(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 November 2016 (10.11.2016)

WIPO | PCT

(10) International Publication Number
WO 2016/179579 A2

(51) International Patent Classification:
A61K 39/395 (2006.01) *G01N 33/53* (2006.01)

(21) International Application Number:
PCT/US2016/031425

(22) International Filing Date:
9 May 2016 (09.05.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/158,182 7 May 2015 (07.05.2015) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

(54) Title: CYTOPLASMIC AND NUCLEAR ANTIBODY COMPOSITIONS AND METHODS OF USE THEREOF

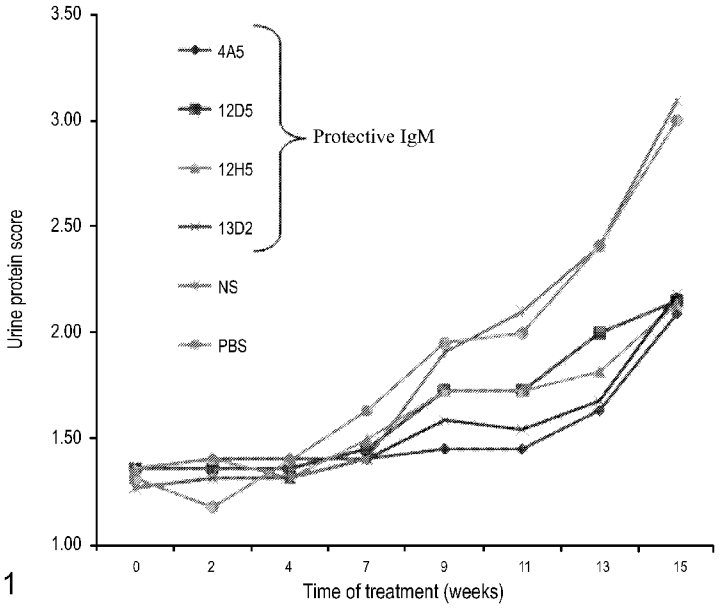

FIG. 1

(57) Abstract: The presently disclosed subject matter relates to cytoplasmic, Fc region, and nuclear antibody compositions and methods of use thereof, particularly wherein the compositions are used as an enrichment product for intravenous immunoglobulins. More particularly, the presently disclosed subject matter relates to methods of use of the disclosed compositions for treating or diagnosing blood related cancers, immune complex-mediated diseases, and certain autoimmune disorders.

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CYTOPLASMIC AND NUCLEAR ANTIBODY COMPOSITIONS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 62/158,182, filed May 7, 2015, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

Embodiments described herein relate to cytoplasmic, Fc region and nuclear antibody compositions and methods of use thereof, particularly wherein the compositions are used alone or as an enrichment product for intravenous immunoglobulins. More particularly, embodiments described herein relate to methods of use of the disclosed compositions for treating or diagnosing blood related cancers, immune complex-mediated diseases, and certain autoimmune disorders.

BACKGROUND

Autoimmune disorders are a heterogeneous group of chronic immune disorders that afflict over 20 million Americans. These disorders are characterized by an inappropriate immune response to self-antigens wherein an individual's own cells or tissues are attacked by components of the immune system. Autoimmune diseases vary in the degree of organ involvement, ranging from organ specific autoimmunity such as the thyroid in autoimmune thyroiditis or where multiple organ systems are compromised such as systemic lupus erythematosus (SLE), rheumatoid arthritis, mixed connective tissue disorder (MCTD), and scleroderma (SSc). They also differ in the specific immune component imparting pathogenesis but T and/or B lymphocytes and other inflammatory cells such as neutrophils and macrophages are commonly implicated in the majority of these disorders. SLE is a clear example of a systemic autoimmune disorder with a strong B cell component, and therefore a good model to examine how B cells can impart autoimmunity. It is characterized by the circulation of autoantibodies and immune complex deposition in various tissues, particularly the kidney glomeruli causing nephritis, a common fatal complication of SLE (Yung & Chan (2008) *Autoimmun Rev.*, 7:317). Hallmark autoantibodies of SLE recognize nuclear cellular components, in particular dsDNA (Winkler *et al.* (1992) *J. Immunol,* 22:1719). It is not understood why antinuclear

antibodies develop, but potential mechanisms include defective apoptosis, defective clearance of apoptotic material, excess necrosis from ongoing chronic damage, and even defects in toll receptor signaling (Theofilopoulos & Lawson (1999) *Ann. Rheum. Dis.* 58 Suppl 1:I49; Pickering *et al.* (2000) *Adv. Immunol.*, 76:227; Potter *et al.* (2003) *J. Immunol.*, 170: 3223). Ultimately, these autoantibodies can trigger an autoimmune cascade characterized by inflammation and tissue destruction. We discovered that certain autoreactive antibodies of the IgM type are protective from development of lupus nephritis and likely other immune-complex mediated disorders (type III hypersensitivity).

An estimated 1.5 million Americans, and at least 5 million people worldwide, have a form of lupus. Lupus is primarily a disease of young women. There are approximately 16,000 new cases of lupus diagnosed each year, with 70% of them developing the systemic form. Of that group, up to 50% develop lupus nephritis and up to 30% of those progress to end-stage renal disease, and require dialysis or a kidney transplant. Over 30% of all SLE deaths occur between the ages of 15-44. Standard treatment aims to suppress immune system. Corticosteroids and powerful immunosuppressants such as high dose cyclophosphamide, mycophenolate mofetil, azathioprine are used. Immunosuppressants especially in the context of the immune dysfunction of SLE can lead to severe complications such as sepsis. Belimumab, the newest FDA approved treatment for lupus nephritis, is only marginally effective and a pivotal study increased deaths in the treatment group and was associated with an increased risk of serious infection. Clearly, a novel approach for lupus nephritis is critically needed.

