med. Virol.*, 50:97-103, 1996.
- Fogeda et al., "In vitro infection of human peripheral blood mononuclear cells by GB virus C/hepatitis G virus," *J. Virol.*, 73(5):4052-4061, 1999.
- GenBank Accession No. AF070476, "GB virus C variant troglodytes, complete genome," 1998.
- GenBank Accession No. AY196904, "Hepatitis G virus isolate 765, complete genome," 2003.
- George SL, et al., "Interactions Between GB Virus Type C and HIV," *Curr Infect Dis Rep.* 4:550-558, 2002.
- Lefrere et al., "Carriage of GB virus C/hepatitis G virus RNA is associated with a slower immunologic, virologic, and clinical progression of human immunodeficiency virus disease in coinfecting persons," *J. Infect. Dis.*, 179:783-789, 1999.
- Loeffler et al., "Antitumor effects of interleukin 2 liposomes and anti-CD3-stimulated T-cells against murine MCA-38 hepatic metastasis," *Cancer Res.*, 51:2127-2132, 1991.
- Moore et al., "Zidovudine and the natural history of the acquired immunodeficiency syndrome," *New England J. Med.*, 324(20):1412-1416, 1991.
- Sabin et al., "Effect of coinfection with hepatitis G virus on HIV disease progression in hemophilic men," *J. Acquir. Immune Defic. Syndr.*, 19:546-549, 1998.
- Sawyer et al., "Possible beneficial effects of neutralizing antibodies and antibody-dependent, cell-mediated cytotoxicity in human immunodeficiency virus infection," *AIDS Res. Hum. Retroviruses*, 6(3):341-356, 1990.
- Schmolke et al., "Identification of hepatitis G virus particles in human serum by E2-specific monoclonal antibodies generated by DNA immunization," *J. Virol.*, 72(5):4541-4545, 1998.
- Seipp et al., "Hepatotropism of GB virus C (GBV-C): GBV-C replication in human hepatocytes and cells of human hepatoma cell lines," *J. Hepatol.*, 30:570-579, 1999.
- Shimizu, "Replication of GB virus C (hepatitis G virus) in interferon-resistant daudi cells," *J. Virol.*, 73:8411-8414, 1999.
- Stapleton et al., "Prospective comparison of whole-blood- and plasma-based hepatitis C virus RNA detection systems: improved detection using whole blood as the source of viral RNA," *J Clin Microbiol.* 37:484-9, 1999.
- Stapleton, "GB virus type C/hepatitis G virus," *Seminars in Liver Disease*, 23(2):137-148, 2003.
- Tacke et al., "Humoral immune response to the E2 protein of hepatitis G virus is associated with long-term recovery from infection and reveals a high frequency of hepatitis G virus exposure among healthy blood donors," *Hepatol.*, 26(6):1626-1633, 1997.
- Thomas et al., "Association of antibody to GB virus C (hepatitis G virus) with viral clearance and protection from reinfection," *J. Infect. Dis.*, 177:539-542, 1998.
- Tillmann et al. "GB virus-C infection in patients infected with the human immunodeficiency virus," *Antiviral Res.* 52:83-90, 2001. Review.
- Tillmann et al., "Infection with GB virus C and reduced mortality among HIV-infected patients," *N Engl J Med.* 345:715-24, 2001.
- Toyoda et al., "Effect of GB virus C/hepatitis G virus coinfection on the course of HIV infection in hemophilia patients in Japan," *J. Acquir. Immune Defic. Syndr.*, 17:209-213, 1998.
- Voirin et al., "Effects of co-infection with hepatitis C virus and GB virus C on CD4 cell count and HIV-RNA level among HIV-infected patients treated with highly active antiretroviral therapy," *AIDS.* 16:1556-9, 2002.
- Xiang et al. "Effect of coinfection with GB virus C on survival among patients with HIV infection," *N Engl J Med.* 345:707-14, 2001.
- Berman et al., "Hiv-1 challenge of chimpanzees immunized with recombinant gp120," *Proc. Natl. Acad. Sci. U S A.*, 85:5200-5204, 1988.
- Mohr et al., "GB Virus Type C Envelope Protein E2 Elicits Antibodies That React with a Cellular Antigen on HIV-1 Particles and Neutralize Diverse HIV-1 Isolates," *J. of Immunology*, 185:4496-4505, 2010.
- Ruprecht et al., "Antibody protection: passive immunization of neonates against oral AIDS virus challenge," *Vaccine*, 21:3370-3373, 2003.
- Trkola et al., "Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies," *Nature Med.*, 11:615-622, 2005.
- Biron et al., "The 2F5 epitope is helical in the HIV-1 entry inhibitor T-20," *Biochemistry*, 44:13602-13611, 2005.
- Coutant et al., "Both lipid environment and pH are critical for determining physiological solution structure of 3-D-conserved epitopes of the HIV-1 gp41-MPER peptide P1," *FASEB Journal*, 22:4338-4351, 2008.
- Dennison et al., "Stable docking of neutralizing human immunodeficiency virus type 1 gp41 membrane-proximal external region monoclonal antibodies 2F5 and 4E10 is dependent on the membrane immersion depth of their epitope regions," *J. Virology*, 83(19): 10211-10223, 2009.
- Ferrantelli et al., "Post-exposure prophylaxis with human monoclonal antibodies prevented SHIV89.6P infection or disease in neonatal macaques," *AIDS*, 17(3): 301-309, 2003.
- Gray et al., "4E10-resistant variants in a human immunodeficiency virus type 1 subtype C-infected individual with an anti-membrane-proximal external region-neutralizing antibody response," *JVirology*, 82(5):2367-2375, 2008.
- Haynes and Alam, "HIV-1 hides an Achilles' heel in virion lipids," *Immunity*, 28:10-12, 2008.
- Haynes et al., "Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies," *Science*, 308:1906-1908, 2005.
- Huarte et al., "Lipid modulation of membrane-bound epitope recognition and blocking by HIV-1 neutralizing antibodies," *FEBS Letters*, 582:3798-3804, 2008.
- Johnston and Fauci, "An HIV vaccine—challenges and prospects," *NEJM*, 359: 888-890, 2008.
- Joos et al., "Long-term multiple-dose pharmacokinetics of human monoclonal antibodies (MAbs) against human immunodeficiency virus type 1 envelope gp120 (MAb 2G12) and gp41 (MAbs 4E10 and 2F5)," *Antimicrob Agents Chemother.*, 50(5):1773-1779, 2006.
- Koff, "Accelerating HIV vaccine development," *Nature*, 464:161-162, 2010.
- Matyas et al., "Lipid binding properties of 4E10, 2F5, WR304 monoclonal antibodies that neutralize HIV-1," *Biochimica et Biophysica Acta*, 1788:660-665, 2009.
- Mehandru et al., "Adjunctive passive immunotherapy in human immunodeficiency virus type 1-infected individuals treated with antiviral therapy during acute and early infection," *J. Virology*, 81(2): 11016-11031, 2007.
- Nakowitsch et al., "HIV-1 mutants escaping neutralization by the human antibodies 2F5, 2G12, and 4E10: in vitro experiments versus clinical studies," *AIDS*, 19(17): 1957-1966, 2005.
- Zhou et al., "Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01," *Science*, 329:811-817, 2010.
- Larios et al., "Characterization of a putative fusogenic sequence in the E2 hepatitis G virus protein," *Arch Biochem Biophys*, 442(2):149-159, 2005.
- McLinden et al., "Characterization of an immunodominant antigenic site on GB virus C glycoprotein E2 that is involved in cell binding," *J. Virol.*, 80(24):12131-12140, 2006.

\* cited by examiner

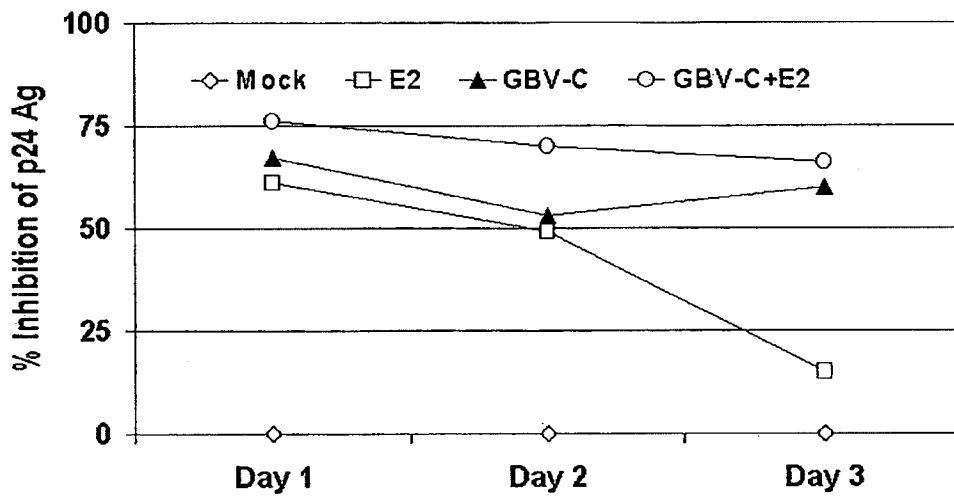


FIG. 1

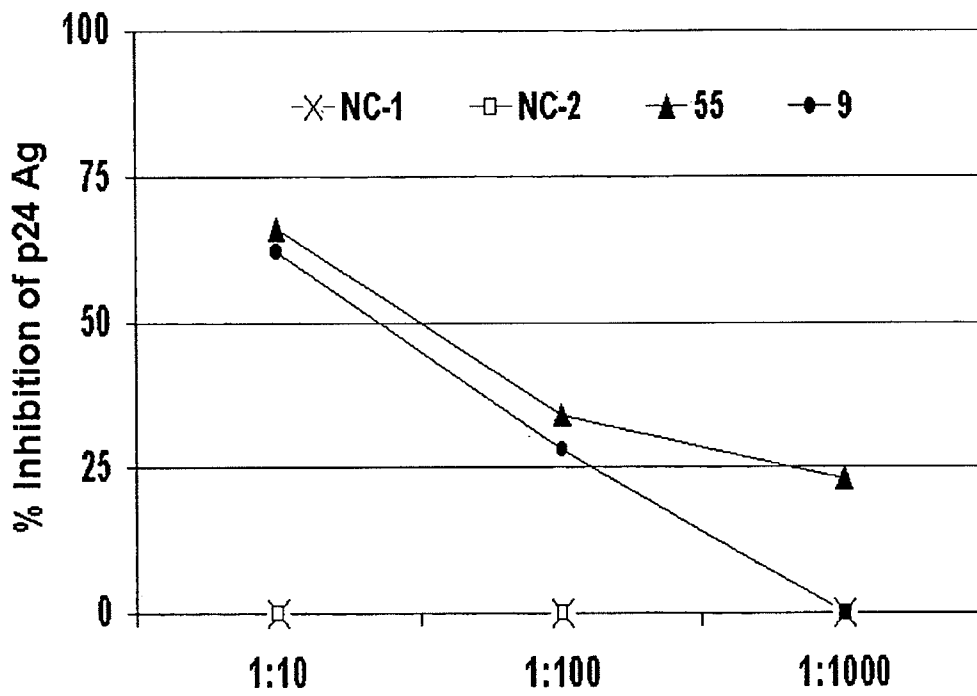


FIG. 2

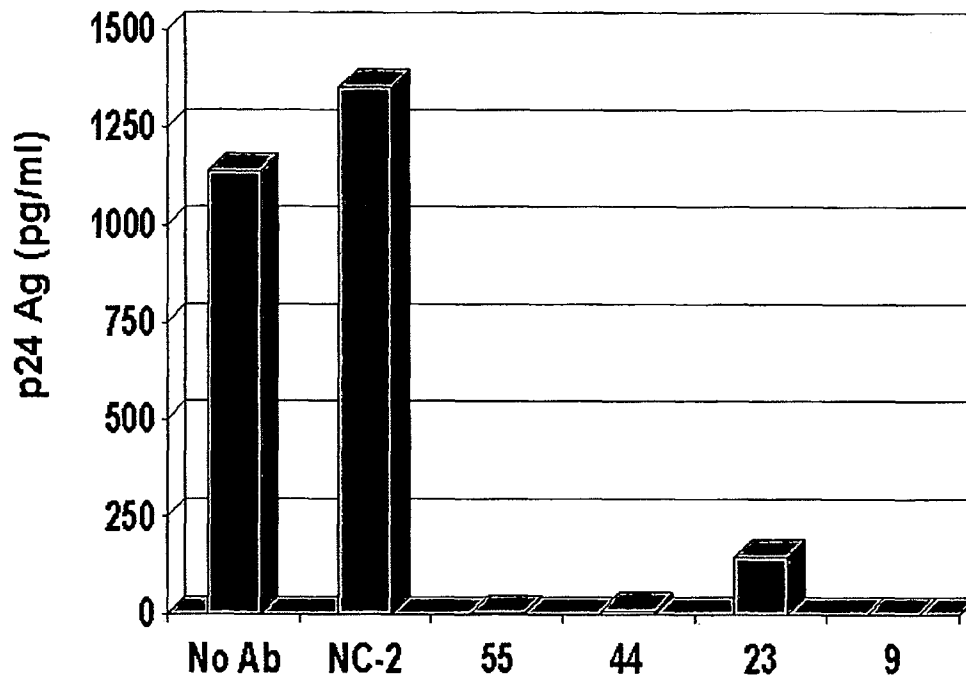


FIG. 3

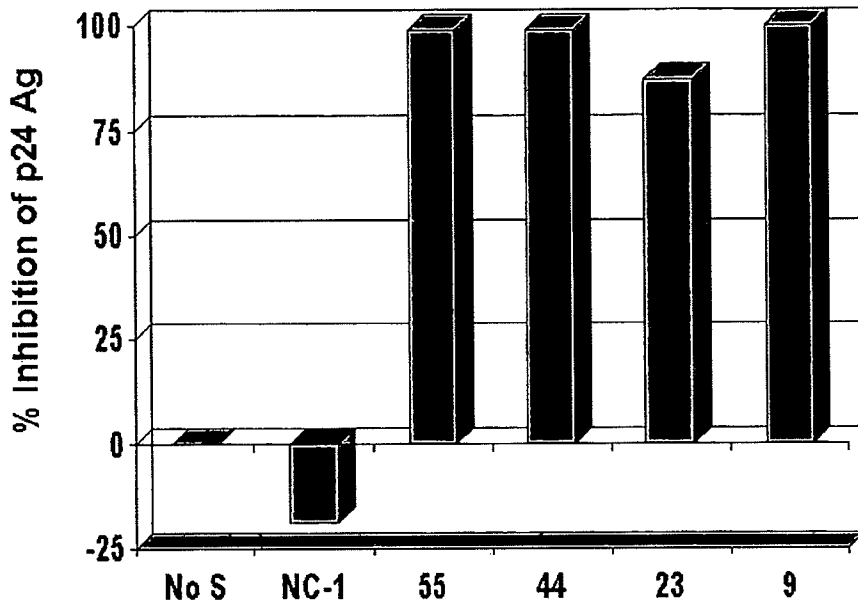


FIG. 4

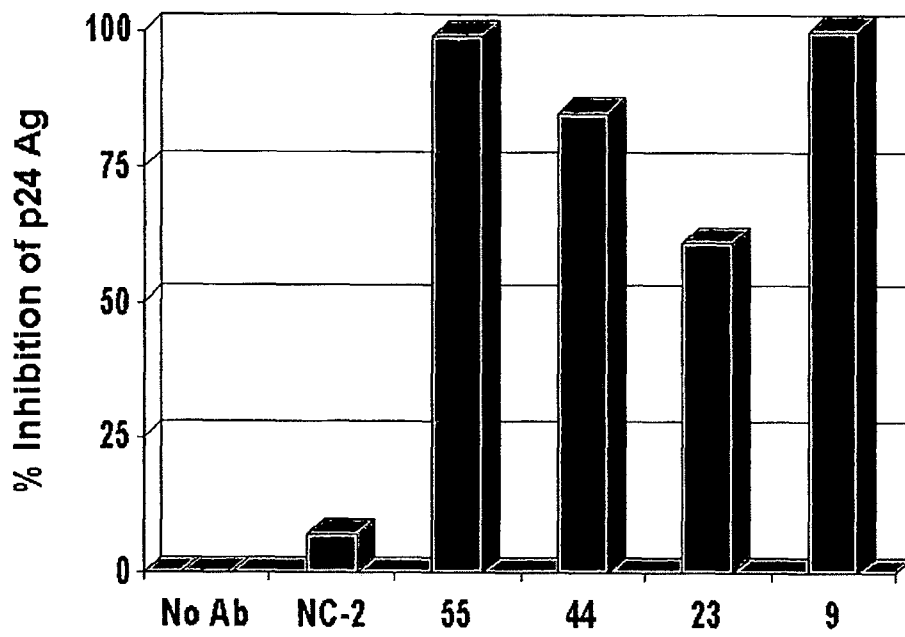


FIG. 5

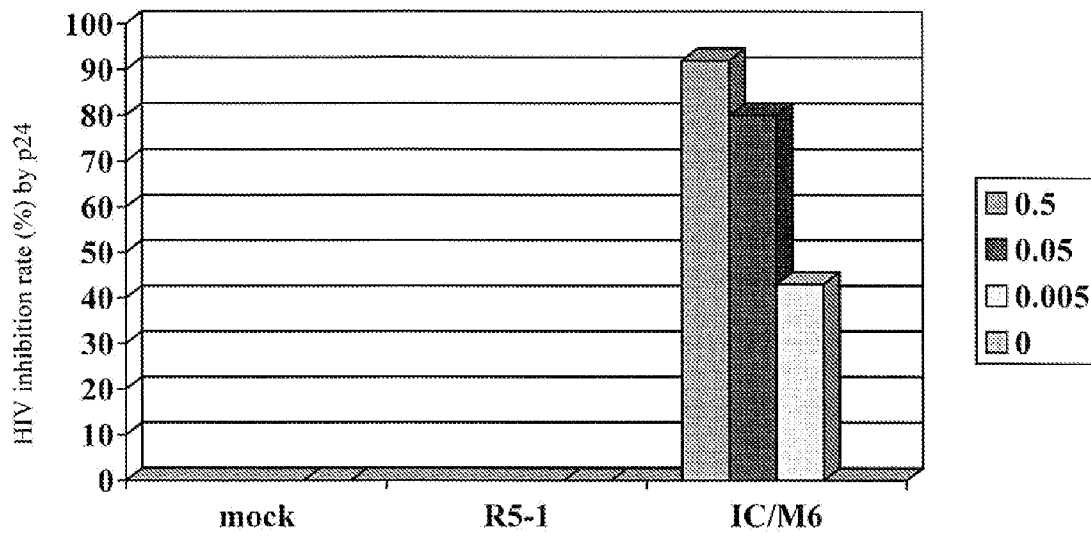
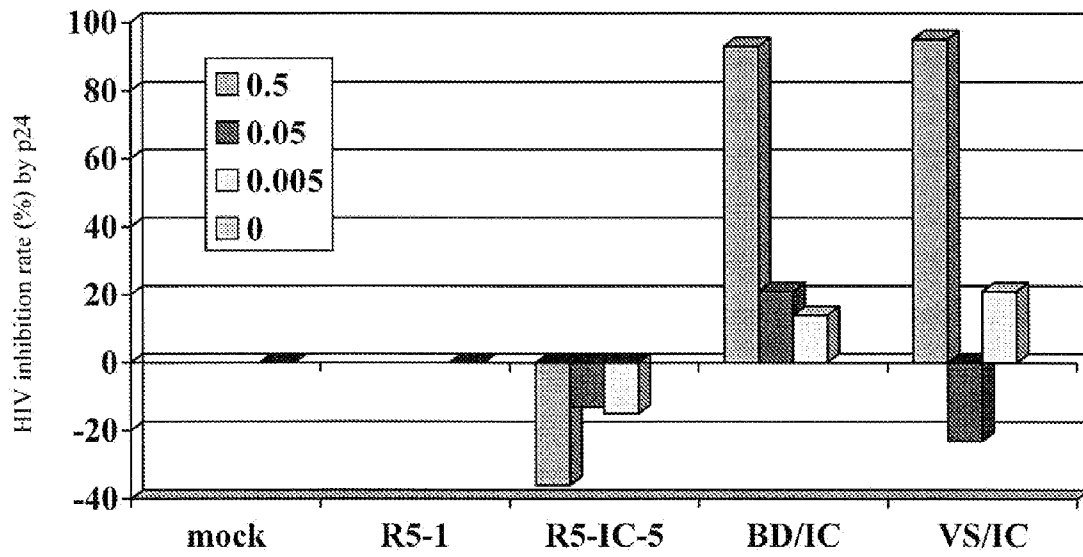


