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| <b>(54) Title:</b> ANTIBODY-BASED TREATMENT OF HIV INFECTION<br><br><b>(57) Abstract</b><br><br>An approach to slowing or preventing the progression of HIV infection in an HIV-exposed individual, and to preventing HIV infection in an individual at risk of HIV infection, entails providing antibody:antigen complex or antibody:antigen:complement complex to the individual in titers adequate to competitively inhibit HIV complexes from binding cells via Fc receptors or complement receptors in lymphoid tissue. This approach employs antibodies that bind to cellular antigens to generate antigen:antibody complexes and/or antigen:antibody:complement complexes, which in turn inhibit or prevent the binding of HIV:antibody complexes or HIV:antibody:complement complexes or HIV:complement complexes to cells in lymph tissue. By restricting the concentration of HIV complexes bound to lymph tissue, the load of virally infected cells decreases, increasing the effectiveness of anti-retroviral agents and increasing virus inactivation in the bloodstream. In addition, the decreased concentration or absence of HIV in lymph tissue hinders infection of CD4 <sup>+</sup> T cells there. |           |                                                                                                                                                                                                                                                                                                                                                   |

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**ANTIBODY-BASED TREATMENT OF HIV INFECTION**Background of the Invention

The present invention relates to the use of antibody which recognizes a cell surface antigen, particularly a red blood cell surface antigen, for the purposes of competitively inhibiting the binding of HIV to Fc receptor- and complement receptor-containing cells in someone at risk of HIV infection or an individual already infected.

Current therapies for HIV infection and immune restoration generally have failed to improve immune function or reduce viral replication fundamentally. Nearly all HIV-infected individuals who have been subjected to existing therapies, including treatment with reverse transcriptase inhibitors, nevertheless have progressed to AIDS. Why conventional therapies cannot stop the clinical advancement to full-blown AIDS in such individuals remains one of the most important questions facing those involved in treating and preventing HIV infection.

Anti-retrovirals, such as AZT and ddI, only reduce the burden of HIV by about one-half to one-tenth of the initial value. See Piatak et al., *Science* 259: 1749 (1993); Holodniy, *J. Cell Biochem. Suppl.* 17E: 3 (1993); S. Aoki et al., *AIDS Res. Hum. Retroviruses* 6: 1331 (1990). While these reverse transcriptase inhibitors should protect new cells from infection, HIV-infected individuals still progress to AIDS while on anti-retrovirals. Johnston et al., *Science* 260: 1286-93 (1993).

One explanation for this failure of known anti-AIDS drugs is the emergence of drug resistance, which researchers generally attribute to a greater and more genetically diverse HIV burden. Viral strains resistant to antiviral drugs emerge after variable periods of antiviral drug therapy. Pantaleo et al., *J. Nat'l Instit. Health Res.* 5: 68 (1993). The reduced sensitivity to anti-retroviral agents is thought to be due to viral genetic selection. Larder et al., *Science* 243: 1731 (1989). Also, an increase in the multiplicity of infection -- the so-called "viral burden" -- decreases the antiviral effectiveness of nucleoside analogs. Sommadossi, *Clin. Infect. Diseases* 16(S1): S7 (1993). Combinations of antiviral drugs are of interest

because they diminish the probability of drug-virus resistance development. Baba et al., *Antimicrob. Agents Chemother.* 31: 1613 (1987). Thus, current modalities for treating HIV patients late in their disease may not be as effective as therapies that attack earlier, when the viral burden is lower and less genetically diverse and when the immune system is still relatively intact.

In addition to anti-retroviral agents that block HIV replication by hindering reverse transcriptase, there are various therapies under development that act on other viral functions. One such therapy attempts to block binding of HIV's gp120 to the CD4 molecule on susceptible cells of the infected individual's immune system. "CD4" is a surface glycoprotein component of certain monocytes and lymphocytes that acts as a receptor for HIV; this receptor provides a means for HIV to infect CD4<sup>+</sup> cells. Unfortunately, clinical trials with a fusion protein composed of soluble CD4 and exotoxin have demonstrated a dose-limiting hepatotoxicity. Hussey et al., *Nature* 331: 78 (1988); Ramont et al., *Ann. Int. Med.* 112: 241 (1990); Ashorn et al., *Proc. Nat'l Acad. Sci. USA*, supra; Brown et al., *J. Infect. Dis.* 167: 21 (1993); Anderson et al., in PROCEEDINGS: 8th INTERNATIONAL CONFERENCE ON AIDS (Amsterdam, 19 - 24 July 1992), at page We47; Brett-Smith et al., *ibid.*, at page B88; Person et al., *ibid.*, at page B90.

Other HIV treatment strategies employ gene therapy, and several of these have succeeded in producing anti-HIV activity in cultured cells. But known genetic therapy approaches are fraught with difficulties related, for example, to obtaining sufficient expression of the desired therapeutic gene in a sufficient number of cells *in vivo*.

Retrovirologists have studied the earliest stages of HIV disease in the hope that information thus obtained may yield valuable clues to new therapeutic strategies. One such clue was that the HIV is not actually latent during the so-called "clinical latency" period of HIV infection. The HIV clinical latency period in question is a period during the course of HIV infection which occurs after acute HIV infection and prior to the onset of ARC/AIDS. During clinical latency, the levels of virus in the peripheral blood are low to absent, the quantity of virus

in the peripheral blood mononuclear cells is low, virus expression is negligible in the few peripheral blood cells infected with HIV, the concentration of CD4<sup>+</sup> peripheral blood lymphocytes is in the normal range, and the HIV infected individual produces HIV-specific antibodies, such as anti-HIV Env (envelope) and anti-HIV p24, and/or cytotoxic lymphocytes which are HIV-specific. Weiss, *Science* 260: 1273 (1993).

It is now known that HIV accumulates quickly after infection in lymphoid organs where the virus replicates actively during clinical latency. Pantaleo et al., *J. Nat'l Instit. Health Res.*, *supra*; *Nature* 362: 355 (1993). During this period, HIV particles actually are located extracellularly in lymph nodes, where large numbers of free virus bind to the surfaces of the cytoplasmic processes of follicular dendritic cells (FDCs) in lymph tissues.

Follicular dendritic cells (FDCs) are cells within lymph node follicles that have Class II major histocompatibility complex molecules on their surface and are capable of presenting antigen to T cells or B cells, thereby eliciting an immune response and/or activating T cells or B cell precursors to replicate. The activation of CD4<sup>+</sup> cells is believed to be necessary for, and facilitates HIV infection of the CD4<sup>+</sup> cells. Pantaleo et al., (1993b), *supra*.

During the early stage of HIV infection, for example, in patients with more than 500 CD4<sup>+</sup> cells per mm<sup>3</sup>, HIV particles are trapped on the villus processes of the FDCs that sit in the germinal centers of lymph nodes. The FDCs surround and are intimately associated with lymphocytes. Under these circumstances, the HIVs displayed on FDCs are potentially a concentrated source of infectious virus that can infect the CD4<sup>+</sup> cells that target to that lymphoid location. See Pantaleo et al., *Proc. Nat'l Acad. Sci. USA* 88: 9838-42 (1991); *New England J. Med.* 328(5): 327-35 (1993); Pantaleo, et. al., *J. Nat'l Instit. Health Res.*, *supra*.

Using the polymerase chain reaction (PCR) to detect HIV DNA and reverse-transcriptase PCR to detect HIV RNA, one study found five to ten times more HIV-infected cells and higher concentrations of both regulatory and structural HIV mRNAs in

lymphoid organs (lymph nodes, adenoids, and tonsils) than in the peripheral blood of a group of "clinically latent" HIV patients. See Graziosi et al., *Clinical Res.* 40: 333A (1992); Pantaleo et al., *Proc. Nat'l Acad. Sci. USA*, supra; Pantaleo, *New England J. Med.* 328(5): 327 (1993). The role of lymphoid organs as reservoirs of HIV has been confirmed by *in situ* hybridization and electron microscopy. Pantaleo et al., *Nature* 362: 355 (1993).

It has been speculated that the majority of HIV particles cling to the FDC processes by way of antibody-, complement- and/or antibody-complement-mediated binding. This speculation is based on knowledge that FDCs contain receptors for both complement and the Fc portion of antibodies. (In this context, "Fc" denotes the portion of an antibody that is responsible for binding to antibody receptors on cells and the Clq component of complement. An "Fc receptor" is a cell-surface glycoprotein that specifically binds the Fc portion of an antibody.) It has been proposed in the same context that the formation of HIV:antibody complexes, HIV:complement complexes and/or HIV:antibody:complement complexes (collectively, "HIV complexes") contribute to the attachment of HIV to the FDC. Pantaleo et al., *Nature* 362: 355 (1993).