Surprisingly, we also discovered that a combination of certain autoantibodies of the IgM subtype protect lymphoma-prone mice from development of high grade T cell lymphoma. The relationship between autoimmunity and cancer has been documented but is controversial, as early reports suggest that autoantibodies could be protective or mere markers of disease. However, recent reports suggest that these autoantibodies may in fact be pathogenic or predispose patients to certain cancers, while others may be truly protective (Strauss *et al.* (2001) *Blood*, 98:194; Guyomard *et al.* (2003) *Br. J. Haematol.* 123:90; Kubota *et al.* (2010) *J. Clin. Pathol.* 63:79; Toubi *et al.*(2007) *Oncol. Rep.* 17:245; Mellemkjar *et al.* (2008) *Arthritis Rheum.* 58:657; Liu *et al.* (2013) *J. Urol.* 189:2262; Dagklis *et al.* (2012) *Leukemia* 26:814). Resolution of this apparent paradox probably lies in distinguishing those autoreactive antibodies that are early markers of

neoplastic processes (Blaes (2012) *Curr. Pharm. Des.* 18:4518), from those that are actually protective as our data indicates for certain autoantibody combinations. This is further complicated by the fact that defects correlated with immune dysregulation are often directly associated with both disease processes, such as defects in apoptotic pathways (Zornig *et al.* (1995) *Oncogene* 10:2397). In addition, immunodeficiencies, acquired or inborn, are often independently associated with both cancer susceptibility and autoimmunity (Cunningham-Rundles *et al.* (2002) *Blood Rev.* 16:61). Because of these reasons, the prevalent opinion is that the significant correlation observed between circulating autoantibodies and malignancy is due to a common co-occurrence rather than these antibodies playing a role in disease protection. However, our data demonstrates that in the case of certain blood cancers, this association is not incidental but rather evidence that certain autoantibodies can bind and selective kill tumor cells.

SUMMARY

In one embodiment, a composition is provided comprising a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity. In a particular embodiment, at least one anti-nuclear specificity is selected from the group consisting of anti-single stranded DNA (anti-ssDNA), anti-double stranded DNA (anti-dsDNA), anti-histone, anti-RNP, anti-Smith (anti-Sm), anti-Ro, anti-La, anti-DNP, anti-Scl-70, and anti-PM/Scl). In another particular embodiment, at least one anti-cytoplasmic specificity selected from the group consisting of anti-Jo-1, anti-phospholipid, anti-phosphocholine, anti-phosphatidylcholine, and anti-Ribosomal-P.

In another embodiment, the composition comprising IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity further comprises IgM antibodies with at least one anti-Fc receptor (of IgG) specificity.

In another embodiment, the composition comprising IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity further comprises at least one IgM antibody capable of activating mesangial cells to induce phagocytosis and at least one IgM antibody capable of activating mesangial cells to secrete cytokines.

In another embodiment, the composition comprising IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity further comprises at least one IgM antibody capable of binding apoptotic cells and at least one IgM antibody capable of binding immune complexes.

In another embodiment, the composition comprising IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity further comprises at least two autoreactive IgM specificities equivalent to specificities of the pathogenic IgG spectra of a disease, selected from the group consisting of a disease associated with Type III hypersensitivity reactions, a disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer.

In another embodiment, the composition comprising IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity further comprises: a) at least one IgM antibody capable of activating mesangial cells to induce phagocytosis and at least one IgM antibody capable of activating mesangial cells to secrete cytokines; b) at least one IgM antibody capable of binding apoptotic cells and at least one IgM antibody capable of binding immune complexes; and c) at least two autoreactive IgM specificities equivalent to specificities of the pathogenic IgG spectra of a disease, selected from the group consisting of a disease associated with Type III hypersensitivity reactions, a disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer.

In another embodiment, any of the compositions described above further comprise IvIg.

In another embodiment, any of the compositions described above comprise IgM antibodies that are humanized and/or in chimeric form.

In a further embodiment, a method is provided for treating a disease by administering a therapeutically effective amount of any of the compositions described above to a subject in need thereof, wherein the disease is selected from the group consisting of a disease associated with Type III hypersensitivity reactions, a disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer. In a particular embodiment, the disease associated with Type III hypersensitivity reactions is selected from the group consisting of nephritis, vasculitis, and some forms of arthritis. In another

particular embodiment, the disease associated with excessive apoptotic debris and inflammation or the autoimmune disease associated with pathogenic IgG autoantibodies is selected from the group consisting of SLE, scleroderma, rheumatoid arthritis, atherosclerosis, and ischemia. In another particular embodiment, the blood cancer is selected from the group consisting of leukemia, B cell lymphoma, and T cell lymphoma.

In a further embodiment, a method for predicting the likelihood of developing nephritis in a subject with SLE is provided, the method comprising the steps of: a) obtaining a sample from the subject; b) measuring levels of protective IgM antibodies and of pathogenic IgG in the sample; and c) determining the ratio of protective IgM antibodies to pathogenic IgG in the sample; wherein a higher level of protective IgM antibodies as compared the level of pathogenic IgG is indicative of a lower likelihood of developing nephritis in the subject with SLE. In a particular embodiment, the protective antibodies as a group have at least three characteristics selected from the group consisting of: 1) being of the IgM type; 2) having the ability to bind antinuclear and/or cytoplasmic antigens; and 3) having the ability to bind apoptotic cells and stimulate cytokine secretion by mesangial cells. In a further particular embodiment, the pathogenic antibodies are of the IgG subtype and bind nuclear and/or certain cytoplasmic antigens. In another embodiment, the protective antibodies and the pathogenic antibodies are measured by a method selected from the group consisting of immunofluorescence techniques with antinuclear antigen assays, mesangial cell cultures, apoptotic binding assays, a specific antigen binding assay, and heavy chain immunoglobulin variable region family determination. In another embodiment, the specific antigen binding assay is ELISA.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing summary, as well as the following detailed description of various embodiments, is better understood when read in conjunction with the drawings provided herein. For the purposes of illustration, there is shown in the drawings exemplary embodiments; however, the presently disclosed subject matter is not limited to the specific methods and instrumentalities disclosed.

FIG. 1 shows that passive transfer of certain autoreactive IgM antibodies protected lupus-prone mice from development of lupus nephritis as measured by levels of protein in the urine.

FIG. 2 shows that IgM autoreactive antibodies with some level of protection display a

variety of specificities. It is expected that specific combinations of protective autoreactive IgM antibodies, with or without an anti-IgG specificity, is an effective treatment for SLE patients with nephritis.

FIG. 3 shows that protective antibodies also vary dramatically in their ability to stimulate IL-6 production by mesangial cells. All depicted treatments were with autoreactive igM antibodies but only two, Hom-1 and WT-1 induced secretion. This was associated with increased proliferation of mesangial cells. Although counterintuitive because of the pro-inflammatory properties of IL-6, antibodies with strongest stimulation of IL-6 production are expected to be a critical component of the combination therapy.

FIG 4. shows that antibodies vary in their ability to stimulate phagocytosis by mesangial cells. We expect that combination therapy is enhanced by inclusion of antibodies with the best ability to induce mesangial cell phagocytosis.

FIG. 5 illustrates that protective antibodies efficiently bind apoptotic cells.

FIG. 6. illustrates that treatment with autoreactive IgM antibodies (high IgM group) can effectively reduce the severity of T cell lymphoma in lymphoma-prone mice. The onset was delayed as well. It is expected that combinations of antibodies with autoreactive specificities is an effective treatment for patients with lymphoma.