FIG. 6





R5-1 = no antibody control;  
R5-IC = IC/no Ab;  
BD/IC and VS/IC.  
All values =  $1 - \text{sample/IC} \times 100 = \% \text{ inhibition}$

FIG. 7

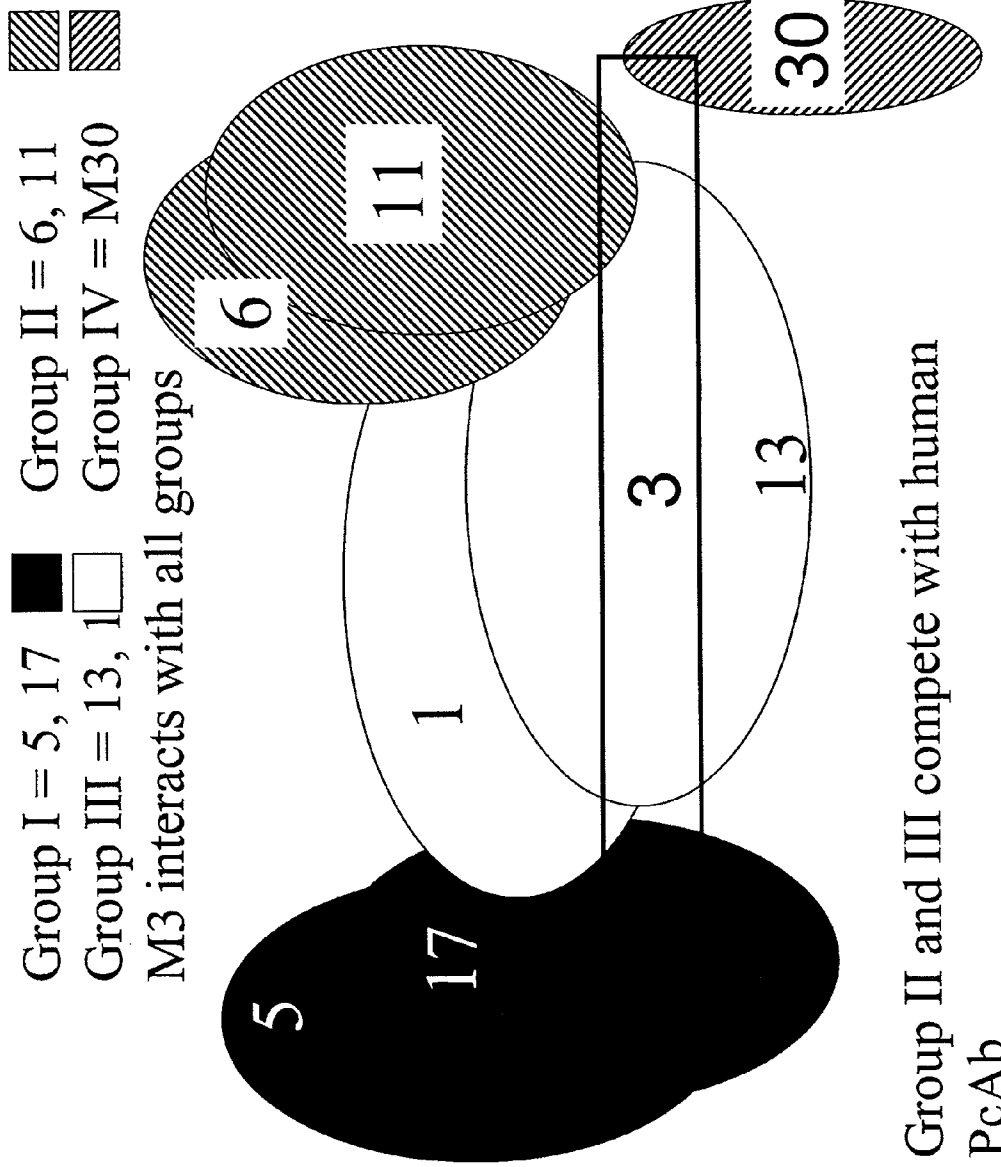


FIG. 8

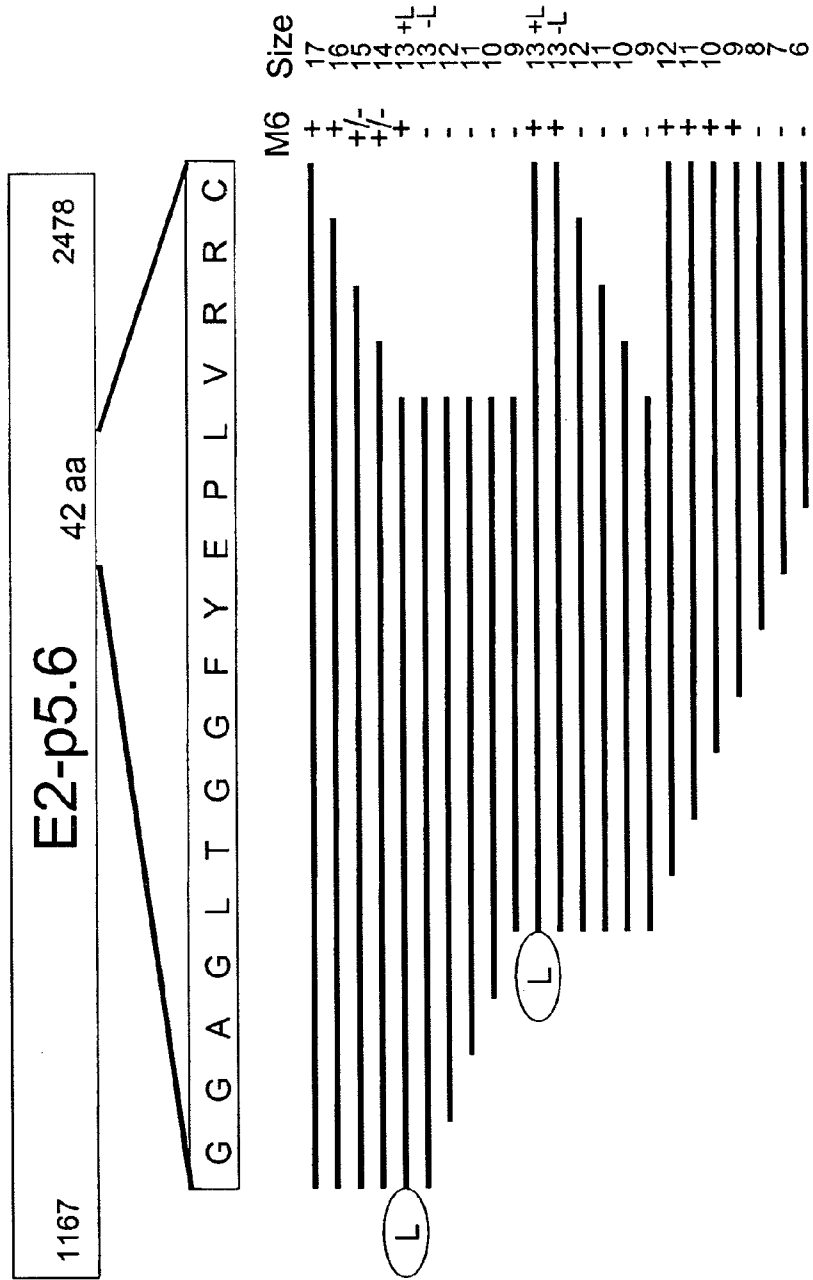


FIG. 9

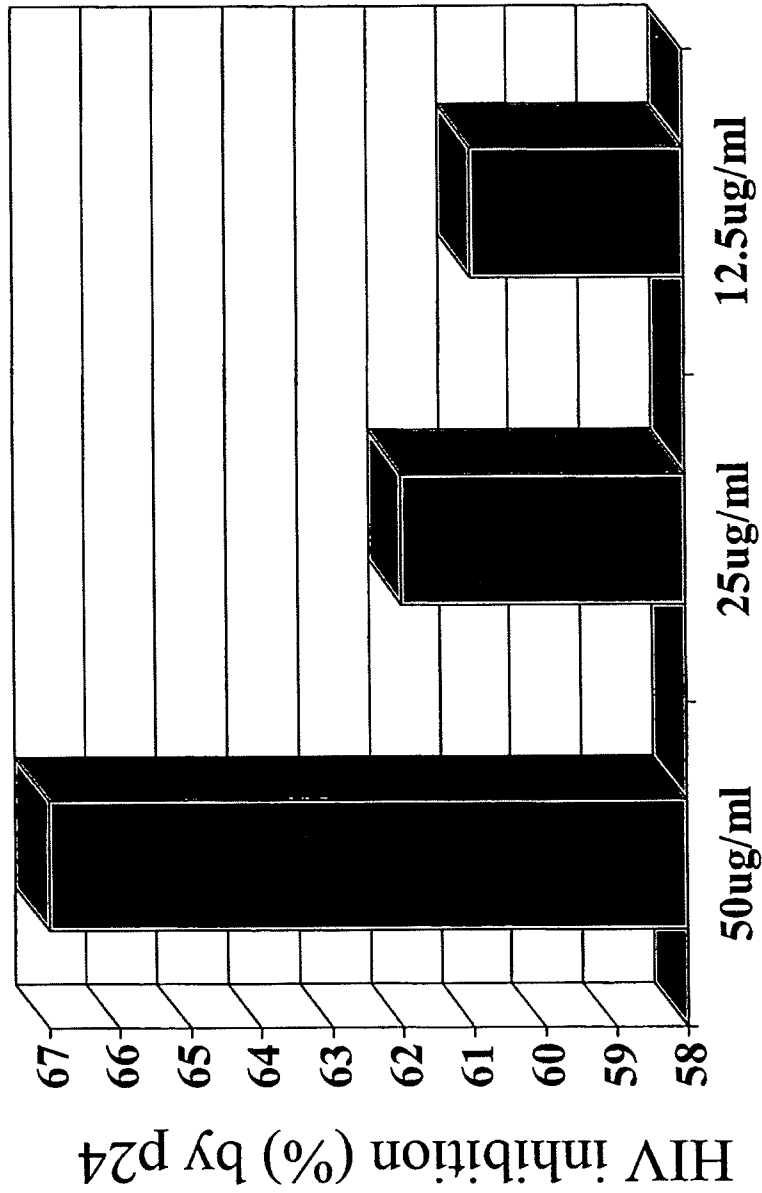


FIG. 10

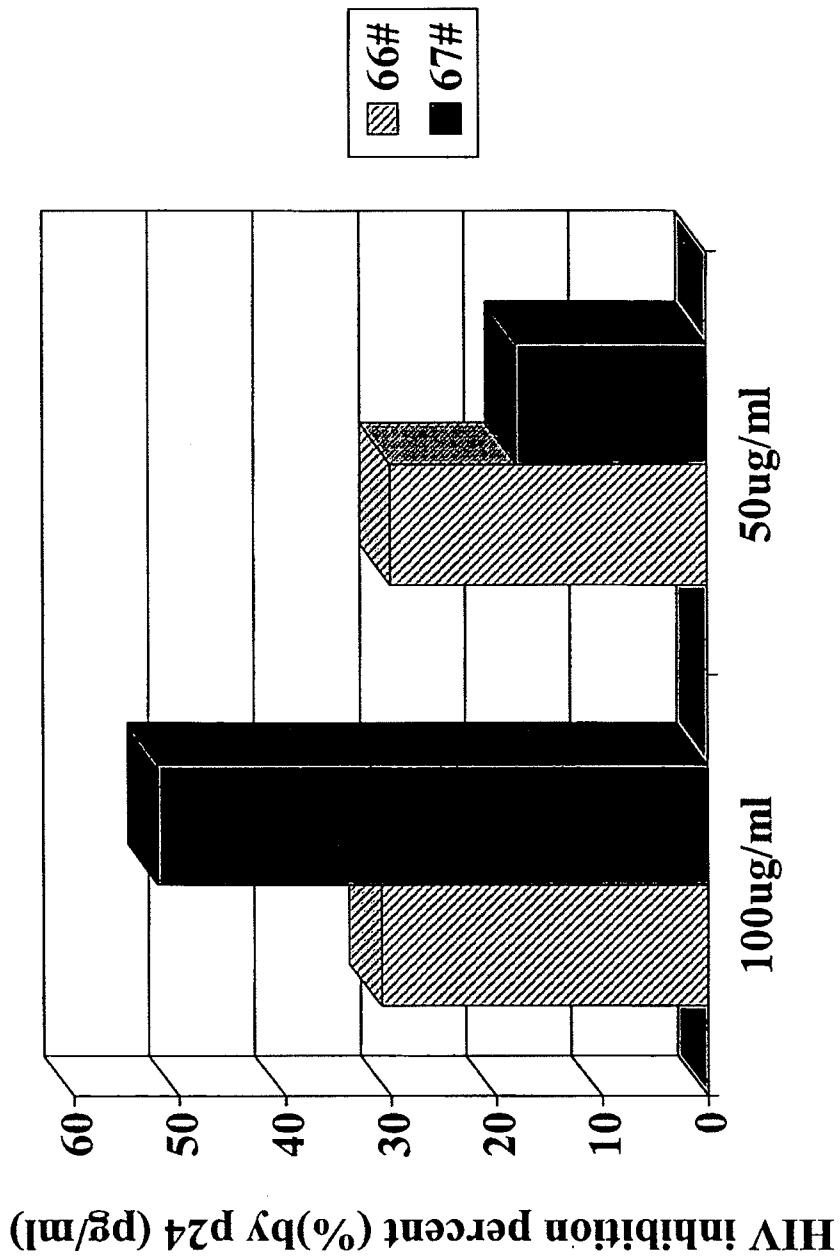


FIG. 11

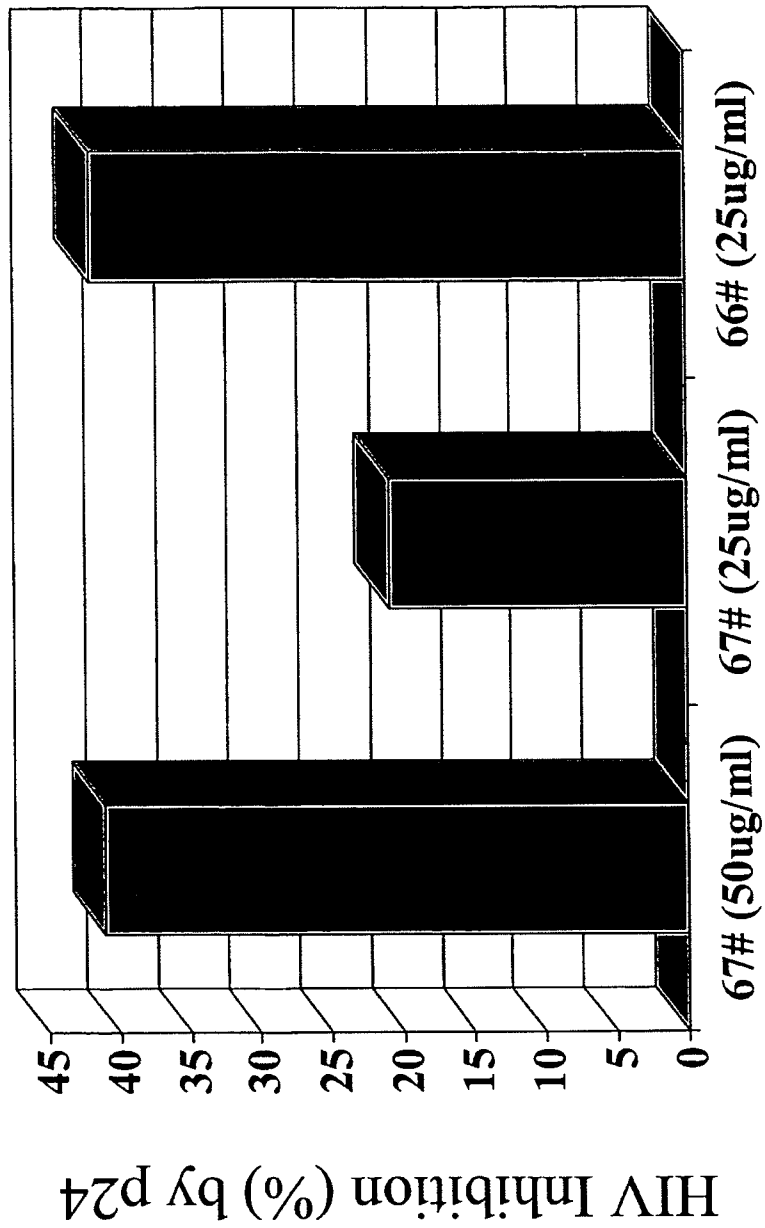


FIG. 12

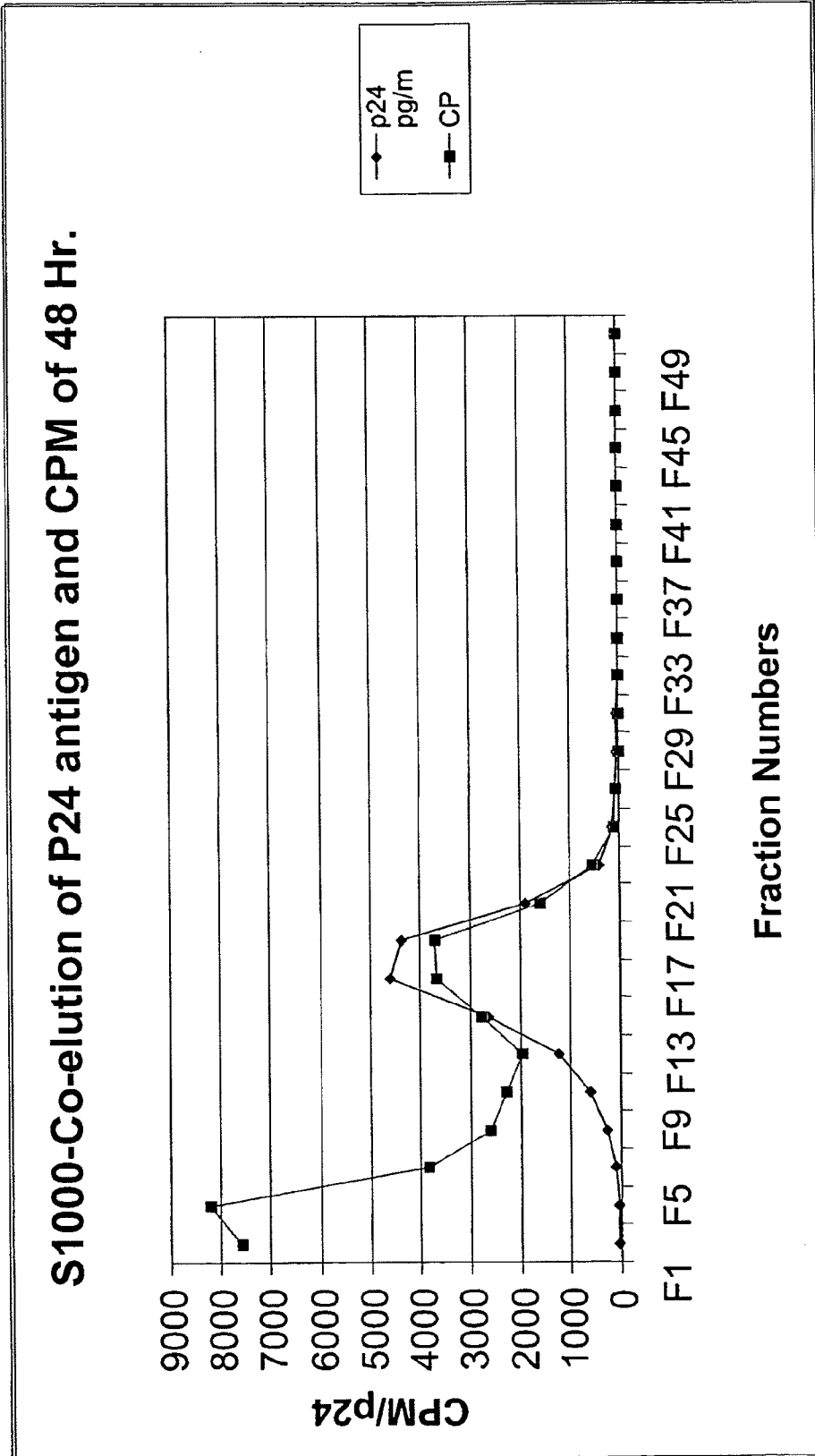


FIG. 13

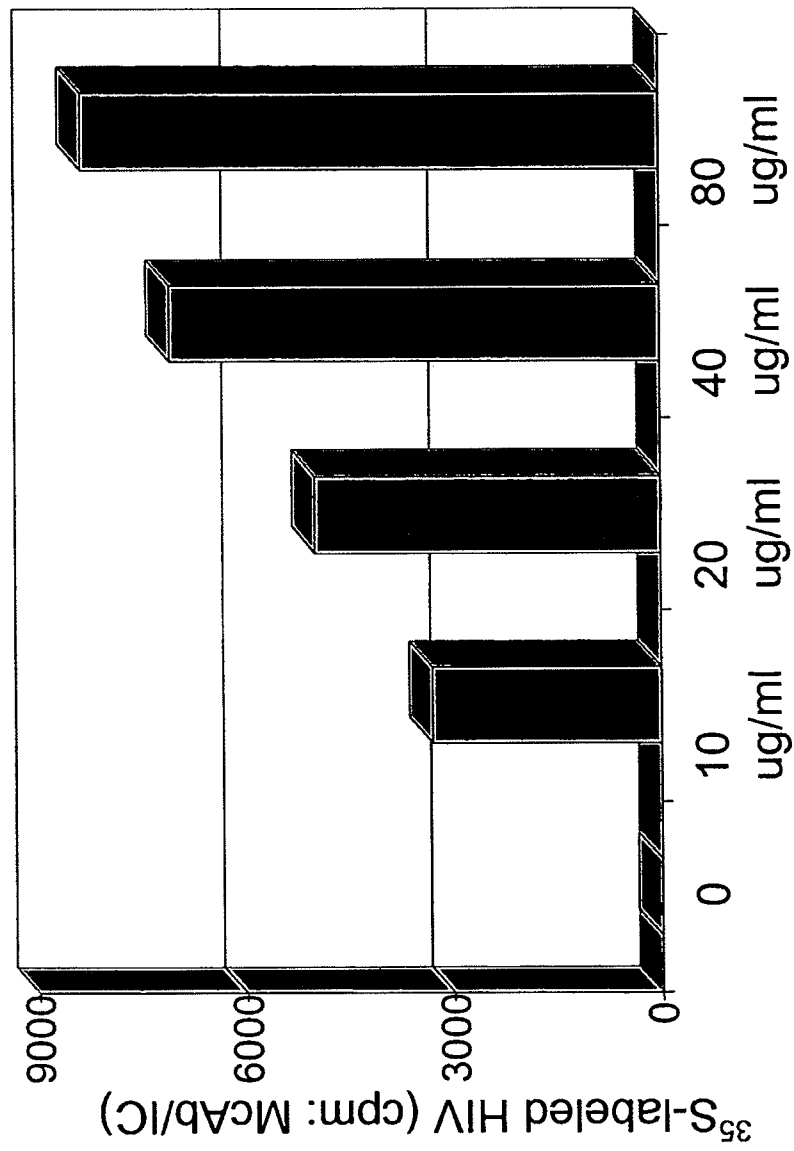


FIG. 14



**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 own rights in this invention pursuant to grant number R01AA12671 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 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; Nerurkar 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  $\geq 500$  cells/mm<sup>3</sup> (Easterbrook, 1999). Evaluation of LTNP's 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 LTNP's (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 (PBMC's) (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, Lefrère 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 (Lefrère 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.

Embodiments 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 antibody may be a polyclonal antibody, a monoclonal 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 monoclonal antibody. In certain embodiments, a GBV-C peptide can be derived from a GBV-C envelope protein. In particular embodiments, the GBV-C envelope protein is an E2 protein.

Embodiments 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 aptamer, an anti-GBV-C antibody, an antibody-like molecule, or other 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.

Embodiments 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, LTGGFYEPLVRRRC (SEQ ID NO:6), GGAGLTGGFYEPLVRRRC (SEQ ID NO:7), or FYEPLVRRRC (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, LTGGFYEPLVRRRC (SEQ ID NO:6), or GGAGLTGGFYEPLVRRRC (SEQ ID NO:7), or FYEPLVRRRC (SEQ ID NO:8).

Embodiments 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 X4 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 GGAGLTGGFY EPLVRR (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 IgG 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 <sup>35</sup>S-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-IgG complexes were immuno-precipitated using staph protein A (PAN-SORBIN®). Data represent HIV cpm precipitated by M6 (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 antigens 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 antigens 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  $\beta$ -chemokines and reducing expression of the HIV co-receptor CCR5, 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 from 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 or 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 (Laskus et al., 1998; Pessoa et al., 1998; Seipp et al., 1999). Recently, there were reports that human peripheral blood mononuclear cells (PBMC's) and interferon-resistant Daudi cells are permissive for GBV-C replication (Fogeda et al., 1999; Shimizu, 1999). In addition, transient replication of GBV-C was described in MT-2 cells (a human T-cell line), and PH5CH (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 there from, 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 containing a 9 amino acid sequence FYEPLVRRRC (SEQ ID NO:8) or derivative thereof is contemplated. In certain embodiments, an antigen comprising a 13 amino acid sequence (LTGGFYEPLVRRRC, SEQ ID NO:6) or a derivative thereof is contemplated. In still further embodiments, an antigen comprising a 17 amino acid sequence (GGAGLTGGFYEPLVRRRC, 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 can be substitutional, 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 asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

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

CODON TABLE						
Amino Acids			Codons			
Alanine	Ala	A	GCA	GCC	GCG	GCU
Cysteine	Cys	C	UGC	UGU		
Aspartic acid	Asp	D	GAC	GAU		
Glutamic acid	Glu	E	GAA	GAG		
Phenylalanine	Phe	F	UUC	UUU		
Glycine	Gly	G	GGA	GGC	GGG	GGU
Histidine	His	H	CAC	CAU		
Isoleucine	Ile	I	AUA	AUC	AUU	
Lysine	Lys	K	AAA	AAG		
Leucine	Leu	L	UUA	UUG	CUA	CUC CUG CUU
Methionine	Met	M	AUG			
Asparagine	Asn	N	AAC	AAU		
Proline	Pro	P	CCA	CCC	CCG	CCU
Glutamine	Gln	Q	CAA	CAG		
Arginine	Arg	R	AGA	AGG	CGA	CGC CGG CGU
Serine	Ser	S	AGC	AGU	UCA	UCC UCG UCU
Threonine	Thr	T	ACA	ACC	ACG	ACU
Valine	Val	V	GUA	GUC	GUG	GUU
Tryptophan	Trp	W	UGG			
Tyrosine	Tyr	Y	UAC	UAU		

In making such changes, the hydrophatic index of amino acids may be considered. The importance of the hydrophatic 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 hydrophatic 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 as 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 their propagation (i.e., a monolayer type of cell growth).

Non-anchorage dependent or suspension cultures from continuous established cell lines 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 polyprotein 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 1 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, 1200, 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 AY196904 or AF070476 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 are 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 exogenous, 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

Promoter and/or Enhancer	
Promoter/Enhancer	References
Immunoglobulin Heavy Chain	Banerji et al., 1983; Gilles et al., 1983; Grosschedl et al., 1985; Atchinson et al., 1986, 1987; Imler et al., 1987; Weinberger et al., 1984; Kiledjian et al., 1988; Porton et al., 1990
Immunoglobulin Light Chain	Queen et al., 1983; Picard et al., 1984
T-Cell Receptor	Luria et al., 1987; Winoto et al., 1989; Redondo et al., 1990
HLA DQ a and/or DQ $\beta$	Sullivan et al., 1987
$\beta$ -Interferon	Goodbourn et al., 1986; Fujita et al., 1987; Goodbourn et al., 1988
Interleukin-2	Greene et al., 1989
Interleukin-2 Receptor	Greene et al., 1989; Lin et al., 1990
MHC Class II 5	Koch et al., 1989
MHC Class II HLA-DRA	Sherman et al., 1989
$\beta$ -Actin	Kawamoto et al., 1988; Ng et al., 1989
Muscle Creatine Kinase (MCK)	Jaynes et al., 1988; Horlick et al., 1989; Johnson et al., 1989
Prealbumin (Transthyretin)	Costa et al., 1988
Elastase I	Omitz et al., 1987
Metallothionein (MTII)	Karin et al., 1987; Culotta et al., 1989
Collagenase	Pinkert et al., 1987; Angel et al., 1987
Albumin	Pinkert et al., 1987; Tronche et al., 1989, 1990
$\alpha$ -Fetoprotein	Godbout et al., 1988; Campere et al., 1989
$\gamma$ -Globin	Bodine et al., 1987; Perez-Stable et al., 1990
$\beta$ -Globin	Trudel et al., 1987
c-fos	Cohen et al., 1987
c-HA-ras	Triesman, 1986; Deschamps et al., 1985
Insulin	Edlund et al., 1985
Neural Cell Adhesion Molecule (NCAM)	Hirsh et al., 1990
$\alpha_1$ -Antitrypsin	Latimer et al., 1990
H2B (TH2B) Histone	Hwang et al., 1990
Mouse and/or Type I Collagen	Ripe et al., 1989
Glucose-Regulated Proteins (GRP94 and GRP78)	Chang et al., 1989
Rat Growth Hormone	Larsen et al., 1986
Human Serum Amyloid A (SAA)	Edbrooke et al., 1989
Troponin I (TN I)	Yutzey et al., 1989
Platelet-Derived Growth Factor (PDGF)	Pech et al., 1989
Duchenne Muscular Dystrophy SV40	Klamut et al., 1990 Banerji et al., 1981; Moreau et al., 1981; Sleight et al., 1985; Firak et al., 1986; Herr et al., 1986; Imbra et al., 1986; Kadesch et al., 1986; Wang et al., 1986; Ondek et al., 1987; Kuhl et al., 1987; Schaffner et al., 1988
Polyoma	Swartzendruber et al., 1975; Vasseur et al., 1980; Katinka et al., 1980, 1981; Tyndell et al., 1981; Dandolo et al., 1983; de Villiers et al., 1984; Hen et al., 1986; Satake et al., 1988; Campbell and/or Villarreal, 1988
Retroviruses	Kriegler et al., 1982, 1983; Levinson et al., 1982; Kriegler et al., 1983, 1984a, b, 1988; Bosze et al., 1986; Miksicsek 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

Promoter and/or Enhancer	
Promoter/Enhancer	References
Papilloma Virus	Campo et al., 1983; Lusky et al., 1983; Spandidos and/or Wilkie, 1983; Spalholz et al., 1985; Lusky et al., 1986; Cripe et al., 1987; Gloss et al., 1987; Hirochika et al., 1987; Stephens et al., 1987; Glue et al., 1988
Hepatitis B Virus	Bulla et al., 1986; Jameel et al., 1986; Shaul et al., 1987; Spandau et al., 1988; Vannice et al., 1988
Human Immunodeficiency Virus	Muesing et al., 1987; Hauber et al., 1988; Jakobovits et al., 1988; Feng et al., 1988; Takebe et al., 1988; Rosen et al., 1988; Berkhout et al., 1989; Laspia et al., 1989; Sharp et al., 1989; Braddock et al., 1989
Cytomegalovirus (CMV)	Weber et al., 1984; Boshart et al., 1985; Foecking et al., 1986
Gibbon Ape Leukemia Virus	Holbrook et al., 1987; Quinn et al., 1989

TABLE 3

Inducible Elements		
Element	Inducer	References
MT II	Phorbol Ester (TFA) Heavy metals	Palmiter et al., 1982; Haslinger et al., 1985; Searle et al., 1985; Stuart et al., 1985; Imagawa et al., 1987; Karin et al., 1987; Angel et al., 1987b; McNeill et al., 1989
MMTV (mouse mammary tumor virus)	Glucocorticoids	Huang et al., 1981; Lee et al., 1981; Majors et al., 1983; Chandler et al., 1983; Lee et al., 1984; Ponta et al., 1985; Sakai et al., 1988
$\beta$ -Interferon	poly(rI)x poly(rc)	Tavernier et al., 1983
Adenovirus 5 E2	EIA	Imperiale et al., 1984
Collagenase	Phorbol Ester (TPA)	Angel et al., 1987a
Stromelysin	Phorbol Ester (TPA)	Angel et al., 1987b
SV40	Phorbol Ester (TPA)	Angel et al., 1987b
Murine MX Gene	Interferon, Newcastle Disease Virus	Hug et al., 1988
GRP78 Gene	A23187	Resendez et al., 1988
$\alpha$ -2-Macroglobulin	IL-6	Kunz et al., 1989
Vimentin	Serum	Rittling et al., 1989
MHC Class I Gene H-2kb	Interferon	Blonar et al., 1989
HSP70	EIA, SV40 Large T Antigen	Taylor et al., 1989, 1990a, 1990b
Proliferin	Phorbol Ester-TPA	Mordacq et al., 1989
Tumor Necrosis Factor	PMA	Hensel et al., 1989
Thyroid Stimulating Hormone $\alpha$ Gene	Thyroid Hormone	Chatterjee et al., 1989

