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(54) Title: ANTIGEN-BASED HETEROPOLYMERS AND METHOD FOR TREATING AUTOIMMUNE DISEASES USING THE SAME

(57) Abstract

Constructs consisting of antigen-based heteropolymers (AHP's) are provided. The antigen-based heteropolymers comprise at least one monoclonal antibody specific for binding to complement receptor (CR1) site on a human or non-human primate erythrocyte, and the anti-CR1 monoclonal antibody is cross-linked to an antigen specific for a target pathogenic autoantibody. Further provided is a method for treating autoimmune diseases in human or non-human primates using the AHP.

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Description

Antigen-Based Heteropolymers and Method For Treating Autoimmune Diseases Using the Same

Technical Field

The present invention relates to antigen-based heteropolymers specific for both a specific receptor site on a primate erythrocyte and a target pathogenic autoantibody. The present invention further relates to methods for treating autoimmune diseases using these antigen-based heteropolymers.

Background Art

Circulating autoantibodies are responsible for much of the pathogenesis associated with a number of autoimmune diseases including, but not limited to, systemic lupus erythematosus (SLE), autoimmune myocarditis, immune complex mediated kidney disease, rheumatoid arthritis, myasthenia gravis, autoimmune anemias, Sjogren's Syndrome, idiopathic thrombocytopenic purpura, various forms of vasculitis, and at least some of the cellular cytotoxicity accompanying Acquired Immune Deficiency Syndrome (AIDS).

Efforts to treat autoantibody-mediated disorders have been only partially successful. Many of the past developed therapies have been based on the use of general immunosuppressive measures, through drugs or therapeutic monoclonal antibodies designed to completely abrogate antibody production. However, to date, a successful treatment has not been designed which targets the specific autoantibodies.

Plasmapheresis, which is designed to remove all the circulating antibodies, has been attempted, but only with limited success. In recent years, a variety of extracorporeal immunoabsorption procedures have been attempted. These procedures are more specific, and attempt to remove only the pathogenic autoantibodies.

This involves allowing the blood or plasma to flow over matrices outside the body which contain the autoantigen which is the natural target of the autoantibodies. These methods are slow, invasive, and expensive, and are associated with several technical problems including the need to perform the procedures repeatedly because of their quantitative inefficiency. The complication of complement activation on the matrices exists. Overall, therapeutic successes have been modest, at test.

Another general, non-specific approach involves aggressive immunosuppressive therapy with corticosteroids, and cytotoxic and nonsteroidal anti-inflammatory drugs. Although in many instances clinical improvements have been obtained, there continues to be significant morbidity and mortality in autoimmune diseases despite theese medications.

Therefore, notwithstanding these advances in autoimmune therapy, there remains to be seen an internal therapy which is specific for the target pathogenic autoantibody and which is both fast and quantitative.

Disclosure of the Invention

Accordingly, it is an objective of the present invention to provide an autoimmune therapy which is highly specific for a target pathogenic autoantibody and which produces fast and quantitative results.

It is a further objective of the present invention to provide a method for treating autoimmune diseases in humans and non-human primates using such an autoimmune therapy.

The above objectives have been met by the present invention, which are called antigen-based heteropolymers. These are specific for both complement receptor site (CR1) on a primate erythrocyte and a target pathogenic autoantibody. More specifically, the above objectives

have been met by the present invention which provides a complex which comprises at least one antigen-based heteropolymer comprising a monoclonal antibody specific for binding to a CR1 site on a primate erythrocyte, wherein the monoclonal antibody is crosslinked to an antigen specific for a target pathogenic autoantibody.

In addition, the objectives have been met by the present invention which further provides a method for treating autoimmune diseases by administering to a human or non-human primate an effective amount of an antigen-based heteropolymer comprised of a monoclonal antibody specific for the CR1 site on a primate erythrocyte, wherein the monoclonal antibody is crosslinked to an antigen which is specific for a target pathogenic autoantibody.

Brief Description of the Invention

Figure 1 is a schematic illustration of the antigen-based heteropolymer complex consisting of a monoclonal antibody bound to the CR1 receptor site on a primate erythrocyte. The monoclonal antibody is also crosslinked to an autoantigen (dsDNA) which in turn, is bound to the target pathogenic autoantibody (anti-dsDNA antibody) .

Figure 2 is a graph showing the kinetics of the antigen-based heteropolymer-mediated binding of IgG anti-dsDNA antibodies to human red blood cells. The counts bound are proportional to the amount of human IgG bound (see below). Ma, Mo, and Ha are the last name initials of SLE patients from whom the plasmas or IgG fractions were taken. The solid symbols indicate incubation with red blood cells and antigen-based heteropolymer, and the above respective antibodies. The open symbols represent incubation with red blood cells and antibodies, without antigen-based heteropolymer.

Figure 3 is a graph showing examination of red blood cells prepared and incubated as described in Figure 2, and probed with ^{125}I-labeled monoclonal antibodies to both human IgG and human IgM. Plasma Ma is representative of samples from patients with severe nephritis and contains almost exclusively IgG anti-dsDNA antibodies at a very high titer. Plasma Va contains both IgG and IgM anti-dsDNA antibodies.

Figure 4 is a graph showing results of control experiments to demonstrate the specificity of binding. Human red blood cells were examined with a four-fold dilution of plasma Ma, or an IgG fraction from plasma Ma was examined. Pl = plasma; Nl = normal; SRBCS = sheep red blood cells; AHP = antigen-based heteropolymer; Ma = patient with severe nephritis.

Figure 5 is a graph showing the results of control experiments which indicate that binding is permissive in normal human serum and verify that the red blood cells are not lysed. A two-fold dilution of plasma Ma (diluted in bovine serum albumin) was compared to a similar dilution of plasma Ma in fresh, normal human serum. PL = plasma; NHSC = normal human serum complement; AHP = antigen-based heteropolymer; IRR irrelevant monoclonal antibody.

Figure 6 is a graph showing the results of dose response experiments illustrating the optimum input of antigen-based heteropolymer to maximize binding of human IgG anti-dsDNA antibodies to 150 μl of red blood cells (50% hematocrit) in an SLE IgG sample. AHP3 was prepared as in Example 1 (see below). AHP2 = contains 1/2 as much dsDNA as AHP3; AHP1 = contains 1/4 as much dsDNA as AHP3.

Figure 7 is a graph showing binding as a function of the concentration of IgG anti-dsDNA antibodies. A 50% dispersion of human red blood cells was examined in a mixture of bovine serum albumin and IgG Mo.

The data (calculated line) was fit to a simple straight line equation by the least squares analysis.

Figure 8 is a graph showing binding as a function of the concentration of IgG anti-dsDNA antibodies using a very high titer anti-dsDNA plasma (Ma).

Figure 9 is a graph showing binding as a function of the concentration of IgG anti-dsDNA antibodies using plasma Ma. (7G9 AHP and 1B4 kHP alone as compared to cocktail containing 7G9 and 1B4 together.)

Best Mode For Carrying Out The Invention

The present invention is based on the unique structural and biophysical properties of the primate red blood cell complement receptor (CR1). The antigen-based heteropolymer complexes of the present invention are specific for both the CR1 site on a primate red blood cell and for a target pathogenic autoantibody.

The antigen-based heteropolymers of the present invention are prepared from monoclonal antibodies which are specific for the CR1 receptor site on a primate erythrocyte. The monoclonal antibodies must also be capable of being crosslinked to an autoantigen which is specific for the target pathogenic autoantibody.

Examples of such monoclonal antibodies used in the present invention include 1B4, HB8592, and 7G9. HB8592 and 1B4 are disclosed in Taylor et al., "Use of heteropolymeric monoclonal antibodies to attach antigens to the C3b receptor of human erythrocytes: A potential therapeutic treatment," Proc. Nat'l. Acad. Sci., 88:3305-3309 (April 1991); Reist et al., "Antigens pre-bound to the primate erythrocyte complement receptor via cross-linked bispecific monoclonal antibody heteropolymers are rapidly cleared from the circulation, "Eur. J. Immunol. 23:3021-3027 (1993). 7G9 is a mAb recently developed in the inventors' laboratory and can be prepared by known

techniques. Other mAbs to CR1 available and useful include 3D9 and E-11 [(previously used by the present inventors in Proc. Nat'l. Acad. Sci., Vol. 91 (July 1992)], and 57F and YZ1 (prepared and reported by Nussenzweig and Fearon, respectively). It is likely that any mAb for CR1 will work successfully in the antigen-based heteropolymer system of the present invention.

The monoclonal antibody is crosslinked to an antigen specific for a target pathogenic autoantibody. The crosslinking can be performed by any efficacious crosslinking method. For example, purified monoclonal antibodies may first be biotinylated. Typically, each monoclonal antibody may contain 5 biotins. The biotinylated purified monoclonal antibody is then crosslinked to a previously biotinylated antigen or autoantigen by the use of streptavidin. Other known methods, such as use of N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP) can be used for crosslinking the monoclonal antibody to the antigen if the antigen has free amino groups. The details of preparation of non-antigen-based heteropolymers can be found in Taylor et al. Proc. Nat'l. Acad. Sci. *supra;* Reist et al. *supra;* and Taylor et al., "In vitro binding and clearance of circulating antigen by bispecific heteropolymer-mediated binding to primate erythrocyte complement receptor," J. Immunol., 148(8):2462-2468 (April 1992).

