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(54) Title: IMMUNOTHERAPY WITH SUBSTANTIALLY HUMAN POLYCLONAL ANTIBODY PREPARATIONS PURIFIED FROM GENETICALLY ENGINEERED BIRDS

(57) Abstract: Substantially human antibodies are provided by genetically modifying a bird. The bird is genetically modified by generating inactive heavy and light chain immunoglobulin loci and integrating at least a portion of the human heavy and light chain immunoglobulin loci. The antibodies purified from serum or egg yolk of immunized bird find use in the treatment of diseases, immunocompromised patients and in case of transplantation.

IMMUNOTHERAPY WITH SUBSTANTIALLY HUMAN POLYCLONAL ANTIBODY PREPARATIONS PURIFIED FROM GENETICALLY ENGINEERED BIRDS

This invention relates to substantially human polyclonal antibody preparations for prophylactic and therapeutic treatment of humans.

The therapy of infectious diseases caused by bacteria, fungi, virus and parasites is largely based on chemotherapy. However, the emergence of drug-resistant organisms requires the continuous development of new antibiotics. At the same time the control of infections is threatened by the emergence of new pathogens. The increasing number of immunocompromised individuals due to malnutrition, AIDS, medical therapies of cancer, autoimmune diseases and organ transplantation decreases the efficacy of antibiotic therapy and increases the difficulty of controlling infections.

Therapies of patients with malignancies and cancer are also based on chemotherapy. However, many of these therapies are ineffective and the mortality of diseased patients is high. Advances in monoclonal antibody technology provided little improvement because of the immunogenicity of the monoclonal antibodies and their lack of potency. Anti-idiotypic antibody responses in patients undergoing monoclonal antibody therapy can render the antibody therapy ineffective.

Therapy of steroid resistant rejection of transplanted organs requires the uses of biological reagents (monoclonal or polyclonal antibody preparations) that reverse the ongoing alloimmune response in the transplant recipient. However, immunogenicity of antibody preparations may render such therapy ineffective and prevent rejection reversal. As a consequence, a transplanted organ may be rejected. Similarly, antibody therapies of autoimmune disease patients are of limited success due to the immunogenicity of antibody preparations. While humanization of antibodies decreases immunogenicity, the effectiveness of such antibodies is limited by anti-idiotypic antibody responses and the lack of potency of monoclonal antibodies. Non-immunogenic, potent reagents for the modulation of immune responses need to be developed.

Polyclonal antibody therapy for the treatment of infectious diseases was introduced at the end of the last century. By the 1930s, serum therapy was used for treatment of bacterial and viral infections including pneumonia, meningitis, scarlet fever, whooping cough, anthrax, botulism, gangrene, tetanus, brucellosis, dysentery, tularemia, diphtheria, measles, poliomyelitis mumps and chickenpox. However, the systemic administration of animal sera caused fevers, chills, and allergic reactions. Serum sickness occurred in 10-50% of treated individuals.

The potential of using antibodies in the treatment of a variety of indications is very high. The ability to specifically bind to a target entity provides diverse opportunities to sequester and destroy the entity. However, as demonstrated above, there have been many impediments to the use of heterologous and humanized antibodies. The limitations of monoclonal antibodies add the additional impediment of reduced affinity. Thus, there is a pressing need to find alternative modalities which provide protection against infectious disease and malignancies or the immunomodulation of transplant recipients and autoimmune disease patients.

Antibody-based therapies in infectious diseases were recently reviewed by A. Casadevall and M. D. Scharff, Clinical Infectious Diseases 1995; 150-161.

The use of antibodies for the treatment of cancer and malignancies was recently reviewed by C. Botti, A. Marinetti, S. Nerini-Molteni, and L Ferrari, Int J Biol Markers 1997; 12(4):141-147; D.R. Anderson, A. Grillo-Lopez, C. Varns, and K.S. Chambers, Biochem Soc Trans 1997; 25(2):705-708; C. Renner, L. Trumper, and M. Pfreundschuh, Leukemia 1997; 11 Suppl 2:S55-59; B. Bodey, S.E. Siegel, and H.E. Kaiser, Anticancer Res 1996; 16(2):661-674.