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 5' 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 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 Cocea, 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 permit site-specific cleavage of the new transcript to expose a polyadenylation site. This signals a specialized endogenous polymerase 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 more stable and are translated more efficiently. Thus, in other embodiments involving eukaryotes, it is preferred that 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 DH5 $\alpha$ , JM109, and KC8, as well as a number of commercially available bacterial hosts such as SURE<sup>®</sup> Competent Cells and SOLOPACK<sup>™</sup> Gold Cells (STRATAGENE<sup>®</sup>, La Jolla). Alternatively, bacterial cells such as *E. coli* LE392 could be used as host cells for phage 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 MAXBAC<sup>®</sup> 2.0 from INVITROGEN<sup>®</sup> and BACPACK<sup>™</sup> BACULOVIRUS EXPRESSION SYSTEM from CLONTECH<sup>®</sup>.

Other examples of expression systems include STRATAGENE<sup>®</sup>'s COMPLETE CONTROL<sup>™</sup> 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<sup>®</sup>, which carries the T-REX<sup>™</sup> (tetracycline-regulated expression) System, an inducible mammalian expression system that uses the full-length CMV promoter. The Tet-On<sup>™</sup> and Tet-Off<sup>™</sup> systems from CLONTECH<sup>®</sup> 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<sup>®</sup> 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.

#### D. 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 encapsidated 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 (Nicolau 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 Bachhawat, 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 it 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 synchronization 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-

tem 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, Ig 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. influenza* pneumonia. In pneumococcal disease it was essential to identify the infecting serotype and obtain the appropriate type specific antiserum. 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  $\lambda$ -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 mimics 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 results 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. Antigenic 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 established 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 <sup>51</sup>Cr release microtoxicity 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 <sup>51</sup>Cr release assay.

In certain aspects, T helper cell responses can be measured by in vitro or in vivo assay with peptides, polypeptides, 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), Gefter et al. (1977), each 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 Falo, 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 leukopheresis 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-AK) has been described as taking lymphocytes by leukopheresis or 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-AK cells has demonstrated that CD8<sup>+</sup> 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/peptide/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', Fab, F(ab')<sub>2</sub>, 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-maleimidobenzoyl-N-hydroxysuccinimide ester, carbodiimide, and bis-biaxotized 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, succinic anhydride, SOCl<sub>2</sub>, or R<sup>1</sup>N=C=NR, where R and R<sup>1</sup> 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*), 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 non-antibody-producing, have high fusion efficiency, and enzyme deficiencies that render them incapable of growing in certain selective media which support the growth of only the desired fused cells (hybridomas).

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-X63/Ag8, X63-Ag8.653, NS1/1.Ag 4 1, Sp210-Ag14, FO, NSO/U, MPC-11, MPC11-X45-GTG 1.7 and S194/5XX0 Bu1; for rats, one may use R210.RCY3, Y3-Ag 1.2.3, IR983F and 4B210; and U-266, GM1500-GRG2, LICR-LON-HMy2 and UC729-6 are all useful in connection with human cell fusions.

One preferred murine myeloma cell 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-azaguanine-resistant mouse murine myeloma SP2/0 non-producer 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  $1 \times 10^{-6}$  to  $1 \times 10^{-8}$ . However, this does not pose a problem, as the viable, fused hybrids are differentiated from the parental, infused cells (particularly the infused 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 azaserine. Aminopterin and methotrexate block de novo synthesis of both purines and pyrimidines, whereas azaserine 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 azaserine 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 by 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  $10^4$  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-GBV-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-PLVRR (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 "VS" 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; Riechmann et al., 1988; Verhoeyen 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 recombi-

nant immunoglobulin 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 human mAbs 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 (Jakobovits 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 a natural immune response, antibody genes accumulate mutations at a high rate (somatic hypermutation). Some of the changes introduced will confer higher affinity, and B-cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., 1992). 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 technique 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, a anti-viral agent, a detectable label, a cytotoxic agent, a chemical, a toxic, 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 immunoassays.

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 (Fv) 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 of 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 FYEPLVRRRC (SEQ ID NO:8). In preferred embodiments, the antigen is contemplated to comprise the 13 amino acid LTGGFYEPLVRRRC (SEQ ID NO:6). In more preferred embodiments, the antigen is contemplated to comprise the 17 amino acid sequence GGAGLTGGFYEPLVRRRC (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-maleimidobencoyl-N-hydroxysuccinimide ester, carbodiimide, and bis-biazotized 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,  $\gamma$ -interferon, GMCSF, BCG, aluminum hydroxide, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, and monophosphoryl lipid A (MPL). RIBI, which contains three components extracted from bacteria, MPL, trehalose dimycolate (TDM) and cell wall skeleton (CWS) in a 2% squalene/Tween 80 emulsion. 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 adjuvants 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/m<sup>2</sup>) (Johnson/Mead, NJ) and cytokines such as  $\gamma$ -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 or 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 of 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; pruritus; 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 HIV-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 medical treatment 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 nonexhaustive 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  $\alpha$ -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 pharmacological 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, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between the respective administrations.

It also is conceivable that more than one administration of a GBV-C based therapeutic agent will be desired. Various combinations may be employed, where a GBV-C based therapeutic is "A" and the other agent is "B," as exemplified below:

A/B/A B/A/B B/B/A A/A/B B/A/A A/B/B B/B/B A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A B/A/B/A B/A/A/B B/B/B/A A/A/A/B B/A/A/A A/B/A/A A/A/B/A A/B/B/B B/A/B/B B/B/A/B

Other combinations are contemplated as well.

#### 1. AZT

A well known, traditional therapy for the treatment of AIDS involves zidovudine (AZT™ available from Burroughs Wellcome). This is one of a class of nucleoside analogues known as dideoxynucleosides which block HIV replication by inhibiting HIV reverse transcriptase. The anti-AIDS drug zidovudine (also known as AZT) may also be used in limited circumstances, mostly in combination with rifampin, as described by Burger et al. (1993).

The compositions and methods disclosed herein will be particularly effective in conjunction with other forms of therapy, such as AZT and/or protease inhibitors that are designed to inhibit viral replication, by maintaining desirable levels of white blood cells. This, in effect, buys the patient the time necessary for the anti-viral therapies to work.

#### 2. HAART

New combination drug therapy has shown promising results in the treatment of HIV-infected patients. Treatment with potent anti-HIV drug combinations is referred to as "highly active anti-retroviral therapy" (HAART), and it has provided clinical improvement, longer survival, and improved quality of life for people infected with HIV during all four stages of HIV disease. Examples of HAART include a protease inhibitor (indinavir, nelfinavir, ritonavir, ritonavir/saquinavir, or saquinavir) combined with two nucleoside analogs (AZT/ddI, d4T/ddI, AZT/ddC, AZT/3TC, or d4T/3TC).

In many instances, it will be desirable to have multiple administrations of the inventive compositions and/or a vaccines, usually not exceeding six administrations or vaccinations, more usually not exceeding four vaccinations. In certain embodiments, one or more, usually at least about three administrations or vaccinations may be provided. The administrations or vaccinations will normally be at from two to twelve week intervals, more usually from three to five week intervals. Periodic boosters at intervals of 1-5 years, usually three years, will be desirable to maintain protective levels of the antibodies. The course of the immunization or treatment may be followed by standard antibody assays. The assays may be performed by labeling with conventional labels, such as radionuclides, enzymes, fluorescents, and the like. These 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.