Many different autoantibodies can theoretically be cleared from the circulation of a primate by using the antigen-based heteropolymers of the present invention. Specifically, certain humans with Hemophilia have been shown to be deficient in factor Viii. Recombinant factor VIII replacement treats this hemophilia. However, eventually some patients develop antibodies against factor VIII, thus interfering with the therapy. The antigen-

based heteropolymer of the present invention prepared with factor VIII provides a therapeutic solution for this problem.

Specifically, an antigen-based heteropolymer comprised of factor VIII crosslinked to a mAb to CR1 specifically binds circulating anti-factor VIII autoantibodies to erythrocyte CR1 and facilitates clearance of said autoantibodies. Factor VIII is allowed to circulate and facilitate blood clotting.

Other autoantibodies which can be cleared by the antigen-based heteropolymer complex of the present invention include, but are not limited to, autoantibodies to the following antigens: the muscle acetylcholine receptor (the antibodies are associated with the disease myasthenia gravis); cardiolipin (associated with the disease lupus); platelet associated proteins (associated with the disease idiopathic thrombocytopenic purpura); the multiple antigens associated with Sjogren's Syndrome; the antigens implicated in the case of tissue transplantation autoimmune reactions; the antigens found on heart muscle (associated with the disease autoimmune myocarditis); the antigens associated with immune complex mediated kidney disease; the dsDNA and ssDNA antigens (associated with lupus nephritis); desmogleins and desmoplakins (associated with pemphigus and pemphigoid); or any other antigen which is well-characterized, can be crosslinked to an anti-CR1 mAb, and is associated with disease pathogenesis.

When the above antigen-based heteropolymer complexes are injected into the circulation of a human or non-human primate, the antigen-based heteropolymer will readily bind to the red blood cells via the monoclonal antibody at a high percentage and in agreement with the number of CR1 sites on the red blood cells. At approximately the same rate, the antigen-based heteropolymer will then bind

to the autoantibody via the antigen, which is crosslinked to the monoclonal antibody. The red blood cells which contain the bound antigen-based heteropolymer autoantibody complex can then act therapeutically by facilitating the neutralization and clearance from the circulation of the bound pathogenic autoantibody. In the present invention, the antigen-based heteropolymers facilitate binding to the red blood cells and subsequently clear the autoantibody from the circulation of human and non-human primates, without also clearing the red blood cells themselves.

The present inventors have found for the first time, that by crosslinking anti-CR1 monoclonal antibodies with an autoantigen, thus generating the antigen-based heteropolymers of the present invention, it is possible to bind specifically to red blood cells the vast majority (ca. 80%-95%, Table 1 and Figure 9) of the autoantibodies (specific for an antigen) found in plasmas from patients with a specific autoimmune disease. Specifically, the examples demonstrate that it is possible to bind specifically to red blood cells the vast majority of the autoantibodies to the dsDNA antigen found in plasmas from patients with systemic lupus erythematosus (SLE).

The present invention utilizes the unique properties of the primate red cell CR1 which enable it to bind and clear complement-opsonized immune complexes from the circulation. The immune complexes cleared via this system are taken up by the liver and spleen (Cornacoff, J. Clin. Invest. (1983)).

The specificity of the binding of the AHP complex to the autoantibodies or antibodies and the red blood cell CR1-antigen-based heteropolymer complex is clear, because when a) the antigen-based heteropolymer is omitted; b) sheep red blood cells which lack the CR1 site are substituted for human red blood cells; c) the

biotinylated autoantigen is omitted in preparing the antigen-based heteropolymer; and d) normal sera or normal IgG is substituted for SLE plasmas, no binding is observed (Figure 4). Further proof of specificity is demonstrated in that no binding is observed if e) the antigen-based heteropolymer is prepared with no monoclonal antibody; f) the antigen-based heteropolymer is prepared with an "irrelevant" monoclonal antibody; and g) excess monomeric monoclonal antibody anti-CR1 is used to inhibit the antigen-based heteropolymer complex (Figure 5). It has also been confirmed that the presence of fresh serum (as a complement source, 25% by volume) does not inhibit specific binding or lyse the red blood cells (Figure 5).

The input range of the antigen-based heteropolymer is determined based on the red blood cell concentration and the number of CR1 epitope sites recognized by the anti-CR1 monoclonal antibodies per red blood cell. If the antigen-based heteropolymer complex is added in excess, a fraction of the antigen-based heteropolymer will not bind to red blood cells, but will instead inhibit the uptake of the autoantibodies by the red blood cell. The reason is that when the free antigen-based heteropolymer is in solution, it will simply compete for available autoantibody with antigen-based heteropolymer bound to red blood cells. Thus, the antigen-based heteropolymer-mediated binding of the autoantibodies to human red blood cells follows a bell-shaped curve when binding is examined as a function of the input antigen-based heteropolymer concentration (Figure 6).

The quantitative effectiveness of the antigen-based heteropolymers of the present invention in facilitating the binding of autoantibodies to red blood cells is demonstrated in the results of Farr assays.

The results of the Farr assays demonstrate that the autoantibodies are indeed specifically absorbed to red blood cells and in a high percentage (> 90% in Table 1) in the presence of the antigen-based heteropolymer complex of the present invention.

It has further been demonstrated that there is a linear relationship between the input of autoantibodies and the level of autoantibodies present on the red blood cells due to antigen-based heteropolymer-mediated binding (Figure 7). In some instances the system shows a saturation because the concentration of the autoantibodies in the plasma is so high that even at the optimum input of antigen-based heteropolymers, not all of the autoantibodies can be bound to the red blood cells under standard conditions (Figure 8). This is also shown in the Farr assays. For example, for a very high titer sera, a fraction of the autoantibody binding activity is not bound to the red blood cells (Table 1) due to its high concentration. These cases are the exception, and for the majority of samples from patients with lupus nephritis (which have pathogenic levels of anti-dsDNA antibodies), the method of the present invention removes > 90% of the anti-dsDNA antibodies.

However, the saturation problem can be solved by using combinations of antigen-based heteropolymer which contain monoclonal antibodies that bind to different sites on CR1. The monoclonal antibodies 7G9 and 1B4 bind to separate and noncompeting sites on red blood cell CR1. Therefore, a "cocktail" containing a mixture of two antigen-based heteropolymers made with respective monoclonal antibodies gives rise to greater binding of autoantibodies to red blood cells. Plasma Ma is one of the highest titer SLE IgG anti-dsDNA plasma studied. The single antigen-based heteropolymer prepared with either anti-CR1 monoclonal antibodies 7G9 or 1B4 alone

cannot absorb all anti-dsDNA antibodies to red blood cells in the undiluted plasma. However, when the "cocktail" is used, more than 90% of the IgG anti-dsDNA in plasma Ma is absorbed to red blood cells (Figure 9). Also, the results of the Farr assay (Table 1) of the plasma supernatants indicate that greater than 90% of the anti-dsDNA antibody binding activity in plasma Ma is removed when it is treated with the antigen-based heteropolymer cocktail and red blood cells.

The present invention further encompasses a method for treating an autoimmune disease comprising administering to a primate an effective amount of an antigen-based heteropolymer complex of the present invention. The route of administration will likely be by intravenous injection into the blood of a human or non-human primate.

An effective amount of the antigen-based heteropolymer complex of the present invention is from 1-10 mg, preferably 5 mg administered once. This dosage should clear up to 2 μg/ml of the autoantibody from the circulation of a primate. In a therapeutic environment, the treatment should be repeated until complete clearance of the pathogenic autoantibody. The antigen-based heteropolymers of the present invention can be used in combination with certain fluids used for intravenous infusions.

Although the prototype studies disclosed herein are carried out using mouse mAbs, currently available technology should allow "humanization" of these mouse mAbs. This will decrease the chance that an immune response to the antigen-based heteropolymer will abrogate its effectiveness in repeated doses.

Red blood cells removed and isolated may also be used as therapeutic agents. Once franked with the antigen-based heteropolymer, these red blood cells can be

reintroduced into the patient, where, in the bloodstream free autoantibodies will bind and be immobilized on the red blood cell, and subsequently cleared in accordance with the body's red blood cell clearing mechanism.

As set forth above, in an alternative embodiment, red blood cells are franked with a "cocktail" of at least two antigen-based heteropolymers, which, in addition to binding to the target autoantibody, also bind to several distinct and non-overlapping sites on CR1 of the primate red blood cell. As some of the following experiments show, by using at least two non-overlapping monoclonal antibodies for binding to CR1 on the red blood cell of primates, the number of heteropolymer complexes that can also bind is increased at a high percentage and in good agreement with the number of available binding sites. This, in turn, allows for more autoantibody binding to the heteropolymer complexes of the present invention. This augments the capability of a relatively small number of red blood cells to bind to a relatively larger amount of autoantibody, and further can facilitate the removal of the autoantibody through the normal primate immune clearance system.

The AHP of the present invention can further be used in the case of clearance of exogenous administered antibodies which become pathogenic.

EXAMPLES

EXAMPLE 1

Monoclonal antibodies and crosslinked AHP anti-CR1/dSDNA complexes.

Three monoclonal antibodies specific for primate CR1, i.e., 1B4, HB8592, and 7G9, were purified by known methods as set forth in Reist et al., *supra*. The purified monoclonal antibodies were then biotinylated by known methods. Typically, each monoclonal antibody would

contain 5 biotins. An amount of 24 μg of biotinylated 7G9 was incubated for 30 minutes at room temperature with 30 μg of streptavidin (SA) in a volume of 48 μl borate saline (BS buffer).

