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(54) **COMPOSITIONS, METHODS FOR TREATMENT, AND DIAGNOSES OF AUTOIMMUNITY-RELATED DISORDERS AND METHODS FOR MAKING SUCH COMPOSITIONS**

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(57) **ABSTRACT**

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The present invention provides compositions and methods useful in the diagnosis and treatment of autoimmunity-related disorders, including cancers and other disorders involving angiogenesis, as well as non-cancer disorders involving a dysfunction in the immune system. In some embodiments, the invention described a plasma assay. In other embodiments, urine assay. In certain other embodiments, the invention provides therapeutic methods comprising removing toxic autoantibodies from the circulation of a patient, e.g., via plasmapheresis, and subsequently infusing the patient with one or more immunoglobulins or immunoglobulin complexes to restore the immune system of the patient to a baseline status whereby the patient's restored immune system either eliminates the source of the disorder (e.g., in the case of cancers) or no longer causes the disease or disorder (e.g., in the case of autoimmune disorders such as multiple sclerosis, psoriasis, latent autoimmune type 1 diabetes in adults (LADA) and the like). Methods of making the high activity IVIG preparation are also provided.

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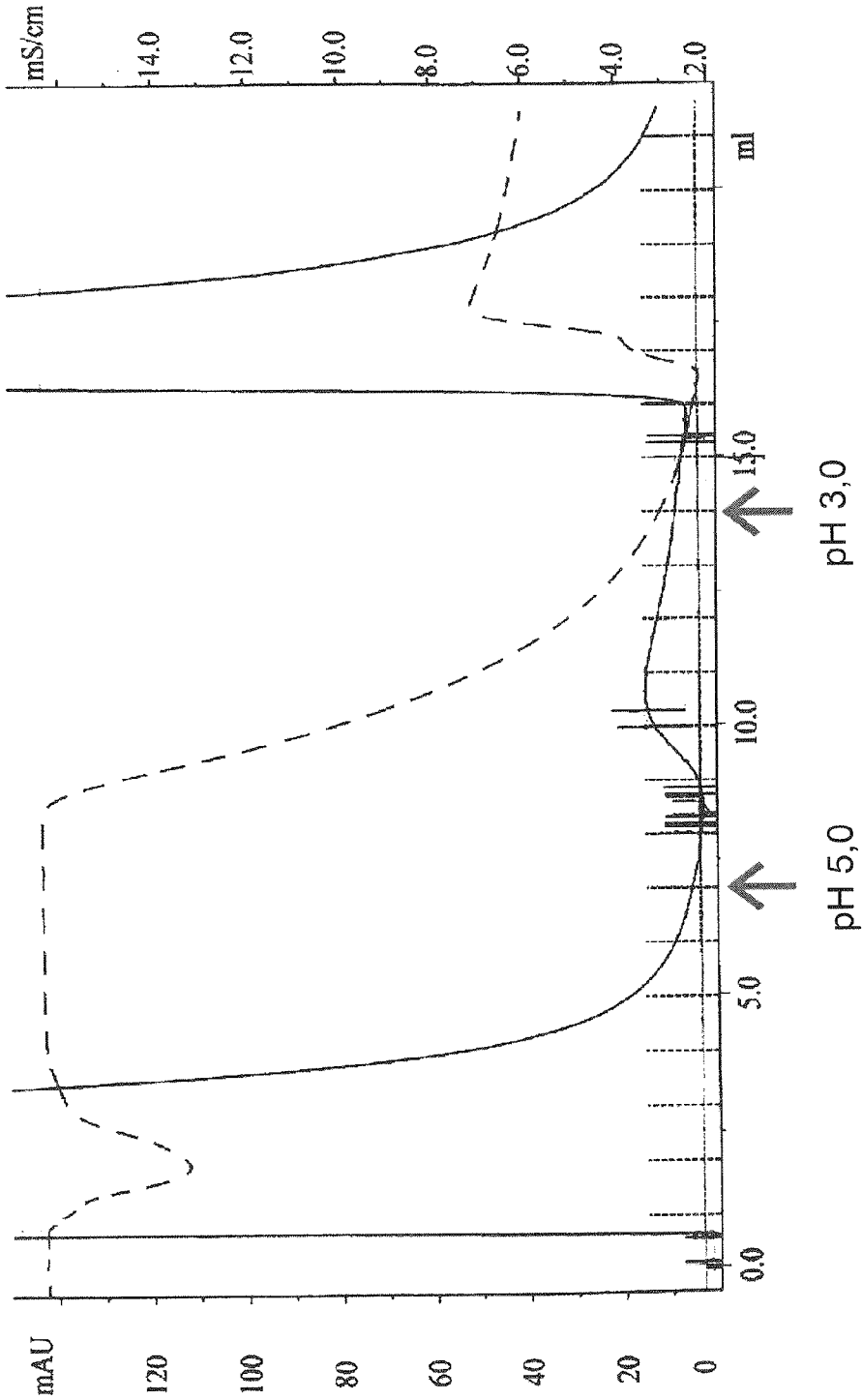