The manner of application may be varied widely. Any of the conventional methods for administration of an antibody or vaccine are applicable. These are believed to include oral application on a solid physiologically acceptable base or in a physiologically acceptable dispersion, parenterally, by injection or the like. The dosage of the anti-GBV-C antibody or vaccine will depend on the route of administration and will vary according to the size of the host.

The anti-GBV-C binding agents, GBV-C infectious nucleic acids and/or GBV-C antigens of the invention may be formulated into a pharmaceutically acceptable composition, see below, or vaccine as neutral or salt forms. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the peptide) and those that are formed with inorganic acids such as, for example, hydrochloro-

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 isopropylamine, 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 5,620,896, 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 gene 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, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredients, its use in 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, fluorescers, 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 excretion. 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 an 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 10-24 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 diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal 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 to 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 parenteral injection (intravenous, intraperitoneal, intramuscular, subcutaneous, intracavity or transdermic). For viral vectors, one generally will prepare a viral vector stock. Depending on the kind of virus and the titer attainable, one will deliver 1 to 100, 10 to 50, 100-1000, or up to  $1 \times 10^4$ ,  $1 \times 10^5$ ,  $1 \times 10^6$ ,  $1 \times 10^7$ ,  $1 \times 10^8$ ,  $1 \times 10^9$ ,  $1 \times 10^{10}$ ,  $1 \times 10^{11}$ , or  $1 \times 10^{12}$  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.

Dosages 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 ml to 5 ml per dose. Repeated doses to deliver the appropriate amount of active compound are common. Both the age 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 type 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 transduced 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-

41

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 antiserum 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 four E2 anti-

42

body-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 used 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 FYEPLVRRRC (SEQ ID NO:8) or in a 17 amino acid sequence of GGAGLTGGFYEPLVRRRC (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  $\times 100$ .

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 Schmolke et al. (1998). M3 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 and immunized 2 New Zealand White rabbits (commercially done by InVivoGen). IgG was purified from serum collected pre-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  $^{35}\text{S}$ -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  $^{35}\text{S}$ -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  $^{35}\text{S}$ -labeled material was incubated in normal mouse IgG overnight at 4° C., and material reacting with IgG non-specifically was removed by precipitation with staph protein 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).

\* \* \*

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. 4,275,149
- U.S. Pat. No. 3,791,932
- U.S. Pat. No. 3,817,837
- U.S. Pat. No. 3,850,752
- U.S. Pat. No. 3,939,350
- U.S. Pat. No. 3,949,064
- U.S. Pat. No. 3,996,345
- U.S. Pat. No. 4,174,384
- U.S. Pat. No. 4,196,265
- U.S. Pat. No. 4,277,437
- U.S. Pat. No. 4,366,241
- U.S. Pat. No. 4,554,101
- U.S. Pat. No. 4,676,980
- 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
- U.S. Pat. No. 5,582,981
- U.S. Pat. No. 5,620,896
- U.S. Pat. No. 5,650,298
- 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
- U.S. Pat. No. 5,843,650
- U.S. Pat. No. 5,843,651
- U.S. Pat. No. 5,843,663
- U.S. Pat. No. 5,846,708
- U.S. Pat. No. 5,846,709
- U.S. Pat. No. 5,846,717
- U.S. Pat. No. 5,846,726
- U.S. Pat. No. 5,846,729
- U.S. Pat. No. 5,846,783
- U.S. Pat. No. 5,849,481
- U.S. Pat. No. 5,849,483

U.S. Pat. No. 5,849,486  
 U.S. Pat. No. 5,849,487  
 U.S. Pat. No. 5,849,497  
 U.S. Pat. No. 5,849,546  
 U.S. Pat. No. 5,849,547  
 U.S. Pat. No. 5,851,770  
 U.S. Pat. No. 5,851,772  
 U.S. Pat. No. 5,853,990  
 U.S. Pat. No. 5,853,992  
 U.S. Pat. No. 5,853,993  
 U.S. Pat. No. 5,856,092  
 U.S. Pat. No. 5,858,652  
 U.S. Pat. No. 5,861,244  
 U.S. Pat. No. 5,863,732  
 U.S. Pat. No. 5,863,753  
 U.S. Pat. No. 5,866,331  
 U.S. Pat. No. 5,866,336  
 U.S. Pat. No. 5,866,337  
 U.S. Pat. No. 5,866,366  
 U.S. Pat. No. 5,871,986  
 U.S. Pat. No. 5,874,563  
 U.S. Pat. No. 5,882,864  
 U.S. Pat. No. 5,900,481  
 U.S. Pat. No. 5,905,024  
 U.S. Pat. No. 5,910,407  
 U.S. Pat. No. 5,912,124  
 U.S. Pat. No. 5,912,145  
 U.S. Pat. No. 5,912,148  
 U.S. Pat. No. 5,916,776  
 U.S. Pat. No. 5,916,779  
 U.S. Pat. No. 5,919,626  
 U.S. Pat. No. 5,919,630  
 U.S. Pat. No. 5,925,517  
 U.S. Pat. No. 5,925,525  
 U.S. Pat. No. 5,925,565  
 U.S. Pat. No. 5,928,862  
 U.S. Pat. No. 5,928,869  
 U.S. Pat. No. 5,928,870  
 U.S. Pat. No. 5,928,905  
 U.S. Pat. No. 5,928,906  
 U.S. Pat. No. 5,929,227  
 U.S. Pat. No. 5,932,413  
 U.S. Pat. No. 5,932,451  
 U.S. Pat. No. 5,935,791  
 U.S. Pat. No. 5,935,819  
 U.S. Pat. No. 5,935,825  
 U.S. Pat. No. 5,939,291  
 U.S. Pat. No. 5,942,391  
 U.S. Pat. No. 5,958,895  
 U.S. Pat. No. 6,004,799  
 U.S. Pat. No. 6,399,763  
 U.S. Pat. No. 6,479,243  
 European Application 03089  
 European Application 320 308  
 European Application 329 822  
 GB Application 2 202 328  
 PCT Application PCT/US87/00880  
 PCT Application PCT/US89/01025  
 PCT Application WO 01/77157  
 PCT Application WO 88/10315  
 PCT Application WO 91/00360  
 PCT Application WO 92/200373  
 PCT Application WO 93/06213  
 PCT Application WO 93/08829  
 Akiyoshi et al., *Am. J. Gastroenterol.*, 94:1627-1631, 1999.  
 Almendro, et al., *J Immunol.*, 157(12):5411-21, 1996.  
 Alter et al., *N. Engl. J. Med.*, 336:741-746, 1997a.

Alter et al., *N. Engl. J. Med.*, 336:747-754, 1997b.  
 Anderson et al., *J. Immunol.*, 142, 1383, 1989.  
 Angel et al., *Mol. Cell. Biol.*, 7:2256, 1987a.  
 Angel et al., *Cell*, 49:729, 1987b.  
 5 Atchison and Perry, *Cell*, 46:253, 1986.  
 Atchison and Perry, *Cell*, 48:121, 1987.  
 Ausubel et al., *In: Current Protocols in Molecular Biology*,  
 Greene Publishing Associates and Wiley Interscience, NY,  
 1989.  
 10 Baichwal and Sugden, *In: Gene Transfer*, Kucherlapati (Ed.),  
 NY, Plenum Press, 117-148, 1986.  
 Banerji et al., *Cell*, 27(2 Pt 1):299-308, 1981.  
 Banerji et al., *Cell*, 33(3):729-740, 1983.  
 Bedzyk et al., *J. Biol. Chem.*, 265:18615, 1990  
 15 Berkhout et al., *Cell*, 59:273-282, 1989.  
 Blonar et al., *EMBO J.*, 8:1139, 1989.  
 Bodine and Ley, *EMBO J.*, 6:2997, 1987.  
 Boshart et al., *Cell*, 41:521, 1985.  
 Bosze et al., *EMBO J.*, 5(7):1615-1623, 1986.  
 20 Braddock et al., *Cell*, 58:269, 1989.  
 Brodeur et al., *In: Monoclonal antibody production tech-  
 niques and applications*, Marcel Dekker, Inc., NY, 51-63,  
 1987.  
 Bukh et al., *J. Inf. Dis.*, 177:855-862, 1998.  
 25 Bulla and Siddiqui, *J. Virol.*, 62:1437, 1986.  
 Burbage et al., *Leuk Res*, 21(7):681-690, 1997.  
 Burger et al., *Antimicrob Agents Chemother.*, 37(7):1426-31,  
 1993.  
 Burton and Woof, *Adv. Immunol.*, 51:1-84, 1992.  
 30 Campbell and Villarreal, *Mol. Cell. Biol.*, 8:1993, 1988.  
 Campbell, *In: Monoclonal Antibody Technology, Laboratory  
 Techniques in Biochemistry and Molecular Biology*, Bur-  
 den and Von Knippenberg (Eds.), Elsevier, Amsterdam,  
 13:71-74/75-83, 1984.  
 35 Campere and Tilghinan, *Genes and Dev.*, 3:537, 1989.  
 Campo et al., *Nature*, 303:77, 1983.  
 Carbonell et al., *FEMS Microbiol. Lett.*, 177(1):75-82, 1999.  
 Cecilia et al., *Journal of Virology*, 72(9):6988-96, 1998.  
 Celander and Haseltine, *J. Virology*, 61:269, 1987.  
 40 Celander et al., *J. Virology*, 62:1314, 1988.  
 Chandler et al., *Cell*, 33:489, 1983.  
 Chandler et al., *Proc Natl. Acad. Sci. USA*, 94(8):3569-601,  
 1997.  
 Chang et al., *Mol. Cell. Biol.*, 9:2153, 1989.  
 45 Chatterjee et al., *Proc Natl. Acad. Sci. U.S.A.*, 86:9114, 1989.  
 Chaudhary et al., *Proc. Nat'l Acad. Sci.*, 87:9491, 1990  
 Chen and Okayama, *Mol. Cell. Biol.*, 7:2745-2752, 1987.  
 Choi et al., *Cell*, 53:519, 1988.  
 Clackson et al., *Nature*, 352:624-628, 1991.  
 50 Cocea, *Biotechniques*, 23(5):814-6, 1997.  
 Cohen et al., *J. Cell. Physiol.*, 5:75, 1987.  
 Costa et al., *Mol. Cell. Biol.*, 8:81, 1988.  
 Cripe et al., *EMBO J*, 6:3745, 1987.  
 Culotta and Hamer, *Mol. Cell. Biol.*, 9:1376, 1989.  
 55 Dandolo et al., *J. Virology*, 47:55-64, 1983.  
 Dawson et al., *J. Med. Virol.* 50:97-103, 1996.  
 de Martino et al., *J. Infect. Dis.*, 178:862-865, 1998.  
 De Villiers et al., *Nature*, 312(5991):242-246, 1984.  
 Deacon et al., *Science* 270:988-991, 1995.  
 60 Deschamps et al., *Science*, 230:1174-1177, 1985.  
 Easterbrook, *J. Infect.* 38:71-73, 1999.  
 Edbrooke et al., *Mol. Cell. Biol.*, 9:1908, 1989.  
 Edlund et al., *Science*, 230:912-916, 1985.  
 Elvander et al., *Acta. Vet. Scand.* 39:251-264, 1998.  
 65 Fechheimer et al., *Proc. Nat'l Acad. Sci. USA*, 84:8463-8467,  
 1987.  
 Feng and Holland, *Nature*, 334:6178, 1988.

Feucht et al., *Hepatology*, 26:491-494, 1997.  
 Firak and Subramanian, *Mol. Cell. Biol.*, 6:3667, 1986.  
 Foecking and Hofstetter, *Gene*, 45(1):101-105, 1986.  
 Fogeda et al., *J. Virol.* 73:4052-4061, 1999.  
 Forster and Symons, *Cell*, 49:211-220, 1987.  
 Fraley et al., *Proc. Natl. Acad. Sci. USA*, 76:3348-3352, 1979.  
 Freshney, In: *Animal Cell Culture: a Practical Approach*,  
 Second Edition, Oxford/NY, IRL Press, Oxford University  
 Press, 1992.  
 Fujita et al., *Cell*, 49:357, 1987.  
 Gale, Jr. et al., *Mol. Cell. Biol.*, 18:5208-5218, 1998  
 Gefter et al., *Somatic Cell Genet.*, 3(2):231-6, 1977.  
 Ghosh and Bachhawat, In: *Liver diseases, targeted diagnosis  
 and therapy using specific receptors and ligands*, (Wu G,  
 Wu C ed.), NY, Marcel Dekker, 87-104, 1991.  
 Gilles et al., *Cell*, 33:717, 1983.  
 Gloss et al., *EMBO J.*, 6:3735, 1987.  
 Godbout et al., *Mol. Cell. Biol.*, 8:1169, 1988.  
 Goding et al., In: *Monoclonal Antibodies: Principles and  
 Practice*, 2d ed., Orlando, Fla., Academic Press, 60-61,  
 65-66, 71-74, 1986.  
 Goodbourn and Maniatis, *Proc. Natl. Acad. Sci. USA*,  
 85:1447, 1988.  
 Goodbourn et al., *Cell*, 45:601, 1986.  
 Gopal, *Mol. Cell. Biol.*, 5:1188-1190, 1985.  
 Gossen et al., *Proc. Natl. Acad. Sci. USA*, 89:5547-5551,  
 1992.  
 Gossen et al., *Science*, 268:1766-69, 1995.  
 Graham and Van Der Eb, *Virology*, 52:456-467, 1973.  
 Greene et al., *Immunology Today*, 10:272, 1989  
 Griffith et al., *EMBO J.*, 12:725-734, 1993.  
 Grosschedl and Baltimore, *Cell*, 41:885, 1985.  
 Harland and Weintraub, *J. Cell Biol.*, 101:1094-1099, 1985.  
 Harlow and Lane, *Antibodies: A Laboratory manual*, Cold  
 Spring Harbor Laboratory, 1988.  
 Haslinger and Karin, *Proc. Natl. Acad. Sci. USA*, 82:8572,  
 1985.  
 Hauber and Cullen, *J. Virology*, 62:673, 1988.  
 Hen et al., *Nature*, 321:249, 1986.  
 Hensel et al., *Lymphokine Res.*, 8:347, 1989.  
 Herr and Clarke, *Cell*, 45:461, 1986.  
 Hirochika et al., *J. Virol.*, 61:2599, 1987.  
 Hirsch et al., *Mol. Cell. Biol.*, 10:1959, 1990.  
 Holbrook et al., *Virology*, 157:211, 1987.  
 Hoon et al., *J. Urol.*, 150(6):2013-2018, 1993.  
 Horlick and Benfield, *Mol. Cell. Biol.*, 9:2396, 1989.  
 Huang et al., *Cell*, 27:245, 1981.  
 Huang et al., *Nature Med.*, 2:1240-1243, 1996.  
 Hug et al., *Mol. Cell. Biol.*, 8:3065, 1988.  
 Humphreys and Glover, *Curr Opin Drug Discov Devel.*, 4(2):  
 172-185, 2001.  
 Hwang et al., *Mol. Cell. Biol.*, 10:585, 1990.  
 Imagawa et al., *Cell*, 51:251, 1987.  
 Imbra and Karin, *Nature*, 323:555, 1986.  
 Imler et al., *Mol. Cell. Biol.*, 7:2558, 1987.  
 Imperiale and Nevins, *Mol. Cell. Biol.*, 4:875, 1984.  
 Jakobovits et al., *Mol. Cell. Biol.*, 8:2555, 1988.  
 Jakobovits et al., *Nature* 362, 255-258, 1993.  
 Jameel and Siddiqui, *Mol. Cell. Biol.*, 6:710, 1986.  
 Jaynes et al., *Mol. Cell. Biol.*, 8:62, 1988.  
 Johnson et al., In: *Biotechnology And Pharmacy*, Pezzuto et  
 al., (Eds.), Chapman and Hall, NY, 1993.  
 Johnson et al., *Mol. Cell. Biol.*, 9:3393, 1989.  
 Jones et al., *Nature*, 321:522-525, 1986.  
 Kadesch and Berg, *Mol. Cell. Biol.*, 6:2593, 1986.  
 Kaneda et al., *Science*, 243:375-378, 1989.  
 Karin et al., *Mol. Cell. Biol.*, 7:606, 1987.

Karin et al., *Mol. Cell. Biol.*, 7:606, 1987.  
 Katinka et al., *Cell*, 20:393, 1980.  
 Kawamoto et al., *Mol. Cell. Biol.*, 8:267, 1988.  
 Kiledjian et al., *Mol. Cell. Biol.*, 8:145, 1988.  
 5 Klamut et al., *Mol. Cell. Biol.*, 10:193, 1990.  
 Koch et al., *Mol. Cell. Biol.*, 9:303, 1989.  
 Kohler and Milstein, *Eur. J. Immunol.*, 6:511-519, 1976.  
 Kohler and Milstein, *Nature*, 256:495-497, 1975.  
 Kozbor, *J. Immunol.*, 133:3001, 1984.  
 10 Kraus et al., *FEBS Lett.*, 428(3):165-70, 1998.  
 Kriegler and Botchan, In: *Eukaryotic Viral Vectors*, Y. Gluz-  
 man, ed., Cold Spring Harbor: Cold Spring Harbor Labo-  
 ratory, NY, 1982.  
 Kriegler and Botchan, *Mol. Cell. Biol.*, 3:325, 1983.  
 15 Kriegler et al., *Cell*, 38:483, 1984.  
 Kriegler et al., *Cell*, 53:45, 1988.  
 Kuhl et al., *Cell*, 50:1057, 1987.  
 Kunz et al., *Nucl. Acids Res.*, 17:1121, 1989.  
 Kwoh et al., *Proc. Natl. Acad. Sci. USA*, 86(4):1173-1177,  
 20 1989.  
 Kyte and Doolittle, *J. Mol. Biol.*, 157:105-132, 1982.  
 Lareyre, et al., *J. Bio. Chem.*, 274(12):8282-90, 1999.  
 Larsen et al., *Proc Natl. Acad. Sci. USA.*, 83:8283, 1986.  
 Laskus et al., *J. Virol.*, 72:3072-3075, 1998.  
 25 Laspia et al, *Cell*, 59:283, 1989.  
 Latimer et al., *Mol. Cell. Biol.*, 10:760, 1990.  
 Leary et al., *J. Med. Virol.*, 48:60-67, 1996.  
 Lee et al., *Mol. Endocrinol.*, 2: 404-411, 1988.  
 Lee et al., *Nature*, 294:228, 1981.  
 30 Lee et al., *Nucleic Acids Res.*, 12:4191-206, 1984.  
 Lefrère et al., *J. Infect. Dis.*, 179:783-789, 1999.  
 Levenson et al., *Hum. Gene Ther.*, 9(8):1233-6, 1998.  
 Levinson et al., *Nature*, 295:79, 1982.  
 Lidor et al., *Am. J. Obstet. Gynecol.*, 177(3):579-585, 1997.  
 35 Lin et al., *Mol. Cell. Biol.*, 10:850, 1990.  
 Linnen et al., *Science*, 271:505-508, 1996.  
 Loeffler et al., *Cancer Res.*, 51:2127, 1991.  
 Luria et al., *EMBO J.*, 6:3307, 1987.  
 Lusky and Botchan, *Proc. Natl. Acad. Sci. USA*, 83:3609,  
 40 1986.  
 Lusky et al., *Mol. Cell. Biol.* 3:1108, 1983.  
 Macejak et al., *Nature*, 353:90-94, 1991.  
 Majors and Varmus, *Proc. Natl. Acad. Sci. USA*, 80:5866,  
 1983.  
 45 Maniatis, et al., *Molecular Cloning, A Laboratory Manual*,  
 Cold Spring Harbor Press, Cold Spring Harbor, N.Y., 1988.  
 Marks et al., *Bio/Technol.*, 10:779-783, 1992.  
 Marks et al., *J. Mol. Biol.*, 222:581-597, 1991.  
 Massuda et al., *Proc Natl Acad Sci USA*, 94(26):14701-  
 50 14706, 1997.  
 McCafferty et al., *Nature*, 348:552-553, 1990.  
 McNeill et al., *Gene*, 76:81, 1989.  
 Miksicek et al., *Cell*, 46:203, 1986.  
 Millstein and Cuello, *Nature*, 305:537-539, 1983.  
 55 Moore et al., *N. Engl. J. Med.*, 324(20):1412-1416, 1991.  
 Mordacq and Linzer, *Genes and Dev.*, 3:760, 1989.  
 Moreau et al., *Nucl. Acids Res.*, 9:6047, 1981.  
 Muesing et al., *Cell*, 48:691, 1987.  
 Nerurkar et al., *J. Med. Virol.*, 56:123-127, 1998.  
 60 Ng et al., *Nuc. Acids Res.*, 17:601, 1989.  
 Nicolau and Rubenstein, In: *Vectors: A survey of molecular  
 cloning vectors and their uses*, Rodriguez and Denhardt  
 (eds.), Stoneham: Butterworth, 493-513, 1988.  
 Nicolau and Sene, *Biochim. Biophys. Acta*, 721:185-190,  
 65 1982.  
 Nomoto et al., *Gene*, 236(2):259-271, 1999.  
 Okamoto et al., *J. Gen. Virol.*, 78:737-745, 1997.