The resulting sample was then incubated with 33 μg of biotinylated dsDNA which was purchased from Dr. W. Emlen of the University of Colorado Medical Center (1 biotin per 30 base pairs in 1 ml of BS buffer) in order to crosslink 7G9 to the biotinylated dsDNA. The process was repeated using 1B4 or HB8592. The anti-CR1/dsDNA complexes formed were used without any other purification.

EXAMPLE 2

Binding assays.

SLE plasmas or IgG fractions from 3 different patients which are denoted by last name initials (i.e., Ma, Mo, and Ha) were incubated by general methods [Taylor et al., "The Interaction of Antibody/DNA Immune Complexes with Complement,"Arthritis and Rheumatism, 30(2):176-185 (February 1987) with red blood cells and AHP (10 μl AHP3 per 150 μl of 50% red cells) (solid symbols in Figure 2) and without AHP (open symbols in Figure 2) for the indicated time period at 37°C as follows: Type O red blood cells were washed and then reconstituted to a 50% hematocrit in one of the above plasmas. The AHP was added in an "equivalence" of ca. 0.6-1.2 μg of anti-CR1 mAb per ml blood based on 500-1000 CR1 receptors per red blood cell. After brief incubation, the sample was centrifuged and the supernatant isolated and saved for an independent assay (Farr assay) of the anti-dsDNA antibodies. The red cell pellets were washed 3 times and then an ^{125}I-labeled monoclonal antibody to human IgG (HB43) was added to the red blood cells to measure uptake of human IgG. (Taylor et al., 88:3305-3309, Proc. Natl.

Acad. Sci. *supra*.) The arrow at the bottom of Figure 2 emphasizes the low level of background binding to red blood cells of all 3 samples after a full 15 minute incubation. The broken lines at the bottom left of the figure are extrapolations to 0 minutes of incubation with the AHP.

Figure 2 demonstrates the results of the kinetics of AHP-mediated binding of IgG anti-dsDNA antibodies to human red blood cells. These results show that the complexes of the present invention bind to red blood cells. The results further show that by virtue of the incorporated dsDNA, the AHP complexes facilitate the specific and rapid binding (ca. 80% complete in ca. 5 minutes at 37°C as shown in Figure 2) to red blood cells of high avidity IgG and/or IgM/anti-dsDNA antibodies found in SLE plasmas. The specificity of the binding for anti-dsDNA antibodies and the anti-CR1/dsDNA AHP complex is clear due to the fact that all the binding is abrogated when the AHP is omitted (open symbols in Figure 2).

EXAMPLE 3

Two SLE samples were prepared as in Example 2 above. However, the samples were incubated with AHP for 15 minutes at 37°C.

Plasma Ma, as shown in Figure 3, is representative of samples from patients with severe nephritis, and as previously reported, has almost exclusively IgG anti-dsDNA antibodies at very high titer (Taylor et al., Arthritis and Rheumatism, (1987)]. The plasma Va was shown in Taylor et al. to have both IgG and IgM anti-dsDNA antibodies.

The red blood cells were probed with ^{125}I-labeled monoclonal antibodies to both human IgG (HB43) and human IgM (HB57).

EXAMPLE 4

Control experiments to demonstrate the specificity of binding.

Plasma Ma, or an IgG fraction from plasma Ma, was prepared as set forth above. The plasma Ma and the IgG fraction from plasma Ma were diluted four-fold. Human red blood cells were used as above in this experiment. Five different samples were prepared as follows: a) AHP omitted; b) sheep red blood cells (SRBCS) lacking the CR1 site were substituted for human red blood cells; c) the biotinylated dsDNA was omitted in preparing the AHP; and d) normal plasma or normal IgG was substituted for SLE plasma (Figure 4).

The results shown in Figure 4 demonstrate that only in the presence of the AHP and human red blood cells (first 2 groups of Figure 4 on the left) is binding evident. If AHP is omitted or the dsDNA antigen is omitted from the AHP, no binding is seen. Further, when sheep red blood cells were used with Ma, or normal human IgG was used in place of Ma, binding was also reduced to background levels.

EXAMPLE 5

In this experiment, a two-fold dilution of plasma Ma (diluted in bovine serum albumin) was compared to the same dilution of plasma Ma in fresh normal human serum. The following four samples were prepared: a) the AHP was prepared with no monoclonal antibody; b) the AHP was prepared with a monoclonal antibody to the dinitrophenol group (23D1, an irrelevant (IRR) antibody); c) excess monomeric 7G9 anti-CR1 was used to inhibit the 7G9/dsDNA AHP; and d) serum prepared with AHP.

The proof of specificity is demonstrated in Figure 5 by the showing that no binding is observed if the AHP is prepared with no monoclonal antibody, the AHP is prepared

with an irrelevant monoclonal antibody, or if excess monomeric 7G9 anti-CR1 is used to inhibit the 7G9/dsDNA AHP. The specificity of the AHP, which contains biotinylated monoclonal antibody 7G9, is demonstrated because excess monoclonal antibody 7G9 is able to compete for sites on CR1 and abrogate all binding. It has also been confirmed that the presence of fresh serum (as a complement source, 25% by volume) does not inhibit specific binding or lyse the red blood cells (Figure 5).

EXAMPLE 6

Farr assay showing quantitative effectiveness of the antigen-based heteropolymers.

The quantitative effectiveness of the antigen-based heteropolymer complexes of the present invention in facilitating binding of target autoantibodies to red blood cells is demonstrated in Table I which shows the results of Farr assays. The Farr assay was performed as in Taylor et al., Arthritis and Rheumatism, *supra*. These assays are specific for anti-dsDNA antibodies. The supernatant samples contain the plasma proteins and the vast majority of IgG, not specific for dsDNA, which did not bind to red blood cells. These assays indicate that the anti-dsDNA binding activity in the supernatants is reduced considerably for most SLE plasma and IgG fractions (more than 90% in Table 1). That is, the IgG anti-dsDNA antibodies are indeed specifically absorbed to the red blood cells in the presence of the AHP complex.

Table 1

Table 1. Efficiency of Removal of Anti-dsDNA Antibody Binding Activity from SLE Plasmas by adsorption to Red Cells in the Presence of a Specific AHP. Analysis of binding of ^{3}H-dsDNA in the Farr Assay.

SLE Plasma[a]	Dilution[b] tested in Farr assay	^{3}H-dsDNA (DPM) in the SN (Unbound Counts) Adsorbed with red cells alone	Adsorbed with red cells +AHP	% Adsorbed[c]
Ma/2	5 25 100	58 270 NL IgG[d]: (1000)	710 950 970	95
Va/1	10 25 100	680 1040 1420 NL IgG[d]: (1800)	1400	90
Co/1	4 20 40	730 1280 1550 NL IgG[d]: (2600)	1250 1830 2300	80
Ha/2	5 20 80 320	30 90 270 390 NL IgG[d]: (1200)	120 360 630 680	85, 95
Ma/1	4 8 16 32	370 470 970 1700 NL IgG[d]: (3000)	1760	90[e]

a) Dilution of SLE plasma incubated with the red cells (± AHP).

b) Dilutions of material not bound to red cells tested in Farr assay.

c) Percent adsorbed calculated based on relative dilution of "+AHP" sample needed to give same level of binding as sample lacking AHP. Unless otherwise specified, 7G9 was used to generate the AHP.

d) In Farr assays, IgG antibodies are precipitated with saturate ammonium sulfate, and then dsDNA not bound to the anti-dsDNA antibodies is measured in the supernatant (SN). The background binding level for normal IgG is given for each assay. A number of dsDNA preparations of different input and specific activity were tested.

e) A "cocktail" of the AEP prepared with both 7G9 and 1B4 was tested. When the two AHP's were each evaluated separately, only 70% of the anti-dsDNA binding activity was adsorbed.

The above results demonstrate that the autoantibodies are indeed specifically absorbed to red blood cells and in a high percentage (> 90%) in the presence of the antigen-based heteropolymer complex of the present invention.

EXAMPLE 7

Dose response experiments.

AHP1, AHP2 and AHP3 were prepared as follows. AHP3 was prepared in accordance with the procedures set forth in Example 1 using 10 μl of AHP3 per 150 μl of 50% red blood cells. AHP1 and AHP2 were prepared accordingly, except that AHP2 contains 1/2 as much dsDNA as AHP3, and AHP1 contains 1/4 as much dsDNA as AHP3.

Dose response experiments were performed to find the optimum input of AHP to maximize binding of human IgG anti-dsDNA antibodies to 150 μl of human red blood cells (50% hematocrit) in an SLE IgG sample.

The AHP-mediated binding of IgG anti-dsDNA antibodies to human red blood cells follows a bell-shaped

curve when binding is examined as a function of the input AHP concentration (Figure 6).

AHP3 was chosen as the exemplified AHP because it facilitates the highest level of binding IgG anti-dsDNA to red blood cells.

This data shows that the input range of AHP is determined based on the red blood cell concentration and the number of CR1 epitope sites (recognized by the anti-CR1 monoclonal antibodies) per red blood cell. It is clear that if the AHP is added in excess, a fraction of the AHP will not bind to the red blood cells and will instead, inhibit uptake of the autoantibodies by the red blood cell. The reason is that the free AHP in solution will simply compete for available autoantibody with AHP bound to red blood cells.

EXAMPLE 8

Dose response experiment as a function of autoantibody concentration.

A 50% dispersion of human red blood cells was examined in mixtures of bovine serum albumin and IgG Mo. Figure 7 shows the results of this binding experiment. The results indicate that there is a linear relationship between counts bound (level of bound IgG) and the input level of SLE IgG anti-dsDNA antibodies due to AHP-mediated binding. The data was calculated to fit a simple straight line equation by least squares analysis.