These HIV complexes can be formed after HIV infection stimulates the production of HIV-specific antibodies that bind to the virus. HIV:antibody complexes may also be formed when HIV particles bind autoantibodies that may be induced by and bind HIV. Complement presumably binds to HIV:antibody complexes via complement binding sites on the antibody molecule. Complement binding to HIV, forming HIV:complement complexes, has also been shown to occur in the absence of antibody. Montefiori, et al., *J. Virol.* 67: 2699 (1993). HIV complexes circulate to the lymph nodes, where they are bound to the FDCs via the Fc and/or complement receptors on these cells. Fc- or complement receptor-containing cells in lymphoid tissues or the reticuloendothelial system (RES) are denoted "complex binding cells" because they bind antibody:antigen, complement:antigen and/or antibody:antigen:complement complexes.

The recent elucidation of a correlation between HIV trapping in lymph nodes and the integrity of the FDC suggests

that the association between HIV and antigen presenting cells in lymphoid tissues may be involved in the immunopathogenic mechanism of lymph node destruction and HIV disease. The presence of HIV in lymphoid organs is intimately related to the destruction of FDCs in lymphoid organs and the destruction of thymus and other lymphoid tissues. The HIV induced destruction of lymphoid tissues is associated with immunosuppression. Yarchoan et al., *Immunology Today* 14: 303 (1993).

As illustrated in Figure 1A, the surfaces of antigen-presenting cells, such as FDCs, and phagocytic cells of the RES, are thought to be a prime location where HIV particles can infect CD4<sup>+</sup> lymphocytes and perhaps macrophages which continuously traffic through the lymphoid tissues. Pantaleo et al., *New Engl. J. Med.* 328: 327 (1993). Progressive infection of CD4<sup>+</sup> T cells occurs over time in lymph tissue and is one of the components leading to the immunodeficiency of AIDS. Spiegel et al., *Am. J. Pathol.* 140: 15 (1992).

#### Summary of the Invention

It is therefore an object of the present invention to block HIV complexes from ever taking up residence in lymphoid tissue by prophylactically inhibiting the binding of HIV complexes to lymphoid tissue in individuals at risk of HIV infection.

It is an additional object of the present invention to provide a method for lowering the concentration of HIV in the lymph tissue of an HIV-exposed individual.

It is a further object of the present invention to prevent the accumulation of HIV in association with FDCs in order to reduce the reservoir of infectious virus in the vicinity of CD4<sup>+</sup> cells and limit infection of those cells.

It is yet another object to provide a method for slowing the rate of infection of CD4<sup>+</sup> cells by reducing the exposure of CD4<sup>+</sup> cells to HIV *in vivo*. By removal of HIV from the vicinity of CD4<sup>+</sup> T cells, the HIV is less able to infect these cells progressively and, hence, the virus is less able to replicate.

It is yet another object of the present invention to lower the load/burden of HIV-infected cells over time so that anti-retroviral agents can be more effective.

It is also an object of the present invention to  
5 release HIV from lymph tissue or block its accumulation in lymph tissue. Release of the virus from lymphoid organs not only allows virus to be inactivated by immune mechanisms and/or physicochemical mechanisms but prevents or retards destruction of FDCs in lymphoid organs and thymus tissue.

10 Another object of the present invention is to slow the progression to AIDS immunodeficiency by decreasing the incidence of exposure of CD4<sup>+</sup> targets cells to HIV particles in lymph tissue, thereby retarding the infection and destruction of new CD4<sup>+</sup> cells which leads to AIDS. This object stabilizes CD4<sup>+</sup> cell  
15 counts by preventing or retarding the HIV mediated drop in CD4<sup>+</sup> cells.

It is yet a further object of the present invention to reduce the association of HIV with FDCs in order to prevent or slow the destruction of lymphoid tissue and the resulting  
20 destruction of the immune system that is associated with progressive HIV infection.

It is still another object of the present invention to provide an approach to lowering HIV resistance to anti-retroviral drugs/agents. By early intervention in the pathogenesis of an  
25 infected individual, limiting the magnitude of the HIV infection, the chance that replicating HIV can become genetically diverse enough to become resistant is greatly reduced.

It is also an object of the present invention to provide an approach to reducing the level of HIV replication in  
30 the infected host, thereby preventing or slowing the appearance of HIV variants that are not neutralized by the immune response of the host.

In accomplishing these and other objects, there has been provided, in accordance with one aspect of the present  
35 invention, a method for slowing the progression of HIV infection in an HIV-exposed individual or preventing HIV infection in an individual at risk of HIV infection, comprising the step of providing the individual with provided complex selected from

antibody:antigen complex and antibody:antigen: complement complex, in a titer adequate to competitively inhibit HIV complexes from binding cells via Fc receptors or complement receptors, wherein the provided complex is given to the patient  
5 prior to any onset of clinically significant idiopathic thrombocytopenic purpura (ITP).

In one preferred embodiment, the method further comprises the step of subjecting the individual to an anti-retroviral therapy, either concurrently with or subsequently to  
10 the providing of the provided complex. In another preferred embodiment, the provided complex is administered in a regimen that comprises an initial dose of the antibody, followed by daily doses capable of competitively inhibiting HIV binding cells via Fc receptors or complement receptors, and thereafter by  
15 administrations of maintenance doses. In yet another preferred embodiment, the method further comprises the step of subjecting the individual to treatment with HIV-specific antibodies, either concurrently with or subsequently to the providing of complex. Such HIV-specific antibodies are selected from the group  
20 consisting of polyclonal antibody that recognizes HIV gp120, polyclonal antibody that recognizes HIV p24, polyclonal antibody that recognizes HIV gp160, monoclonal antibody that recognizes HIV gp120, monoclonal antibody that recognizes HIV p24, monoclonal antibody that recognizes HIV gp160, polyclonal  
25 antibody that recognizes TN antigen, polyclonal antibody that recognizes sialyl TN antigen, monoclonal antibody that recognizes TN antigen and monoclonal antibody that recognizes sialyl TN antigen. In another preferred embodiment, the method further comprises the step of subjecting the individual to treatment with  
30 an HIV vaccine, either concurrently or subsequently to the providing of provided complex.

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  
35 description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit

and scope of the invention will become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

The present invention can be understood more fully by reference to the following drawings, where:

FIGURE 1 is a drawing illustrating the competitive inhibition of HIV-complex binding to follicular dendritic cells. FIGURE 1A is a drawing showing how the surfaces of antigen-presenting cells, such as FDCs, and phagocytic cells of the RES, serve as a prime location where HIV particles can infect CD4<sup>+</sup> lymphocytes and perhaps macrophages which continuously traffic through the lymphoid tissues.

FIGURE 1B is a drawing representing the mechanism by which antibody:antigen or antibody:antigen:complement complex, such as WinRho complex, competitively inhibits the binding of HIV complexes to Fc receptor- or complement receptor-containing cells, such as FDCs, in lymphoid tissues. WinRho<sup>TM</sup> and WinRho-SD<sup>TM</sup> (hereinafter "WinRho") are two examples of anti-Rh antibody for used in this method; both have been tested clinically and shown to be safe. WinRho binds to Rh antigen-containing red blood cells to form immune complexes which contain Fc receptor binding sites and bind complement. Immune complexes bound to red blood cells induce red blood cell lysis and fragmentation. These red blood cell fragment complexes bind complement and Fc receptors on FDCs and competitively inhibit the binding of HIV complexes to FDCs.

FIGURE 2 is a flow chart illustrating the temporal administration of antibody complex or WinRho therapy during the progression of HIV infection. The diagram shows the progression of HIV infection to morbidity and mortality. The right side depicts information obtained by monitoring the blood of infected individuals. The left side summarizes information on the presumed role of lymphoid organs in disease progression. The open arrows depict sites of provided antibody complex or WinRho action. The double lines indicate blockage of the progression of HIV infection and the slowing or prevention of the onset of AIDS resulting from intervention with antibody complex or WinRho therapy.

Detailed Description of the Preferred Embodiments

The present invention exploits to advantage the phenomena that are believed to underlie the binding of HIV complexes to the cytoplasmic processes of FDCs in lymph tissues and, more generally, to complex-binding cells in lymph tissues and the reticuloendothelial system, thereby to prevent the binding of HIV complexes to these cells/tissues, or to displace HIV from these cells/tissues by means of adequate titers of a particulate antibody:antigen complex. The "reticuloendothelial system" (RES) is composed of lymphoid tissues widely distributed throughout the body that are infiltrated by a class of mononuclear cells of the monocyte-macrophage series. Thus, by provision of particulate antibody:antigen complexes to HIV-infected individuals or individuals at risk of HIV infection, HIV complexes are competitively blocked from accumulating in lymph nodes and the RES or HIV complexes are cleared from these reservoirs. In addition, the administration of antibodies in accordance with the present invention may immunomodulate cells of the immune system (e.g., T-cells or macrophages), preventing certain T cell responses, such as the release of cytokines, which are essential to HIV-mediated suppression of the immune system.