- Ondek et al., *EMBO J.*, 6:1017, 1987.  
 Ornitz et al., *Mol. Cell. Biol.*, 7:3466, 1987.  
 Palmiter et al., *Nature*, 300:611, 1982.  
 Pech et al., *Mol. Cell. Biol.*, 9:396, 1989.  
 Pelletier et al., *Nature*, 334(6180):320-325, 1988.  
 Perez-Stable and Constantini, *Mol. Cell. Biol.*, 10:1116, 1990.  
 Pessoa et al., *Hepatology*, 27:877-880, 1998.  
 Picard and Schaffner, *Nature*, 307:83, 1984.  
 Pinkert et al., *Genes and Dev.*, 1:268, 1987.  
 Ponta et al., *Proc. Natl. Acad. Sci. USA*, 82:1020, 1985.  
 Porton et al., *Mol. Cell. Biol.*, 10:1076, 1990.  
 Potter et al., *Proc. Nat'l Acad. Sci. USA*, 81:7161-7165, 1984.  
 Queen and Baltimore, *Cell*, 35:741, 1983.  
 Quinn et al., *Mol. Cell. Biol.*, 9:4713, 1989.  
 Redondo et al., *Science*, 247:1225, 1990.  
 Reisman and Rotter, *Mol. Cell. Biol.*, 9:3571, 1989.  
 Remington's Pharmaceutical Sciences, 18th Ed. Mack Printing Company, 1389-1404, 1990,  
 Resendez Jr. et al., *Mol. Cell. Biol.*, 8:4579, 1988.  
 Ridgeway, In: *Vectors: A survey of molecular cloning vectors and their uses*, Rodriguez and Denhardt (Eds.), Stoneham: Butterworth, 467-492, 1988.  
 Riechmann et al., *Nature*, 332:323-327, 1988.  
 Rinaldo, Jr. et al., *Infect. Immun.*, 14:660-666, 1976.  
 Ripe et al., *Mol. Cell. Biol.*, 9:2224, 1989.  
 Rippe et al., *Mol. Cell. Biol.*, 10:689-695, 1990.  
 Rittling et al., *Nuc. Acids Res.*, 17:1619, 1989.  
 Robertson et al., *Arch. Virol.*, 143:2493-2503, 1998.  
 Rosen et al., *Cell*, 41:813, 1988.  
 Rowland-Jones, *J. Infect.*, 38:67-70, 1999.  
 Sabin et al., *J. Acquir. Immune Defic. Syndr.*, 19:546-547, 1998.  
 Sakai et al., *Genes and Dev.*, 2:1144, 1988.  
 Sambrook et al., In: *Molecular Cloning: A Laboratory Manual*, Vol. 1, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 7(7)19-17.29, 1989.  
 Satake et al., *J. Virology*, 62:970, 1988.  
 Sawyer et al., *AIDS Res. Hum. Retroviruses*, 6(3):341-356, 1990.  
 Schaffner et al., *J. Mol. Biol.*, 201:81, 1988.  
 Schmolke et al., *J. Virol.*, 72(5):4541-5, 1998.  
 Searle et al., *Mol. Cell. Biol.*, 5:1480, 1985.  
 Seipp et al., *J. Hepatology*, 30:570-579, 1999.  
 Sharp and Marciniak, *Cell*, 59:229, 1989.  
 Shaul and Ben-Levy, *EMBO J.*, 6:1913, 1987.  
 Sherman et al., *Mol. Cell. Biol.*, 9:50, 1989.  
 Shimizu, *J. Virol.*, 73:8411-8414, 1999.  
 Simons et al., *J. Virol.*, 70:6126-6135, 1996.  
 Simons et al., *Nature Med.*, 1:564-569, 1995a.

- Simons et al., *Proc. Natl. Acad. Sci. USA*, 92:3401-3405, 1995b.  
 Sleight and Lockett, *J. EMBO*, 4:3831, 1985.  
 Spalholz et al., *Cell*, 42:183, 1985.  
 5 Spandau and Lee, *J. Virology*, 62:427, 1988.  
 Spandidos and Wilkie, *EMBO J.*, 2:1193, 1983.  
 Stephens and Hentschel, *Biochem. J.*, 248:1, 1987.  
 Stuart et al., *Nature*, 317:828, 1985.  
 Sullivan and Peterlin, *Mol. Cell. Biol.*, 7:3315, 1987.  
 10 Suresh et al., *Methods in Enzymology*, 121:210-228, 1986.  
 Swartzendruber and Lehman, *J. Cell. Physiology*, 85:179, 1975.  
 Tacke et al., *Hepatology*, 26:1626-1633, 1997.  
 Takebe et al., *Mol. Cell. Biol.*, 8:466, 1988.  
 Tanaka et al., *J. Hepatology*, 27:1110-1112, 1997.  
 15 Tavernier et al., *Nature*, 301:634, 1983.  
 Taylor and Kingston, *Mol. Cell. Biol.*, 10:165, 1990a.  
 Taylor and Kingston, *Mol. Cell. Biol.*, 10:176, 1990b.  
 Taylor et al., *J. Biol. Chem.*, 264:15160, 1989.  
 Taylor et al., *Science* 285:107-110, 1999.  
 20 Temin, In: *Gene Transfer*, Kucherlapati (ed.), NY: Plenum Press, 149-188, 1986.  
 Thiesen et al., *J. Virology*, 62:614, 1988.  
 Thomas et al., *J. Infect. Dis.*, 177:539-542, 1998.  
 25 Toyoda et al., *J. Acquir. Immune Defic. Syndr.*, 17:209-213, 1998.  
 Traunecker et al., *EMBO*, 10:3655-3659, 1991.  
 Treisman, *Cell*, 42:889, 1985.  
 Tronche et al., *Mol. Biol. Med.*, 7:173, 1990.  
 Trudel and Constantini, *Genes and Dev.*, 6:954, 1987.  
 30 Tsumaki et al., *J. Biol. Chem.*, 273(36):22861-22864, 1998.  
 Tur-Kaspa et al., *Mol. Cell. Biol.*, 6:716-718, 1986.  
 Tyndell et al., *Nuc. Acids Res.*, 9:6231, 1981.  
 Vannice and Levinson, *J. Virology*, 62:1305, 1988.  
 Vasseur et al., *Proc Natl. Acad. Sci. USA*, 77:1068, 1980.  
 35 Verhoeyen et al., *Science*, 239:1534-1536, 1988.  
 Wang and Calame, *Cell*, 47:241, 1986.  
 Waterhouse et al., *Nucl. Acids Res.*, 21:2265-2266, 1993.  
 Weber et al., *Cell*, 36:983, 1984.  
 40 Weinberger et al. *Mol. Cell. Biol.*, 8:988, 1984.  
 Winoto and Baltimore, *Cell*, 59:649, 1989.  
 Wu and Wu, *Biochem.*, 27:887-892, 1988.  
 Wu and Wu, *J. Biol. Chem.*, 262:4429-4432, 1987.  
 Wu et al., *Biochem. Biophys. Res. Commun.*, 233(1):221-6, 1997.  
 45 Wu et al., *J. Med. Virol.*, 52:83-85, 1997.  
 Xiang et al., *J. Viral Hepat.*, 6:S16-S22, 1999.  
 Yang et al., *Proc. Nat'l Acad. Sci. USA*, 87:9568-9572, 1990.  
 Yützey et al., *Mol. Cell. Biol.*, 9:1397, 1989.  
 Zhao-Emonet, et al., *Biochim. Biophys. Acta*, 1442(2-3):109-19, 1998.

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 8

<210> SEQ ID NO 1  
 <211> LENGTH: 9377  
 <212> TYPE: DNA  
 <213> ORGANISM: Hepatitis GB virus C  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (534)..(9065)

<400> SEQUENCE: 1

ccccccccc ggcactgggt gcaagcccca gaaaccgacg cctactgaag tagacgtaat 60

-continued

ggccccgcgc cgaaccggcg accggccaaa aggtggtgga tgggtgatga cagggttggt	120
aggtcgtaaa tcccggctcat cctggtagcc actataggtg ggtcttaagg ggaggctacg	180
gtccctcttg cgcataatgga ggaaaagcgc acggtccaca ggtggttggtc ctaccgggtg	240
aataaggacc cggcgctagg cacgcogtta aaccgagccc gttactcccc tgggcaaacg	300
acgcccacgt acggteccacg tcgcccttca atgtctctct tgaccaatag gcgtagccgg	360
cgagttgaca aggaccagtg ggggcccgggc gggaggggga aggaccccc cegctgcct	420
tccccgggag gcgggaaatg catggggcca cccagctccg cggcggccta cagccggggt	480
agcccaagaa ccttcgggtg agggcgggtg gcatttcttt tcctataaccg atc atg	536
	Met
	1
gca gtc ctt ctg ctc cta ctc gtg gtg gag gcc ggg gct att tta gcc	584
Ala Val Leu Leu Leu Leu Val Val Glu Ala Gly Ala Ile Leu Ala	
	5 10 15
ccg gcc acc cat gct tgt agc gcg aaa ggg caa tat tts ctc aca aac	632
Pro Ala Thr His Ala Cys Ser Ala Lys Gly Gln Tyr Xaa Leu Thr Asn	
	20 25 30
tgt tgc gcc ctg gag gac ata ggc ttc tgc ctg gag ggc gga tgc ctg	680
Cys Cys Ala Leu Glu Asp Ile Gly Phe Cys Leu Glu Gly Gly Cys Leu	
	35 40 45
gtg gct ctg ggg tgc acc att tgc acc gac cgc tgc tgg cca ctg tat	728
Val Ala Leu Gly Cys Thr Ile Cys Thr Asp Arg Cys Trp Pro Leu Tyr	
	50 55 60 65
cag gcg ggt ttg gcc gtg cgg ccc ggc aag tcc gcc gcc cag ttg gtg	776
Gln Ala Gly Leu Ala Val Arg Pro Gly Lys Ser Ala Ala Gln Leu Val	
	70 75 80
ggg gaa ctc ggt agt ctc tac ggg ccc ttg tgc gtc tgc gct tat gtg	824
Gly Glu Leu Gly Ser Leu Tyr Gly Pro Leu Ser Val Ser Ala Tyr Val	
	85 90 95
gcc ggg atc ctg ggg ctt ggg gag gtc tac tgc ggg gtc ctc acc gtc	872
Ala Gly Ile Leu Gly Leu Gly Glu Val Tyr Ser Gly Val Leu Thr Val	
	100 105 110
ggg gtg gcg ttg acg cgc agg gtc tac ccg gtc ccg aac ctg acg tgt	920
Gly Val Ala Leu Thr Arg Arg Val Tyr Pro Val Pro Asn Leu Thr Cys	
	115 120 125
gca gta gag tgt gag ttg aag tgg gaa agt gag ttt tgg aga tgg act	968
Ala Val Glu Cys Glu Leu Lys Trp Glu Ser Glu Phe Trp Arg Trp Thr	
	130 135 140 145
gaa cag ctg gcc tca aac tac tgg att ctg gaa tac ctc tgg aag gtg	1016
Glu Gln Leu Ala Ser Asn Tyr Trp Ile Leu Glu Tyr Leu Trp Lys Val	
	150 155 160
cct ttc gac ttt tgg cgg gga gtg atg agc ctt act cct ctc ttg gtg	1064
Pro Phe Asp Phe Trp Arg Gly Val Met Ser Leu Thr Pro Leu Leu Val	
	165 170 175
tgc gtg gcg gcc ctc ctc ctg ctg gag cag cgt att gtc atg gtc ttc	1112
Cys Val Ala Ala Leu Leu Leu Leu Glu Gln Arg Ile Val Met Val Phe	
	180 185 190
ctc ctg gtc act atg gcg ggc atg tgc caa ggc gcg ccc gcc tca gtg	1160
Leu Leu Val Thr Met Ala Gly Met Ser Gln Gly Ala Pro Ala Ser Val	
	195 200 205
ttg ggg tca cgg cct ttc gag gcc ggg ttg act tgg cag tct tgt tct	1208
Leu Gly Ser Arg Pro Phe Glu Ala Gly Leu Thr Trp Gln Ser Cys Ser	
	210 215 220 225
tgc agg tgc aac ggg tcc cgc gtg ccg acg ggg gag agg gtt tgg gaa	1256
Cys Arg Ser Asn Gly Ser Arg Val Pro Thr Gly Glu Arg Val Trp Glu	
	230 235 240

-continued

cgt ggg aac gtc aca ctt ttg tgt gac tgc ccc aac ggt cct tgg gtg	1304
Arg Gly Asn Val Thr Leu Leu Cys Asp Cys Pro Asn Gly Pro Trp Val	
245 250 255	
tgg gtc ccg gcc ctt tgc cag gca atc gga tgg ggc gac cct atc act	1352
Trp Val Pro Ala Leu Cys Gln Ala Ile Gly Trp Gly Asp Pro Ile Thr	
260 265 270	
cat tgg agc cac gga caa aat cag tgg ccc ctt tct tgt ccc caa ttt	1400
His Trp Ser His Gly Gln Asn Gln Trp Pro Leu Ser Cys Pro Gln Phe	
275 280 285	
gtc tac ggc gcc gtt tca gtg acc tgc gtg tgg ggt tct gtg tct tgg	1448
Val Tyr Gly Ala Val Ser Val Thr Cys Val Trp Gly Ser Val Ser Trp	
290 295 300 305	
ttt gct tcc act ggg ggt cgc gac tcc aag gtt gat gtg tgg agt ttg	1496
Phe Ala Ser Thr Gly Gly Arg Asp Ser Lys Val Asp Val Trp Ser Leu	
310 315 320	
gtt cca gtt ggc tct gcc agc tgc acc ata gcc gca ctg gga tct tcg	1544
Val Pro Val Gly Ser Ala Ser Cys Thr Ile Ala Ala Leu Gly Ser Ser	
325 330 335	
gat cgc gac aca gtg gtt gag ctg tcc gag tgg gga att ccc tgc gcc	1592
Asp Arg Asp Thr Val Val Glu Leu Ser Glu Trp Gly Ile Pro Cys Ala	
340 345 350	
act tgt atc ctg gac agg cgg cct gcc tcg tgt ggc acc tgt gtg agg	1640
Thr Cys Ile Leu Asp Arg Arg Pro Ala Ser Cys Gly Thr Cys Val Arg	
355 360 365	
gac tgc tgg ccc gag acc ggg tcg gta cgt ttc cca ttc cac agg tgt	1688
Asp Cys Trp Pro Glu Thr Gly Ser Val Arg Phe Pro Phe His Arg Cys	
370 375 380 385	
ggc gcg gga ccg agg ctg acc aga gac ctt gag gct gtg ccc ttc gtc	1736
Gly Ala Gly Pro Arg Leu Thr Arg Asp Leu Glu Ala Val Pro Phe Val	
390 395 400	
aat agg aca act ccc ttc acc ata agg ggg ccc ctg ggc aac cag ggg	1784
Asn Arg Thr Thr Pro Phe Thr Ile Arg Gly Pro Leu Gly Asn Gln Gly	
405 410 415	
cga ggc aac ccg gtg cgg tcg ccc ttg ggt ttt ggg tcc tac acc atg	1832
Arg Gly Asn Pro Val Arg Ser Pro Leu Gly Phe Gly Ser Tyr Thr Met	
420 425 430	
acc aag atc cga gac tcc tta cac ttg gtg aaa tgt ccc acc cca gcc	1880
Thr Lys Ile Arg Asp Ser Leu His Leu Val Lys Cys Pro Thr Pro Ala	
435 440 445	
att gag cct ccc acc gga acg ttt ggg ttc ttc cca gga gtc ccc ccc	1928
Ile Glu Pro Pro Thr Gly Thr Phe Gly Phe Phe Pro Gly Val Pro Pro	
450 455 460 465	
ctt aac aac tgc atg ctt ctc ggc act gag gtg tca gag gta ttg ggt	1976
Leu Asn Asn Cys Met Leu Leu Gly Thr Glu Val Ser Glu Val Leu Gly	
470 475 480	
ggg gcg ggc ctc act ggg ggg ttt tac gaa cct ctg gtg cgg cgg tgt	2024
Gly Ala Gly Leu Thr Gly Gly Phe Tyr Glu Pro Leu Val Arg Arg Cys	
485 490 495	
tca gag ctg atg ggt cgg cgg aat ccg gtc tgc ccg ggg ttt gca tgg	2072
Ser Glu Leu Met Gly Arg Arg Asn Pro Val Cys Pro Gly Phe Ala Trp	
500 505 510	
ctc tct tcg gga cgg cct gat ggg ttc ata cat gtt cag ggc cac ttg	2120
Leu Ser Ser Gly Arg Pro Asp Gly Phe Ile His Val Gln Gly His Leu	
515 520 525	
cag gag gtg gat gcg ggc aac ttc att ccg ccc cca cgc tgg ttg ctc	2168
Gln Glu Val Asp Ala Gly Asn Phe Ile Pro Pro Arg Trp Leu Leu	
530 535 540 545	
ttg gac ttt gta ttt gtc ctg tta tac ctg atg aag ctg gca gag gca	2216
Leu Asp Phe Val Phe Val Leu Leu Tyr Leu Met Lys Leu Ala Glu Ala	
550 555 560	

-continued

cgg ttg gtc ccg ctg atc ctc ctc ctg cta tgg tgg tgg gtg aac cag	2264
Arg Leu Val Pro Leu Ile Leu Leu Leu Leu Trp Trp Trp Val Asn Gln	
565 570 575	
ttg gcg gtc ctt gkt gtg scg gct gck crc gcc gcc gtg gct gga gag	2312
Leu Ala Val Leu Xaa Val Xaa Ala Xaa Xaa Ala Val Ala Gly Glu	
580 585 590	
gtg ttt gcg ggc cct gcc ttg tcc tgg tgt ctg gcc cta ccc ttc gtg	2360
Val Phe Ala Gly Pro Ala Leu Ser Trp Cys Leu Gly Leu Pro Phe Val	
595 600 605	
agt atg atc ctg ggg cta gca aac ctg gtg ttg tac ttc cgc tgg atg	2408
Ser Met Ile Leu Gly Leu Ala Asn Leu Val Leu Tyr Phe Arg Trp Met	
610 615 620 625	
ggg cct caa cgc ctg atg ttc ctc gtg ttg tgg aag ctc gct cgg ggg	2456
Gly Pro Gln Arg Leu Met Phe Leu Val Leu Trp Lys Leu Ala Arg Gly	
630 635 640	
gct ttc ccg ctg gca tta ctg atg ggg att tcc gcc act cgc ggc cgc	2504
Ala Phe Pro Leu Ala Leu Leu Met Gly Ile Ser Ala Thr Arg Gly Arg	
645 650 655	
acc tct gtg ctt ggc gcc gaa ttc tgc ttt gat gtc acc ttt gaa gtg	2552
Thr Ser Val Leu Gly Ala Glu Phe Cys Phe Asp Val Thr Phe Glu Val	
660 665 670	
gac acg tca gtc ttg ggt tgg gtg gtt gct agt gtg gtg gct tgg gcc	2600
Asp Thr Ser Val Leu Gly Trp Val Val Ala Ser Val Val Ala Trp Ala	
675 680 685	
ata gcg ctc ctg agc tct atg agc gcg ggg ggg tgg aag cac aaa gcc	2648
Ile Ala Leu Leu Ser Ser Met Ser Ala Gly Gly Trp Lys His Lys Ala	
690 695 700 705	
ata atc tat agg acg tgg tgt aaa ggg tac cag gcy ctt cgc cag cgc	2696
Ile Ile Tyr Arg Thr Trp Cys Lys Gly Tyr Gln Xaa Leu Arg Gln Arg	
710 715 720	
gtg gtg cgt agc ccc ctc ggg gag ggg cgg ccc acc aag ccg ctg acg	2744
Val Val Arg Ser Pro Leu Gly Glu Gly Arg Pro Thr Lys Pro Leu Thr	
725 730 735	
ata gcc tgg tgt ctg gcc tct tac atc tgg ccg gac gct gtg atg ttg	2792
Ile Ala Trp Cys Leu Ala Ser Tyr Ile Trp Pro Asp Ala Val Met Leu	
740 745 750	
gtg gtt gtg gcc atg gtc ctc ctc ttc gcc ctt ttc gac gcg ctc gat	2840
Val Val Val Ala Met Val Leu Leu Phe Gly Leu Phe Asp Ala Leu Asp	
755 760 765	
tgg gcc ttg gag gag ctc ctt gtg tgc cgg cct tgc ttg cgt cgt ttg	2888
Trp Ala Leu Glu Glu Leu Leu Val Ser Arg Pro Ser Leu Arg Arg Leu	
770 775 780 785	
gca agg gtg gtg gag tgt tgt gtg atg gcg gcc gag aag gcc act acc	2936
Ala Arg Val Val Glu Cys Cys Val Met Ala Gly Glu Lys Ala Thr Thr	
790 795 800	
gtc cgg ctt gtg tcc aag atg tgc gcg aga ggg gcc tac ctg ttt gac	2984
Val Arg Leu Val Ser Lys Met Cys Ala Arg Gly Ala Tyr Leu Phe Asp	
805 810 815	
cac atg ggg tgc ttc tgc cgc gcg gtc aag gag cgc ttg ctg gag tgg	3032
His Met Gly Ser Phe Ser Arg Ala Val Lys Glu Arg Leu Leu Glu Trp	
820 825 830	
gac gcg gct ttg gag mcc ctg tca ttc act agg acg gac tgt cgc atc	3080
Asp Ala Ala Leu Glu Xaa Leu Ser Phe Thr Arg Thr Asp Cys Arg Ile	
835 840 845	
ata cga gac gcc gcc agg acc ctg agc tgc gcc caa tgc gtc atg gcc	3128
Ile Arg Asp Ala Ala Arg Thr Leu Ser Cys Gly Gln Cys Val Met Gly	
850 855 860 865	