DETAILED DESCRIPTION

The presently disclosed subject matter is described with specificity to meet statutory requirements. However, the description itself is not intended to limit the scope of this patent. Rather, the inventors have contemplated that the claimed subject matter might also be embodied in other ways, to include different steps or elements similar to the ones described in this document, in conjunction with other present or future technologies. Moreover, although the term "step" may be used herein to connote different aspects of methods employed, the term should not be interpreted as implying any particular order among or between various steps herein disclosed unless and except when the order of individual steps is explicitly described.

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs.

DEFINITIONS

As used herein, "activation of specific B cells" is understood as administration of an agent or combination of agents to stimulate the production of antibodies by a specific B cell or population of B cells. For example, the administration of an agent or combination of agents to stimulate the production of germline IgM antibodies that specifically bind dsDNA.

An "agent" is understood herein to include a therapeutically active compound or a potentially therapeutic active compound. An agent can be a previously known or unknown compound. As used herein, an agent is typically a non-cell based compound, however, an agent can include a biological therapeutic agent, e.g., peptide or nucleic acid therapeutic, cytokine, antibody, etc.

An "agonist" is understood herein as a chemical substance capable of initiating the same reaction or activity typically produced by the binding of an endogenous substance or ligand to its receptor. An "antagonist" is understood herein as a chemical substance capable of inhibiting the reaction or activity typically produced by the binding of an endogenous substance (e.g., an endogenous agonist) to its receptor to prevent signaling through a receptor or to prevent downstream signaling that is the normal result of activation of the receptor. The antagonist can bind directly to the receptor or can act through other proteins or factors required for signaling, antigenonists and antagonists can modulate some or all of the activities of the endogenous substance or ligand that binds to the receptor. Antagonists are typically characterized by determining the amount of the antagonist is required to inhibit the activity of the endogenous agonist. For example, an inhibitor at 0.01-, 0.1-, 1-, 5-, 10-, 50-, 100-, 200-, 500-, or 1000-fold molar concentration relative to the agonist can inhibit the activity of the agonist by at least 10%, 20%, 30%, 40%, 50%, 60%. 70%, 80%, 90%, or more.

As used herein "amelioration" or "treatment" is understood as meaning to lessen or decrease at least one sign, symptom, indication, or effect of a specific disease or condition. For example, amelioration or treatment of lupus nephritis can include prevention of progression of at least one sign or symptom from a diagnostic class of lupus nephritis to the next higher pathological designation, or decrease in signs or symptoms of inflammation associated with lupus nephritis as determined by the presence or absence of cytokines, inflammatory complexes, or activated macrophages, either in serum or kidney. In an embodiment, amelioration or treatment includes

delay or prevention of the progression from one diagnostic class to the next diagnostic class. Amelioration and treatment can be viewed as a continuum and need not be understood as distinct activities.

As used herein, "antibody" is understood as a globular plasma protein having a molecular weight of about 15OkDa, also known as an immunoglobulin. The basic functional unit of each antibody is an immunoglobulin (Ig) monomer (containing only one Ig unit); secreted antibodies can also be dimeric with two Ig units as with IgA, or pentameric with five Ig units, like mammalian IgM. The Ig monomer is a "Y"-shaped molecule that consists of four polypeptide chains; two identical heavy chains and two identical light chains connected by disulfide bonds. Each chain is composed of structural domains called Ig domains. These domains contain about 70-110 amino acids and are classified into different categories (for example, variable or IgV, and constant or IgC) according to their size and function. They have a characteristic immunoglobulin fold in which two beta sheets create a binding site, held together by interactions between conserved cysteines and other charged amino acids. As used herein, "antibody" also includes fragments, e.g., proteolytic fragments, of antibodies including Fab fragments, Fc fragments, and $F(ab)_2$ fragments.

As used herein, "antibody" can also include any of a number of single or multi chain containing a paired V_H/V_L domain that specifically binds an antigen. A single -chain variable fragment (scFv) is a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFvs can be made by transplanting V_H and V_L regions, from naturally occurring or synthetic (e.g., humanized), into the desired scFv sequence, or transplanting of CDRs from desired antibodies into an antibody framework present in an scFv sequence. Antigen binding portions (i.e., V_H/V_L pairs) can be optimized for the desired binding characteristics using methods such as antibody phage display (see, e.g., Antibody Phage Display: Methods and Protocols, Edited by P. M. O'Brien and R. Aitken, Humana Press, c. 2002, incorporated herein by reference). It is understood that scFvs can be modified to include sequences to facilitate multimerization of the single chains, either by expressing tandem scFvs from a single promoter, or by including sequences

to allow for cross-linking, to include antigens to allow for multimerization by binding to a divalent antibody. Another possibility is the creation of scFvs with linker peptides that are too short for the two variable regions to fold together (about five amino acids), forcing scFvs to dimerize. This type is known as diabodies. Diabodies have been shown to have dissociation constants up to 40-fold lower than corresponding scFvs, corresponding to a much higher affinity to their target. Still shorter linkers (one or two amino acids) lead to the formation of trimers, so-called triabodies or tribodies and tetrabodies have also been produced (see, e.g., Adams, et al., (1998). British journal of cancer 77: 1405-12; Le Gall (1999). FEBS Letters 453: 164-168; and Mathew, (2004) Stroke; a journal of cerebral circulation 35: 2335-9, each incorporated herein by reference).

As used herein, the term "antigen" refers to a molecule that is bound by an antibody paired V_H/V_L domain. Typically, antigens are capable of raising an antibody response in vivo. An antigen can be a peptide, protein, nucleic acid, lipid, carbohydrate, hapten, or other molecule. Antigens can be non-self, e.g., from a pathogen, or in the case of various autoimmune diseases or disorders, antigens can include self-antigens.

As used herein, an "antibody with autoreactive specificity" is understood as an antibody that specifically binds an autoantigen in the absence of proteins. The antibody binds the autoantigen with at least a 10-fold, 20-fold, 50-fold, 100- fold, 200-fold, 500-fold, 1000-fold, 2000-fold, 500-fold, or more preference over non-specific antigens.

As used herein, "autoimmune" response is understood as the production of antibodies against self-antigens including, but not limited to, polypeptides, nucleic acids, and combinations thereof. B-cells capable of producing autoantibodies are typically cleared during development in a clonal selection process. An "autoimmune" response producing "autoantibodies" typically results in the development of a disease or disorder, including but not limited to the various forms of lupus including lupus nephritis.