The use of polyclonal antibody preparations for the treatment of transplant rejection was recently reviewed by N. Bonnefoy-Berard and J.P. Revillard, J Heart Lung Transplant 1996; 15(5):435-442; C. Colby, C.A. Stoukides, and T.R. Spitzer, Ann Pharmacother 1996; 30(10):1164-1174; M.J. Dugan, T.E. DeFor, M. Steinbuch, and A.H. Filipovich, Ann Hematol 1997; 75(1-2):41-46.

The use of polyclonal antibody therapies for autoimmune diseases has been described by W. Cendrowski, Boll Ist Sieroter Milan 1997; 58(4):339-343; L.K. Kastrukoff, D.R. McLean, and T.A. McPherson, Can J Neurol Sci 1978; 5(2):175-178; J.E. Walker, M.M Hoehn, and N. Kashiwagi, J Neurol Sci 1976; 29(2-4):303-309.The depletion of fat cells using antibody preparations has been described by L. De Clercq, J. Mourot, C. Genart, V. Davidts, and C. Boone, J Anim Sci 1997; 75(7):1791-1797; J.T. Wright and G.J. Hausman, Obes Res 1995; 3(3):265-272.

The isolation and characterization of avian embryonic stem cells has been described by R.J Etches, M.E. Clark, A. Toner, G. Liu and A.M Verrinder Gibbins, Mol Reprod Dev 1996, 45: 291–298; B. Pain, M.E. Clark, M. Shen, H. Nakazawa, M. Sakurai, J. Smarut and R.J. Etches, Development 1996; 122: 2339-2348; I. Chang, D.K. Jeong, Y.H. Hong, T. S. Park, Y.K Moon, T Ohno and J.E. Han, Cell Biology Intl 1997; 21(8):495-499.

Production of antibodies from transgenic animals is described in U.S. Patent Nos. 5,814,318; 5,545,807; and 5,570,429. Homologous recombination for chimeric mammalian hosts is exemplified in U.S. Patent No. 5,416,260. A method for introducing DNA into an embryo is described in U.S. Patent No. 5,567,607. Maintenance and expansion of embryonic stem cells is described in U.S. Patent No. 5,453,357. Maintenance and expansion of avian embryonic stem cells is described U.S. Patents Nos. 5,340,740, and 5,656,479.

Methods are provided for the production of substantially human antibodies to a specific antigen, where a transgenic domestic birds comprising genetically altered light and heavy chain immunoglobulin loci and at least a portion of human light and heavy chain immunoglobulin loci is provided. The method employs stepwise modification of a domestic bird in which the antibody repertoir is diversified predominantly by gene conversion (including but not limited to chicken, turkey, ducks, goose, and quail). The method involves replacement of endogenous elements of the immunoglobulin loci with the corresponding human counterparts, in particular, replacement of one or several exons encoding constant regions of heavy and light chain and one or several variable region elements including the one proximal to the D region locus. In birds, where antibody diversity is generated predominantly by gene conversion, replacement of the V region most proximal to the D region with a human V region element will result in expression of the human V element in the majority of immunoglobulins. This genetic engineering is followed by breeding hosts of the same

species and selecting for a host which is capable of responding to immunization with production of substantially human antibodies with host glycosylation, the immunoglobulin having at least a functional portion of the human heavy chain. Alternatively, one may proceed through the same process as earlier, where the other avian locus is modified to provide effectively homozygous birds for human immunoglobulin. Birds expressing the substantially human immunoglobulin proteins are used for the generation of polyclonal antibody preparations by immunization with immunogens of interest, particularly, immunogens which initiate production of antibody which has therapeutic activity. After purification of the antibodies from egg yolk or antiserum, such antibody preparations may be used, by themselves or in combination with other reagents for the depletion of infectious reagents, malignant cells, cancers, undesirable target cells or immunomodulation.