EXAMPLE 9

A similar experiment as in experiment 8 was performed except, in this case, a very high titer anti-dsDNA plasma (Ma) was examined. When the sample was used and diluted, it was clear that not all the endogenous IgG anti-dsDNA is bound by the red blood cells. Therefore, in some instances, the system shows saturation because

the concentration of IgG anti-dsDNA antibodies in the SLE plasma is so high that even at optimum input of AHP, not all the IgG anti-dsDNA antibodies can be bound to the red blood cells under standard conditions (Figure 8). This is also shown in the Farr assays (below) where it has been found that for very high titer plasma, a substantial fraction of the anti-dsDNA antibody binding activity is not bound to the red blood cells (see Table 1). Fortunately, these cases are the exceptions. For the majority of SLE plasma from patients with lupus nephritis (and pathogenic levels of anti-dsDNA antibodies) which were examined, the method does remove greater than 90% of the anti-dsDNA antibodies.

EXAMPLE 10

Binding as a function of the concentration of IgG anti-dsDNA antibodies using ARP "cocktail."

Combinations of AHP were prepared which contain monoclonal antibodies that bind to different sites on CR1. The monoclonal antibodies 7G9 and 1B4 bind to separate and non-competing sites on the CR1 site of the red blood cell. These cocktails produced contain a mixture of the two AHP's made with these respective monoclonal antibodies. The results as shown in Figure 9 show that use of a "cocktail" gives rise to even greater binding of IgG anti-dsDNA antibodies to red blood cells.

As set forth above, plasma Ma is one of the highest titer SLE IgG anti-dsDNA plasma which has been studied. The single AHP prepared with either anti-CR1 monoclonal antibody 7G9 or 1B4 alone cannot absorb all the anti-dsDNA antibodies to red blood cells in the undiluted plasma. However, when the "cocktail" is used, the continued linearity in the binding (even in the undiluted plasma) suggests that it is possible to absorb to red blood cells, more than 90% of the IgG anti-dsDNA in

plasma Ma (Figure 9). This was confirmed in the Farr assays (see Table 1) of the plasma supernatants which indicated that greater than 90% of the anti-dsDNA antibody binding activity in plasma ma is removed when it is treated with the AHP "cocktail" and human red blood cells.

The AHP prepared with anti-CR1 monoclonal antibody 1B4 showed slightly better binding than the AHP prepared with monoclonal antibody 7G9. However, saturation at the highest input of Ma was seen in both cases. When the cocktail containing both AHP's was used, on the other hand, binding was enhanced considerably which suggests that all the IgG anti-dsDNA can be bound to red blood cells (Figure 9).

Claims

1. An antigen-based heteropolymer (AHP) complex comprising a monoclonal antibody specific for binding to complement receptor (CR1) site on a primate erythrocyte, wherein said monoclonal antibody is crosslinked to an antigen specific for a target pathogenic antibody or autoantibody.

2. The AHP of Claim 1, wherein the monoclonal antibodies are selected from the group consisting of 1B4, HB8592, and 7G9.

3. The AHP of Claim 1, wherein the target antibody or autoantibody is selected from the group consisting of antibodies or autoantibodies to the following antigens: factor VIII, muscle acetylcholine receptor, cardiolipin, platelet associated proteins, antigens associated with Sjogren's Syndrome, double stranded deoxyribonucleic acid (dsDNA), and single stranded DNA (ssDNA).

4. The AHP of Claim 1, wherein said antigen is selected from the group consisting of factor VIII, muscle acetylcholine receptor, cardiolipin, platelet associated proteins, antigens associated with Sjogren's Syndrome, double stranded deoxyribonucleic acid (dsDNA), and single stranded DNA (ssDNA).

5. An AHP cocktail, comprising at least two AHP's wherein said AHP comprises a monoclonal antibody specific for binding to complement receptor (CR1) site on a primate erythrocyte, and wherein said monoclonal antibody is crosslinked to an antigen specific for a target pathogenic antibody or autoantibody.

6. A method for treating an autoimmune disease comprising the steps of:

1) administering to a human or non-human primate a clinically effective amount of an AHP, said AHP comprising a monoclonal antibody specific for complement receptor (CR1) site on a primate erythrocyte, and wherein said monoclonal antibody is crosslinked to an antigen which is specific for a target pathogenic antibody or autoantibody;

2) allowing said AHP to bind to at least one competing CR1 site and to said pathogenic antibody or autoantibody; and

3) permitting said bound AHP to be cleared from circulation of said human or non-human primate.

7. The method of Claim 6, wherein the monoclonal antibody is selected from the group consisting of 1B4, HB8592, and 7G9.

8. The AHP of Claim 6, wherein the target antibody or autoantibody is selected from the group consisting of antibodies or autoantibodies to the following antigens: factor VIII, muscle acetylcholine receptor, cardiolipin, platelet associated proteins, antigens associated with Sjogren's Syndrome, double stranded deoxyribonucleic acid (dsDNA), and single stranded DNA (ssDNA).

9. The AHP of Claim 6, wherein said antigen is selected from the group consisting of factor VIII, muscle acetylcholine receptor, cardiolipin, platelet associated proteins, antigens associated with Sjogren's Syndrome, double stranded deoxyribonucleic acid (dsDNA), and single stranded DNA (ssDNA).

10. The method of Claim 6, wherein the AHP is administered intravenously to a human or non-human primate in a clinically effective amount.

11. The method of Claim 10, wherein said AHP is administered intravenously to a human in a clinically effective amount of 1-10 mg.

12. The method of Claim 6, wherein said administration of said clinically effective amount of AHP is repeated until the pathogenic antibody or autoantibody is completely cleared from circulation of said human or non-human primate.

13. The method of Claim 6, wherein said target pathogenic antibody or autoantibody is cleared from a circulatory system of a primate and said primate erythrocyte is recirculated through the circulatory system.

14. A method for treating an autoimmune disease comprising the steps of:

1) administering to a human or non-human primate an effective amount of an AHP cocktail comprising at least two AHP'S, wherein each AHP comprises a monoclonal antibody specific for complement receptor (CR1) site on a primate erythrocyte, and wherein said monoclonal antibody is crosslinked to an antigen which is specific for a target pathogenic antibody or autoantibody;

2) allowing said AHP cocktail to bind to at least one competing CR1 site and to said pathogenic antibody or autoantibody; and

3) permitting said bound AHP cocktail to be cleared

from circulation of said human or non-human primate.

15. A method for treating an autoimmune disease comprising the steps of:

1) franking human or non-human primate erythrocytes with an AHP, said AHP comprising a monoclonal antibody specific for complement receptor (CR1) site on a primate erythrocyte, and wherein said monoclonal antibody is crosslinked to an antigen which is specific for a target pathogenic antibody or autoantibody;

2) administering to a human or non-human primate a clinically effective amount of the AHP-franked erythrocytes;

3) allowing said franked AHP to bind to said pathogenic antibody or autoantibody; and

4) permitting said bound AHP to be cleared from circulation of said human or non-human primate.