FIG. 1

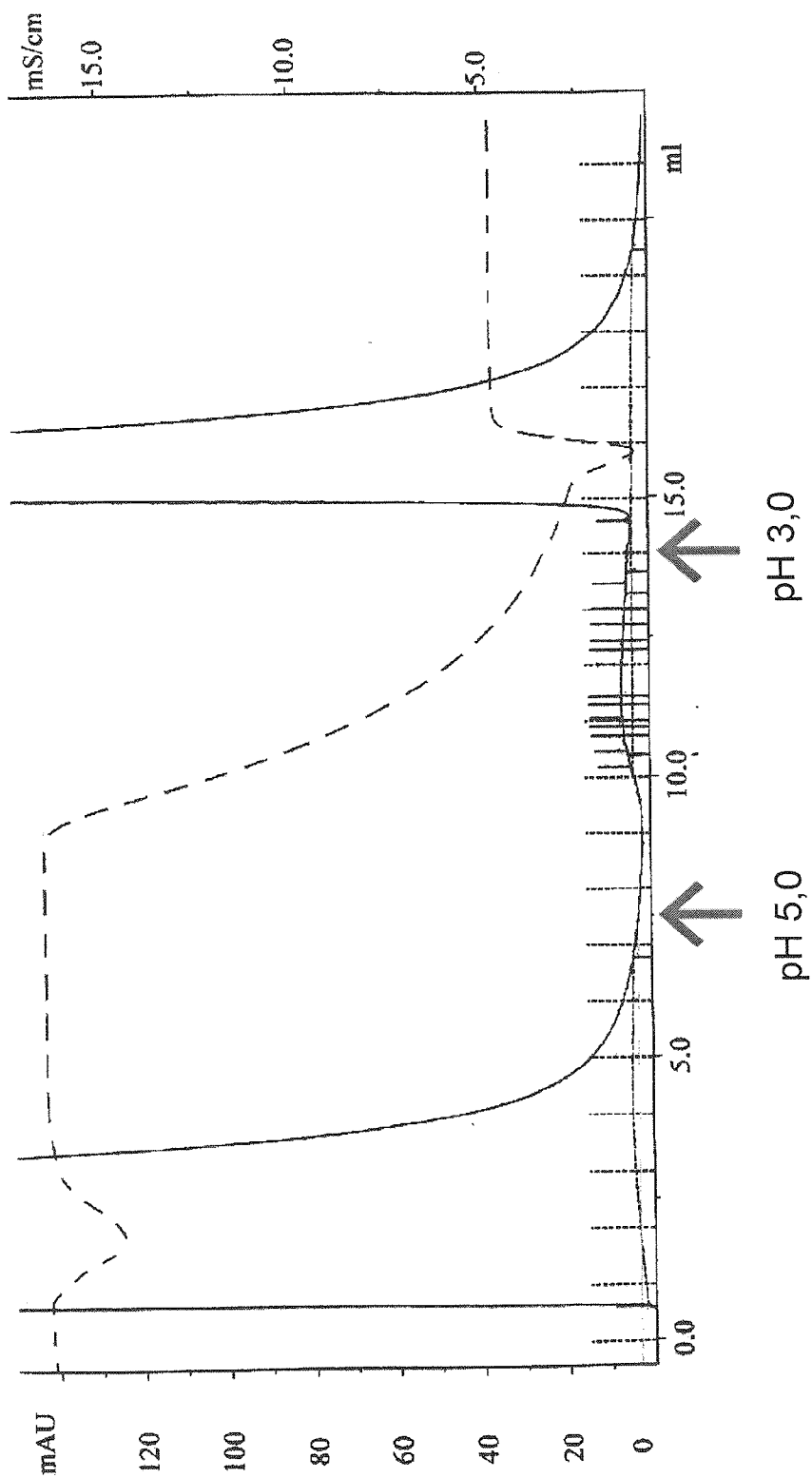


FIG. 2

COMPOSITIONS, METHODS FOR TREATMENT, AND DIAGNOSES OF AUTOIMMUNITY-RELATED DISORDERS AND METHODS FOR MAKING SUCH COMPOSITIONS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. provisional application No. 61/254,072, filed Oct. 22, 2009, and 61/306,718, filed Feb. 22, 2010, both of which are incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The present invention is in the fields of medicine, immunology and pharmacology, particularly in the areas of medical therapeutics and diagnostics. More particularly, the present invention provides compositions and methods useful in the treatment of diseases and disorders, particularly autoimmunity-related diseases and disorders, including cancers and other disorders involving autoimmune-related angiogenesis, as well as non-cancer disorders involving a dysfunction in the immune system such as multiple sclerosis, psoriasis, diabetes (including latent autoimmune type 1 diabetes in adults (LADA)) and the like. The invention also provides analytical tools for diagnosing diseases and disorders that have an autoimmune origin. Another aspect of the present invention relates to pharmaceutical compositions comprising immunoglobulins of high activity, and methods for determining the activity levels of immunoglobulins in the pharmaceutical preparations. The present invention further provides a novel method for purification of a highly effective intravenous immunoglobulin (IVIG), wherein the resultant highly effective IVIG retains as much of its useful therapeutic characteristics in the donated bodily fluid that is the process input.

[0004] 2. Related Art