Methods are provided for producing substantially human antibodies in a heterologous bird by immunizing the bird with an immunogen. The bird is characterized by being capable of producing substantially human polypeptide antisera upon exposure to an immunogenic substance; and retaining its capability of rearranging the immunoglobulin locus and recombining the V, (D_H), J and C regions to produce substantially human protein antibodies, which include at least one human immunoglobulin constant region and/or at least one human variable (V) region element. Of particular interest are constant regions of the subclasses of C_α or C_γ, including any of the $C_\gamma$subclasses 1, 2, 3 and 4. DNA fragments encoding human constant regions and variable elements are integrated into the genome by homologous recombination and replace the corresponding endogenous elements.

Various birds, particularly domestic birds, which can provide reasonable amounts of immunoglobulin in egg yolk or antiserum may be employed. Of particular interest are chickens. In birds, diversification of the antibody repertoir is accomplished predominantly by gene conversion. Replacement of the V region element proximal to the D region with a human V region element will result in the expression of the human V region element in the majority of immunoglobulins. The described genetic engineering approach is substantially easier than other approaches that have been performed with mice. In mice, however, diversification of the antibody repertoir is accomplished predominantly by gene rearrangement.

Genetically engineered birds may be generated from genetically engineered embryonic stem cells. Alternatively, genetically engineered birds may be generated using nuclear transfer (cloning) technology. Host cells which may be grown and expanded in culture and do not have a rearranged genome, are transformed (genetically modified) by the introduction of DNA fragments into the cells, where the fragments become integrated into the host. Introduction may be by a variety of methods, including bare DNA, transfection with a viral vector, fusion, biolistics, liposomes, etc. The particular method can be selected in accordance with the purpose of the introduction of the DNA and the efficiency of integration. Functional immunoglobulin light and heavy chain loci can be modified by homologous recombination, by replacing at least a portion of the host heavy chain constant region with at least a functional portion of the human heavy chain constant region and if desired, analogously, the host light chain constant region with a human light chain constant region. Of particular interest is also the replacement of the V region most proximal to the D region with a human V region element. In this way, while some portions of the immunoglobulin are host sequences, the antibodies do not cause a strong immune response in view of the great variety of variable regions in the antisera. In birds, where antibody diversity is generated predominantly by gene conversion, replacement of the V region most proximal to the D region with a human V region element can result in expression of the human V element in the majority of immunoglobulins. Alternatively, a rearranged substantially human VDJ element derived from the human heavy chain locus and/or a substantially human VJ element derived from the human light chain locus is inserted into the birds immunoglobulin locus in such a way that expressed immunoglobulin heavy and light chains are produced exclusively using these DNA elements. In birds, where antibody diversity is generated predominantely by gene conversion, the inserted VDJ and/or VJ elements are diversified by gene conversion, which result in a highly diversified substantially human antibody repertoir. For the replacement of constant regions and to provide functional heavy regions, it is of particular interest to include at least about 2 of the 3 domains C_{H1}, C_{H2}, and C_{H3}, of the constant region, particularly including C_{H3}. Desirably the antisera which are produced in the bird have a binding affinity of at least about 10^{-7}M.

For integration at a predetermined site, constructs may be prepared which include, in sequence, the DNA fragment for integration and a first marker gene

bordered by homologous sequences of at least about 30nt and a second marker gene, whereby homologous integration results in loss of the second marker gene. By having the second marker gene providing negative selection--cells with the second marker gene are selected against and removed from the cell mixture; by having the first marker gene providing positive selection--cells having the first marker gene are retained--by using a medium to which the second marker gene is sensitive. In this manner, those cells in which the construct is randomly integrated can be decreased. By following with a medium selective for the first marker gene, cells not retaining the first marker gene can be decreased. In this way, the remaining cells should be those having homologous recombination. Desirably, the cells are in a rapidly proliferating status, rather than a non-proliferating status. By employing a growth medium, such as RPMI1640 or DMEM, supplemented with serum and growth factors, a growth cycle can be induced.