FIG. 1

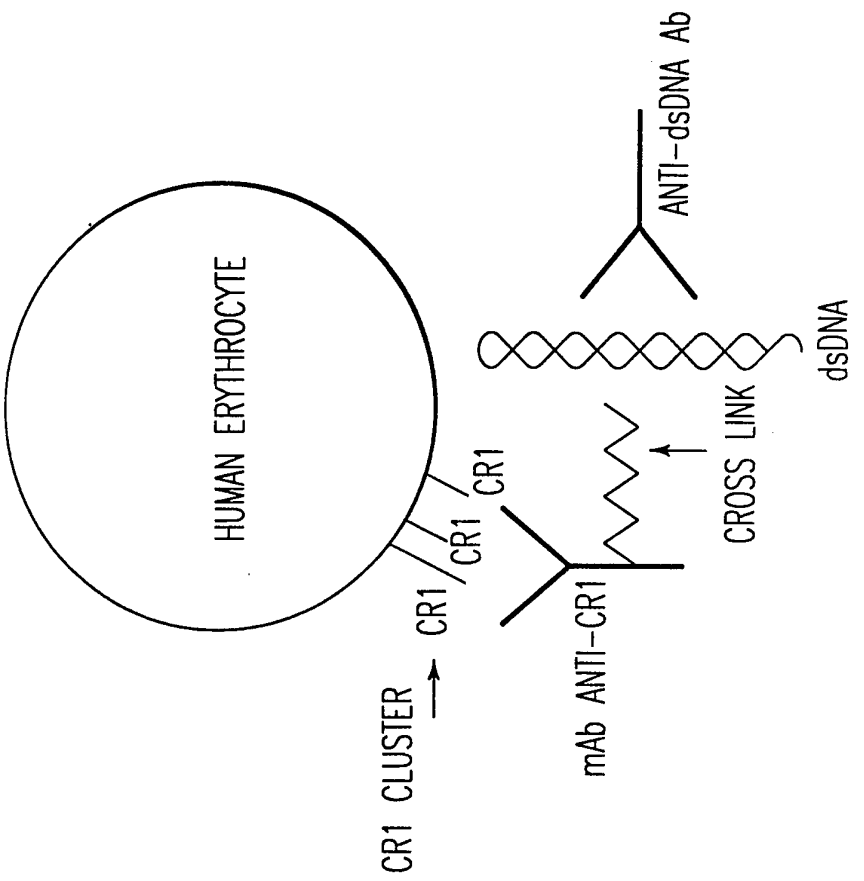

HUMAN ERYTHROCYTE
CR1
CR1
CR1
CR1 CLUSTER
mAb ANTI-CR1
CROSS LINK
dsDNA
ANTI-dsDNA Ab

FIG. 2

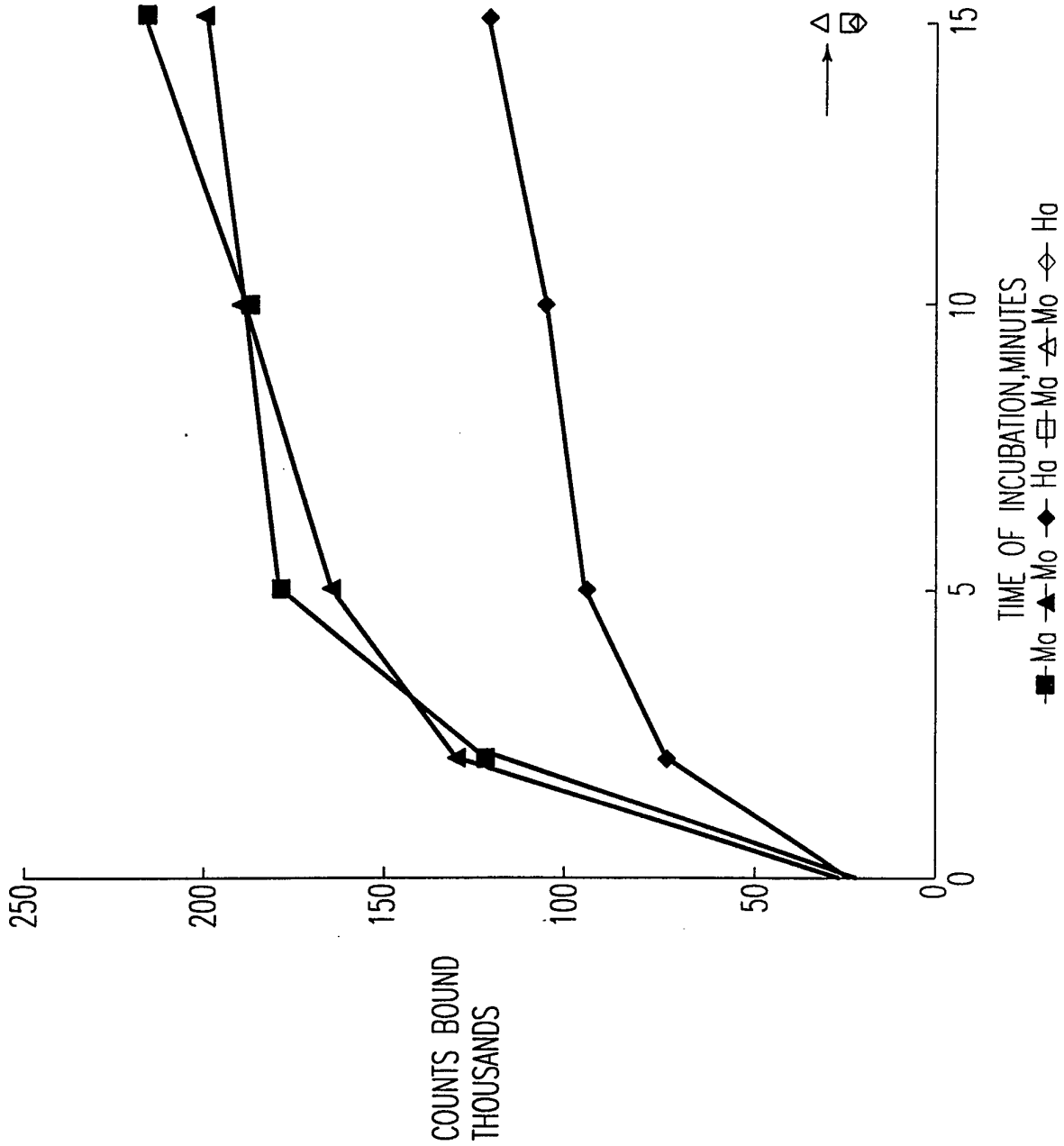