As used herein, "class switching" or "isotype switching" refers to a biological process occurring after activation of the B cell, which allows the cell to produce different classes of antibody (IgA, IgE, or IgG). The different classes of antibody, and thus effector functions, are defined by the constant (C) regions of the immunoglobulin heavy chain. Initially, naïve B cells express only cell- surface IgM and IgD with identical antigen binding regions. Each isotype is adapted for a distinct function, therefore, after activation, an antibody with a IgG, IgA, or IgE

effector function might be required to effectively eliminate an antigen. Class switching allows different daughter cells from the same activated B cell to produce antibodies of different isotypes. Only the constant region of the antibody heavy chain changes during class switching; the variable regions, and therefore antigen specificity, remain unchanged. Thus the progeny of a single B cell can produce antibodies, all specific for the same antigen, but with the ability to produce the effector function appropriate for each antigenic challenge. Class switching is triggered by cytokines; the isotype generated depends on which cytokines are present in the B cell environment. Class switching occurs in the heavy chain gene locus by a mechanism called class switch recombination (CSR). This process results in an immunoglobulin gene that encodes an antibody of a different isotype.

As used herein, "changed as compared to a control" sample or subject is understood as having a level of the analyte or diagnostic or therapeutic indicator to be detected at a level that is statistically different than a sample from a normal, untreated, non-transgenic or other control geneotype, or control sample. Control samples include, for example, cells in culture, one or more laboratory test animals, or one or more human subjects. Methods to select and test control samples is within the ability of those in the art. An analyte can be a naturally occurring substance that is characteristically expressed or produced by the cell or organism (e.g., IgM or IgG antibodies, antibodies with a defined specificity) or a substance produced by a reporter construct (e.g, β-galactosidase or lucif erase). Change as compared to a control can be a change in the presence or severity of at least one sign or symptom of lupus nephritis as set forth in the classification table herein. Depending on the method used for detection the amount (or level) and measurement of the change can vary. Determination of statistical significance is within the ability of those skilled in the art.

As used herein, a "complementarity determining region" (CDR) is a short amino acid sequence found in the variable domains of antigen binding pocket (e.g. immunoglobulin and T cell receptor) proteins that complements an antigen and therefore provides a binding pocket with its specificity for that particular antigen. Each polypeptide chain of an antigen receptor contains three CDRs (CDRl, CDR2 and CDR3). Since the antigen binding pockets are typically composed of two polypeptide chains, there are six CDRs for each antigen receptor that can come into contact with the antigen (each heavy and light chain contains three CDRs), twelve CDRs on a single antibody

molecule and sixty CDRs on a pentameric IgM molecule. Since most sequence variation associated with immunoglobulins and T cell receptors are found in the CDRs, these regions are sometimes referred to as hypervariable domains. Among these, CDR3 shows the greatest variability as it is encoded by a recombination of the VJ (VDJ in the case of heavy chain) regions.

A "competition assay" as used herein is any type of test in which the binding of two agents, typically two antibodies, to the same agent is tested simultaneously in a single reaction mixture. For example, in a competition assay, a human antibody and a mouse antibody that bind to the same antigen are combined at various ratios (e.g., 100:1, 50:1, 25:1, 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, 1:10, 1:25, 1:50, 1:100) and contacted with the antigen to which both antibodies bind under conditions where the antibody to be detected is present in excess of the amount of antigen present. Specific binding of one of the antibodies is detected. If the binding of the detected antibody is decreased with increasing amounts of the not directly detected antibody, the antibodies are said to compete for binding. The antibodies can compete equally for binding to the antigen, e.g., when the antibodies are mixed at a 1:1 ratio, the amount of the detected antibody detected decreases by about half. As used herein, a first antibody competes for binding with a second antibody to a specific antigen when the presence of the first antibody decreases the binding of the second antibody by at least about 10%, or the second antibody decreases the binding of the first antibody by at least about 10%, when the antibodies are present in a mixture at about a 1:1 ratio. Similarly, a competition assay can be performed using two antigens to compete for binding to one antibody.

"Contacting a cell" is understood herein as providing an agent to a test cell or cell to be treated in culture or in an animal, such that the agent or isolated cell can interact with the surface of the test cell or cell to be treated, potentially be taken up by the test cell or cell to be treated, and have an effect on the test cell or cell to be treated. The agent or isolated cell can be delivered to the cell directly (e.g., by addition of the agent to culture medium or by injection into the cell or tissue of interest), or by delivery to the organism by an enteral or parenteral route of administration for delivery to the cell by circulation, lymphatic, or other means.

As used herein, "detecting", "detection" and the like are understood that an assay performed for identification of a specific analyte in a sample or a product from a reporter construct in a sample or one or more specific signs or symptoms of lupus nephritis. The amount (or level) of analyte

detected in the sample or the change in one or more signs or symptoms of a disease or condition can be none or below the level of detection of the assay or method.

As used herein, a "diagnostic marker" is understood as one or more signs or symptoms of a disease or condition that can be assessed, preferably quantitatively to monitor the progress or efficacy of a disease treatment or prophylactic treatment or method. A diagnostic marker can be one or more of the diagnostic classification criteria set forth in the classification table herein.

As used herein, the terms "effective" and "effectiveness" includes both pharmacological effectiveness and physiological safety. Pharmacological effectiveness refers to the ability of the treatment to result in a desired biological effect in the patient. Physiological safety refers to the level of toxicity, or other adverse physiological effects at the cellular, organ and/or organism level (often referred to as side -effects) resulting from administration of the treatment. On the other hand, the term "ineffective" indicates that a treatment does not provide sufficient pharmacological effect to be therapeutically useful, even in the absence of deleterious effects, at least in the unstratified population. (Such a treatment may be ineffective in a subgroup that can be identified by the expression profile or profiles.) "Less effective" means that the treatment results in a therapeutically significant lower level of pharmacological effectiveness and/or a therapeutically greater level of adverse physiological effects, e.g., greater liver toxicity.

Thus, in connection with the administration of a drug, a drug which is "effective against" a disease or condition indicates that administration in a clinically appropriate manner results in a beneficial effect for at least a statistically significant fraction of patients, such as a improvement of symptoms, a cure, a reduction in disease signs or symptoms, extension of life, improvement in quality of life, or other effect generally recognized as positive by medical doctors familiar with treating the particular type of disease or condition.

As used herein, "enriched" is understood increasing the relative portions of some desirable quality, attribute, or agent to a mixture, e.g., enriching a population of antibodies for the presence of IgM antibodies for example relative to the amount of IgG antibodies in the sample or relative to the amounts normally present in sera preparations. A sample may be enriched for a particular agent by adding the isolated agent to the mixture, e.g., addition of a monoclonal IgM antibody to a pharmaceutically acceptable carrier, or by selecting the source of the sample, e.g., a subject

deficient in somatic hypermutation and antibody class switching to provide an enriched population of IgM antibodies or B cells for generation of hybridoma cells to express IgM antibodies.