After the cells have been transformed or transfected, the cells are put in a selective medium in accordance with the marker employed, usually an antibiotic resistance or the tk gene. The cells may be expanded in culture and then cloned using nuclear transfer technology. Individual cells in clones may then be screened for the desired genetic modification. Conveniently, PCR may be used to identify that the desired modification, deletion or integration, has taken place. Alternatively, when embryonic stem cells have been used, genetically engineered cells can be used directly to generate embryos and, subsequently, live offspring.

The genetic modifications may be a single modification or, if desired, after expansion of cells having the first modification, the cells may then be subjected to a second modification. For example, after replacing the heavy chain constant regions, one can replace the light chain constant regions.

Where an individual modification occurs, one can use a single marker for positive selection and use the same marker repetitively. Where two or more modifications to the same cell are generated, different positive selection markers must be used, in order to independently select at each stage. As already indicated, there are numerous antibiotic resistance genes, which genes may be used in combination, allowing for selection at each stage. Genes useful for selection include neo, tet, cam, tk, pen, mtx, among others.

After the host cells have been modified and demonstrated to have the desired modification, the cells may then be fused with enucleated nuclear transfer unit

cells, e.g. oocytes, embryonic stem cells, or other cells which are totipotent and capable of forming a functional neonate. Fusion is performed in accordance with conventional techniques which are well established. See, for example, Cibelli et al., Science (1998) 280:1256. Alternatively, enucleation of oocytes and nuclear transfer can be performed by microsurgery using injection pipettes. See, for example, Wakayama et al., Nature (1998) 394:369. The resulting functional egg cells are then cultivated in an appropriate medium and transferred into irradiated eggs. Alternatively, when embryonic stem cells have been used, genetically modified cells are transferred directly into irradiated eggs.

The resulting genetically modified birds may then be used for breeding with other genetically modified hosts. For example, birds having an altered heavy chain immunoglobulin locus may be bred with birds having an altered light chain immunoglobulin locus to breed a host capable of producing substantially human polypeptide immunoglobulins. The hemizygous siblings containing the two mutated genes are then bred to produce homozygous siblings. Homozygosity may be readily determined by the absence of the undesired gene sequences. After each breeding, the host is assayed for the presence of the genetic modification in its cells, particularly the germ cells, and may be bred to a further generation, usually not more than three generations, to ensure that the modification is stably maintained through successive generations. The genomes of the various offspring may be analyzed for the maintenance of the genetic modifications or, as appropriate, the offspring may be analyzed for the biological change which the genetic modification generated.

Once the bird has been generated, the bird may now be used to produce antibodies under a variety of conditions. Depending upon the use of the antibodies, antigens, immunogens comprising a hapten covalently bonded to an antigen, organisms, e.g., viruses and unicellular organisms, alive, attenuated or dead, fragments of organisms, organelles, cells, particularly human cells or fragments of cells, or the like may be used. Thus the antibodies may be directed to an antigen, a small organic molecule or a cell, where the various entities may be endogenous or exogenous to the human host. The immunization composition may be administered in any convenient manner, with or without an adjuvant, and may be administered in accordance with a predetermined schedule. The affinity for the immunization composition may then be monitored and the antibodies collected when the antibodies

have the desired specificity and affinity. The affinity of the antisera can be at least 10^{-7}, usually at least 10^{-8}, preferably at least 10^{-9}, or higher.

For some applications, one may use birds in which the V element proximal to the D regions has been replaced with various human V region elements. In this way, different immune responses to the same immunogen can be obtained from the different birds, where the variable region sequence may be as a result of gene conversion, providing different alleles. The antibody preparations from the different birds may be mixed to provide a broader repertoire of antibodies. Up to 10 or more different hosts may be employed, depending on the antigen of interest.