[0005] Autoimmune and inflammatory diseases affect more than fifty million Americans. The immune system functions as the body's major defense against diseases caused by invading organisms. This complex system fights disease by killing invaders such as bacteria, viruses, parasites or cancerous cells while leaving the body's normal tissues unharmed. The immune system's ability to distinguish the body's normal tissues, or self, from foreign or cancerous tissue, or non-self, is an essential feature of normal immune system function. A second essential feature is memory, the ability to remember a particular foreign invader and to mount an enhanced defensive response when the previously encountered invader returns. The loss of recognition of a particular tissue as self and the subsequent immune response directed against that tissue produce serious illness.

[0006] Inflammation is involved in a large number of physiological and pathological conditions affecting animals and humans. Inflammatory responses can usually be traced to an immune response to an antigen, allergen, irritant, endotoxin or to tissue damage. The process is complex, involving a large number of components, many of which display pleiotropic effects, many of which are amplifiers or inhibitors of other components. While many instances of an inflammatory response are well controlled and self-limited, many patho-

logic conditions arise from uncontrolled or inappropriate responses, resulting in both acute and chronic conditions.

[0007] The immune system when operating normally is involved in precise functions such as recognition and memory of, specific response to, and clearance of, foreign substances (chemical and cellular antigens) that either penetrate the protective body barriers of skin and mucosal surfaces (transplanted tissue and microorganisms such as bacteria, viruses, parasites) or arise de novo (malignant transformation). The arsenal of the immune response is composed of two major types of lymphocytes that are either B-lymphocytes (B cells, responsible for producing antibodies which attack the invading microorganisms) or the T-lymphocytes (T cells, responsible for eliminating the infected or abnormal target cells) in cooperation with macrophages.