As used herein, the term "epitope" refers to a unit of structure conventionally bound by an immunoglobulin V_H/V_L pair. Epitopes define the minimum binding site for an antibody, and thus represent the target of specificity of an antibody.

A "germline antibody" is an antibody that arises exclusively from V(D)J recombination of CDR regions present in the germline of the individual producing the antibodies without somatic hypermutation or class switching. They are polyreactive, tend to use VH families proximal to the JH region and in spite of binding auto antigens, they constitute a small fraction of the normal repertoire. B cells secreting JH region -proximal germline autoantibodies are predominantly expressed in the neonatal stage of development but represent a small portion of the B cell population (2-15% of which only a small fraction are specifically against ds-DNA) present in an adult unless elicited by a specific antigen. Nuclear autoantigens like dsDNA, are not typically available to the immune system of normal subjects; therefore the presence of antibodies to such antigens, and B-cells expressing antibodies to such antigens would be uncommon in a normal subject. Moreover, cells expressing germline IgM antibodies would be expected to undergo class switching upon stimulation to produce IgG antibodies rather than IgM antibodies upon activation. Therefore, autoreactive IgM antibodies in germline configuration antibodies are unusual even in patients chronically displaying autoantigens due to cell death and inflammation.

"Humanized antibodies" or "chimeric antibodies" are a type of monoclonal antibody that have been synthesized using recombinant DNA technology to circumvent the clinical problem of immune response to foreign antigens. The standard procedure of producing monoclonal antibodies yields mouse antibodies. Although murine antibodies are very similar to human ones there are differences, and the human immune system recognizes mouse antibodies as foreign, rapidly removing them from circulation and causing systemic inflammatory effects. Humanized antibodies are produced by operably linking the DNA that encodes the binding portion of a monoclonal mouse antibody with human antibody-producing framework and constant regions. Humanized antibodies are typically expressed in culture, however, the method of making the antibodies is not a limitation of the composition. Methods for generation of constructs for the expression of humanized

antibodies, and methods of expression of humanized antibodies are well known in the art. (e.g., Hay et al.,

"Bacteriophage cloning and Escherichia coli expression of a human IgM Fab," Hum. Antibod. Hybridomas, 3:81-85 (1992); and Pascalis et al., Grafting of "abbreviated" complementarity- determining regions containing specificity-determining residues essential for ligand contact to engineer a less immunogenic humanized monoclonal antibody. Journal of Immunology, 2002. 169: 3076-3084, both incorporated herein by reference.)

"Hypermutation" is understood as the process of random mutation in rapidly proliferating, stimulated B -cells to produce a population of cells expressing antibodies with differences in antigen binding specificities. The process depends on the enzyme Activation-Induced (Cytidine) Deaminase, or AID which causes the deamination of cytidine to uracil in the DNA. The uracil in DNA can either be replicated over to yield C to T or G to A transition mutations, or can be removed and the patch resynthesized by an error prone DNA repair mechanism. The end result of this mutational process is to generate variation in the DNA sequence, and subsequently in the antibody polypeptide sequence.

As used herein, "identity" is understood as the percent of matching of nucleic acid or amino acid sequence over at least a portion of a nucleic acid or amino acid sequence. Sequence identity can be determined by those of skill in the art, for example, by computer programs that compare sequences such as BLAST or ClustalW. Identity is typically expressed as a percent, for example 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100%. Identity can be determined for the length of a domain (e.g., for a CDR, for an antibody variable domain) or over the length of an entire peptide (e.g., for a light chain or heavy chain). Determining identity is well within the ability of those of skill in the art. As used herein, "isolated" or "purified" when used in reference to a polypeptide means that a natural polypeptide or protein has been removed from its normal physiological environment (e.g., protein isolated from plasma or tissue) or is synthesized in a non-natural environment (e.g., artificially synthesized in a heterologous system), and optionally further removed from the artificial synthetic environment. Thus, an "isolated" or "purified" polypeptide can be in a cell-free solution or placed in a different cellular environment (e.g., expressed in a heterologous cell type or heterologous organism). The term "purified" does not imply that the polypeptide is the only polypeptide present, but that it is essentially free (about 90-95%, up to 99-100% pure) of cellular or

organismal material naturally associated with it, and thus is distinguished from naturally occurring polypeptide. Similarly, an isolated nucleic acid is removed from its normal physiological environment. "Isolated" when used in reference to a cell means the cell is in culture (i.e., not in an animal), either cell culture or organ culture, of a primary cell or cell line. Cells can be isolated from a normal animal, a transgenic animal, an animal having spontaneously occurring genetic changes, and/or an animal having a genetic and/or induced disease or condition.

As used herein, "kits" are understood to contain at least one non-standard laboratory reagent for use in the methods of the invention in appropriate packaging and with instructions for use, or a composition of the invention in appropriate packaging. A therapeutic kit can include one or more anti-dsDNA IgM germline antibodies and a device for delivery of the antibody such as a syringe, or a solution for reconstitution of the antibody when the antibody is provided as a dry powder. A diagnostic kit can include reagents for detection of IgM antibodies, and for differentiation of germline antibodies from antibodies that have undergone somatic hypermutation. The kit can further include any other components required to practice the method of the invention, as dry powders, concentrated solutions, or ready to use solutions. In some embodiments, the kit comprises one or more containers that contain reagents for use in the methods of the invention; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding reagents.

The term "label" or "detectable label" as used herein refers to any atom or molecule which can be used to provide a readily detectable (preferably quantifiable) signal, and which can be attached to a nucleic acid or protein. Labels may provide signals detectable by fluorescence, radioactivity, colorimetry, gravimetry, X-ray diffraction or absorption, magnetism, enzymatic activity, and the like. Various methods of labeling polypeptides and glycoproteins are known in the art and may be used. Examples of labels for include, but are not limited to, the following: radioisotopes (e.g., ^{3}H), fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase), biotinyl groups, predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce

potential steric hindrance. In others, the label is part of the fusion protein, e.g. Green Fluorescent Protein (GFP), Yellow Fluorescent Protein (YFP).

"Lupus nephritis" is acute or chronic renal impairment that may develop in conjunction with SLE, leading to acute or end-stage renal failure. Classification of the stages of lupus nephritis into classes was performed by the International Society of Nephrology/Renal Pathology Society (ISN/RPS) in 2003. The classification method was published in Weening *et al.* (2004) *Am. Soc. Nephrol.* 15:241-250, which is incorporated herein by reference. A summary table reproduced from Weening *et al.* is provided below as Table 1. Other more simplified classification systems are provided in the Examples.