250
200
150
100
50
0
0
5
10
15
COUNTS BOUND
THOUSANDS
TIME OF INCUBATION,MINUTES
Ma
Mo
Ha
Ma
Mo
Ha

FIG. 3

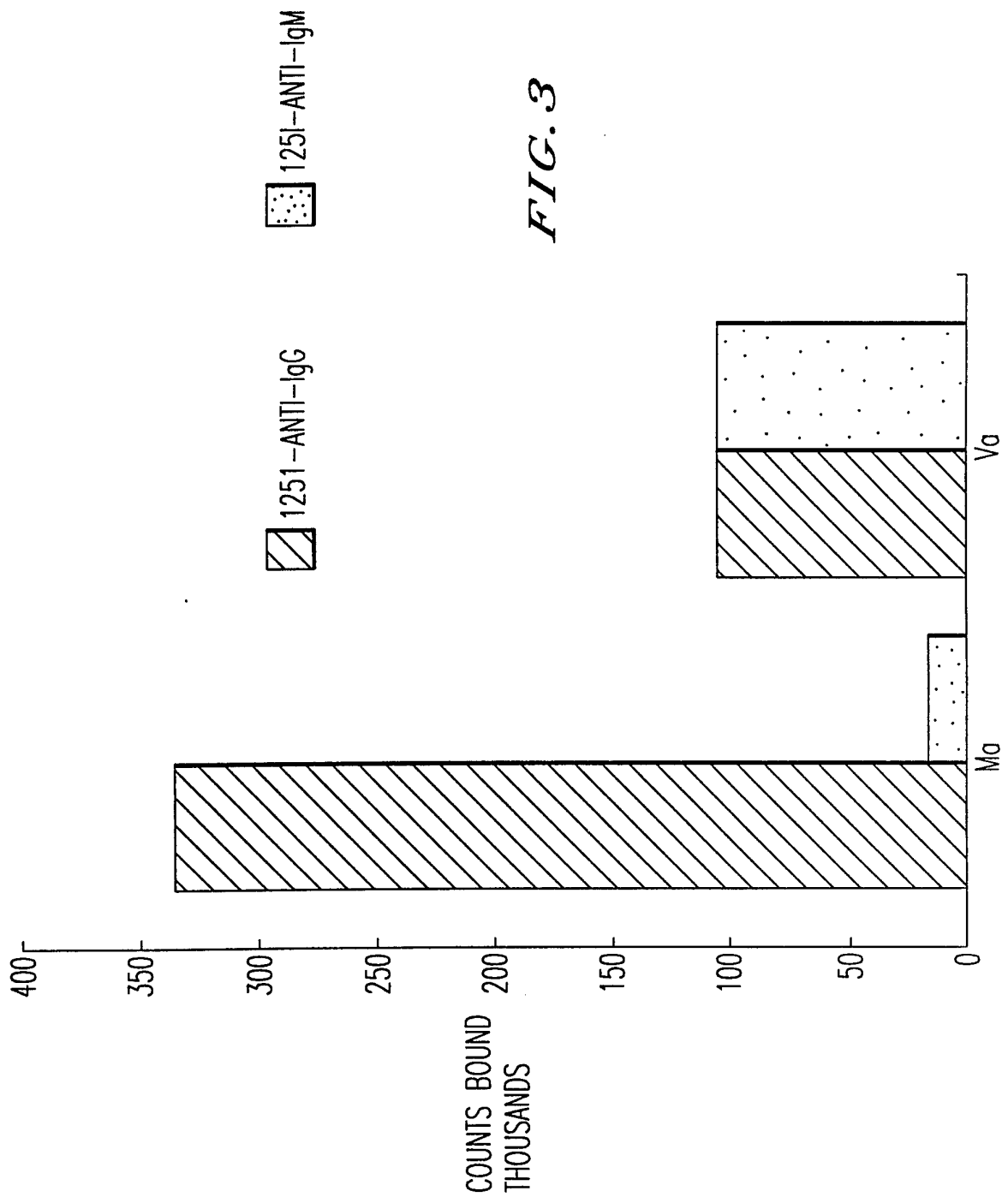
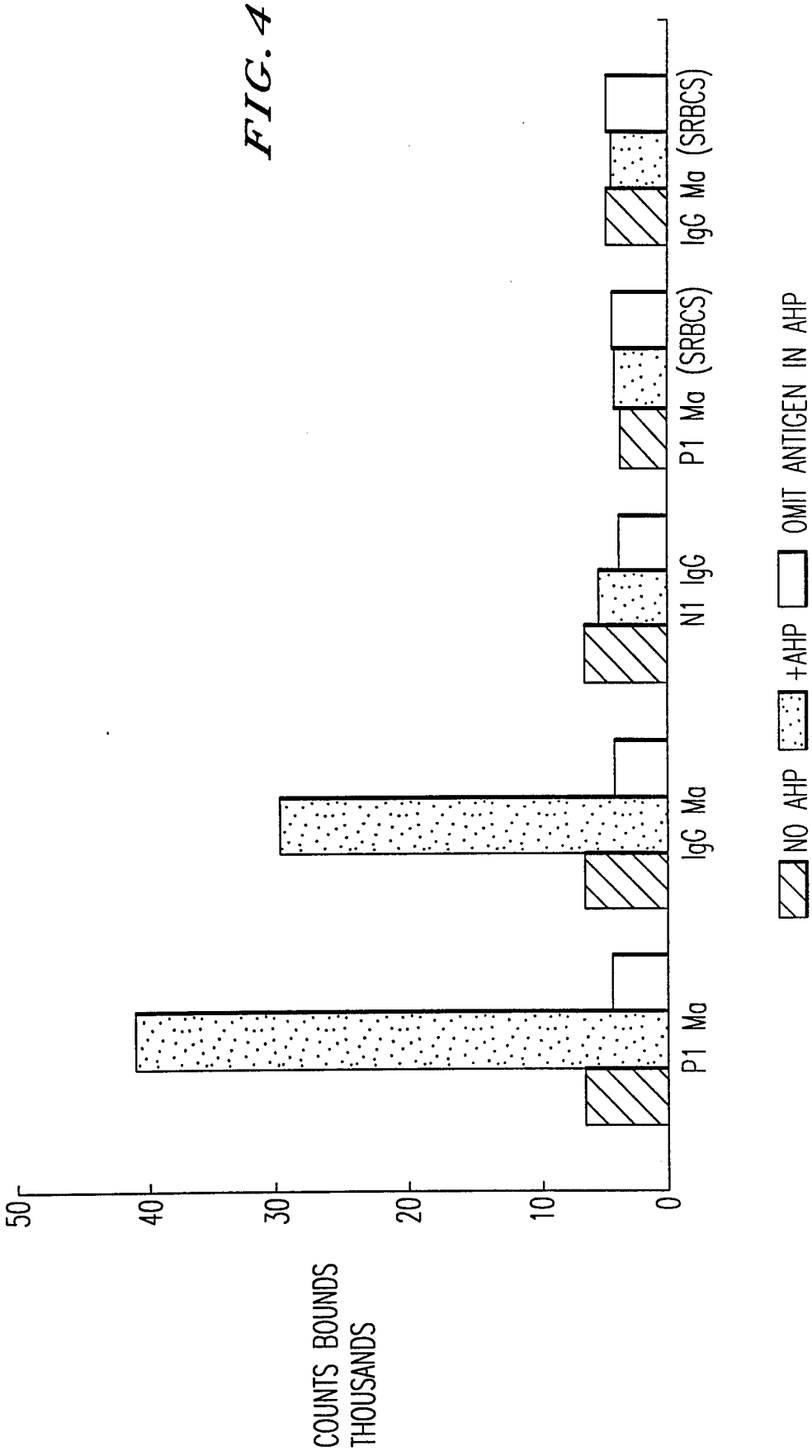