[0008] An autoimmune disease results from an inappropriate immune response directed against a self antigen (an autoantigen), which is a deviation from the normal state of self-tolerance. Self-tolerance arises when the production of T cells and B cells capable of reacting against autoantigens has been prevented by events that occur in the development of the immune system during early life. Several mechanisms are thought to be operative in the pathogenesis of autoimmune diseases, against a backdrop of genetic predisposition and environmental modulation. In general, antibodies (particularly, but not exclusively, IgG antibodies), acting as cytotoxic molecules or as a part of immune complexes, are the principal mediators of various autoimmune diseases, many of which can be debilitating or life-threatening.

[0009] The development and progression of certain forms of cancer and other diseases or disorders is similarly often associated with a pathogenic disturbance in the body's homeostasis. For example, certain forms of neoplastic diseases are associated with increased angiogenesis. In general, angiogenesis is a process of formation of new blood vessels in mammals and other animals. It is inherent to many activities of a normal human or animal body. Angiogenesis is vital for cellular growth and development, as well as wound-healing. Angiogenesis is also a necessary process for tumor growth.

[0010] Tumor progression is dependent on a number of sequential steps, including tumor-vascular interactions and recruitment of blood vessels. It is known that human and animal tumors produce a defined set of proangiogenic factors, which are typically offset by certain antiangiogenic factors produced in the normal mammalian body. When the proangiogenic and antiangiogenic activities are balanced, tumor mass cannot expand beyond a limited size, and the development of most mammalian cancers is arrested at a dormant mass of about 1-2 mm³ or smaller; cancers of this size often elude clinical detection and are cleared by the normal immune system of the mammal without any outward manifestation of the disease. However, due to a poorly understood molecular switch governed by various genetic and epigenetic factors, some tumours become excessively proangiogenic, which enables them to overproduce proangiogenic factors that overcome the antiangiogenic factors being produced by the normal mammalian body, thereby disturbing the homeostatic situation; in such cases, the tumors are able to recruit and sustain their own blood supply via the process of angiogenesis, resulting in the growth of the cancer into a palpable or otherwise clinically detectable tumor.

[0011] A vast number of pro- and anti-angiogenic factors have been described. Examples of proangiogenic factors include fibroblast growth factors, vascular endothelial growth

factors, colony stimulating factors, interleukins, platelet-derived growth factors, angiopoietins, tumor-necrosis factors, matrix metalloproteinases (MMPs) and, in particular, transforming growth factor beta 1 (TGF- β 1), intercellular adhesion molecules (ICAMs), hepatocyte growth factor, nerve growth factor, connective tissue growth factor, tenascin R, prolactin, growth hormone, placental lactogen, insulin-like growth factor 1, thymidine-phosphorylase, and the like. Examples of antiangiogenic factors include interferons, tissue inhibitors of metalloproteinases (TIMPs), plasminogen, collagen, fibronectin, prolactin, growth hormones, thrombospondins, and fragments thereof. Among the most characterized antiangiogenic factors is angiostatin, a proteolytic fragment of plasminogen. As long as the expression, secretion or generation of pro- and antiangiogenic factors remains in equilibrium in the animal body, tumors will remain dormant. In certain diseases or disorders, however, this equilibrium in the activity of pro- and antiangiogenic factors is disrupted, which in turn can disturb the angiogenic balance resulting in the growth of new blood vessels, which can lead to angiogenesis-mediated pathologies.

[0012] Diagnosing and monitoring an activity of a disease or a disorder with autoimmune origin are both problematic in patients. Diagnosis is problematic because the spectrum of autoimmune diseases is often broad and ranges from subtle or vague symptoms to life threatening multi-organ failure. In addition, other diseases can be mistaken for autoimmune diseases, and vice versa. To further complicate a difficult diagnosis, symptoms of many autoimmune diseases may occur in combination with each other, and may continually evolve over the course of the disease. New symptoms in previously unaffected organs can develop over time. Testing of these highly variable diseases can therefore be complex, and is often misunderstood.

[0013] Monitoring disease activity is also problematic in caring for patients with malfunctions of the immune system. Some autoimmune diseases progress in a series of flares, or periods of acute illness, followed by remissions. In order to minimize devastating consequences of systemic organ damage often associated with autoimmune disorders, earlier and more accurate detection of disease flares would not only expedite appropriate treatment, but would reduce the frequency of unnecessary interventions. From an investigative standpoint, the ability to uniformly describe the activity of disease in individual organ systems or as a general measure is an invaluable research tool. Furthermore, a measure of disease activity can be used as a response variable in a therapeutic trial.