"Lymphoid tissues" or "lymph tissues" refer to lymph nodes, tonsils, spleen and adenoids. An "antigen" is any molecule that induces the formation of antigen-specific antibodies. Further, an antigen can be bound to the antigen-binding site of an antibody. Such an antigen may be haptenic or complete, or soluble or particulate, but most preferably is expressed in particulate form.

No one has proposed a method for inhibiting the binding of HIV complexes to lymphoid tissue *in vivo*. Competitive inhibition of HIV complex-binding to lymphoid tissue would block HIV complex from binding to lymphoid tissue and/or displace HIV complex already bound to lymphoid tissue. The phrase "competitive inhibition" in relation to HIV complex-binding refers to both: (1) the blocking of HIV complex from binding to lymphoid tissues and (2) the displacement of HIV complex bound to lymphoid tissues. Figure 1B illustrates the mechanism by which antibody:antigen or antibody:antigen:complement complex,

such as WinRho complex, competitively inhibits the binding of HIV complexes to Fc receptor- or complement receptor-containing cells in lymphoid tissues.

5 Removing HIV from its sequestered position in lymph tissue, in accordance with the present invention, achieves the following: (1) lowers the concentration of HIV in lymph tissues, (2) lowers over time the number and concentration of infected cells in lymph tissue, (3) prevents the virus from directly or indirectly destroying much of the FDC network, and (4) prevents  
10 progressive infection of CD4<sup>+</sup> T cells by limiting both the exposure of the cells to HIV and the activation of immune cells.

Removal of HIV complexes from their reservoirs in lymphoid tissue lowers the concentration of HIV in lymph tissue. Such withdrawal of HIV from its prime vantage point for  
15 activating and/or infecting the CD4<sup>+</sup> lymphocytes and macrophages that are trafficking through the lymphoid tissues stops the progressive infection and subsequent destruction of the CD4<sup>+</sup> T cells that leads to the immunodeficiency of AIDS. The progression of HIV infection is slowed by reducing the efficiency  
20 of HIV infection of CD4<sup>+</sup> T-cells and macrophages thereby preserving an uninfected cell population capable of sustaining immune function. Preventing the binding of HIV complexes to the FDCs significantly reduces the exposure of CD4<sup>+</sup> cells to HIV, which slows the rate of infection of those cells. Slowing the  
25 rate of CD4<sup>+</sup> cell infection by HIV will slow progression to frank AIDS. As illustrated in Figure 2, the present invention's method of providing antibody complex or WinRho therapy blocks the progression of HIV infection and slows or prevents the onset of AIDS.

30 More specifically, it has been discovered that HIV infection can be counteracted with antibodies that bind to cell surface antigens to form complexes that can competitively inhibit binding of HIV complexes to FDCs and other complex-binding cells. In the present context, the term "antibody" denotes any protein  
35 containing an antigen binding domain and an Fc domain that is capable of forming an "immune complex," i.e., an antibody:antigen complex or antibody:antigen:complement complex. "Complement" is

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comprised of several components that combine with an antigen:antibody complex to form immune complexes.

More specifically, an "antibody" for purposes of the present invention is any protein that contains an antigen-binding site and an Fc domain that is capable of forming an immune complex. "Immune complex" refers to an antibody:antigen complex or antibody:antigen:complement complex. The term "antibody complex" denotes either (a) antibody bound to antigen or (b) antibody bound to antigen plus complement, which can bind the antibody or antigen. An antibody within the present invention can be of the type that recognizes antigens present in the host individual or antigens present on cells co-administered with the antibody or antigen present on cells administered to the individual, as described in greater detail below.

In the present description, an "antibody" can be either a polyclonal antibody, obtained from human plasma, or a monoclonal antibody produced in accordance with conventional technology, so long as the antibody binds a cell-surface antigen and contains an Fc domain capable of binding Fc receptors on human cells. Monoclonal antibodies can be produced in various ways, using well-understood techniques detailed, for example, in ANTIBODIES: A LABORATORY MANUAL 726 (Cold Spring Harbor Publications 1988), which is hereby incorporated by reference.

Furthermore, an "antibody" can be any antibody fragment that contains antigen binding site(s) and a functional Fc domain, such that the antibody is capable of forming complexes that bind to Fc receptor- and/or complement receptor-containing cells to the exclusion of HIV complexes. Fragments of this sort can be produced by methods well known in the art. See Skerra et al., *Science* 240: 1038-1041 (1988) and King et al., *Biochemical J.* 290: 723-729 (1991), the contents of each of which are hereby incorporated by reference.

More generally, an "antibody" can be any polypeptide, natural or synthetic, that has the ability to bind both an antigen (preferably a cell surface antigen) and an Fc receptor. By the same token, an "antibody" can be a polypeptide that contains an antigen binding site and is capable of binding complement in a form capable of binding complement receptor.

Exemplary of such polypeptides is a so-called "half antibody molecule," which is a single heavy:light chain pair.

An "antibody" for use in the present invention also can be an anti-idiotypic antibody produced by methods well-known to the art. See Cozenza, *Eur. J. Immunol.* 6: 114 (1976), and ANTIBODIES: A LABORATORY MANUAL 726 (Cold Spring Harbor Publications, 1988), the respective contents of which are hereby incorporated by reference.

An "antibody" employed in accordance with the present invention can be a chimeric antibody. By one approach in this regard, such a chimeric antibody is engineered by cloning recombinant DNA containing the promoter, leader, and variable-region sequences from a mouse antibody gene and the constant-region exons of a human antibody gene. The antibody encoded by such a recombinant gene is a mouse-human chimera. Such a chimeric antibody's specificity is determined by the variable region derived from mouse DNA; its isotype and Fc receptor binding site, which is determined by the constant region, is derived from human DNA. See Verhoeyn et al., *BioEssays* 8: 74 (1988), the contents of which are hereby incorporated by reference.

In another embodiment, the antibody used in the present invention is a "humanized" antibody, produced by techniques well-known in the art. See, for example, Carter et al., *Proc. Nat'l Acad. Sci. USA* 89: 4285-289 (1992); Singer et al., *J. Immun.* 150: 2844-2857 (1992) and Mountain et al., *Biotechnol. Genet. Eng. Rev.* 10: 1-142 (1992), the respective contents of which are hereby incorporated by reference. Thus, mouse complementary determining regions ("CDRs") can be transferred from heavy and light V-chains of the mouse Ig into a human V-domain, followed by the replacement of some human residues in the framework regions of the murine counterparts. "Humanized" antibodies in accordance with this invention are suitable for use in *in vivo* therapeutic methods.

A suitable antibody for the present invention can recognize an antigen that is present in the body of a subject, where the antigen preferably is present on the surface of cells in the subject. Preferably, when using an antibody recognizing

endogenous antigen, the antigen-containing cells will be present in the lymphatic or vascular system so that cells, cell fragments and/or cell antigen:antibody complexes will have access to lymph tissue.

5 Another suitable antibody is one that recognizes an antigen that is not endogenous to the individual requiring treatment. When such an antigen target is not present *in vivo*, the suitable antibody recognizes the exogenous antigen present on cells or cell fragments and is capable of forming antibody  
10 complex *ex vivo*. The appropriate antibody can generate adequate antigen:antibody complex and or antibody:antigen: complement complexes that competitively inhibit HIV complex binding to FDCs.

Relative non-toxicity is a further criterion for antibodies and antibody dosages suitably applied in accordance  
15 with in the present invention. For example, an effective dose of antibody recognizing a red cell antigen should not cause life-threatening hemolysis.

By the therapeutic approach of the present invention, HIV can be prevented from accumulating in lymph tissue or can be  
20 displaced from lymphoid-tissue and RES reservoirs in HIV-infected individuals. In particular, the present invention can be applied to slow the progress of HIV infection prior to any clinically significant immune thrombocytopenic purpura (ITP).

ITP is a blood disorder characterized by abnormally low  
25 platelet counts due to circulating antibodies that bind platelets and mediate their destruction by the immune system. "Clinically significant ITP" refers to a patient who has a platelet count generally less than  $50 \times 10^9$  platelets/liter but patients with less than  $30 \times 10^9$  platelets/liter are nearly always treated for  
30 their ITP. Therefore, "prior to any onset of clinically significant ITP" means that an individual has a platelet count generally greater than  $50 \times 10^9$  / liter but always greater than  $30 \times 10^9$  / liter.

Exemplary candidates for treatment according to the  
35 present invention include but are not limited to: (1) newborns suspected of HIV infection because their mothers are HIV antibody-positive; (2) Center for Disease Control (CDC) HIV Group I individuals who are in the acute infection stage yet prior to

any onset of clinically significant ITP (i.e. have a platelet count generally greater than  $50 \times 10^9$  / liter but always greater than  $30 \times 10^9$  / liter); (3) CDC HIV Group II patients who are in the asymptomatic infection stage, including clinical latency yet prior to any onset of clinically significant ITP; (4) CDC HIV Group III patients who have persistent generalized lymphadenopathy yet prior to any onset of clinically significant ITP; (5) CDC HIV Group IV patients who have AIDS, including secondary infectious diseases, cancers and other conditions, yet prior to any onset of clinically significant ITP; (6) patients in any of CDC HIV Groups I through IV yet prior to any onset of clinically significant ITP; (7) children who have been exposed to HIV, yet prior to any onset of clinically significant ITP; (8) individuals who are at risk of HIV infection, for example through occupational exposure occasioned by needle sticks. In any event, the present invention preferably is applied to an HIV-exposed individual or a person at risk of being infected by HIV yet prior to any onset of clinically significant ITP.