-continued

ttg ccc gtg gtg gct agg cgc ggc gat gag gtc ctg att ggg gtc ttt	3176
Leu Pro Val Val Ala Arg Arg Gly Asp Glu Val Leu Ile Gly Val Phe	
870 875 880	
cag gat gtg aac cac ttg cct ccg ggg ttt gyt cct aca gcg cct gtt	3224
Gln Asp Val Asn His Leu Pro Pro Gly Phe Xaa Pro Thr Ala Pro Val	
885 890 895	
gtc atc cgt cgg tgc gga aag ggc ttc ctc ggg gtc act aag gct gcc	3272
Val Ile Arg Arg Cys Gly Lys Phe Leu Gly Val Thr Lys Ala Ala	
900 905 910	
ttg act ggt cgg gat cct gac tta cac cca gga aac gtc atg gtt ttg	3320
Leu Thr Gly Arg Asp Pro Asp Leu His Pro Gly Asn Val Met Val Leu	
915 920 925	
ggg acg gct acc tcg cgc agc atg gga acg tgc tta aac ggg ttg ctg	3368
Gly Thr Ala Thr Ser Arg Ser Met Gly Thr Cys Leu Asn Gly Leu Leu	
930 935 940 945	
ttc acg aca ttc cat ggg gct tct tcc cga acc att gcg aca cct gtg	3416
Phe Thr Thr Phe His Gly Ala Ser Ser Arg Thr Ile Ala Thr Pro Val	
950 955 960	
ggg gcc ctt aac cca agg tgg tgg tcg gcc agt gat gac gtc acg gtc	3464
Gly Ala Leu Asn Pro Arg Trp Trp Ser Ala Ser Asp Asp Val Thr Val	
965 970 975	
tat ccc ctc ccc gat gga gct aac tcg ttg gtt ccc tgc tcg tgt cag	3512
Tyr Pro Leu Pro Asp Gly Ala Asn Ser Leu Val Pro Cys Ser Cys Gln	
980 985 990	
gct gag tcc tgt tgg gtc aty cga tcc gat ggg gct ctt tgc cat ggc	3560
Ala Glu Ser Cys Trp Val Xaa Arg Ser Asp Gly Ala Leu Cys His Gly	
995 1000 1005	
ttg agc aag ggg gac aag gta gaa ctg gac gtg gcc atg gag gtt gct	3608
Leu Ser Lys Gly Asp Lys Val Glu Leu Asp Val Ala Met Glu Val Ala	
1010 1015 1020 1025	
gac ttt cgt ggg tcg tct ggg tct cct gtc cta tgc gac gag ggg cac	3656
Asp Phe Arg Gly Ser Ser Gly Ser Pro Val Leu Cys Asp Glu Gly His	
1030 1035 1040	
gct gta gga atg ctc gtg tcc gtc ctt cat tcg ggg ggg agg gtg acc	3704
Ala Val Gly Met Leu Val Ser Val Leu His Ser Gly Gly Arg Val Thr	
1045 1050 1055	
gcg gct cga ttc act cgg ccg tgg acc caa gtc cca aca gac gcc aag	3752
Ala Ala Arg Phe Thr Arg Pro Trp Thr Gln Val Pro Thr Asp Ala Lys	
1060 1065 1070	
act acc act gag cca ccc ccg gtg cca gct aaa ggg gtt ttc aaa gag	3800
Thr Thr Thr Glu Pro Pro Pro Val Pro Ala Lys Gly Val Phe Lys Glu	
1075 1080 1085	
gct cct ctt ttc atg cca aca ggg gcg ggg aaa agc aca cgc gtc cct	3848
Ala Pro Leu Phe Met Pro Thr Gly Ala Gly Lys Ser Thr Arg Val Pro	
1090 1095 1100 1105	
ttg gag tat gga aac atg ggg cac aag gtc ctg att ctc aac ccg tcg	3896
Leu Glu Tyr Gly Asn Met Gly His Lys Val Leu Ile Leu Asn Pro Ser	
1110 1115 1120	
gtt gcc act gtg agg gcc atg ggc cct tac atg gag agg ctg gcg ggg	3944
Val Ala Thr Val Arg Ala Met Gly Pro Tyr Met Glu Arg Leu Ala Gly	
1125 1130 1135	
aaa cat cct agc att ttc tgt gga cac gac aca aca gct ttc aca cgg	3992
Lys His Pro Ser Ile Phe Cys Gly His Asp Thr Thr Ala Phe Thr Arg	
1140 1145 1150	
atc acg gac tct cca ttg acg tac tct acc tat ggg agg ttt ctg gcc	4040
Ile Thr Asp Ser Pro Leu Thr Tyr Ser Thr Tyr Gly Arg Phe Leu Ala	
1155 1160 1165	
aac ccg agg cag atg ctg agg gga gtt tcc gtg gtc atc tgt gat gag	4088
Asn Pro Arg Gln Met Leu Arg Gly Val Ser Val Val Ile Cys Asp Glu	
1170 1175 1180 1185	

-continued

tgc cac agt cat gac tca act gtg ttg ctg ggt ata ggc agg gtc agg Cys His Ser His Asp Ser Thr Val Leu Leu Gly Ile Gly Arg Val Arg 1190 1195 1200	4136
gac gtg gcg cgg ggg tgt gga gtg caa tta gtg ctc tac gct act gcg Asp Val Ala Arg Gly Cys Gly Val Gln Leu Val Leu Tyr Ala Thr Ala 1205 1210 1215	4184
act ccc ccg ggc tcg cct atg act cag cat cca tcc ata att gag aca Thr Pro Pro Gly Ser Pro Met Thr Gln His Pro Ser Ile Ile Glu Thr 1220 1225 1230	4232
aag ctg gac gtt ggt gag atc ccc ttt tat ggg cat ggt atc ccc ctc Lys Leu Asp Val Gly Glu Ile Pro Phe Tyr Gly His Gly Ile Pro Leu 1235 1240 1245	4280
gag cgt atg agg act ggt cgc cac ctt gta ttc tgc cat tcc aag gcg Glu Arg Met Arg Thr Gly Arg His Leu Val Phe Cys His Ser Lys Ala 1250 1255 1260 1265	4328
gag tgc gag aga ttg gcc ggc cag ttc tcc gcg cgg ggg gtt aat gcc Glu Cys Glu Arg Leu Ala Gly Gln Phe Ser Ala Arg Gly Val Asn Ala 1270 1275 1280	4376
atc gcc tat tat agg ggt aag gac agt tcc atc atc aaa gac gga gac Ile Ala Tyr Tyr Arg Gly Lys Asp Ser Ser Ile Ile Lys Asp Gly Asp 1285 1290 1295	4424
ctg gtg gtt tgt gcg aca gac gcg ctc tct acc ggg tac aca gga aac Leu Val Val Cys Ala Thr Asp Ala Leu Ser Thr Gly Tyr Thr Gly Asn 1300 1305 1310	4472
ttc gat tct gtc acc gac tgt ggg ttg gtg gtg gag gag gtc gtt gag Phe Asp Ser Val Thr Asp Cys Gly Leu Val Val Glu Glu Val Val Glu 1315 1320 1325	4520
gtg acc ctt gat ccc acc att acc att tcc ttg cgg act gtc cct gct Val Thr Leu Asp Pro Thr Ile Thr Ile Ser Leu Arg Thr Val Pro Ala 1330 1335 1340 1345	4568
tcg gct gaa ttg tcg atg cag cgg cgc gga cgc acg ggg aga ggt cgg Ser Ala Glu Leu Ser Met Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg 1350 1355 1360	4616
tcg ggc cgc tac tac tac gct ggg gtc ggt aag gct ccc gcg ggg gtg Ser Gly Arg Tyr Tyr Tyr Ala Gly Val Gly Lys Ala Pro Ala Gly Val 1365 1370 1375	4664
gtg cgg tct ggt ccg gtc tgg tcg gca gtg gaa gct gga gtg acc tgg Val Arg Ser Gly Pro Val Trp Ser Ala Val Glu Ala Gly Val Thr Trp 1380 1385 1390	4712
tat gga atg gaa cct gac ttg aca gca aac ctt ctg aga ctt tac gac Tyr Gly Met Glu Pro Asp Leu Thr Ala Asn Leu Leu Arg Leu Tyr Asp 1395 1400 1405	4760
gac tgc cct tac acc gca gcc gtc gca gct gac att ggt gaa gcc gcg Asp Cys Pro Tyr Thr Ala Ala Val Ala Ala Asp Ile Gly Glu Ala Ala 1410 1415 1420 1425	4808
gtg ttc ttt gcg ggc ctc gcg ccc ctc agg atg cat ccc gat gtt agc Val Phe Phe Ala Gly Leu Ala Pro Leu Arg Met His Pro Asp Val Ser 1430 1435 1440	4856
tgg gca aaa gtt cgc ggc gtc aat tgg ccc ctc ctg gtg ggt gtt cag Trp Ala Lys Val Arg Gly Val Asn Trp Pro Leu Leu Val Gly Val Gln 1445 1450 1455	4904
cgg acg atg tgt ccg gaa aca ctg tct ccc ggc ccg tcg gac gac cct Arg Thr Met Cys Arg Glu Thr Leu Ser Pro Gly Pro Ser Asp Asp Pro 1460 1465 1470	4952
cag tgg gca ggt ctg aaa ggc ccg aat cct gtc cca cta ctg ctg agg Gln Trp Ala Gly Leu Lys Gly Pro Asn Pro Val Pro Leu Leu Leu Arg 1475 1480 1485	5000

-continued

tgg ggc aat gat ttg cca tca aaa gtg gcc ggc cac cac ata gtt gac	5048
Trp Gly Asn Asp Leu Pro Ser Lys Val Ala Gly His His Ile Val Asp	
1490 1495 1500 1505	
gat ctg gtc cgt cgg ctc ggt gtg gcg gag gga tac gtg cgc tgt gat	5096
Asp Leu Val Arg Arg Leu Gly Val Ala Glu Gly Tyr Val Arg Cys Asp	
1510 1515 1520	
gct ggr ccc atc ctc atg gtg ggc ttg gcc ata gcg ggc gcc atg atc	5144
Ala Xaa Pro Ile Leu Met Val Gly Leu Ala Ile Ala Gly Gly Met Ile	
1525 1530 1535	
tac gcc tct tac act ggg tcg cta gtg gtg gta aca gac tgg gat gtg	5192
Tyr Ala Ser Tyr Thr Gly Ser Leu Val Val Val Thr Asp Trp Asp Val	
1540 1545 1550	
aag gga ggt ggc aat ccc ctt tat agg agt ggt gac cag gcc acc cct	5240
Lys Gly Gly Gly Asn Pro Leu Tyr Arg Ser Gly Asp Gln Ala Thr Pro	
1555 1560 1565	
caa ccc gtg gtg cag gtc ccc ccg gta gac cat cgg ccg ggg ggg gag	5288
Gln Pro Val Val Gln Val Pro Pro Val Asp His Arg Pro Gly Gly Glu	
1570 1575 1580 1585	
tct gcg cca cgg gat gcc aag aca gtg aca gat gcg gtg gca gcc atc	5336
Ser Ala Pro Arg Asp Ala Lys Thr Val Thr Asp Ala Val Ala Ala Ile	
1590 1595 1600	
cag gtg aac tgc gat tgg tct gtg atg acc ctg tcg atc ggg gaa gtc	5384
Gln Val Asn Cys Asp Trp Ser Val Met Thr Leu Ser Ile Gly Glu Val	
1605 1610 1615	
ctc acc ttg gct cag gct aag aca gcc gag gcc tac gca gct act tcc	5432
Leu Thr Leu Ala Gln Ala Lys Thr Ala Glu Ala Tyr Ala Ala Thr Ser	
1620 1625 1630	
agg tgg ctc gct ggc tgc tac acg ggg acg cgg gcc gtc ccc act gta	5480
Arg Trp Leu Ala Gly Cys Tyr Thr Gly Thr Arg Ala Val Pro Thr Val	
1635 1640 1645	
tca att gtt gac aag ctc ttc gcc ggg ggt tgg gcc gcc gtg gtg ggt	5528
Ser Ile Val Asp Lys Leu Phe Ala Gly Gly Trp Ala Ala Val Val Gly	
1650 1655 1660 1665	
cac tgt cac agc gtc att gct gcg gcg gtg gct gcc tat gga gct tct	5576
His Cys His Ser Val Ile Ala Ala Val Ala Ala Tyr Gly Ala Ser	
1670 1675 1680	
cga agt cct cca ctg gcc gcg gcg gcg tcc tac ctc atg ggg ttg ggc	5624
Arg Ser Pro Pro Leu Ala Ala Ala Ala Ser Tyr Leu Met Gly Leu Gly	
1685 1690 1695	
gtc gga ggc aac gca cag gcg cgc ttg gct tca gct ctt cta ctg ggg	5672
Val Gly Gly Asn Ala Gln Ala Arg Leu Ala Ser Ala Leu Leu Leu Gly	
1700 1705 1710	
gct gct ggt acg gct ctg ggg acc cct gtc gtg gga ctc acc atg gcg	5720
Ala Ala Gly Thr Ala Leu Gly Thr Pro Val Val Gly Leu Thr Met Ala	
1715 1720 1725	
ggg gcc ttc atg ggc ggt gcc agc gtg tcc ccc tcc ctc gtc act gtc	5768
Gly Ala Phe Met Gly Gly Ala Ser Val Ser Pro Ser Leu Val Thr Val	
1730 1735 1740 1745	
cta ctt ggg gct gtg gga ggt tgg gag ggc gtt gtc aac gct gcc agt	5816
Leu Leu Gly Ala Val Gly Gly Trp Glu Gly Val Val Asn Ala Ala Ser	
1750 1755 1760	
ctc gtc ttc gac ttc atg gct ggg aaa ctt tca aca gaa gac ctt tgg	5864
Leu Val Phe Asp Phe Met Ala Gly Lys Leu Ser Thr Glu Asp Leu Trp	
1765 1770 1775	
tat gcc atc ccg gta ctc act agt cct ggr gcg ggc ctc gcg ggg att	5912
Tyr Ala Ile Pro Val Leu Thr Ser Pro Xaa Ala Gly Leu Ala Gly Ile	
1780 1785 1790	
gcc ctt ggt ctg gtt ttg tac tca gca aac aac tct ggc act acc aca	5960
Ala Leu Gly Leu Val Leu Tyr Ser Ala Asn Asn Ser Gly Thr Thr Thr	
1795 1800 1805	

-continued

tgg ctg aac cgt ctg ctg acg acg ttg cca cgg tca tct tgc ata ccc Trp Leu Asn Arg Leu Leu Thr Thr Leu Pro Arg Ser Ser Cys Ile Pro 1810 1815 1820 1825	6008
gac agc tac ttc caa cag gct gac tac tgc gac aag gtc tcg gca atc Asp Ser Tyr Phe Gln Gln Ala Asp Tyr Cys Asp Lys Val Ser Ala Ile 1830 1835 1840	6056
gtg cgc cgc ctg agc ctt act cgc acc gtg gtg gcc ctg gtc aac agg Val Arg Arg Leu Ser Leu Thr Arg Thr Val Val Ala Leu Val Asn Arg 1845 1850 1855	6104
gag cct aag gtg gat gag gtc cag gtg ggg tac gtc tgg gat ctg tgg Glu Pro Lys Val Asp Glu Val Gln Val Gly Tyr Val Trp Asp Leu Trp 1860 1865 1870	6152
gag tgg gtg atg cgc cag gtg cgc atg gtg atg tct aga ctc cgg gcc Glu Trp Val Met Arg Gln Val Arg Met Val Met Ser Arg Leu Arg Ala 1875 1880 1885	6200
ctc tgc cct gtg gtg tca ctc ccc ttg tgg cac tgc ggg gag ggg tgg Leu Cys Pro Val Val Ser Leu Pro Leu Trp His Cys Gly Glu Gly Trp 1890 1895 1900 1905	6248
tcc ggt gaa tgg ctt ctc gat ggg cac gtg gag agt cgt tgt ctg tgc Ser Gly Glu Trp Leu Leu Asp Gly His Val Glu Ser Arg Cys Leu Cys 1910 1915 1920	6296
ggg tgt gta atc acc ggc gac gtc ctc aat ggg caa ctc aaa gat cca Gly Cys Val Ile Thr Gly Asp Val Leu Asn Gly Gln Leu Lys Asp Pro 1925 1930 1935	6344
gtt tac tct acc aag ctg tgc agg cac tac tgg atg gga act gtg ccg Val Tyr Ser Thr Lys Leu Cys Arg His Tyr Trp Met Gly Thr Val Pro 1940 1945 1950	6392
gtc aac atg ctg ggc tac ggg gaa acc tca cct ctt ctc gcc tct gac Val Asn Met Leu Gly Tyr Gly Glu Thr Ser Pro Leu Leu Ala Ser Asp 1955 1960 1965	6440
acc ccg aag gtg gta ccc ttc ggg acg tcg ggg tgg gct gag gtg gtg Thr Pro Lys Val Val Pro Phe Gly Thr Ser Gly Trp Ala Glu Val Val 1970 1975 1980 1985	6488
gtg acc cct acc cac gtg gtg atc agg cgc acg tcc tgt tac aaa ctg Val Thr Pro Thr His Val Val Ile Arg Arg Thr Ser Cys Tyr Lys Leu 1990 1995 2000	6536
ctt cgc cag caa att ctt tca gca gct gta gct gag ccc tac tac gtt Leu Arg Gln Gln Ile Leu Ser Ala Val Ala Glu Pro Tyr Tyr Val 2005 2010 2015	6584
gat ggc att ccg gtc tct tgg gag gct gac gcg aga gcg ccg gcc atg Asp Gly Ile Pro Val Ser Trp Glu Ala Asp Ala Arg Ala Pro Ala Met 2020 2025 2030	6632
gtc tac ggt ccg ggc caa agt gtt acc att gat ggg gag cgc tac acc Val Tyr Gly Pro Gly Gln Ser Val Thr Ile Asp Gly Glu Arg Tyr Thr 2035 2040 2045	6680
ctt ccg cac cag ttg cgg atg cgg aat gtg gcg ccc tct gag gtt tca Leu Pro His Gln Leu Arg Met Arg Asn Val Ala Pro Ser Glu Val Ser 2050 2055 2060 2065	6728
tct gag gtc agc atc gag atc ggg acg gag act gaa gac tca gaa ctg Ser Glu Val Ser Ile Glu Ile Gly Thr Glu Thr Glu Asp Ser Glu Leu 2070 2075 2080	6776
act gag gcc gat ttg cca cca gcg gct gct gcc ctc caa gcg ata gag Thr Glu Ala Asp Leu Pro Pro Ala Ala Ala Leu Gln Ala Ile Glu 2085 2090 2095	6824
aat gct gcg aga att ctc gaa ccg cac atc gat gtc ayc atg gag gat Asn Ala Ala Arg Ile Leu Glu Pro His Ile Asp Val Xaa Met Glu Asp 2100 2105 2110	6872