125I-ANTI-IgM
125I-ANTI-IgG
COUNTS BOUND
THOUSANDS
400
350
300
250
200
150
100
50
0
Ma
Va

FIG. 4


COUNTS BOUNDS
THOUSANDS
50
40
30
20
10
0
P1 Ma
IgG Ma
N1 IgG
P1 Ma (SRBCS)
IgG Ma (SRBCS)
NO AHP
+AHP
OMIT ANTIGEN IN AHP

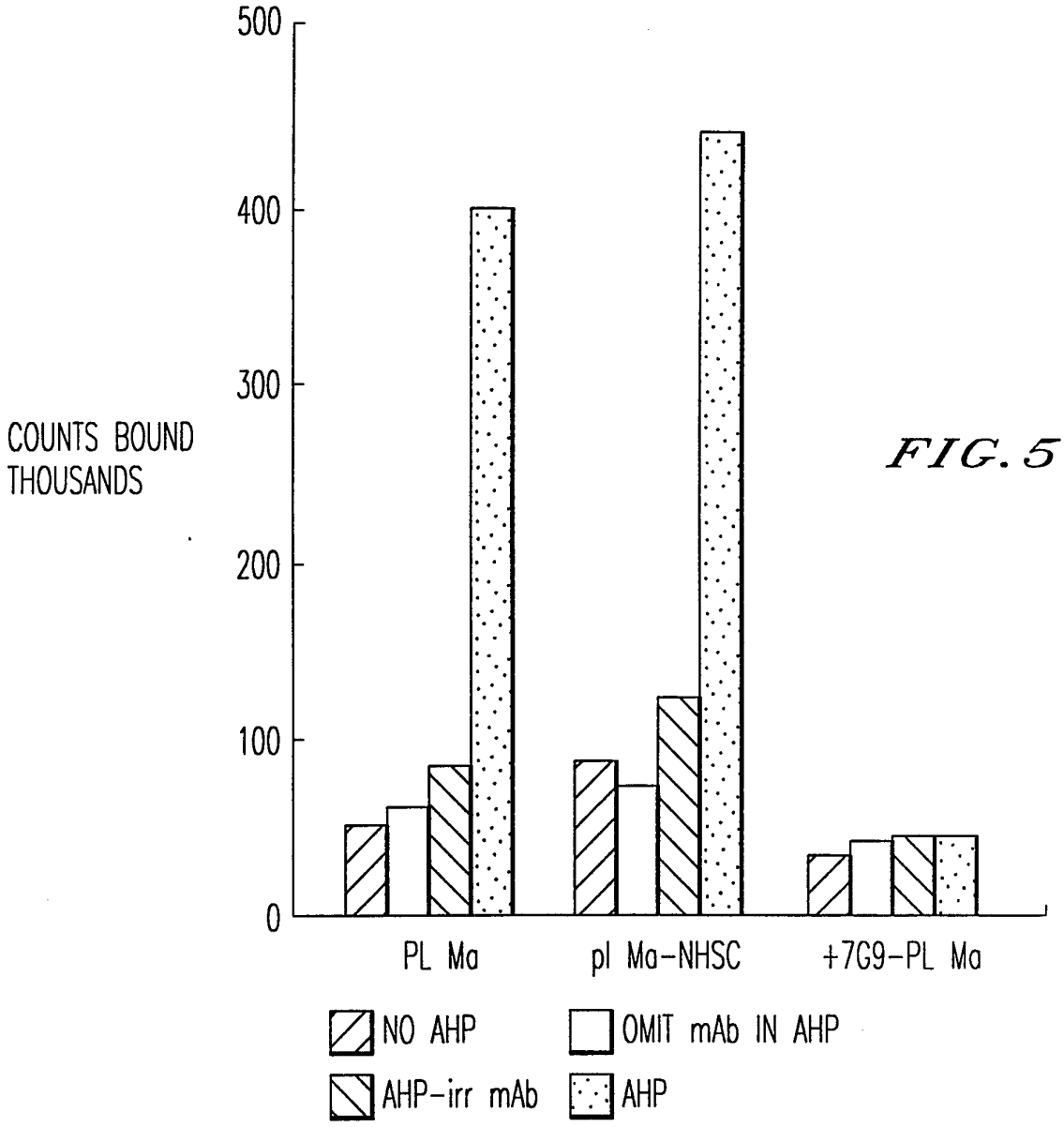

COUNTS BOUND
THOUSANDS
500
400
300
200
100
0
PL Ma
pl Ma-NHSC
+7G9-PL Ma
NO AHP
OMIT mAb IN AHP
AHP-irr mAb
AHP

FIG. 5

FIG. 6

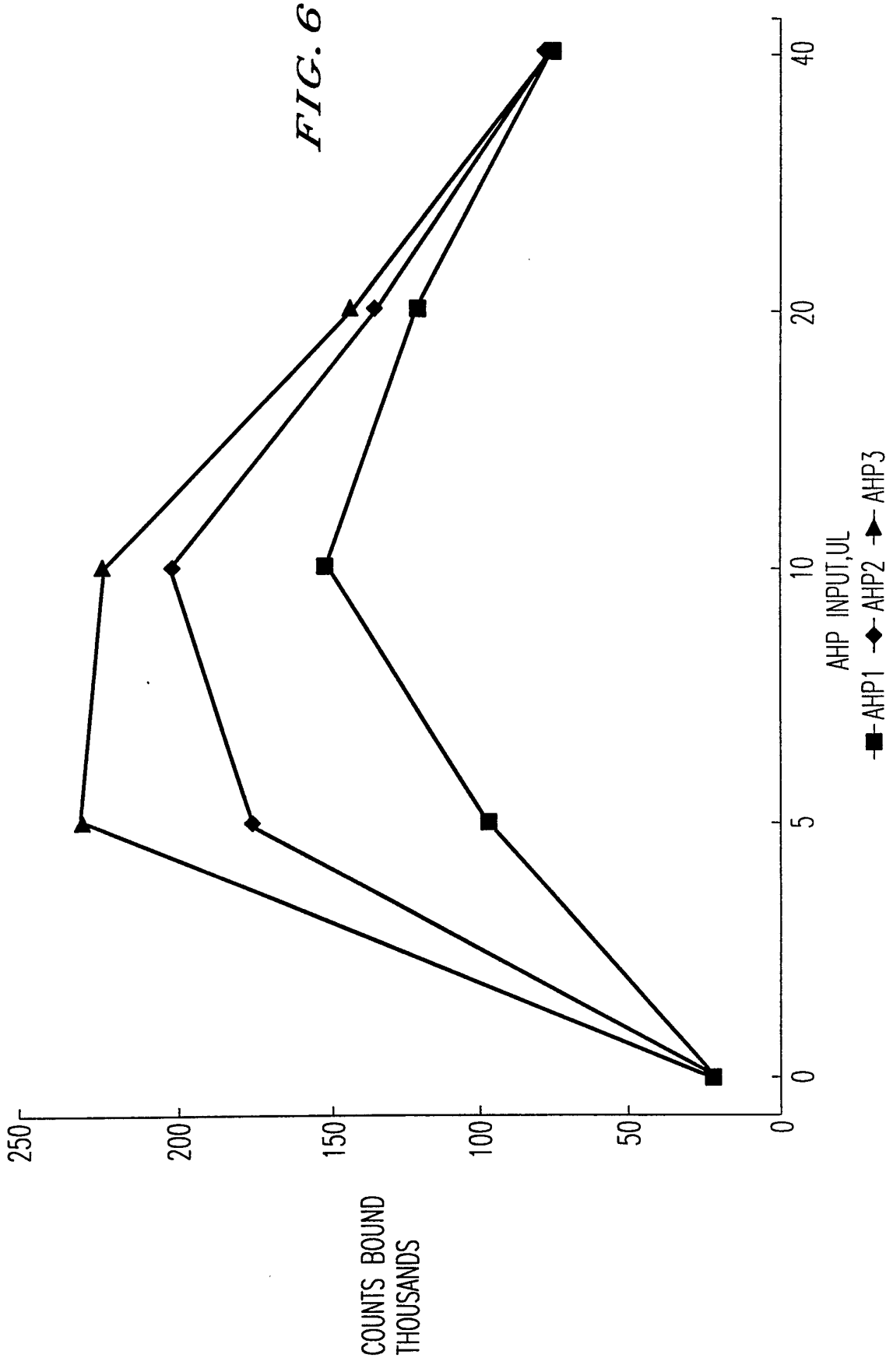
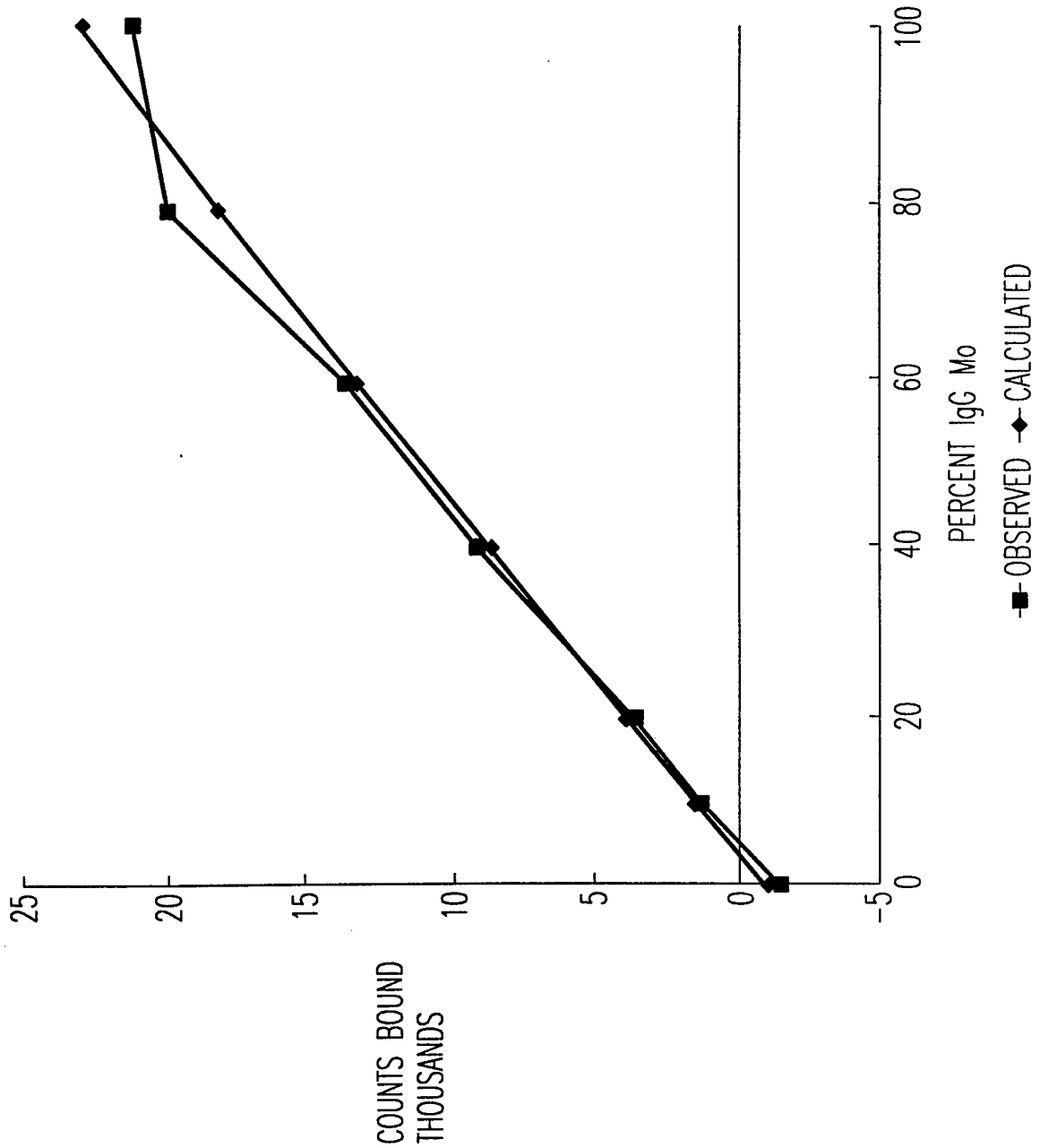