In one preferred embodiment of the present invention, antibodies to cellular antigens are used to slow the progression of HIV infection. In another preferred embodiment of the present invention, immune complex is provided prophylactically to HIV-exposed individuals who are at risk of HIV infection, for example, by virtue of acute occupational exposure, thereby positioning antibody complexes in lymphoid tissues to block any HIV complexes from binding to lymphoid tissues.

In this description, the phrase "providing complex" denotes administration of any of the following: (a) an antibody capable of forming either an antibody:antigen or antibody:antigen:complement complex in an individual, (b) both an antibody and antigen to an individual so that either antibody:antigen complexes or antibody:antigen: complement complexes are formed in the individual, (c) antibody:antigen complexes formed ex vivo or (d) antigen in an immunization regimen prior to treatment with antigen so that the immunized individual produces antibodies that form complexes with the provided antigen. The phrase "formed ex vivo" means that the components are allowed to form an immune complex outside of the human body before being provided to an

individual. For each of the aforementioned approaches to providing complex, the complexes are able competitively to inhibit binding of HIV complexes to Fc receptor- and/or complement receptor-containing cells *in vivo*.

5           The pharmaceutical composition of the present invention is advantageously administered in the form of injectable compositions. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers include, among others, water, saline, certain  
10 buffers and other compounds described, for example, in THE MERCK INDEX (Merck & Co., Rahway, New Jersey). Slow release formulations or slow release apparatus may be utilized for continuous administration. For instance, the composition may contain about 10 mg of human serum albumin and from about 5 to  
15 70 micrograms per kilogram of any antibody for "providing complex" per milliliter of phosphate buffer containing NaCl. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like as described, for instance, in REMINGTON'S  
20 PHARMACEUTICAL SCIENCES (15th ed.) and in THE NATIONAL FORMULARY XIV (14th ed.), the contents of which are hereby incorporated by reference. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water,  
25 alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Antigen provided in an immunization regimen is administered in various adjuvants known to the art. Preservatives include  
30 antimicrobial, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the pharmaceutical composition are adjusted according to routine skills in the art. See GOODMAN & GILMAN'S PHARMACOLOGICAL BASES OF THERAPEUTICS (8th ed.).

35           Particularly preferred pharmaceutical compositions of the present invention are those that, in addition to competitively inhibiting binding to Fc receptor- and/or complement-receptor containing cells *in vivo*, are also relatively

safe at appropriate dosage levels and have a satisfactory duration of effect. The quantity of the compound of the present invention necessary for effective therapy will depend upon many different factors, including the means of administration, target  
5 site, physiological state of the patient, other medicants administered, etc. Thus, treatment dosages should be titrated to optimize safety and efficacy.

Preferentially, the treatment will be initiated while the individual is asymptomatic and prior to any onset of  
10 clinically significant ITP; that is, the individual has a platelet count generally greater than  $50 \times 10^9$  / liter but always greater than  $30 \times 10^9$  / liter. Treatment after onset of clinically significant ITP is not contraindicated, however.

The administered antibody forms an antibody complex in  
15 vivo that competitively binds Fc receptor- and/or complement receptor-containing cells in lymph tissue and prevents or reduces the binding of HIV complexes to FDCs in lymph tissue and other complex binding cells.

One example of the antibodies to be provided is  
20 antibodies to Rh factor (D antigen) in Rh<sup>+</sup> individuals. Rh antigen, also called "Rh factor" or "D antigen," is a type of blood group antigen found on the surface of human erythrocytes. The majority of people possess this Rh or D antigen and are said to be Rh<sup>+</sup>. Persons who do not have the Rh factor on their red  
25 blood cells are Rh<sup>-</sup>. The plasma of Rh<sup>-</sup> persons does not contain antibodies against the Rh antigen, but such antibodies are made when Rh<sup>+</sup> blood is given to Rh<sup>-</sup> individuals. Anti-Rh antibodies bind to Rh<sup>+</sup> red blood cells.

In accordance with the present invention, administering  
30 a mixture comprised of human anti-Rh antibody and Rh<sup>+</sup> red blood cells competitively will inhibit binding of HIV-complexes to lymphoid tissue or the RES in Rh<sup>-</sup> individuals infected with HIV. In either case, this approach will decrease the destruction of the FDC network, limits the progressive infection of CD4<sup>+</sup>  
35 lymphocytes, and will slow the progression of HIV infection. WinRho<sup>TM</sup> or WinRho-SD<sup>TM</sup> are two examples of anti-Rh antibody for

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use in this method; both have been tested clinically and shown to be safe.

WinRho is a human polyclonal antibody to Rh factor. WinRho is derived from the plasma of individuals with high natural levels of this antibody or from Rh<sup>-</sup> individuals who have been immunized with red blood cells from Rh<sup>+</sup> donors. WinRho is currently used in Rh<sup>-</sup> women who are at risk of developing Rh antibodies which might harm their Rh<sup>+</sup> fetus.

It has further been shown that Rh<sup>-</sup> individuals can be immunized with Rh-antigen (or Rh<sup>+</sup> red blood cells) thereby causing induction of antibody to the antigen to be produced in the Rh<sup>-</sup> individual. Subsequently, repeated doses of Rh antigen given to said immunized individuals permits formation of antibody:antigen complexes that compete with HIV complexes for binding to lymphoid and RES cells.

HIV<sup>+</sup> adult patients with ITP have been treated with WinRho or Anti-D antibody in an effort to increase their platelet counts and decrease their tendency for bleeding. Bussel et al., *Blood* 77: 1884 (1991); Rossi et al., *Haematologica* 72: 529 (1987); Oksenhendler et al., *Blood* 71: 1499 (1988). The object of the present invention is unrelated to the treatment of thrombocytopenia. Therefore, the method of the present invention for preventing HIV infection or competitively inhibiting the binding of HIV complexes to Fc receptor- or complement receptor-containing cells in lymphoid tissues is instituted prior to any onset of clinically significant ITP.

The antibody or antibody complex, such as WinRho or WinRho:antigen complex, is injected into an HIV-infected individual as early as possible after the initial stages of infection. The injection may be any of intravenously, intramuscularly, subcutaneously or intraperitoneally. Preferably, the injection will be intravenous to maximize the availability of complex in lymph tissue in the shortest time. Preferentially, the treatment will be initiated while the individual is asymptomatic and prior to any onset of clinically significant ITP. The goal of the therapy is to competitively inhibit the binding of HIV complexes to FDCs in lymph tissue and thereby

reduce the efficiency of HIV infection of CD4<sup>+</sup> T-cells and macrophages. WinRho does this by binding to Rh antigen-containing red blood cells to form immune complexes which contain Fc receptor binding sites and which bind complement. Immune  
5 complexes bound to red blood cells induce red blood cell lysis and fragmentation. These red blood cell fragment complexes bind complement and Fc receptors on FDCs and competitively inhibit the binding of HIV complexes to FDCs. The mechanisms by which red blood cell fragment complexes competitively inhibit HIV-complexes  
10 from binding to FDCs is illustrated in Figure 1B.

In another preferred embodiment of the present invention, the slowing of the progression of HIV infection is achieved by combining the administration of antibody:complexes along with other HIV therapies so that the HIV therapy works more  
15 efficiently. For example, the antibody of the present invention may be used in concert with such antiviral therapies as zidovudine (AZT), HIV-specific antibodies, or anti-HIV therapeutic vaccines. Combining the therapy of the instant invention with other antiviral regimens not only allows the  
20 antiviral to work efficiently due to the decreased viral load, but also diminishes the probability of viral resistance. See Baba *et al.*, *supra*.

A measurable indication of the effectiveness of the antibody or WinRho therapy in the HIV-infected individual will  
25 be prevention or reduction of the lymphadenopathy associated with HIV-infection. Another indicator of the operativeness of this invention is a reduction in the presence of HIV in lymph tissue, as measured, for example, by microscopy, *in situ* hybridization or PCR. Administration of antibody complex to HIV-exposed  
30 individuals achieves the following: (1) lowers the concentration of HIV particles from lymph nodes and (2) at least temporarily increases the relative amount of HIV particles in the bloodstream and/or non-lymphoid body tissues, where the infection process is less optimal and where virus inactivation proceeds.