[0014] There is at present no cure for autoimmune diseases. However, there are a number of traditional approaches to treating autoimmune-related disorders and cancers that are known in the art. Among traditional treatments for patients with autoimmune diseases is an intravenous immunoglobulin (IVIG) therapy. Such therapy is typically accomplished by the intravenous administration to the patient of therapeutic preparations of normal polyspecific immunoglobulins, typically IgG immunoglobulins, obtained from pooled plasma or sera derived from up to thousands of healthy blood donors. Currently used commercially available preparations are made of intact IgG with a distribution of subclasses corresponding to that seen in normal serum and have a half-life of three weeks in vivo for IgG1, IgG2 and IgG4, and somewhat less for IgG3. Most of the preparations contain only traces of IgA, IgM and of Fc-dependent IgG aggregates. Owing to the large

number of donors, the immunoglobulins used in IVIG therapy usually represent a wide spectrum of the expressed normal human IgG repertoire, including antibodies to external antigens, autoreactive antibodies and anti-antibodies (including anti-idiotypic antibodies). IVIG has been widely used for correction of immune deficiencies such as X-linked agammaglobulinemia, hypogammaglobulinemia, and acquired compromised immunity conditions, for treating various inflammatory and autoimmune diseases, and even cancer. U.S. Pat. No. 5,965,130 discloses the use of IVIG therapy for inhibition of tumor metastasis. However, the therapeutic effects of this treatment were disclosed in this patent to be short-lived, lasting between two weeks and three months, which thus does not provide long-term curative potential. Moreover, using these traditional approaches to achieve a long-term cure (even if that were possible) would likely be prohibitively expensive given the costs associated with researching, developing, manufacturing and obtaining regulatory approval for biological therapeutics such as IVIG. For at least these reasons, the use of IVIG in generally treating neoplastic diseases is not widespread.

[0015] The standard IVIG manufacturing process contains the following steps commonly used by most manufacturers: (a) Removal of Factor VIII and Factor IX using cryoprecipitation and ion exchange; (b) a series of cold alcohol processes (Cohn and Oncley cold ethanol process or variants including the Kistler & Nitschmann cold ethanol fractionation process) and absorption that results in a solution containing greater than 99% IgG; (c) a series of steps using low pH (<5.0), high temperature incubation (>30° C.) and harsh chemicals including solvents and detergents; (d) some manufacturers use a small amount of detergent (lubricant) and a filter that will remove any remaining viruses; (e) concentration by ultrafiltration to remove water; (f) a last sterile filtration to remove microbial contaminants; (g) adjust to proper pH (typically 4-6) and add stabilizers and fill; and (h) incubation at 30° C. for 2 weeks.

[0016] U.S. Pat. No. 6,932,969 discloses a method for preparing Ig fractions having reactivity to pathologic autoantibodies against actin, myosin, basic myelin protein, and tubulin. However, this method does not recognize a formation of pathologic autoantibodies against antiangiogenic factors and therefore it cannot be efficiently applied in the treatment of diseases with angiogenesis disorders.

[0017] WO 2008/006187 A2 discloses a method treatment of diseases with angiogenesis disorders having an autoimmune mechanism in their origin. In this method, a patient is administered a protein complex containing an angiogenic factor (or a portion thereof) and an immunomodulating moiety, which can either act as an immunostimulator or an immunosuppressor. Administration of the disclosed protein complex is described to result in a modulation of an immune response to the angiogenic factor in question. The main disadvantage of this method is the need of predefining an angiogenic factor which concentration exceed the normal level and for which there is an elevated levels of autoantibodies produced, and the need to identify (or even produce) a particular antibody, often a monoclonal antibody, that is specific for the predefined angiogenic factor—this need often raises the difficulty and the attendant costs of the procedure.

[0018] The primary goal in manufacturing IVIG for clinical use is to produce a safe product that retains as much of the useful therapeutic characteristics of the IgG in the donated plasma that is the process input. Safety focuses on the deac-