250
200
150
100
50
0
COUNTS BOUND
THOUSANDS
0
5
10
20
40
AHP INPUT,UL
AHP1
AHP2
AHP3

FIG. 7


COUNTS BOUND
THOUSANDS
25
20
15
10
5
0
-5
0
20
40
60
80
100
PERCENT IgG Mo
OBSERVED
CALCULATED

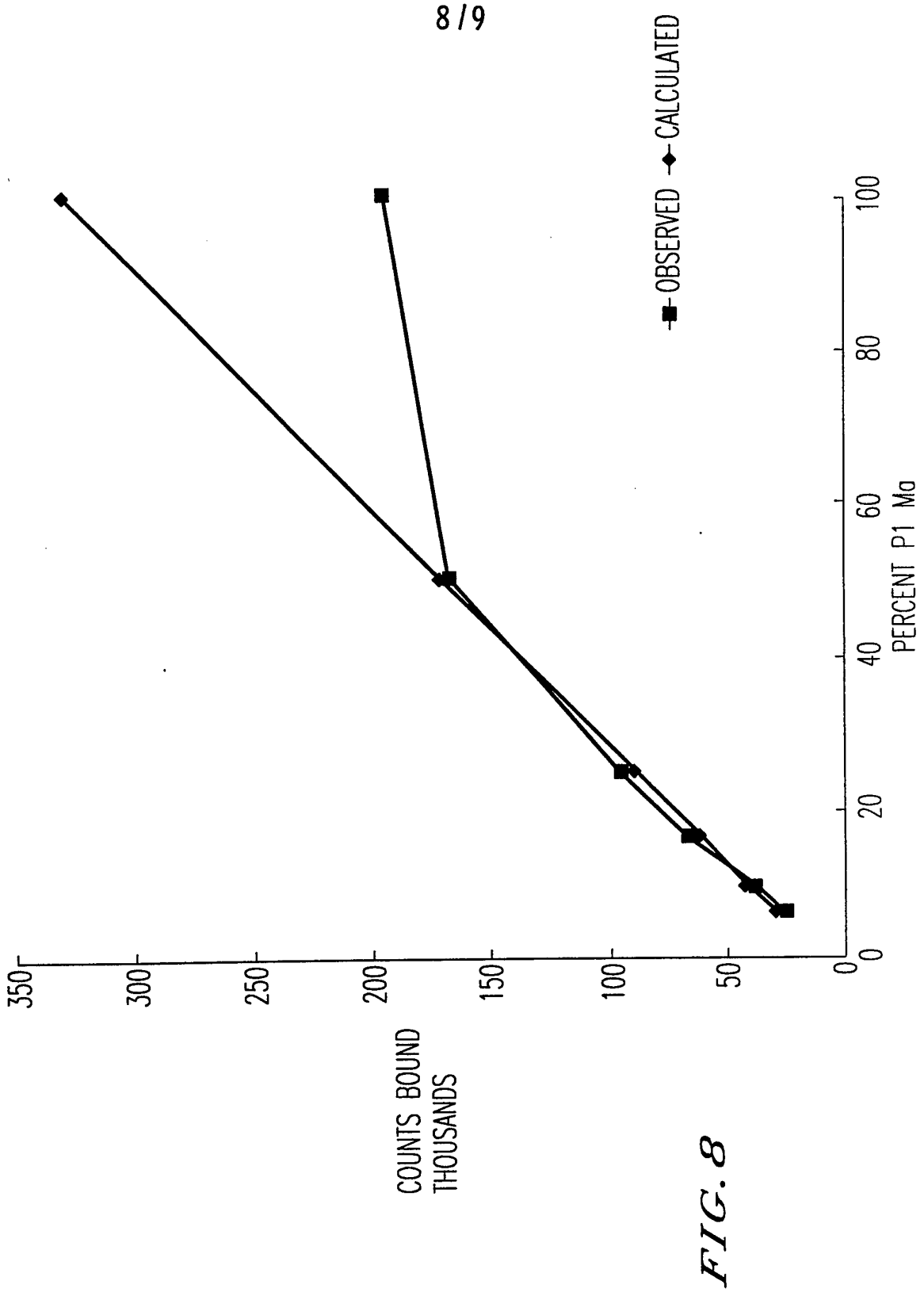

OBSERVED
CALCULATED
COUNTS BOUND
THOUSANDS
350
300
250
200
150
100
50
0
0
20
40
60
80
100
PERCENT P1 Mo

FIG. 8

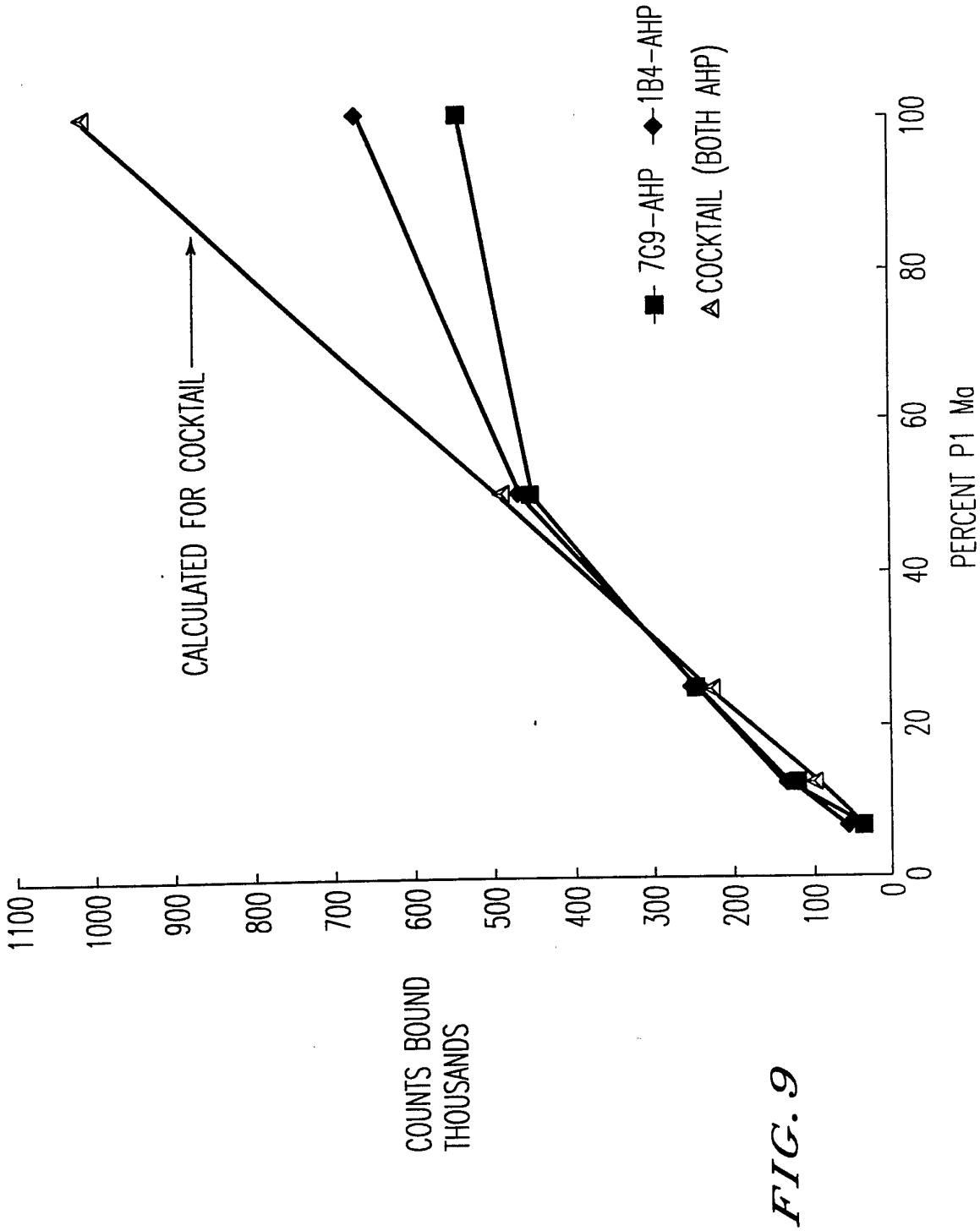

CALCULATED FOR COCKTAIL
7C9-AHP
1B4-AHP
COCKTAIL (BOTH AHP)
COUNTS BOUND
THOUSANDS
PERCENT P1 Mo
1100
1000
900
800
700
600
500
400
300
200
100
0
0
20
40
60
80
100

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02165

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/153.1, 173.1, 178.1, 184.1, 93.1, 93.21; 530/388.7, 389.6, 391.1, 391.7.

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 practicable, search terms used)

APS, DIALOG

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	W. PAUL, "FUNDAMENTAL IMMUNOLOGY", 3rd Ed., published 1993 by Raven Press (New York), pages 917, 926-927, see entire document.	1-15
Y	JOURNAL OF IMMUNOLOGY, Vol. 148, No. 8, issued 15 April 1992, Taylor et al., "In vivo binding and clearance of circulating antigen by bispecific heteropolymer-mediated binding to primate erythrocyte complement receptor," pages 2462-2468, see entire document.	1-15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
"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)	"&"	document member of the same patent family
"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		

Date of the actual completion of the international search 10 MAY 1995	Date of mailing of the international search report 26 MAY1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Deborah Reese for ROBERT D. BUDENS Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02165

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF CLINICAL INVESTIGATION, Vol. 84. issued September 1989, Kimberly et al., "In vivo handling of soluble complement fixing Ab/dsDNA Immune Complexes in Chimpanzees" pages 962-970, see entire document.	1-15
Y	JOURNAL OF IMMUNOLOGICAL METHODS, Vol. 132, issued 1990, Emlen et al., "A new ELISA for the detection of double-stranded DNA antibodies," pages 91-101, see entire document.	1-15
Y	BLOOD, Vol. 57, No. 1, issued 1981, Mannucci et al., "Precipitating antibodies to factor VIII/von Willebrand factor in von Willebrand's disease: Effects on replacement therapy," pages 25-31, see Abstract.	3-4, 8-9
Y	JOURNAL OF NEUROIMMUNOLOGY, Vol. 19, No. 3, issued 1988, Brown et al., "Clonotypic analysis of the antibody response to the acetylcholine receptor in experimental autoimmune myasthenia gravis," pages 205-222, see Abstract.	3-4, 8-9
Y	JOURNAL OF NEUROIMMUNOLOGY, Vol. 14, No. 2, issued 1987, Morel et al., "Increased binding of anti-acetylcholine receptor antibodies to thymic antigen in patients with myasthenia gravis and other autoimmune diseases compared to those with myasthenia gravis alone," pages 221-226, see Abstract.	3-4, 8-9
Y	CLINICAL AND EXPERIMENTAL IMMUNOLOGY, Vol. 75, No. 2, issued 1989, Sutjita et al, "A common anti-cardiolipin antibody idiotype in autoimmune disease identification using a mouse monoclonal antibody directed against a naturally-occuring anti-phospolipid antibody," pages 211-216, see Abstract.	3-4, 8-9
Y	THE JOURNAL OF INFECTIOUS DISEASES, Vol. 157, No. 1, issued JANUARY 1988, Harris et al., "Use of an enzyme-linked immunosorbent assay and of inhibition studies to distinguish between antibodies to cardiolipin from patients with syphilis or autoimmune disorders," pages 23-31, see Abstract.	3-4, 8-9
Y	JOURNAL OF IMMUNOLOGICAL METHODS, Vol. 133, issued 1990, Meilof et al., "The detection of anti-Ro/SS-A and anti-La/ss-B antibodies: A comparison of counterimmunoelectrophoresis with immunoblot, ELISA, and RNA-precipitation assays," pages 215-226, see Abstract.	3-4, 8-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02165

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ARCHIVES OF DERMATOLOGY, Vol. 123, No. 3, issued 1987, Falanga et al., "Antinuclear and anti-single-stranded DNA antibodies in morphea and generalized morphea," pages 350-353, see Abstract.	3-4, 8-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02165

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

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:

Please See Extra Sheet.

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/02165

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 35/18, 39/00, 39/395, 39/44; C07H 21/02, 21/04; C07K 16/00, 16/18, 16/28, 16/44, 19/00.

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/153.1, 173.1, 178.1, 184.1, 93.1, 93.21; 530/388.7, 389.6, 391.1, 391.7.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s)1-4 and 6-13, drawn to antigen-based heteropolymers and methods of use.
Group II, claim(s) 5 and 14, drawn to antigen-based heteropolymer cocktails and method of use.
Group III, claim 15, drawn to a method of treatment using franked erythrocytes.
The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I and II are directed to products differing in their composition, i.e., Group I directed to particular antigen-based heteropolymers and group II directed to mixtures of two or more antigen-based heteropolymers, each of which may be directed to multiple antigens.

The inventions listed as Groups I-III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the methods of groups I-III utilize different products and mechanisms of biological activity.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked by a special technical feature within the meaning of PCT Rule 13.2 so as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Group IV, directed to Factor VIII
Group V, directed to muscle acetylcholine receptor.
Group VI, directed to cardiolipin
Group VII, directed to platelet associated proteins
Group VIII, directed to antigens associated with Sjogren's Syndrome.
Group IX, directed to dsDNA.
Group X, directed to ssDNA.

The claims are deemed to correspond to the species listed above in the following manner:

Claims 3-4 and 8-9 are related to each of species IV-X

The following claims are generic: 1-2, 5-7 and 10-15.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: the antigens of groups IV-X are molecules differing in structure and related to distinct diseases having differing etiologies and pathologies.

If no additional fees are paid, the invention will be limited to the claims of Group I and Species IV (Factor VIII).