-continued

tgc agt aca ccc tct ctc tgt ggt agt agc cga gag atg cct gtg tgg Cys Ser Thr Pro Ser Leu Cys Gly Ser Ser Arg Glu Met Pro Val Trp 2115 2120 2125	6920
gga gaa gac ata ccc cgc act cca tgc cct gca ctt atc tcg gtt acg Gly Glu Asp Ile Pro Arg Thr Pro Ser Pro Ala Leu Ile Ser Val Thr 2130 2135 2140 2145	6968
gag agc agc tca gat gag aag acc ctg tcg gtg acc tcc tcg cag gag Glu Ser Ser Ser Asp Glu Lys Thr Leu Ser Val Thr Ser Ser Gln Glu 2150 2155 2160	7016
gac acc ccg tcc tca gac tca ttt gaa gtc atc caa gag tct gat act Asp Thr Pro Ser Ser Asp Ser Phe Glu Val Ile Gln Glu Ser Asp Thr 2165 2170 2175	7064
gct gaa tca gag gaa agc gtc ttc aac gtg gct ctt tcc gta cta aaa Ala Glu Ser Glu Glu Ser Val Phe Asn Val Ala Leu Ser Val Leu Lys 2180 2185 2190	7112
gcc tta ttt cca cag agc gat gcc aca cga aag cta acg gtt aag atg Ala Leu Phe Pro Gln Ser Asp Ala Thr Arg Lys Leu Thr Val Lys Met 2195 2200 2205	7160
tct tgc tgt gtt gag aag agc gta aca cgc ttc ttt tct tta ggg ttg Ser Cys Cys Val Glu Lys Ser Val Thr Arg Phe Phe Ser Leu Gly Leu 2210 2215 2220 2225	7208
acc gtg gct gac gtg gct agc ctg tgt gag atg gag atc cag aac cat Thr Val Ala Asp Val Ala Ser Leu Cys Glu Met Glu Ile Gln Asn His 2230 2235 2240	7256
aca gcc tat tgt gac aag gtg cgc act ccg ctc gaa ttg caa gtt ggg Thr Ala Tyr Cys Asp Lys Val Arg Thr Pro Leu Glu Leu Gln Val Gly 2245 2250 2255	7304
tgc ttg gtg ggc aat gaa ctt acc ttt gaa tgt gac aag tgt gag gca Cys Leu Val Gly Asn Glu Leu Thr Phe Glu Cys Asp Lys Cys Glu Ala 2260 2265 2270	7352
cgc caa gag acc ctt gcc tcc ttc tcc tac ata tgg tcc ggg gtc cca Arg Gln Glu Thr Leu Ala Ser Phe Ser Tyr Ile Trp Ser Gly Val Pro 2275 2280 2285	7400
ctt act cgg gcc act ccg gcc aaa cca cca gtg gtg agg ccg gtg ggg Leu Thr Arg Ala Thr Pro Ala Lys Pro Pro Val Val Arg Pro Val Gly 2290 2295 2300 2305	7448
tcc ttg ttg gtg gca gac acc acc aag gtc tac gtg acc aat ccg gac Ser Leu Leu Val Ala Asp Thr Thr Lys Val Tyr Val Thr Asn Pro Asp 2310 2315 2320	7496
aat gtt ggg agg agg gtt gac aag gtg act ttc tgg cgc gct cct cgg Asn Val Gly Arg Arg Val Asp Lys Val Thr Phe Trp Arg Ala Pro Arg 2325 2330 2335	7544
gta cac gac aag ttc ctc gtg gac tcg atc gag cgc gct cgg aga gct Val His Asp Lys Phe Leu Val Asp Ser Ile Glu Arg Ala Arg Arg Ala 2340 2345 2350	7592
gct caa ggc tgc cta agc atg ggt tac act tat gag gag gca ata agg Ala Gln Gly Cys Leu Ser Met Gly Tyr Thr Tyr Glu Glu Ala Ile Arg 2355 2360 2365	7640
act gtt agg ccg cat gct gcc atg ggc tgg gga tct aag gtg tcg gtc Thr Val Arg Pro His Ala Ala Met Gly Trp Gly Ser Lys Val Ser Val 2370 2375 2380 2385	7688
aag gac ttg gcc acc cct gcg ggg aag atg gct gtt cat gac cgg ctt Lys Asp Leu Ala Thr Pro Ala Gly Lys Met Ala Val His Asp Arg Leu 2390 2395 2400	7736
cag gag ata ctt gaa ggg act ccg gtc cct ttt acc ctg act gtc aaa Gln Glu Ile Leu Glu Gly Thr Pro Val Pro Phe Thr Leu Thr Val Lys 2405 2410 2415	7784
aag gag gtg ttc ttc aaa gat cgt aag gag gag aag gcc ccc cgc ctc Lys Glu Val Phe Phe Lys Asp Arg Lys Glu Glu Lys Ala Pro Arg Leu 2420 2425 2430	7832

-continued

att gtg ttc ccc ccc ctg gac ttc cgg ata gct gaa aag ctc att ctg Ile Val Phe Pro Pro Leu Asp Phe Arg Ile Ala Glu Lys Leu Ile Leu 2435 2440 2445	7880
gga gac cgg ggg cgg gtt gca aag gcc ggt gtt ggg ggg gct tac gcc Gly Asp Pro Gly Arg Val Ala Lys Ala Gly Val Gly Gly Ala Tyr Ala 2450 2455 2460 2465	7928
ttc cag tac acc ccc aac cag cgg gtt aag gag atg cta aag ctg tgg Phe Gln Tyr Thr Pro Asn Gln Arg Val Lys Glu Met Leu Lys Leu Trp 2470 2475 2480	7976
gaa tca aag aag acc cgg tgc gcc atc tgt gtg gat gcc act tgc ttc Glu Ser Lys Lys Thr Pro Cys Ala Ile Cys Val Asp Ala Thr Cys Phe 2485 2490 2495	8024
gac agt agc att act gar gag gac gtg gca cta gag aca gag ctt tac Asp Ser Ser Ile Thr Glu Glu Asp Val Ala Leu Glu Thr Leu Leu Tyr 2500 2505 2510	8072
gcc ctg gcc tcg gac cat cca gaa tgg gtg cgc gcc ctg ggg aaa tac Ala Leu Ala Ser Asp His Pro Glu Trp Val Arg Ala Leu Gly Lys Tyr 2515 2520 2525	8120
trt gcc tct ggc aca atg gtg acc cgg gaa ggg gtg cca gtg ggc gag Xaa Ala Ser Gly Thr Met Val Thr Pro Glu Gly Val Pro Val Gly Glu 2530 2535 2540 2545	8168
agg tat tgt agg tcc tcg ggt gtg ttg acc aca agt gct agc aac tgt Arg Tyr Cys Arg Ser Ser Gly Val Leu Thr Thr Ser Ala Ser Asn Cys 2550 2555 2560	8216
ttg acc tgc tac atc aaa gtg aga gcc gcc tgt gag agg atc gga ctg Leu Thr Cys Tyr Ile Lys Val Arg Ala Ala Cys Glu Arg Ile Gly Leu 2565 2570 2575	8264
aaa aat gtc tcg ctt ctc atc gcg ggc gat gac tgc tta att gtg tgc Lys Asn Val Ser Leu Leu Ile Ala Gly Asp Asp Cys Leu Ile Val Cys 2580 2585 2590	8312
gag agg cct gta tgc gac cct tgc gag gcc ctg ggc cga acc ctg gct Glu Arg Pro Val Cys Asp Pro Cys Glu Ala Leu Gly Arg Thr Leu Ala 2595 2600 2605	8360
tcg tac ggg tac gcg tgt gag ccc tcg tat cac gct tca ctg gac aca Ser Tyr Gly Tyr Ala Cys Glu Pro Ser Tyr His Ala Ser Leu Asp Thr 2610 2615 2620 2625	8408
gcc ccc ttc tgc tcc act tgg ctc gct gag tgc aat gcg gat ggg raa Ala Pro Phe Cys Ser Thr Trp Leu Ala Glu Cys Asn Ala Asp Gly Xaa 2630 2635 2640	8456
agg cat ttc ttc ctg acc acg gac ttt cgg aga cca ctc gct cgc atg Arg His Phe Phe Leu Thr Thr Asp Phe Arg Arg Pro Leu Ala Arg Met 2645 2650 2655	8504
tcg agc gag tac agt gac cct atg gct tcg gcc att ggt tac att ctc Ser Ser Glu Tyr Ser Asp Pro Met Ala Ser Ala Ile Gly Tyr Ile Leu 2660 2665 2670	8552
ctc tac ccc tgg crt ccc atc aca cgg tgg gtc atc atc ccg cat gtg Leu Tyr Pro Trp Xaa Pro Ile Thr Arg Trp Val Ile Ile Pro His Val 2675 2680 2685	8600
cta aca tgc gct tct tcc cgg ggt ggt ggc aca csg tct gat cgg gtt Leu Thr Cys Ala Ser Ser Arg Gly Gly Gly Thr Xaa Ser Asp Pro Val 2690 2695 2700 2705	8648
tgg tgt cag gtt cat ggt aac tac tac aag ttt ccc ctg gac aaa ctg Trp Cys Gln Val His Gly Asn Tyr Tyr Lys Phe Pro Leu Asp Lys Leu 2710 2715 2720	8696
cct aac atc atc gtg gcc ctc cac gga cca gca gcg ttg agg gtt acc Pro Asn Ile Ile Val Ala Leu His Gly Pro Ala Ala Leu Arg Val Thr 2725 2730 2735	8744

-continued

---

```

gca gac aca acc aaa aca aag atg gag gct ggg aag gtt ctg agc gac      8792
Ala Asp Thr Thr Lys Thr Lys Met Glu Ala Gly Lys Val Leu Ser Asp
      2740                      2745                      2750

ctc aag ctc cct ggt cta gcc gtc cac cgc aag aag gcc ggg gca ttg      8840
Leu Lys Leu Pro Gly Leu Ala Val His Arg Lys Lys Ala Gly Ala Leu
      2755                      2760                      2765

cga aca cgc atg ctc cgg tgc cgc ggt tgg cgc gag ttg gct agg ggc      8888
Arg Thr Arg Met Leu Arg Ser Arg Gly Trp Ala Glu Leu Ala Arg Gly
      2770                      2775                      2780                      2785

ctg ttg tgg cat cca gga ctc cgg ctt cct ccc cct gag att gct ggt      8936
Leu Leu Trp His Pro Gly Leu Arg Leu Pro Pro Pro Glu Ile Ala Gly
      2790                      2795                      2800

atc cca ggg ggt ttc cct ctg tcc ccc ccc tac atg ggg gtg gtt cat      8984
Ile Pro Gly Gly Phe Pro Leu Ser Pro Pro Tyr Met Gly Val Val His
      2805                      2810                      2815

caa ttg gat ttc aca gcs cag cgg agt cgc tgg cgg tgg ttg ggg ttc      9032
Gln Leu Asp Phe Thr Xaa Gln Arg Ser Arg Trp Arg Trp Leu Gly Phe
      2820                      2825                      2830

tta gcc ctg ctc atc gta gcg ctc ttt ggg tga actaaattca tctggtgcgg      9085
Leu Ala Leu Leu Ile Val Ala Leu Phe Gly
      2835                      2840

caaggttgag cggtgatca cgctcaagg aggttccgc cctccccgc ccagggttct      9145

ccccgctggg taaaagggc ccggccttgg gaggcattgg ggttactaac cccctggcag      9205

ggttaacgcc tgatggtgct aatgcactgc cgcttcggcg gcgggtcgct acctatagc      9265

gtaatcogtg actacgggct gctcgagag ccctccccgg atggggcaca gtgcactgtg      9325

atctgaaggg gtgcaccccg gtaagagctc ggcccaaagg ccgggttcta ct      9377

```

```

<210> SEQ ID NO 2
<211> LENGTH: 2843
<212> TYPE: PRT
<213> ORGANISM: Hepatitis GB virus C
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (30)..(2823)
<223> OTHER INFORMATION: xaa = anything

```

```

<400> SEQUENCE: 2

```

```

Met Ala Val Leu Leu Leu Leu Val Val Glu Ala Gly Ala Ile Leu
  1           5           10          15

Ala Pro Ala Thr His Ala Cys Ser Ala Lys Gly Gln Tyr Xaa Leu Thr
  20           25           30

Asn Cys Cys Ala Leu Glu Asp Ile Gly Phe Cys Leu Glu Gly Gly Cys
  35           40           45

Leu Val Ala Leu Gly Cys Thr Ile Cys Thr Asp Arg Cys Trp Pro Leu
  50           55           60

Tyr Gln Ala Gly Leu Ala Val Arg Pro Gly Lys Ser Ala Ala Gln Leu
  65           70           75           80

Val Gly Glu Leu Gly Ser Leu Tyr Gly Pro Leu Ser Val Ser Ala Tyr
  85           90           95

Val Ala Gly Ile Leu Gly Leu Gly Glu Val Tyr Ser Gly Val Leu Thr
  100          105          110

Val Gly Val Ala Leu Thr Arg Arg Val Tyr Pro Val Pro Asn Leu Thr
  115          120          125

Cys Ala Val Glu Cys Glu Leu Lys Trp Glu Ser Glu Phe Trp Arg Trp
  130          135          140

Thr Glu Gln Leu Ala Ser Asn Tyr Trp Ile Leu Glu Tyr Leu Trp Lys
  145          150          155          160

```

-continued

---

Val Pro Phe Asp Phe Trp Arg Gly Val Met Ser Leu Thr Pro Leu Leu  
 165 170 175  
 Val Cys Val Ala Ala Leu Leu Leu Leu Glu Gln Arg Ile Val Met Val  
 180 185 190  
 Phe Leu Leu Val Thr Met Ala Gly Met Ser Gln Gly Ala Pro Ala Ser  
 195 200 205  
 Val Leu Gly Ser Arg Pro Phe Glu Ala Gly Leu Thr Trp Gln Ser Cys  
 210 215 220  
 Ser Cys Arg Ser Asn Gly Ser Arg Val Pro Thr Gly Glu Arg Val Trp  
 225 230 235 240  
 Glu Arg Gly Asn Val Thr Leu Leu Cys Asp Cys Pro Asn Gly Pro Trp  
 245 250 255  
 Val Trp Val Pro Ala Leu Cys Gln Ala Ile Gly Trp Gly Asp Pro Ile  
 260 265 270  
 Thr His Trp Ser His Gly Gln Asn Gln Trp Pro Leu Ser Cys Pro Gln  
 275 280 285  
 Phe Val Tyr Gly Ala Val Ser Val Thr Cys Val Trp Gly Ser Val Ser  
 290 295 300  
 Trp Phe Ala Ser Thr Gly Gly Arg Asp Ser Lys Val Asp Val Trp Ser  
 305 310 315 320  
 Leu Val Pro Val Gly Ser Ala Ser Cys Thr Ile Ala Ala Leu Gly Ser  
 325 330 335  
 Ser Asp Arg Asp Thr Val Val Glu Leu Ser Glu Trp Gly Ile Pro Cys  
 340 345 350  
 Ala Thr Cys Ile Leu Asp Arg Arg Pro Ala Ser Cys Gly Thr Cys Val  
 355 360 365  
 Arg Asp Cys Trp Pro Glu Thr Gly Ser Val Arg Phe Pro Phe His Arg  
 370 375 380  
 Cys Gly Ala Gly Pro Arg Leu Thr Arg Asp Leu Glu Ala Val Pro Phe  
 385 390 395 400  
 Val Asn Arg Thr Thr Pro Phe Thr Ile Arg Gly Pro Leu Gly Asn Gln  
 405 410 415  
 Gly Arg Gly Asn Pro Val Arg Ser Pro Leu Gly Phe Gly Ser Tyr Thr  
 420 425 430  
 Met Thr Lys Ile Arg Asp Ser Leu His Leu Val Lys Cys Pro Thr Pro  
 435 440 445  
 Ala Ile Glu Pro Pro Thr Gly Thr Phe Gly Phe Phe Pro Gly Val Pro  
 450 455 460  
 Pro Leu Asn Asn Cys Met Leu Leu Gly Thr Glu Val Ser Glu Val Leu  
 465 470 475 480  
 Gly Gly Ala Gly Leu Thr Gly Gly Phe Tyr Glu Pro Leu Val Arg Arg  
 485 490 495  
 Cys Ser Glu Leu Met Gly Arg Arg Asn Pro Val Cys Pro Gly Phe Ala  
 500 505 510  
 Trp Leu Ser Ser Gly Arg Pro Asp Gly Phe Ile His Val Gln Gly His  
 515 520 525  
 Leu Gln Glu Val Asp Ala Gly Asn Phe Ile Pro Pro Pro Arg Trp Leu  
 530 535 540  
 Leu Leu Asp Phe Val Phe Val Leu Leu Tyr Leu Met Lys Leu Ala Glu  
 545 550 555 560  
 Ala Arg Leu Val Pro Leu Ile Leu Leu Leu Leu Trp Trp Trp Val Asn  
 565 570 575

-continued

---

Gln Leu Ala Val Leu Xaa Val Xaa Ala Xaa Xaa Ala Ala Val Ala Gly  
 580 585 590

Glu Val Phe Ala Gly Pro Ala Leu Ser Trp Cys Leu Gly Leu Pro Phe  
 595 600 605

Val Ser Met Ile Leu Gly Leu Ala Asn Leu Val Leu Tyr Phe Arg Trp  
 610 615 620

Met Gly Pro Gln Arg Leu Met Phe Leu Val Leu Trp Lys Leu Ala Arg  
 625 630 635 640

Gly Ala Phe Pro Leu Ala Leu Leu Met Gly Ile Ser Ala Thr Arg Gly  
 645 650 655

Arg Thr Ser Val Leu Gly Ala Glu Phe Cys Phe Asp Val Thr Phe Glu  
 660 665 670

Val Asp Thr Ser Val Leu Gly Trp Val Val Ala Ser Val Val Ala Trp  
 675 680 685

Ala Ile Ala Leu Leu Ser Ser Met Ser Ala Gly Gly Trp Lys His Lys  
 690 695 700

Ala Ile Ile Tyr Arg Thr Trp Cys Lys Gly Tyr Gln Xaa Leu Arg Gln  
 705 710 715 720

Arg Val Val Arg Ser Pro Leu Gly Glu Gly Arg Pro Thr Lys Pro Leu  
 725 730 735

Thr Ile Ala Trp Cys Leu Ala Ser Tyr Ile Trp Pro Asp Ala Val Met  
 740 745 750

Leu Val Val Val Ala Met Val Leu Leu Phe Gly Leu Phe Asp Ala Leu  
 755 760 765

Asp Trp Ala Leu Glu Glu Leu Leu Val Ser Arg Pro Ser Leu Arg Arg  
 770 775 780

Leu Ala Arg Val Val Glu Cys Cys Val Met Ala Gly Glu Lys Ala Thr  
 785 790 795 800

Thr Val Arg Leu Val Ser Lys Met Cys Ala Arg Gly Ala Tyr Leu Phe  
 805 810 815

Asp His Met Gly Ser Phe Ser Arg Ala Val Lys Glu Arg Leu Leu Glu  
 820 825 830

Trp Asp Ala Ala Leu Glu Xaa Leu Ser Phe Thr Arg Thr Asp Cys Arg  
 835 840 845

Ile Ile Arg Asp Ala Ala Arg Thr Leu Ser Cys Gly Gln Cys Val Met  
 850 855 860

Gly Leu Pro Val Val Ala Arg Arg Gly Asp Glu Val Leu Ile Gly Val  
 865 870 875 880

Phe Gln Asp Val Asn His Leu Pro Pro Gly Phe Xaa Pro Thr Ala Pro  
 885 890 895

Val Val Ile Arg Arg Cys Gly Lys Gly Phe Leu Gly Val Thr Lys Ala  
 900 905 910

Ala Leu Thr Gly Arg Asp Pro Asp Leu His Pro Gly Asn Val Met Val  
 915 920 925

Leu Gly Thr Ala Thr Ser Arg Ser Met Gly Thr Cys Leu Asn Gly Leu  
 930 935 940

Leu Phe Thr Thr Phe His Gly Ala Ser Ser Arg Thr Ile Ala Thr Pro  
 945 950 955 960