Antibody preparations can be obtained by fractionating egg yolk from genetically engineered birds expressing human sequence immunoglobulins. Alternatively, antibodies may be purified from serum. A concentrated immunoglobulin fraction may be prepared by chromatography (affinity, ionic exchange, gel filtration, etc.), selective precipitation with salts such as ammonium sulfate, organic solvents such as ethanol, or polymers such as polyethylene glycol.

The fractionated antibodies may be dissolved or diluted in non-toxic, non-pyrogenic media suitable for intravenous administration in humans, for instance, sterile buffered saline. In some applications, antibody preparations may be applied directly onto epithelium. For such applications, fractionated antibodies may be dissolved in a water soluble gel like KY-jelly and the like.

The antibody preparations used for administration are generally characterized by containing a polyclonal antibody population, having immunoglobulin concentrations from 0.1 to 100 mg/ml, more usual from 1 to 10 mg/ml. The antibody preparation may contain immunoglobulins of various isotypes. Alternatively, the antibody preparation may contain antibodies of only one isotypes, or a number of selected isotypes.

In most instances the antibody preparation contain unmodified immunoglobulins. Alternatively, the immunoglobulin fraction may be subject to treatment such as enzymatic digestion (e.g. with pepsin, papain, plasmin, glycosidases, nucleases, etc.), heating, etc, and/or further fractionated.

The antibody preparations can be administered into the vascular system, conveniently intravenously by injection or infusion via a catheter implanted into an appropriate vein. The antibody preparation is administered at an appropriate rate, generally ranging from about 10 minutes to about 24 hours, more commonly from

about 30 minutes to about 6 hours, in accordance with the rate at which the liquid can be accepted by the patient. For applications on epithelial surfaces antibody preparations are dissolved in a water based gel like KY-jelly or the like.

Administration of the effective dosage may occur in a single infusion or in a series of infusions. Repeated infusions may be administered once a day, once a week, once a month, or once every three months, depending on the half-life of the antibody preparation and the clinical indication.

Administration of an antibody preparation against an infectious agent as monotherapy or in combination with chemotherapy results in elimination of infectious particles. A single administration of antibodies decreases the number of infectious particles 10 to 100 fold, more commonly more than 1000-fold. Similarly, antibody therapy in patients with malignant disease as monotherapy or in combination with chemotherapy reduces the number of malignant cells 10 to 100 fold, or more than 1000-fold. Therapy may be repeated over an extended amount of time to assure the complete elimination of infectious particles, malignant cells, etc. In some instances, therapy with antibody preparations can be continued for extended amounts of time in the absence of detectable amounts of infectious particles or undesirable cells. Similarly, the use of antibody therapy for the modulation of immune responses may consist of a single or multiple administrations of therapeutic antibodies. Therapy may be continued for extended amounts of time in the absence of any disease symptoms.

The subject treatment may be employed in conjunction with chemotherapy at dosages sufficient to inhibit infectious disease or malignancies. In autoimmune disease patients or transplant recipients antibody therapy may be employed in conjunction with immunosuppressive therapy at dosages sufficient to inhibit immune reactions.

The following examples are offered by way of illustration and not by way of limitation.

Generation of transgenic chickens expressing substantially human immunoglobulin

Chicken embryonic stem cells are isolated and cultured as described by Pain et al. (Development 122, 2339-2348; 1996). Chicken embryos are obtained from eggs immediately after they are layed. The entire blastoderm is removed by gentle aspiration, embryos are slowly dissociated mechanically and cells are seeded in ESA complete medium on inactivated STO feeder cells. ESA medium is composed of