Table 1. International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 Classification of Lupus Nephritis.

Class I	**Minimal mesangial lupus nephritis**
	Normal glomeruli by light microscopy, but mesangial immune deposits by immunofluorescence
Class II	**Mesangial proliferative lupus nephritis**
	Purely mesangial hypercellularity of any degree or mesangial matrix expansion by light microscopy, with mesangial immune deposits
	May be a few isolated subepithelial or subendothelial deposits visible by immunofluorescence or electron microscopy, but not by light microscopy
Class III	**Focal lupus nephritis**[a]
	Active or inactive focal, segmental or global endo- or extracapillary glomerulonephritis involving <50% of all glomeruli, typically with focal subendothelial immune deposits, with or without mesangial alterations
Class III (A)	Active lesions: focal proliferative lupus nephritis
Class III (A/C)	Active and chronic lesions: focal proliferative and sclerosing lupus nephritis
Class III (C)	Chronic inactive lesions with glomerular scars: focal sclerosing lupus nephritis
Class IV	**Diffuse lupus nephritis**[b]
	Active or inactive diffuse, segmental or global endo- or extracapillary glomerulonephritis involving ≥50% of all glomeruli, typically with diffuse subendothelial immune deposits, with or without mesangial alterations. This class is

	divided into diffuse segmental (IV-S) lupus nephritis when ≥50% of the involved glomeruli have segmental lesions, and diffuse global (IV-G) lupus nephritis when ≥50% of the involved glomeruli have global lesions. Segmental is defined as a glomerular lesion that involves less than half of the glomerular tuft. This class includes cases with diffuse wire loop deposits but with little or no glomerular proliferation
Class IV-S (A)	Active lesions: diffuse segmental proliferative lupus nephritis
Class IV-G (A)	Active lesions: diffuse global proliferative lupus nephritis
Class IV-S (A/C)	Active and chronic lesions: diffuse segmental proliferative and sclerosing lupus nephritis
	Active and chronic lesions: diffuse global proliferative and sclerosing lupus nephritis
Class IV-S (C)	Chronic inactive lesions with scars: diffuse segmental sclerosing lupus nephritis
Class IV-G (C)	Chronic inactive lesions with scars: diffuse global sclerosing lupus nephritis
Class V	**Membranous lupus nephritis**
	Global or segmental subepithelial immune deposits or their morphologic sequelae by light microscopy and by immunofluorescence or electron microscopy, with or without mesangial alterations
	Class V lupus nephritis may occur in combination with class III or IV in which case both will be diagnosed
	Class V lupus nephritis show advanced sclerosis
Class VI	**Advanced sclerosis lupus nephritis**
	≥90% of glomeruli globally sclerosed without residual activity

[a] Indicate the proportion of glomeruli with active and with sclerotic lesions.
[b] Indicate the proportion of glomeruli with fibrinoid necrosis and/or cellular crescents.

"Monoclonal antibody" or "mAb" as used herein is a monospecific antibody produced by immortalized immune cells that are all clones of a single parent cell. Monoclonal antibody expressing cells are typically made by fusing myeloma cells with the spleen cells from a mouse that has been immunized with the desired antigen. The coding sequences of mouse monoclonal antibodies can be modified to include the CDR antigen binding portions from mouse in the context of a human antibody. Such antibodies are particularly useful for therapeutic applications in humans.

"Obtaining" is understood herein as manufacturing, purchasing, or otherwise coming into possession of.

"Operably linked" as used herein is understood as joining in a manner such that each component in the linkage performs the desired activity. For example, coding sequences for mouse CDRs can be operably linked to the coding sequences for human constant chains and frameworks by fusing the sequences in frame, and the chimeric sequence can further be operably linked to a promoter such that the expression of the chimeric protein is controlled by the promoter sequence, which can be a constitutive or inducible promoter.

The phrase "pharmaceutically acceptable carrier" is art recognized and includes a pharmaceutically acceptable material, composition or vehicle, suitable for administering compounds of the present invention to mammals. The carriers include liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. For example, pharmaceutically acceptable carriers for administration of cells typically is a carrier acceptable for delivery by injection, and do not include agents such as detergents or other compounds that could damage the cells to be delivered. Some examples of materials which can serve as pharmaceutically acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as propylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically acceptable antioxidants include: water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, α-tocopherol, and the like; and metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Formulations of the present invention include those suitable for intravenous, oral, nasal, topical, transdermal, buccal, sublingual, intramuscular, intraperotineal, rectal, vaginal and/or parenteral administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient that can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound that produces a therapeutic effect.

As used herein, "plurality" is understood to mean more than one. For example, a plurality refers to at least two, three, four, five, or more.

As used herein, "prevention" is understood as delaying the onset of at least one sign or symptom of a disease in a subject prone to the disease or condition. Prevention does not require that the disease or condition never develop in the subject. Prevention can include administration of multiple doses of an agent, for example, multiple doses of anti-dsIgM antibody, can be administered to a subject having a family history of lupus or having lupus with no detectable renal involvement, to delay the onset of at least one sign or symptom of lupus nephritis.

As used herein, "prone to" as in a subject prone to a specific disease or condition, such as SLE or lupus nephritis, refers to a subject more likely than the general population to develop the disease or condition. For example, a subject prone to SLE or lupus nephritis is a woman of non-European descent who is between the ages of about 15 to about 50. A woman having close relatives having autoimmune diseases or conditions, particularly lupus, may be further prone to the disease. A subject having previously been diagnosed with lupus erythematosus or other localized or systemic form of lupus is prone to lupus nephritis. One of skill in the art, such as a physician, can identify a subject prone to SLE or lupus nephritis.

As used herein, "providing" is understood as to supply or make available.

"Reporter construct" as used herein is understood to be an exogenously inserted gene, often present on a plasmid, with a detectable gene product, under the control of a promoter sequence. Preferably, the gene product is easily detectable using a quantitative method. Common reporter genes include lucif erase and beta-galactosidase. The reporter construct can be transiently inserted into the cell by transfection or infection methods. Alternatively, stable cell lines can be made using methods well known to those skilled in the art, or cells can be obtained from transgenic animals expressing a reporter construct. The specific reporter gene or method of detection is not a limitation of the invention.