#### **Production of Antibodies for Use in the Method:**

35 Antibodies for use in this invention may be produced by stimulating plasma donors with cell surface proteins, such as the erythrocyte D antigen, glycophorin or Band III. Erythrocyte

antigens, number over 100 and comprise some 20 blood group systems. The ABO blood group and the Rh system are just two of the best known blood group systems. Any known or identified cell surface antigen, such as cell surface receptors or binding proteins or the extracellular portion of transmembrane proteins or idiotypes, may be used to stimulate the production of antibody. Antibodies for use in this invention include those antibodies that recognize antigens present in the individual to be treated; such antibodies are called autoantibodies.

The human plasma to be used in the process of producing antibodies may be from humans with a high-titer of particular antibodies to particular human antigens, such as Rh factor or cells surface receptors on migratory human cells. The antibody-containing serum or plasma is then isolated by methods generally known for the production of sera or plasma immunoglobulin enriched preparations obtained from plasma. Antibody preparations can be obtained from plasma via Cohn fractionation. See Cohn et al., *J. Am. Chem. Soc.* 68: 459 (1946); Oncley et al., *J. Am. Chem. Soc.* 71: 451 (1949); Canadian patent No. 1,168,152 (issued May 29, 1984), the respective contents of which are hereby incorporated by reference.

For example, human plasma containing the desired antibodies is diluted with an equal volume of pyrogen-free distilled water prior to fractionation on a DEAE-Sephadex A-50 column. The antibody eluted from this column is stabilized with sodium chloride and glycine before ultrafiltration and subsequent freeze-drying. The final concentrated solution following filtration usually contains about 91% antibody. The purified plasma is treated with a solvent detergent process which results in efficient viral inactivation. This aseptic protocol yields human antibodies that are sterile, non-pyrogenic and nontoxic. Canadian patent No. 1,168,152, *supra*.

WinRho-SD<sup>TM</sup> is produced by stimulating Rh<sup>-</sup> plasma donors with Rh<sup>+</sup> red blood cells, a process similar to that used to prepare hyperimmune polyclonal antibody products for protection against tetanus, rabies and hepatitis B. WinRho-SD<sup>TM</sup> is then isolated from the stimulated plasma by a purification procedure used for several years in the manufacture of WinRho<sup>TM</sup>, a product

licensed in Canada for prevention of Rh-disease in the newborn. WinRho-SD<sup>TM</sup> differs from WinRho<sup>TM</sup> in that it is treated with a solvent detergent process which results in efficient viral inactivation. Bowman et al., CMA J. 123: 1121 (1980) and Canadian patent No. 1168152, which issued May 29 1984, both of which are incorporated in their entirety herein by reference.

#### Providing Antibody Complex

Various protocols may be followed for providing antibody in an HIV-exposed individual or an individual at risk of HIV infection. The first period of treatment is referred to as the induction period. During the induction period, the complex is provided in a dose, the lower limit of which is determined differently for the two major classes of treated individuals; these two groups are: (1) the HIV-infected individual and (2) the individual at risk of HIV infection. For HIV-infected individuals, the lower dose limit is that which does any of the following: (1) measurably reduces the concentration of virus in lymph tissue or keeps lymph nodes clear of virus, as determined by lymph node or spleen biopsy, (2) releases HIV into the circulation from HIV reservoirs in lymphoid tissues, as indicated by a measured increase in circulating virus, virus RNA or viral antigens, (3) stabilizes CD4<sup>+</sup> cell counts (i.e., prevention or retardation of CD4<sup>+</sup> cell count drop) or (4) prevents or retards the destruction of FDCs in lymphoid organs and thymus tissue. See Yarchoan et al., *Immunology Today* 14: 303 (1993).

For individuals at risk of HIV infection, the dosage lower limit of provided antibody complex is that which either measurably reduces the concentration of marker complex in lymph tissue or keeps lymph nodes clear of marker complex. A "marker complex" is any detectably labelled immune complex that may be used for determining the lower dose in such individuals at risk of HIV infection. For example, doses of radioactive WinRho or radioactive WinRho complex may be provided to Rh<sup>+</sup> individuals. This labelled WinRho complex is chased from lymphoid tissue by administering cold or unlabelled WinRho complex. The dosage lower limit of provided WinRho complex is that dose which clears lymph nodes of radioactive WinRho complex (i.e. marker complex).

The unlabelled complex's ability to competitively inhibit marker complex binding to lymph tissues may be determined by lymph node or spleen biopsy or in vivo imaging. The term "in vivo imaging" refers to any method which permits the detection of a labelled marker complex which contains either antibody or complement of the present invention or fragment thereof that specifically binds to Fc receptor- or complement receptor-containing cells. An imaging effective amount of marker complex must be provided prior to or after providing the non-marker antibody complex of the present invention. The phrase "an imaging effective amount of marker complex" refers to the amount of administered labeled marker complex that is sufficient to enable detection of complex bound in lymphoid tissues.

Alternatively, the lower dosage limit of provided complex is that which produces any of: (a) detectable hemolysis in the recipient, as measured by plasma hemoglobin concentrations, (b) red blood cell breakdown products present in the circulation or (c) the presence of damaged red blood cells, as determined by microscopy.

Doses of provided marker complex will depend on the type and intensity of radioactive label as well as the method of imaging. Dosage of antibody or antibody complex can vary from 0.01  $\mu\text{g/kg}$  to 100  $\mu\text{g/kg}$ , preferably 5  $\mu\text{g/kg}$  to 70  $\mu\text{g/kg}$ , when free antibody is administered. When antibody is provided in the form of ex vivo formed complex, higher doses may be tolerated.

For purposes of in vivo imaging, the type of detection instrument available is a major factor in selecting a given label. For instance, radioactive isotopes and paramagnetic isotopes are particularly suitable for in vivo imaging in detecting marker complex. The type of instrument used will guide the selection of the radionuclide. For instance, the radionuclide chosen must have a type of decay which is detectable for a given type of instrument. However, any conventional method for visualizing diagnostic imaging can be utilized in accordance with determining doses appropriate for preventing HIV infection.

During the induction period, the complex is provided in a dose, the upper limit of which is determined similarly for all treated individuals. For both the HIV-exposed individual and

the individual at risk of HIV infection, the upper dose limit is that which is unacceptably toxic. For example, a dose of antibody that recognizes a red blood cell antigen is relatively nontoxic if the dose fails to produce an unacceptable level of hemolysis in the individual. While the determination of clinically unacceptable levels of hemolysis is subjectively made by the treating physician, generally a decrease in hemoglobin equal to or greater than 2 grams per deciliter is considered clinically significant. In clinical studies, 40 to 60 micrograms-per-kilogram dosages of WinRho-SD<sup>TM</sup> resulted in a mean maximum decrease of 1.70 grams per deciliter.

Doses associated with acceptable toxicity can vary from between 0.1  $\mu\text{g/kg/day}$  to 150  $\mu\text{g/kg/day}$ , preferably 5  $\mu\text{g/kg/day}$  to 70  $\mu\text{g/kg/day}$ . Administration of antibody continues during the induction period in the HIV infected individual until either the presence of HIV in lymph nodes is cleared or the presence of HIV in the peripheral blood increases or until general toxicity increases to a clinically unacceptable level. In an HIV exposed individual, administration of antibody continues during the induction period in the individual at risk of HIV infection until labelled marker is prevented from binding to lymphoid tissue or the presence of marker complex in lymph nodes is cleared (in a labelled marker complex, followed by unlabelled marker complex determination) or the presence of marker complex in the peripheral blood increases or until general toxicity increases to an unacceptably high level.

The presence of HIV in lymphoid organs can be identified and measured by *in situ* hybridization and the polymerase chain reaction (PCR). Fox, C.H., et al., *Journal of Infectious Disease* 164:1051-7 (1991); and Spiegel, H. et al., *American Journal of Pathology* 140:15-22 (1992); Pantaleo, G., *supra*; and Shibata, D., *supra*. Alternatively, HIV may be detected in lymph tissues using either immunohistochemistry to detect HIV antigens, or electron microscopy to identify typical retrovirus particles. Tenner-Racz et al., *Am. J. Pathol.* 123: 9 (1986).

The presence of HIV in peripheral blood can be detected and measured using various known means. The following are three

examples of means for detecting and measuring HIV in peripheral blood: (1) PCR specific for HIV DNA, (2) reverse transcriptase PCR specific for HIV RNA, or (3) HIV-1 p24 core antigen quantitation using commercial enzyme immunoassays. Graziosi et al., *supra*; Pantaleo et al., *Proc. Nat'l Acad. Sci. USA, supra*; Pantaleo, *New Eng. J. Med., supra*; Ho et al., *New Eng. J. Med.* 321: 1621 (1989); Shibata et al., *American Journal of Pathology* 135:697-702 (1989).

Maintenance treatment is administered as needed, with the lower and upper limits of dose determined similar to that described for the initial dose. For example, in HIV-infected individuals, antibody complex is provided as needed to: (1) keep the HIV spilling from the lymph node into the peripheral blood and (2) maintain the presence of HIV low or absent in lymph nodes. In individuals at risk of HIV infection, antibody complex is provided as needed to: (1) maintain the presence of marker complex low or absent in lymph nodes or (2) keep the marker complex spilling from the lymph node into the peripheral blood.