MEM medium containing 10% FCS, 2% chicken serum, 1% bovine serum albumin, 10 ng/ml ovalbumin, 1 mM sodium pyruvate, 1% non-essential amino acids, 1 μM of each nucleotide adenosine, guanosine, cytidine, uridine, thymidine, 0.16 mM β-mercaptoethanol, ESA complete medium is supplemented with 10 ng/ml bFGF, 20 ng/ml h-IGF-1, 1% vol/vol avian-SCF and 1% vol/vol h-LIF, 1% vol/vol h-IL-11. Cell cultures are incubated wt 37°C in 7.5 CO_2 and 90% humidity. After 48 hours fresh blastodermal cells are added to the culture in half of the original volume of ESA complete medium. After an additional incubation for three days, the culture medium is partially (50%) replaced with fresh ESA complete medium, and totally every day thereafter. For cell harvesting, cultures are washed with PBS and incubated in a pronase solution (0.025% w/v). Dissociated cells are transfected with various linearized DNA constructs containing human immunoglobulin locus elements. Tranfected cells are incubated with STO feeder cells (as described above) in the presence of selective antibiotics. Cells are transferred onto fresh feeder cells twice per week. Antibiotic resistant cells are isolated and the appropriate integration of human immunoglobulin gene fragments at the corresponding chicken immunoglobulin gene loci is confirmed by PCR.

Subsequently, genetically modified cells are injected into recipient embryos. As recipient embryos, freshly laid eggs are irradiated (6Gy - Cobalt source). Between 100 to 200 genetically modified cells are injected into the subgerminal cavity using a micropipet. The window in the egg shell is closed and the eggs are incubated. Somatic chimerism of hatched chickens is evaluated by PCR. The observed frequency of somatic chimerism is about 40%. Germ-line chimerism is assessed by mating of somatic chimeras. About 5% of the somatic chimeras are also germline chimeras. Transgenic chicken expressing modified immunoglobulin heavy chains containing the constant regions of human immunoglobulin G1 are mated with chicken expressing modified immunoglobulin light chains containing the constant region of human immunoglobulin kappa chain. Offsprings are screened for expression of immunoglobulin molecules containing human heavy and light chain elements.

Binding of human antibodies expressed in chickens to Hepatitis B surface antigen

Genetically engineered chickens (as described above) are immunized intramuscularly with purified Hepatitis B surface antigen (HBsAg) (5μg in

incomplete Freund's adjuvant) on day 0, 14 and day 28. On day 35 animals are bled and serum is prepared. ELISA plates (NUNC, Denmark) are coated with 1 μg/ml HBsAg in PBS for 1 hour at room temperature. Subsequently, available binding sites are blocked by incubation with 1% non-fat dry milk (NFM) in PBS (300 μl/well). Chicken serum is diluted in PBS/1%NFM and added to the coated wells. After an incubation of 1 hour, the plates are washed 3 times with PBS/0.05% Tween 20 and bound Ig is detected using goat anti-human Ig conjugated with horseradish peroxidase. Conjugated goat antibody is detected using o-phenylenediamine dihydrochloride (Sigma) at 1 mg/ml. The colorimetric reaction is stopped by addition of 1 M HCl solution and the absorbance is measured at 490 nm. As a control, serum from non-immunized chicken is used. Serum from non-immunized chickens does not react with HBsAg. At a dilution of 1:250 the optical density measured in uncoated and HBsAg coated wells is below 0.2. In contrast, serum from immunized chickens contains substantially human antibodies reactive with HBsAg. At a serum dilution of 1:250 the measured optical density is 2.3. Upon further dilution of the serum the measured optical density declines to 0.1 (at a dilution of 25600). No antibodies reactive with a goat anti-chicken IgG-HRP conjugate can be detected. This demonstrates that the genetically engineered chickens produce substantially human anti-HBsAg antibodies following immunization.