Val Gly Ala Leu Asn Pro Arg Trp Trp Ser Ala Ser Asp Asp Val Thr  
 965 970 975

Val Tyr Pro Leu Pro Asp Gly Ala Asn Ser Leu Val Pro Cys Ser Cys  
 980 985 990

-continued

---

Gln Ala Glu Ser Cys Trp Val Xaa Arg Ser Asp Gly Ala Leu Cys His  
 995 1000 1005

Gly Leu Ser Lys Gly Asp Lys Val Glu Leu Asp Val Ala Met Glu Val  
 1010 1015 1020

Ala Asp Phe Arg Gly Ser Ser Gly Ser Pro Val Leu Cys Asp Glu Gly  
 1025 1030 1035 1040

His Ala Val Gly Met Leu Val Ser Val Leu His Ser Gly Gly Arg Val  
 1045 1050 1055

Thr Ala Ala Arg Phe Thr Arg Pro Trp Thr Gln Val Pro Thr Asp Ala  
 1060 1065 1070

Lys Thr Thr Thr Glu Pro Pro Pro Val Pro Ala Lys Gly Val Phe Lys  
 1075 1080 1085

Glu Ala Pro Leu Phe Met Pro Thr Gly Ala Gly Lys Ser Thr Arg Val  
 1090 1095 1100

Pro Leu Glu Tyr Gly Asn Met Gly His Lys Val Leu Ile Leu Asn Pro  
 1105 1110 1115 1120

Ser Val Ala Thr Val Arg Ala Met Gly Pro Tyr Met Glu Arg Leu Ala  
 1125 1130 1135

Gly Lys His Pro Ser Ile Phe Cys Gly His Asp Thr Thr Ala Phe Thr  
 1140 1145 1150

Arg Ile Thr Asp Ser Pro Leu Thr Tyr Ser Thr Tyr Gly Arg Phe Leu  
 1155 1160 1165

Ala Asn Pro Arg Gln Met Leu Arg Gly Val Ser Val Val Ile Cys Asp  
 1170 1175 1180

Glu Cys His Ser His Asp Ser Thr Val Leu Leu Gly Ile Gly Arg Val  
 1185 1190 1195 1200

Arg Asp Val Ala Arg Gly Cys Gly Val Gln Leu Val Leu Tyr Ala Thr  
 1205 1210 1215

Ala Thr Pro Pro Gly Ser Pro Met Thr Gln His Pro Ser Ile Ile Glu  
 1220 1225 1230

Thr Lys Leu Asp Val Gly Glu Ile Pro Phe Tyr Gly His Gly Ile Pro  
 1235 1240 1245

Leu Glu Arg Met Arg Thr Gly Arg His Leu Val Phe Cys His Ser Lys  
 1250 1255 1260

Ala Glu Cys Glu Arg Leu Ala Gly Gln Phe Ser Ala Arg Gly Val Asn  
 1265 1270 1275 1280

Ala Ile Ala Tyr Tyr Arg Gly Lys Asp Ser Ser Ile Ile Lys Asp Gly  
 1285 1290 1295

Asp Leu Val Val Cys Ala Thr Asp Ala Leu Ser Thr Gly Tyr Thr Gly  
 1300 1305 1310

Asn Phe Asp Ser Val Thr Asp Cys Gly Leu Val Val Glu Glu Val Val  
 1315 1320 1325

Glu Val Thr Leu Asp Pro Thr Ile Thr Ile Ser Leu Arg Thr Val Pro  
 1330 1335 1340

Ala Ser Ala Glu Leu Ser Met Gln Arg Arg Gly Arg Thr Gly Arg Gly  
 1345 1350 1355 1360

Arg Ser Gly Arg Tyr Tyr Tyr Ala Gly Val Gly Lys Ala Pro Ala Gly  
 1365 1370 1375

Val Val Arg Ser Gly Pro Val Trp Ser Ala Val Glu Ala Gly Val Thr  
 1380 1385 1390

Trp Tyr Gly Met Glu Pro Asp Leu Thr Ala Asn Leu Leu Arg Leu Tyr  
 1395 1400 1405

-continued

---

Asp Asp Cys Pro Tyr Thr Ala Ala Val Ala Ala Asp Ile Gly Glu Ala  
 1410 1415 1420  
 Ala Val Phe Phe Ala Gly Leu Ala Pro Leu Arg Met His Pro Asp Val  
 1425 1430 1435 1440  
 Ser Trp Ala Lys Val Arg Gly Val Asn Trp Pro Leu Leu Val Gly Val  
 1445 1450 1455  
 Gln Arg Thr Met Cys Arg Glu Thr Leu Ser Pro Gly Pro Ser Asp Asp  
 1460 1465 1470  
 Pro Gln Trp Ala Gly Leu Lys Gly Pro Asn Pro Val Pro Leu Leu Leu  
 1475 1480 1485  
 Arg Trp Gly Asn Asp Leu Pro Ser Lys Val Ala Gly His His Ile Val  
 1490 1495 1500  
 Asp Asp Leu Val Arg Arg Leu Gly Val Ala Glu Gly Tyr Val Arg Cys  
 1505 1510 1515 1520  
 Asp Ala Xaa Pro Ile Leu Met Val Gly Leu Ala Ile Ala Gly Gly Met  
 1525 1530 1535  
 Ile Tyr Ala Ser Tyr Thr Gly Ser Leu Val Val Val Thr Asp Trp Asp  
 1540 1545 1550  
 Val Lys Gly Gly Gly Asn Pro Leu Tyr Arg Ser Gly Asp Gln Ala Thr  
 1555 1560 1565  
 Pro Gln Pro Val Val Gln Val Pro Pro Val Asp His Arg Pro Gly Gly  
 1570 1575 1580  
 Glu Ser Ala Pro Arg Asp Ala Lys Thr Val Thr Asp Ala Val Ala Ala  
 1585 1590 1595 1600  
 Ile Gln Val Asn Cys Asp Trp Ser Val Met Thr Leu Ser Ile Gly Glu  
 1605 1610 1615  
 Val Leu Thr Leu Ala Gln Ala Lys Thr Ala Glu Ala Tyr Ala Ala Thr  
 1620 1625 1630  
 Ser Arg Trp Leu Ala Gly Cys Tyr Thr Gly Thr Arg Ala Val Pro Thr  
 1635 1640 1645  
 Val Ser Ile Val Asp Lys Leu Phe Ala Gly Gly Trp Ala Ala Val Val  
 1650 1655 1660  
 Gly His Cys His Ser Val Ile Ala Ala Ala Val Ala Ala Tyr Gly Ala  
 1665 1670 1675 1680  
 Ser Arg Ser Pro Pro Leu Ala Ala Ala Ala Ser Tyr Leu Met Gly Leu  
 1685 1690 1695  
 Gly Val Gly Gly Asn Ala Gln Ala Arg Leu Ala Ser Ala Leu Leu Leu  
 1700 1705 1710  
 Gly Ala Ala Gly Thr Ala Leu Gly Thr Pro Val Val Gly Leu Thr Met  
 1715 1720 1725  
 Ala Gly Ala Phe Met Gly Gly Ala Ser Val Ser Pro Ser Leu Val Thr  
 1730 1735 1740  
 Val Leu Leu Gly Ala Val Gly Gly Trp Glu Gly Val Val Asn Ala Ala  
 1745 1750 1755 1760  
 Ser Leu Val Phe Asp Phe Met Ala Gly Lys Leu Ser Thr Glu Asp Leu  
 1765 1770 1775  
 Trp Tyr Ala Ile Pro Val Leu Thr Ser Pro Xaa Ala Gly Leu Ala Gly  
 1780 1785 1790  
 Ile Ala Leu Gly Leu Val Leu Tyr Ser Ala Asn Asn Ser Gly Thr Thr  
 1795 1800 1805  
 Thr Trp Leu Asn Arg Leu Leu Thr Thr Leu Pro Arg Ser Ser Cys Ile  
 1810 1815 1820

-continued

---

Pro Asp Ser Tyr Phe Gln Gln Ala Asp Tyr Cys Asp Lys Val Ser Ala  
 1825 1830 1835 1840

Ile Val Arg Arg Leu Ser Leu Thr Arg Thr Val Val Ala Leu Val Asn  
 1845 1850 1855

Arg Glu Pro Lys Val Asp Glu Val Gln Val Gly Tyr Val Trp Asp Leu  
 1860 1865 1870

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 Gln 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 Glu 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

Val Asp Gly Ile Pro Val Ser Trp Glu Ala Asp Ala Arg Ala Pro Ala  
 2020 2025 2030

Met Val Tyr Gly Pro Gly Gln Ser Val Thr Ile Asp Gly Glu Arg Tyr  
 2035 2040 2045

Thr Leu Pro His Gln Leu Arg Met Arg Asn Val Ala Pro Ser Glu Val  
 2050 2055 2060

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 Glu 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

Leu Thr Val Ala Asp Val Ala Ser Leu Cys Glu Met Glu Ile Gln Asn  
 2225 2230 2235 2240



-continued

---

His Thr Ala Tyr Cys Asp Lys Val Arg Thr Pro Leu Glu Leu Gln Val  
 2245 2250 2255

Gly Cys Leu Val Gly Asn Glu Leu Thr Phe Glu Cys Asp Lys Cys Glu  
 2260 2265 2270

Ala Arg Gln Glu Thr Leu Ala Ser Phe Ser Tyr Ile Trp Ser Gly Val  
 2275 2280 2285

Pro Leu Thr Arg Ala Thr Pro Ala Lys Pro Pro Val Val Arg Pro Val  
 2290 2295 2300

Gly Ser Leu Leu Val Ala Asp Thr Thr Lys Val Tyr Val Thr Asn Pro  
 2305 2310 2315 2320

Asp Asn Val Gly Arg Arg Val Asp Lys Val Thr Phe Trp Arg Ala Pro  
 2325 2330 2335

Arg Val His Asp Lys Phe Leu Val Asp Ser Ile Glu Arg Ala Arg Arg  
 2340 2345 2350

Ala Ala Gln Gly Cys Leu Ser Met Gly Tyr Thr Tyr Glu Glu Ala Ile  
 2355 2360 2365

Arg Thr Val Arg Pro His Ala Ala Met Gly Trp Gly Ser Lys Val Ser  
 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

Leu Gly Asp Pro Gly Arg Val Ala Lys Ala Gly Val Gly Gly Ala Tyr  
 2450 2455 2460

Ala Phe Gln Tyr Thr Pro Asn Gln Arg Val Lys Glu Met Leu Lys Leu  
 2465 2470 2475 2480

Trp Glu Ser Lys Lys Thr Pro Cys Ala Ile Cys Val Asp Ala Thr Cys  
 2485 2490 2495

Phe Asp Ser Ser Ile Thr Glu Glu Asp Val Ala Leu Glu Thr Glu Leu  
 2500 2505 2510

Tyr Ala Leu Ala Ser Asp His Pro Glu Trp Val Arg Ala Leu Gly Lys  
 2515 2520 2525

Tyr Xaa Ala Ser Gly Thr Met Val Thr Pro Glu Gly Val Pro Val Gly  
 2530 2535 2540

Glu Arg Tyr Cys Arg Ser Ser Gly Val Leu Thr Thr Ser Ala Ser Asn  
 2545 2550 2555 2560

Cys Leu Thr Cys Tyr Ile Lys Val Arg Ala Ala Cys Glu Arg Ile Gly  
 2565 2570 2575

Leu Lys Asn Val Ser Leu Leu Ile Ala Gly Asp Asp Cys Leu Ile Val  
 2580 2585 2590

Cys Glu Arg Pro Val Cys Asp Pro Cys Glu Ala Leu Gly Arg Thr Leu  
 2595 2600 2605

Ala Ser Tyr Gly Tyr Ala Cys Glu Pro Ser Tyr His Ala Ser Leu Asp  
 2610 2615 2620

Thr Ala Pro Phe Cys Ser Thr Trp Leu Ala Glu Cys Asn Ala Asp Gly  
 2625 2630 2635 2640

Xaa Arg His Phe Phe Leu Thr Thr Asp Phe Arg Arg Pro Leu Ala Arg  
 2645 2650 2655

-continued

Met Ser Ser Glu Tyr Ser Asp Pro Met Ala Ser Ala Ile Gly Tyr Ile  
 2660 2665 2670  
 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 Ser Arg Gly Gly Gly Thr Xaa Ser Asp Pro  
 2690 2695 2700  
 Val Trp Cys Gln Val His Gly Asn Tyr Tyr Lys Phe Pro Leu Asp Lys  
 2705 2710 2715 2720  
 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 Lys Val Leu Ser  
 2740 2745 2750  
 Asp Leu Lys Leu Pro Gly Leu Ala Val His Arg Lys 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 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  
 2820 2825 2830  
 Phe Leu Ala Leu Leu Ile Val Ala Leu Phe Gly  
 2835 2840

<210> SEQ ID NO 3  
 <211> LENGTH: 936  
 <212> TYPE: DNA  
 <213> ORGANISM: Hepatitis GB virus C  
 <220> FEATURE:  
 <221> NAME/KEY: CDS  
 <222> LOCATION: (1)..(936)

<400> SEQUENCE: 3

acc ata gcc gca ctg gga tct tcg gat cgc gac aca gtg gtt gag ctc	48
Thr Ile Ala Ala Leu Gly Ser Ser Asp Arg Asp Thr Val Val Glu Leu	
1 5 10 15	
tcc gag tgg gga att ccc tgc gcc act tgt atc ctg gac agg cgg cct	96
Ser Glu Trp Gly Ile Pro Cys Ala Thr Cys Ile Leu Asp Arg Arg Pro	
20 25 30	
gcc tcg tgt ggc acc tgt gtg agg gac tgc tgg ccc gag acc ggg tcg	144
Ala Ser Cys Gly Thr Cys Val Arg Asp Cys Trp Pro Glu Thr Gly Ser	
35 40 45	
gta cgt ttc cca ttc cac agg tgt ggc gcg gga ccg agg ctg acc aga	192
Val Arg Phe Pro Phe His Arg Cys Gly Ala Gly Pro Arg Leu Thr Arg	
50 55 60	
gac ctt gag gct gtg ccc ttc gtc aat agg aca act ccc ttc acc ata	240
Asp Leu Glu Ala Val Pro Phe Val Asn Arg Thr Thr Pro Phe Thr Ile	
65 70 75 80	
agg ggg ccc ctg ggc aac cag ggg cga ggc aac ccg gtg cgg tcg ccc	288
Arg Gly Pro Leu Gly Asn Gln Gly Arg Gly Asn Pro Val Arg Ser Pro	
85 90 95	
ttg ggt ttt ggg tcc tac acc atg acc aag atc cga gac tcc tta cac	336
Leu Gly Phe Gly Ser Tyr Thr Met Thr Lys Ile Arg Asp Ser Leu His	
100 105 110	
ttg gtg aaa tgt ccc acc cca gcc att gag cct ccc acc gga acg ttt	384
Leu Val Lys Cys Pro Thr Pro Ala Ile Glu Pro Pro Thr Gly Thr Phe	
115 120 125	

-continued

---

```

ggg ttc ttc cca gga gtc ccc ccc ctt aac aac tgc atg ctt ctc ggc      432
Gly Phe Phe Pro Gly Val Pro Pro Leu Asn Asn Cys Met Leu Leu Gly
    130                      135                      140

act gag gtg tca gag gta ttg ggt ggg gcg ggc ctc act ggg ggg ttt      480
Thr Glu Val Ser Glu Val Leu Gly Gly Ala Gly Leu Thr Gly Gly Phe
    145                      150                      155                      160

tac gaa cct ctg gtg cgg cgg tgt tca gag ctg atg ggt cgg cgg aat      528
Tyr Glu Pro Leu Val Arg Arg Cys Ser Glu Leu Met Gly Arg Arg Asn
    165                      170                      175

ccg gtc tgc ccg ggg ttt gca tgg ctc tct tcg gga cgg cct gat ggg      576
Pro Val Cys Pro Gly Phe Ala Trp Leu Ser Ser Gly Arg Pro Asp Gly
    180                      185                      190

ttc ata cat gtt cag ggc cac ttg cag gag gtg gat gcg ggc aac ttc      624
Phe Ile His Val Gln Gly His Leu Gln Glu Val Asp Ala Gly Asn Phe
    195                      200                      205

att ccg ccc cca cgc tgg ttg ctc ttg gac ttt gta ttt gtc ctg tta      672
Ile Pro Pro Pro Arg Trp Leu Leu Leu Asp Phe Val Phe Val Leu Leu
    210                      215                      220

tac ctg atg aag ctg gca gag gca cgg ttg gtc ccg ctg atc ctc ctc      720
Tyr Leu Met Lys Leu Ala Glu Ala Arg Leu Val Pro Leu Ile Leu Leu
    225                      230                      235                      240

ctg cta tgg tgg tgg gtg aac cag ttg gcg gtc ctt gkt gtg scg gct      768
Leu Leu Trp Trp Trp Val Asn Gln Leu Ala Val Leu Xaa Val Xaa Ala
    245                      250                      255

gck crc gcc gcc gtg gct gga gag gtg ttt gcg ggc cct gcc ttg tcc      816
Xaa Xaa Ala Ala Val Ala Gly Glu Val Phe Ala Gly Pro Ala Leu Ser
    260                      265                      270

tgg tgt ctg gcc cta ccc ttc gtg agt atg atc ctg ggg cta gca aac      864
Trp Cys Leu Gly Leu Pro Phe Val Ser Met Ile Leu Gly Leu Ala Asn
    275                      280                      285

ctg gtg ttg tac ttc cgc tgg atg ggt cct caa cgc ctg atg ttc ctc      912
Leu Val Leu Tyr Phe Arg Trp Met Gly Pro Gln Arg Leu Met Phe Leu
    290                      295                      300

gtg ttg tgg aag ctc gct cgg ggg
Val Leu Trp Lys Leu Ala Arg Gly      936
    305                      310

```

```

<210> SEQ ID NO 4
<211> LENGTH: 312
<212> TYPE: PRT
<213> ORGANISM: Hepatitis GB virus C
<220> FEATURE:
<221> NAME/KEY: MOD_RES
<222> LOCATION: (253)..(258)
<223> OTHER INFORMATION: xaa = anything

```

```

<400> SEQUENCE: 4

```

```

Thr Ile Ala Ala Leu Gly Ser Ser Asp Arg Asp Thr Val Val Glu Leu
  1           5           10           15

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           80

Arg Gly Pro Leu Gly Asn Gln Gly Arg Gly Asn Pro Val Arg Ser Pro
    85           90           95

```

-continued

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 Gln Gly His Leu Gln Glu Val Asp Ala Gly Asn 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 Gln Leu Ala Val Leu Xaa Val Xaa Ala  
 245 250 255  
 Xaa Xaa Ala Ala Val Ala Gly Glu 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  
 Leu Val Leu Tyr Phe Arg Trp Met Gly Pro Gln Arg Leu Met Phe Leu  
 290 295 300  
 Val Leu Trp Lys Leu Ala Arg Gly  
 305 310

<210> SEQ ID NO 5  
 <211> LENGTH: 9  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 Peptide

<400> SEQUENCE: 5

Leu Thr Gly Gly Phe Tyr Glu Pro Leu  
 1 5

<210> SEQ ID NO 6  
 <211> LENGTH: 17  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic  
 Peptide

<400> SEQUENCE: 6

Gly Gly Ala Gly Leu Thr Gly Gly Phe Tyr Glu Pro Leu Val Arg Arg  
 1 5 10 15

Cys

<210> SEQ ID NO 7  
 <211> LENGTH: 13  
 <212> TYPE: PRT  
 <213> ORGANISM: Artificial Sequence  
 <220> FEATURE:  
 <223> OTHER INFORMATION: Description of Artificial Sequence: Synthetic



UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 8,858,946 B2  
APPLICATION NO. : 12/179399  
DATED : October 14, 2014  
INVENTOR(S) : Jack T. Stapleton et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

On title page, item (56) References Cited - Other Publications, delete the 3rd reference on page 2 "Chakraborty et al., "Viral coinfections among African children infected with human immunodeficiency virus type 1," *Clin. Infect. Dis.* 36:922-924." and replace with --Chakraborty et al., "Viral coinfections among African children infected with human immunodeficiency virus type 1," *Clin. Infect. Dis.* 36:922-924, 2003.-- therefor.

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



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