Maintenance doses depend on the clinical situation and range between 25% and 300% of the total induction dose. The maintenance treatments may be altered to define the optimal maintenance dose. Because the initial dose varies based on the individual response, the actual infused maintenance doses vary considerably. For example, when using an anti-D antibody preparation recognizing Rh<sup>+</sup> erythrocytes, the preferred range is between 10 and 150 µg/kg/infusion.

Another embodiment of the present invention entails providing a mixture of antibody plus antigen-containing cells made *ex vivo*. Such a mixture would be injected into an HIV-infected patient or an individual at risk of HIV infection. The antigen:antibody complexes in the mixture would be expected to bind to the patient's FDCs, thereby competitively inhibiting HIV:antibody binding. The *ex vivo* formed antigen:antibody complexes have the advantage of reducing toxicity as compared to direct administration of antibody alone.

When the antibody complex is formed *ex vivo* before being provided to the individual, an increased dose may be found to be relatively nontoxic. Higher doses of *ex vivo* formed

antibody complex are less toxic because the administered antibodies are in the form of complex and have fewer free antigen binding sites available to bind the patient's cellular antigens. For example, when providing the ex vivo formed complex composed of Anti-D or WinRho and Rh<sup>+</sup> red blood cells, there is less free anti-D antibody available to bind to an individual's red blood cells to mediate anemia. Thus, there is less hemolysis in Rh<sup>+</sup> individuals given anti-D antibody:antigen complex formed ex vivo compared to direct administration of antibody alone.

Another embodiment of the present invention involves immunizing an HIV-exposed immunocompetent Rh<sup>-</sup> individual or an Rh<sup>-</sup> individual at risk of HIV infection with purified antigen or antigen containing cells (e.g., Rh antigen or Rh<sup>+</sup> red blood cells) to raise an antigen specific antibody. Such a treated individual could then be transfused with the antigen containing cells which then bind to the circulating antigen-specific antibody to yield antibody:antigen and antibody:antigen:complement complexes. The complexes competitively inhibit HIV complexes from binding to lymphoid tissue.

The aforementioned administration of (1) antibody or (b) antibody:antigen complex or (c) antigen in an immunized individual can be combined with either currently approved anti-retroviral therapy, anti-retroviral therapy in clinical trials or HIV therapies in development. The combined use of antibody:antigen complexes and anti-retroviral therapy, in accordance with the present invention produces the following effects: (1) decreases the production of virus from cells, (2) protects new cells from becoming infected by removing virus from the vicinity of its target cells, (3) increases the effectiveness of the anti-retroviral therapy by limiting the number of infected cells, (4) slows the onset of resistance to the anti-retroviral therapy because the rate of resistance is proportional to the number of virally infected cells and (5) delays or prevents a rapid divergence of HIV in the infected individual, thereby delaying or preventing the appearance of HIV variants which are resistant to neutralization by the individual's immune system.

The combined method of the present invention's ability to limit the number of virally infected cells has the effect of slowing the viral infection of cells, which slows the progression to AIDS. Also, by limiting the number of cells infected with HIV, the efficacy of other anti-retroviral therapies increases. Sommadossi, *supra*; Pantaleo, *J. Nat'l Instit. Health Res., supra*; Larder, *supra*; and Baba, *supra*.

Anti-retrovirals for the use in HIV disease include: nucleoside analogs, 3'-azidothymidine (AZT, zidovudine, Retrovir), 2',3'-dideoxycytidine (DDC, Zalcitabine, HIVID), and 2', 3'-dideoxyinosine (ddI, didanosine, Videx). Johnston, M.I. and Hoth, D.F. *Science* 260: 1286 (1993); Yarchoan, et al., *Immunology Today* 14: 303 (1993); didehydrodideoxythymidine (D4T) and phosphonoformate (foscarnet). Sande et al. (eds.) (1992) *AIDS* W.B. Saunders 1992 Philadelphia; and Ribavirin. Baba et al., *supra*. Other anti-retroviral therapies include: (1) reverse transcriptase inhibitors, (2) non-nucleoside inhibitors of HIV reverse transcriptase (tetrahydro-imidazo[4,5,1-jk][1,4]-benzodiazepin-2(1H)-one (TIBO, R82913), 11-cyclopropyl-7-methyl-dipyrido-[2,3-b:3'3'-f]1,4-diazepin-6H-5-one (BI-RG-587, nevirapine), pyridones (L-697,661 and L-696,229), and bis(heteroaryl)piperazines (BHAPs, U-87201E, Atevirdine Mesylate, ATV), R-89439 (an  $\alpha$ -anilino-phenylacetamine ( $\alpha$ -APA) derivative, a second generation BHAP U-90,152, Acyclovir, Vidarabine, Idoxuridine, Triflurioline, Amantadine, Rimantadine, and the Interferons. Johnston et al., *Science, supra*; Mandel et al., *PRINCIPLES & PRACTICE OF INFECTIOUS DISEASE* (3rd ed. 1990); (3) HIV proteins which block entry of the HIV into cells; (4) nucleic acid-based therapeutics, such as antisense oligonucleotides, that block or otherwise target transcription or translation of virally encoded nucleic acids; and (5) protease inhibitors.

The combined use of antibody:antigen complexes and either HIV-specific antibodies (e.g., polyclonal antibodies or monoclonal antibodies that recognize HIV gp120, HIV gp160, HIV gp24, TN antigen or sialyl-TN antigen) or vaccines is likely to decrease the production of virus from cells and protect new cells from becoming infected. See Speigel et al., *supra*. These combined therapies employ the administration anti-retroviral

therapy either concurrently with or subsequently to said providing of said complex.

HIV-specific antibodies include HIV-neutralizing antibodies to gp120, see Wigzell, *FASEB J.* 5: 2406 (1991), and antibodies to TN or sialyl-TN-specific antigens, see O'Boyle et al., *Cancer Res.* 52: 5663 (1992), Hansen et al., *J. Virol.* 65: 6461 (1991), and Hansen et al., *ibid.* 64: 2833 (1990). Vaccines include (1) live, attenuated HIV strains; (2) inactivated HIV; (3) HIV protein subunit immunogens, such as HIV gp120, gp160, or peptides of HIV proteins; (4) multivalent HIV protein subunit immunogen mixtures; (5) subunit immunogens in live vectors (e.g., vaccinia, Salmonella, Calmette-Guerin bacillus, poliovirus, rhinovirus and adenovirus); (6) anti-idiotypic antibody to CD4 or gp120;

(7) intracellular immunization or gene therapy that would make host CD4<sup>+</sup> cells resistant to HIV infection by introducing an HIV resistance gene into CD4<sup>+</sup> immune cells; (8) cDNAs encoding HIV proteins; (9) immunization with host proteins (e.g., CD4 and MHC molecules) to make the HIV incorporate host MHC proteins when budding from infected cells and (10) immunization with TN-specific or sialyl-TN-specific antigens. See Haynes, *Science* 260: 1279 (1993), and O'Boyle et al, *supra*, Hansen et al. (1992, 1991), *supra*.

HIV-infected Rh<sup>+</sup> candidates for therapy pursuant to the present invention may be treated with the following regimen: (A) an initial dose of 5 to 70 µg/kg of antibody followed by daily doses of 5 to 70 µg/kg/day until lymph nodes were cleared of HIV. Thereafter, chronic administration of daily maintenance doses ranging between 5 and 70 µg/kg are provided as needed to prevent or reduce lymphadenopathy. The progression of HIV infection in an HIV-infected individual is considered slowed when an HIV-infected individual has been treated with the antibody-based complex therapy of the present invention and achieves either a decrease in the quantity of HIV particles in their lymph nodes, an increase in circulating HIV, a reduction of HIV-infected cells in lymph tissues or peripheral circulation or a block in the progressive destruction of lymph tissue or a block in the loss of immune function.

Rh<sup>+</sup> individuals at risk of HIV infection are candidates for the therapy of the present invention. They may be treated with the following regimen: (A) an initial dose of 5 to 70  $\mu\text{g/kg}$  of antibody followed by daily doses of 5 to 70  $\mu\text{g/kg/day}$ .  
5 Thereafter, chronic administration of daily maintenance doses ranging between 5 and 70  $\mu\text{g/kg}$  are provided as needed to prevent HIV infection. Prevention of HIV infection is measured by prevention of HIV viremia, a lack of an HIV-specific immune response or an absence of HIV infected cells in the treated  
10 individual.