Complement mediated cytotoxicity of virus infection cell line using human antibodies

A human liver carcinoma cell line expressing HBsAg is labeled with 0.1 mCi 51Cr in 100 ul PBS for 1 hr at 37°C. Two thousand 51Cr-lableled cells are incubated with serum from genetically engineered chickens expressing anti-HBsAg immunoglobulin (see above). After two hours at 37°C the release of 51Cr into the supernatant is determined by measuring radioactivity using a scintillation counter. For the determination of maximum release, 1% Triton X100 is added. The degree of cell lysis is calculated as follows: %Lysis = CPM experimental ±CPMspontaneous / CPMtotal ± CPM spontaneous. Incubation of labeled cells with serum (diluted 1:30) from non-immunized chickens does not result in cell lysis (<10%). However, incubation of cells with serum from immunized chickens does cause 75% cell lysis. Inactivation of complement in the serum by heat treatment (56°C for 30 minutes) renders the serum from immunized chickens inactive. These results demonstrate that

substantially human antibodies produced by genetically engineered chickens bind to HBsAg-positive cells and cause complement dependent lysis.

Treatment of animal with infection.

Substantially human immunoglobulin is purified from egg yolk of genetically engineered chickens. SCID-mice are injected with one million human liver carcinoma cells expressing HBsAg. Subsequently, 20 μg immunoglobulin is injected peritoneally once per day. Animals treated with antibodies isolated from non-immunized chicken serum die after about 60 days. This is similar to untreated recipients of liver carcinoma cells. In contrast, mice treated with antibodies isolated from immunized chicken serum survive for more than 200 days. This demonstrates that human antibodies produced in genetically engineered chicken are capable of eliminating human carcinoma cells from SCID-mice.

It is evident from the above results that by using genetically engineered chickens expressing substantially human immunoglobulin genes, polyclonal antibody preparations against antigens, infectious particles, cancer cells, and the like can be generated. Such polyclonal antibody preparation may be used to treat patients suffering from an infectious disease or a malignancy. The antisera may also be used to modulate an immune response by elimination of cell sub-populations, cytokines, or the like. The human antibody preparation has a substantially reduced capacity of engendering an immune response in human patients, as compared to heterologous antisera, it will have few side effects and it can be used safely with positive results.

All of the references cited herein are incorporated herein.by reference as if each reference was individually wholly incorporated. It will be apparent to one of ordinary skills in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A polyclonal antisera composition from a genetically modified bird, comprising substantially human immunoglobulin protein molecules, wherein said substantially human immunoglobulin protein molecules comprise at least a portion of a human heavy chain polypeptide and specifically bind to an immunogen.

2. A polyclonal antisera composition according to Claim 1, wherein said genetically modified bird is a chicken, turkey, qail, duck, pheasant or goose.

3. A polyclonal antisera according to Claim 1, wherein said portion of human heavy chain immunoglobulin locus comprises at least one constant region element.

4. A polyclonal antisera composition according to Claim 1, wherein said portion of human heavy chain immunoglobulin locus comprises at least one variable region element.

5. The polyclonal antisera composition according to Claim 4, wherein said variable region element is the variable region element proximal to the D region.

6. A polyclonal antisera composition according to Claim 1, wherein said portion of the human heavy chain locus comprises a rearranged VDJ element.

7. The polyclonal antisera composition according to Claim 1, wherein said immunogen comprises a disease-causing organism or antigenic portion thereof.

8. The polyclonal antisera composition according to Claim 1, wherein said immunogen is an antigen endogenous to humans.

9. The polyclonal antisera composition according to Claim 1, wherein said immunogen is an antigen exogenous to humans.

10. A genetically modified bird comprising at least a portion of human heavy chain immunoglobulin genes integrated by homologous recombination into its genome, wherein said portion of human heavy chain immunoglobulin genes rearranges in frame with heavy chain immunoglobulin sequences endogenous to said bird to encode functional, substantially human antibody molecules that comprise at least in part human heavy chain immunoglobulin polypeptide sequences, and wherein said bird produces said functional, substantially human antibody molecules when immunized.

11. A genetically modified bird comprising at least a portion of human light chain immunoglobulin genes integrated by homologous recombination into its genome, wherein said portion of human light chain immunoglobulin genes rearranges in frame with sequences endogenous to said bird to encode functional, substantially human antibody molecules that comprise at least in part human light chain immunoglobulin polypeptide sequences.