Another preferred embodiment of the present invention relates to the treatment of Rh<sup>-</sup>, HIV-infected patients or individuals at risk of HIV infection with a mixture of WinRho plus Rh<sup>+</sup> red blood cells. The antigen:antibody complexes in the  
15 mixture would be expected to bind to the FDCs of the Rh<sup>-</sup> patient thereby competitively inhibiting HIV:antibody binding. Alternatively, an Rh<sup>-</sup> individual may be immunized with Rh<sup>+</sup> red blood cells to raise an Rh-specific antibody. Such a patient could then be transfused with Rh<sup>+</sup> red blood cells which will bind  
20 to the circulating Rh-specific antibody to yield antibody:antigen complexes that competitively inhibit HIV:antibody complexes from binding to FDCs.

Rh<sup>-</sup>, HIV-exposed individuals or Rh<sup>-</sup> individuals at risk of HIV infection are given anti-Rh antibody therapy by one of the  
25 following three regimens: (1) an antibody which is not anti-Rh factor; (2) a mixture of WinRho plus Rh<sup>+</sup> red blood cells; (3) WinRho followed by Rh<sup>+</sup> red blood cells or (4) immunization of said individual with Rh<sup>+</sup> red blood cells to raise an Rh-specific antibody. Any of these regimens are provided to such an  
30 individual prior to any onset of clinically significant ITP.

The dosages administered in a mixture of antibody plus antigen are based on the amount of antibody, and therefore Fc, contained in the complex. Similar to the doses administered for Rh<sup>+</sup> HIV-exposed individuals, the mean initial maintenance dose  
35 of WinRho was 5 to 70  $\mu\text{g/kg/infusion}$ . Any significant increase in the presence of HIV in peripheral blood, as measured by either PCR to detect HIV DNA or reverse transcriptase PCR to detect HIV

RNA and a significantly decreased quantity of HIV in lymph tissues, as assayed by either PCR or in situ hybridization, indicates the slowing of the progression of HIV infection in the HIV-exposed individual.

5                   Alternatively, the Rh<sup>-</sup> individuals may be immunized with Rh<sup>+</sup> red blood cells so that they raise Rh-specific antibodies. These patients are then transfused with Rh<sup>+</sup> red blood cells. The transfused cells bind to the circulating Rh-specific antibody yielding antibody:antigen complexes which  
10 compete with HIV:antibody complexes for binding of FDCs.

Without further elaboration, it is believed that one skilled in the art can, using the preceding description, utilize the present invention to its fullest extent.

What Is Claimed Is:

1. A method for slowing the progression of HIV infection in an HIV-exposed individual or preventing HIV infection in an individual at risk of HIV infection, comprising  
5 the step of providing said individual with provided complex selected from antibody:antigen complex and antibody:antigen: complement complex, in a titer adequate to competitively inhibit HIV complexes from binding cells via Fc receptors or complement  
10 receptors, wherein said provided complex is given to said patient prior to any onset of clinically significant ITP.

2. The method according to claim 1, wherein said provided complex is administered prior to HIV infection to an individual at risk of HIV infection.

15 3. The method according to claim 1, wherein said antibody is an autoantibody.

4. The method according to claim 1, wherein said provided complex is administered after the HIV exposure.

5. The method according to claim 1, wherein said individual belongs to any of CDC HIV Groups I through IV.

20 6. The method according to claim 1, wherein said provided complex comprises an antibody that recognizes a cellular antigen.

7. The method according to claim 6, wherein said cellular antigen is a red blood cell antigen.

25 8. The method according to claim 7, wherein said red blood cell antigen is Rh factor.

9. The method according to claim 7, wherein said red blood cell antigen is glycophorin or band III protein.

30 10. The method according to claim 1, wherein said provided complex is administered in a regimen that comprises an initial dose of said antibody, followed by daily doses capable of competitively inhibiting HIV binding cells via Fc receptors or complement receptors, and thereafter by administrations of maintenance doses.

35 11. The method of claim 1, further comprising the step of subjecting said individual to an anti-retroviral therapy, either concurrently with or subsequently to said providing of said provided complex.

12. The method of claim 11, wherein said therapy comprises administering to said individual a nucleoside analog.

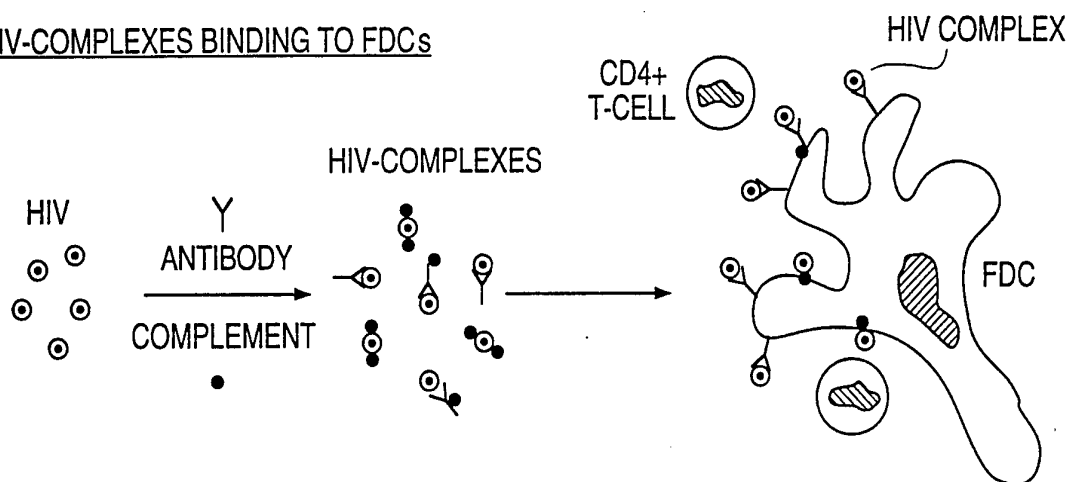
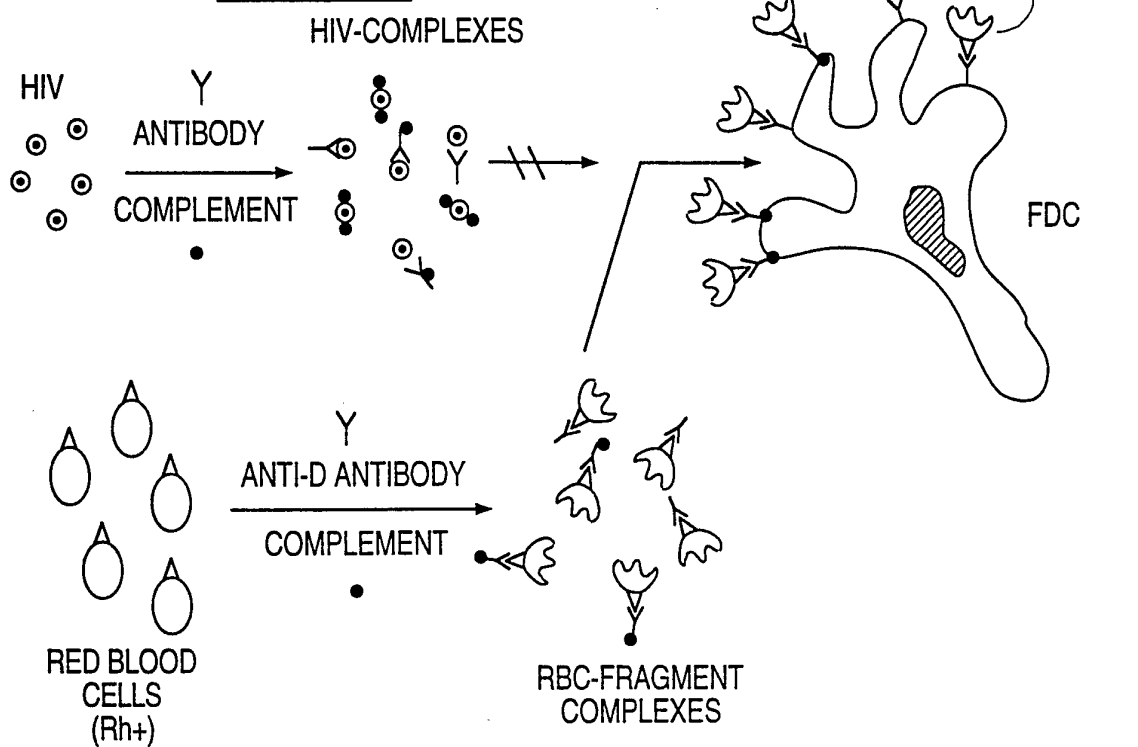
13. The method of claim 11, wherein said anti-retroviral therapy comprises administering an anti-retroviral agent selected from the group consisting of 3'-azidothymidine, 2',3'-dideoxyinosine and 2',3'-dideoxycytidine.

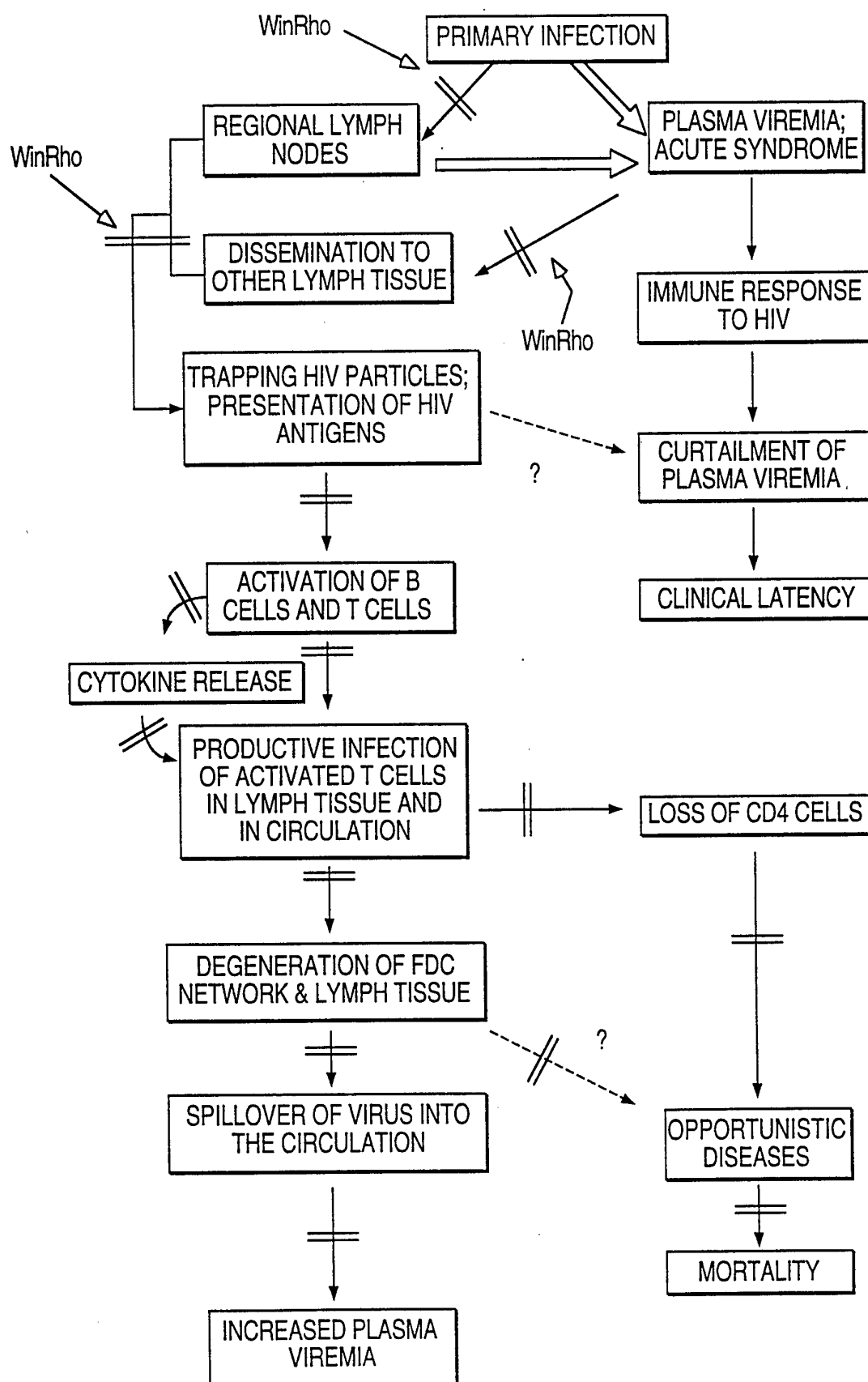
14. The method of claim 1, further comprising the step of subjecting said individual to treatment with HIV-specific antibodies, either concurrently with or subsequently to said providing of said provided complex.

15. The method of claim 14, wherein said HIV-specific antibodies are selected from the group consisting of polyclonal antibody that recognizes HIV gp120, polyclonal antibody that recognizes HIV p24, polyclonal antibody that recognizes HIV gp160, monoclonal antibody that recognizes HIV gp120, monoclonal antibody that recognizes HIV p24, monoclonal antibody that recognizes HIV gp160, polyclonal antibody that recognizes TN antigen, polyclonal antibody that recognizes sialyl TN antigen, monoclonal antibody that recognizes TN antigen and monoclonal antibody that recognizes sialyl TN antigen.

16. The method of claim 1, further comprising the step of subjecting said individual to treatment with an HIV vaccine, either concurrently or subsequently to said providing of said provided complex.

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**FIG. 1A**HIV-COMPLEXES BINDING TO FDCs**FIG. 1B**RBC-COMPLEXES BINDING TO FDCs AND  
COMPETITIVELY INHIBITING HIV-COMPLEX  
FROM BINDING

2/2  
**FIG. 2**

## INTERNATIONAL SEARCH REPORT

International Application No.  
PCT/US 94/08312

## A. CLASSIFICATION OF SUBJECT MATTER

A 61 K 39/42, A 61 K 39/21

According to International Patent Classification (IPC) or to both national classification and IPC 6

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A 61 K

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

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

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                          | Relevant to claim No. |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A          | CHEMICAL ABSTRACTS, vol. 114,<br>no. 11, issued 1991, March 18<br>(Columbus, Ohio, USA),<br>O.ANDERSEN et al. "Conglutinin<br>binds HIV-1 envelope glyco-<br>protein gp160 and inhibits<br>its interaction with cell<br>membrane CD4"<br>page 557, no. 99 782p;<br>& Scand.J.Immunol. 1991,<br>33(1), 81-8. | 1                     |
| A          | ---<br>CHEMICAL ABSTRACTS, vol. 113,<br>no. 23, issued 1990,<br>December 03,<br>(Columbus, Ohio, USA),<br>I.SEKIGAWA et al. "CD4 immuno-<br>adhesin, but not recombinant<br>soluble CD4, blocks syncytium                                                                                                   | 1                     |

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

## \* Special categories of cited documents :

- \* "A" document defining the general state of the art which is not considered to be of particular relevance
- \* "E" earlier document but published on or after the international filing date
- \* "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \* "O" document referring to an oral disclosure, use, exhibition or other means
- \* "P" document published prior to the international filing date but later than the priority date claimed

\* "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\* "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\* "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\* "&" document member of the same patent family

Date of the actual completion of the international search  
23 December 1994

Date of mailing of the international search report

01 -02- 1995

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SCHNASS e.h.

# INTERNATIONAL SEARCH REPORT

-2-

International Application No  
PCT/US 94/08312

| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT |                                                                                                                                                                                                                                                                                         |                       |
|------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| Category *                                           | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                      | Relevant to claim No. |
| A                                                    | <p>formation by human immuno-<br/>deficiency virus type<br/>2-infected lymphoid cells",<br/>page 543, no. 209 496n;<br/>&amp; J.Virol. 1990, 64(10),<br/>5194-8.</p> <p>--</p> <p>WO, A, 91/06 575<br/>(REPLIGEN CORPORATION)<br/>16 May 1991 (16.05.91),<br/>abstract.</p> <p>----</p> | <p>1, 14,<br/>15</p>  |

## INTERNATIONAL SEARCH REPORT

International application

PCT/US 94/08312

**Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

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

1. ☒ Claims Nos.: 1-16  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Though all the claims deal with a subject excluded from patentability by Article 17(2)(a)i) with Rule 39.1.iv) PCT they have all been searched.
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# ANHANG

zum internationalen Recherchen-  
bericht über die internationale  
Patentanmeldung Nr.

# ANNEX

to the International Search  
Report to the International Patent  
Application No.

# AN JEXE

au rapport de recherche inter-  
national relatif à la demande de brevet  
international n°

PCT/US 94/08312 SAE 97346

In diesem Anhang sind die Mitglieder  
der Patentfamilien der im obenge-  
nannten internationalen Recherchenbericht  
angeführten Patentdokumente angegeben.  
Diese Angaben dienen nur zur Unter-  
richtung und erfolgen ohne Gewähr.

This Annex lists the patent family  
members relating to the patent documents  
cited in the above-mentioned inter-  
national search report. The Office is  
in no way liable for these particulars  
which are given merely for the purpose  
of information.

La présente annexe indique les  
membres de la famille de brevets  
relatifs aux documents de brevets cités  
dans le rapport de recherche inter-  
national visée ci-dessus. Les renseigne-  
ments fournis sont donnés à titre indica-  
tif et n'engagent pas la responsabilité  
de l'Office.

| Im Recherchenbericht<br>angeführtes Patentdokument<br>Patent document cited<br>in search report<br>Document de brevet cité<br>dans le rapport de recherche | Datum der<br>Veröffentlichung<br>Publication<br>date<br>Date de<br>publication | Mitglied(er) der<br>Patentfamilie<br>Patent family<br>member(s)<br>Membre(s) de la<br>famille de brevets | Datum der<br>Veröffentlichung<br>Publication<br>date<br>Date de<br>publication |
|------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| WO A1 9106575                                                                                                                                              | 16-05-91                                                                       | keine - none - rien                                                                                      |                                                                                |