12. A genetically modified bird according to Claim 10 or 11, wherein said bird is a chicken, turkey, quail, duck, pheasant or goose.

13. A genetically modified bird according to Claim 10 or 11, wherein said portion of human heavy chain immunoglobulin genes comprises at least one constant region element.

14. A genetically modified bird according to Claim 10 or 11, wherein said portion of human heavy chain immunoglobulin genes further comprises at least one variable region element.

15. A genetically modified bird according to Claim 14, wherein said variable region element is the variable region element proximal to the D region.

16. A genetically modified bird according to Claim 14, wherein said portion of the human heavy chain immunoglobulin genes further comprises a rearranged VDJ element inserted into the birds immunoglobulin locus.

17. A method for neutralizing an antigenic entity in a human body component, said method comprising: contacting said body component with an antisera composition according to Claim 1, whereby said substantially human immunoglobulin protein molecules in said antisera composition specifically bind and neutralize said antigenic entity.

18. The method according to Claim 17, wherein said antigenic entity is from an organism that causes an infectious disease.

19. The method according to Claim 17, wherein said antigenic entity is a cell surface molecule.

20. The method according to Claim 19, wherein said cell surface molecule is from a lymphocyte or an adipocyte.

21. The method according to Claim 19, wherein said antigenic entity is a human cytokine or a human chemokine.

22. The method according to Claim 17, wherein said antigenic entity is a cell surface molecule on a malignant cancer cell.

23. A method of producing a genetically modified bird comprising human immunoglobulin genes integrated by homologous recombination into its genome, wherein said bird produces functional, substantially human antibody molecules comprised at least in part of human immunoglobulin polypeptide sequences when immunized, said method comprising:

producing a first mutated bird comprising heavy chain immunoglobulin loci

where constant and/or variable region elements are replaced with at least a portion of the human heavy chain immunoglobulin locus by genetic alteration of embryonic stem cells, injecting said embryonic stem cells into a recipient embryo to produce a first genetically modified bird, and isolating a first line of germline transgenic genetically modified birds by breeding;

producing a second mutated bird comprising light chain immunoglobulin loci where constant and/or variable region elements are replaced with at least a portion of the human light chain immunoglobulin locus by genetic alteration of embryonic stem cells, injecting said embryonic stem cells into a recipient embryo to produce a second genetically modified bird, and isolating a second line of germline transgenic genetically modified birds by breeding;

breeding first and second line of genetically modified birds and selecting birds capable of producing substantially human antisera.

24. A method of producing a genetically modified bird comprising human immunoglobulin genes integrated by homologous recombination into its genome, wherein said bird produces functional, substantially human antibody molecules comprised at least in part of human immunoglobulin polypeptide sequences when immunized, said method comprising:

producing a genetically modified bird comprising heavy and light chain immunoglobulin loci where constant and/or variable region elements are replaced with at least a portion or the human heavy and/or light chain immunoglobulin locus by genetic alteration of a cell nucleus of said animal, introducing said cell nucleus into an enucleated nuclear transfer unit cell to provide a embryonic cell stem cell, introducing said nuclear transfer unit cell into a female recipient host to produce mutated neonate; and

breeding mature mutated neonates and selecting birds capable of producing substantially human antisera and at least substantially incapable of producing endogenous antisera.

25. The method according to Claim 23 or 24, wherein said nuclear transfer unit cell is an oocyte.

26. A method according to Claim 23 or 24, wherein said heavy chain locus comprises at least one constant region element.

27. A method according to Claim 23 or 24, wherein said heavy chain locus comprises at least one variable region element.

28. A method according to Claim 23 or 24, wherein said heavy chain locus comprises the variable region element proximal to the D region.

29. A method according to Claim 23 or 24, wherein said heavy chain locus comprises a rearranged human VDJ element.