A "sample" as used herein refers to a biological material that is isolated from its environment (e.g., blood, cells, or tissue from an animal, or conditioned media or cells from tissue culture) and is suspected of containing, or known to contain an analyte or a characteristic physiological, histological, or morphological characteristic diagnostic for or indicative of a disease or condition (e.g., a kidney biopsy to be assayed for particular histological characteristics of lupus nephritis such as those provided herein). A sample can also be a partially purified fraction of a tissue or bodily fluid (e.g., serum, or fractionated serum). A reference sample can be a "normal" sample, from a donor not having the disease or condition fluid, or from a normal tissue in a subject having the disease or condition (e.g., normal tissue vs. tumor tissue). A reference sample can also be from an untreated donor or cell culture not treated with an active agent (e.g., no treatment or administration of vehicle only) and/or stimulus. A reference sample can also be taken at a "zero time point" prior to contacting the cell or subject with the agent or cell to be tested.

"Small molecule" as used herein is understood as a compound, typically an organic compound, having a molecular weight of no more than about 1500 Da, 1000 Da, 750 Da, or 500 Da. In an embodiment, a small molecule does not include a polypeptide or nucleic acid. As used herein, "specifically binds" is understood as binding the specific target antigen with a higher relative affinity (e.g., as determined by a competition assay) than to a non-specific antigen, e.g., at least 10-fold higher, at least 10^2-fold higher, at least 10^3-fold higher, at least 10^4-fold higher, or at least 10^5-fold higher.

A "subject" as used herein refers to living organisms. In certain embodiments, the living organism is a human. In certain embodiments, the living organism is an animal. In certain embodiments, the subject is a mammal such as primate including a non-human primate. In certain

embodiments, the subject is a domesticated mammal. Accordingly, examples of subjects include humans, monkeys, dogs, cats, mice, rats, cows, horses, goats, and sheep. A human subject may also be referred to as a patient. Subjects can also include non-mammals. A subject "suffering from or suspected of suffering from" a specific disease, condition, or syndrome has a sufficient number of risk factors or presents with a sufficient number or combination of signs or symptoms of the disease, condition, or syndrome such that a competent individual would diagnose or suspect that the subject was suffering from the disease, condition, or syndrome. Methods for identification of subjects suffering from or suspected of suffering from conditions such as lupus and/ or lupus nephritis is within the ability of those in the art for example using the classification criteria set forth herein. Subjects suffering from, and suspected of suffering from, a specific disease, condition, or syndrome are not necessarily two distinct groups.

"Systemic lupus erythematosus" (SLE or lupus) is a chronic autoimmune connective tissue disease that can affect any part of the body. As occurs in other autoimmune diseases, the immune system attacks the body's cells and tissue, resulting in inflammation and tissue damage.

Autoantibodies present in subjects suffering from SLE include antibodies that specifically bind to dsDNA, ssDNA, RNA, ribonucleoprotein complexes such as RNPs that form the Smith (Sm) antigen, anti-nuclear antigens (ANA), and phospholipids. SLE most often harms the heart, joints, skin, lungs, blood vessels, liver, kidneys, and nervous system. The course of the disease is unpredictable, with periods of illness (called flares) alternating with remissions. The disease occurs nine times more often in women than in men, especially between the ages of 15 and 50, and is more common in those of non-European descent. SLE is treatable through addressing its symptoms, mainly with corticosteroids and immunosuppressants; there is currently no cure. SLE can be fatal, although with recent medical advances, fatalities are becoming increasingly rare. Survival for people with SLE in the United States, Canada, and Europe is approximately 95% at five years, 90% at 10 years, and 78% at 20 years.

"Therapeutically effective amount," as used herein refers to an amount of an agent which is effective, upon single or multiple dose administration to the cell or subject, in prolonging the survivability of the patient with such a disorder beyond that expected in the absence of such treatment.

An agent can be administered to a subject, either alone or in combination with one or more therapeutic agents, as a pharmaceutical composition in mixture with conventional excipient, e.g., pharmaceutically acceptable carrier, or therapeutic treatments such as radiation.

The pharmaceutical agents may be conveniently administered in unit dosage form and may be prepared by any of the methods well known in the pharmaceutical arts, e.g., as described in Remington's Pharmaceutical Sciences (Lippincott Williams & Wilkins; Twenty first Edition, 2005). Formulations for parenteral administration may contain as common excipients such as sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. In particular, biocompatible, biodegradable lactide polymer, lactide/glycolide copolymer, or polyoxyethylene-polyoxypropylene copolymers may be useful excipients to control the release of certain agents.

It will be appreciated that the actual preferred amounts of active compounds used in a given therapy will vary according to e.g., the specific compound being utilized, the particular composition formulated, the mode of administration and characteristics of the subject, e.g., the species, sex, weight, general health and age of the subject. Optimal administration rates for a given protocol of administration can be readily ascertained by those skilled in the art using conventional dosage determination tests conducted with regard to the foregoing guidelines. Doses, for example, would typically fall within the range of about 500μg/kg/week to 10 mg/kg/week.

The term "transfection" as used herein refers to the introduction of a transgene into a cell. The term "transgene" as used herein refers to any nucleic acid sequence which is introduced into the genome of a cell by experimental manipulations. Transfection may be accomplished by a variety of means known to the art including calcium phosphate-DNA co-precipitation, DEAE-dextran-mediated transfection, polybrene-mediated transfection, electroporation, microinjection, liposome fusion, lipofection, protoplast fusion, retroviral infection, biolistics (i.e., particle bombardment) and the like.

The term "stable transfection" or "stably transfected" refers to the introduction and integration of a transgene into the genome of the transfected cell. The term "stable transfectant" refers to a cell which has stably integrated one or more transgenes into the genomic DNA.

The term "transient transfection" or "transiently transfected" refers to the introduction of one or more transgenes into a transfected cell in the absence of integration of the transgene into the

host cell's genome. The term "transient transfectant" refers to a cell which has transiently integrated one or more transgenes.

As used herein, "V(D)J recombination" refers to the process of somatic recombination of immunoglobulins, also known as V(D)J recombination, involves the generation of a unique immunoglobulin variable region. The variable region of each immunoglobulin heavy or light chain is encoded in several gene segments. These segments are called variable (V), diversity (D), and joining (J) segments. V, D and J segments are found in Ig heavy chains, but only V and J segments are found in Ig light chains. Multiple copies of the V, D, and J gene segments exist, and are tandemly arranged in the genomes of mammals. In the bone marrow, each developing B cell assembles an immunoglobulin variable region by randomly selecting and combining one V, one D, and one J gene segment (or one V and one J segment in the light chain). As there are multiple copies of each type of gene segment, and different combinations of gene segments can be used to generate each immunoglobulin variable region, this process generates a huge number of antibodies, each with different paratopes, and thus different antigen specificities.

The term "wild-type" refers to a gene or gene product which has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild- type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product which displays modifications (e.g. deletions, substitutions, etc.) in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted that naturally-occurring mutants can be isolated; these are identified by the fact that they have altered characteristics when compared to the wild-type gene or gene product.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or subrange from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

Unless specifically stated or obvious from context, as used herein, the term "or " is understood to be inclusive.

Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term "about" is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean, antibodyout can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

The term "IL-6" refers to interleukin 6, which is an interleukin acting both as a pro-inflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages and other immune cells.

The term "phagocytosis" refers to the process that cells use to ingest or engulf other cells, particles or immune complexes.

The term "immune complex" refers to an antigen and antibody complex of interlocking antigens and antibodies forming a network of molecules that can be difficult to clear in certain conditions.

In order to classify the processes by which the immune system produces cellular damage, immunologists have divided immune responses into four broad classes (Type I, II, III and IV) (Roitt, I.M., *et al.*, *Immunology*, C.V. Mosby, N.Y., 1985, p. 19.1).

Type I responses are also called immediate hypersensitivity reactions and include those diseases which produce the symptoms classically associated with "allergies" or the "allergic syndrome" including allergic rhinitis (hay fever), allergic asthma, allergic conjunctivitis, and allergic reactions to insect stings or foods. In order for Type I hypersensitivity to occur, a specialized sequence of events within mast cells and basophils must be triggered by immunoglobulin E (IgE) antibodies that have been manufactured within the body.

The hypersensitivity states characterized by types II, III and IV hypersensitivity are distinguished from type I hypersensitivity by many distinct and diverse features. In type I hypersensitivity, the sensitizing agent (allergen) is not a part or component of the host body. Types II, III and IV hypersensitivity, by contrast, may have immune responses directed towards antigens located on cells and molecules that are normal constituents of the body. Such immune responses toward normal constituents of the body are termed "autoimmune diseases" and constitute a medically important class of diseases distinct from allergic diseases.

Type II hypersensitivity occurs when IgG or IgM antibodies bind to antigens located on the surfaces of cells. Such binding is mediated by the antibodies' Fab arms which contain specific structures that recognize cell surface antigens. Upon binding, the Fc regions of IgG or IgM interact with the complement system (a family of inflammatory and cell-killing molecules) or immune system "killer" cells bearing IgG or IgM Fc receptors. Some examples of diseases in which type II hypersensitivity reactions predominate include transfusion reactions, hemolytic disease of the newborn, autoimmune hemolytic anemias, hyperacute graft rejection, Goodpasture's syndrome, myesthenia gravis and other conditions.

Type III hypersensitivity is produced when complexes or aggregates of antibodies (usually IgG or IgM) and soluble antigens form in abnormally large amounts and activate the complement inflammatory system. As used herein, the term "Type III hypersensitivity reactions" refers to the process whereby insoluble immune complexes form in the blood and are deposited in various tissues which can trigger an immune response and inflammation. Some examples of diseases in which type III hypersensitivity reactions are pathogenically important (i.e., diseases associated with type III hypersensitivity reactions) include nephritis, vasculitis, and some forms of arthritis.

Type IV hypersensitivity (delayed-type hypersensitivity), by contrast to the other three hypersensitivity reactions, is triggered primarily by T cells having specialized T cell receptors able to recognize and bind to the specific sensitizing antigen on a cell's surface. Diseases in which type IV hypersensitivity is believed to play an important pathogenic role are frequently termed "T-cell mediated" to reflect the unique role played by the T-cell in recognizing the sensitizing antigen. These diseases include multiple sclerosis, rheumatoid arthritis, juvenile onset diabetes mellitus, ulcerative colitis, and regional enteritis (Crohn's disease), among others.

The term "mesangial" refers to kidney myofibroblasts cells at the glomerulus and along the vascular pole of the glomerulus with contractile and phagocytic properties.

ANTIBODY COMPOSITIONS

In one embodiment, the presently disclosed subject matter is directed to compositions comprising a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity. In some embodiments, at least one anti-nuclear specificity is selected from the group consisting of anti-single stranded DNA (anti-ssDNA), anti-double stranded DNA (anti-dsDNA), anti-histone, anti-RNP, anti-Smith (anti-Sm), anti-Ro, anti-La, anti-DNP, anti-Scl-70, and anti-PM/Scl. In other embodiments, at least one anti-cytoplasmic specificity is selected from the group consisting of anti-Jo-1, anti-phospholipid, anti-phosphocholine, anti-phosphatidylcholine, or anti-Ribosomal-P. In still other embodiments, the combination further comprises IvIg. In other embodiments, IgM antibodies are humanized and/or in chimeric form.

In another embodiment, the presently disclosed subject matter is directed to compositions comprising a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity, further comprising IgM antibodies with at least one anti-Fc receptor of IgG specificity. In still other embodiments, the combination further comprises IvIg. In other embodiments, IgM antibodies are humanized and/or in chimeric form.

In another embodiment, the presently disclosed subject matter is directed to compositions comprising a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity, further comprising at least one IgM antibody capable of activating mesangial cells to induce phagocytosis and at least one IgM antibody capable of activating mesangial cells to secrete cytokines. In still other embodiments, the combination further comprises IvIg. In other embodiments, IgM antibodies are humanized and/or in chimeric form.

In another embodiment, the presently disclosed subject matter is directed to compositions comprising a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity, further comprising at least one IgM antibody capable of binding apoptotic cells and at least one IgM antibody capable of binding immune complexes. In still other embodiments, the combination further comprises IvIg. In other embodiments, IgM antibodies are humanized and/or in chimeric form.

In another embodiment, the presently disclosed subject matter is directed to compositions comprising a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity, further comprising at least two autoreactive IgM specificities equivalent to specificities of the pathogenic IgG spectra of a disease from the group consisting of a disease associated with Type III hypersensitivity reactions, a disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer. In still other embodiments, the combination further comprises IvIg. In other embodiments, IgM antibodies are humanized and/or in chimeric form.

In another embodiment, the presently disclosed subject matter is directed to compositions comprising: 1) a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity; 2) at least one IgM antibody capable of activating mesangial cells to induce phagocytosis and at least one IgM antibody capable of activating mesangial cells to secrete cytokines; 3) at least one IgM antibody capable of binding apoptotic cells and at least one IgM antibody capable of binding immune complexes; and 4) at least two autoreactive IgM specificities equivalent to specificities of the pathogenic IgG spectra of a disease selected from the group consisting of a disease associated with Type III hypersensitivity reactions, an autoimmune disease associated with pathogenic IgG autoantibodies, a disease associated with excessive apoptotic debris and inflammation, and a blood cancer. In still other embodiments, the combination further comprises IvIg. In other embodiments, IgM antibodies are humanized and/or in chimeric form.

METHODS OF TREATMENT

In another embodiment, the presently disclosed subject matter is directed to methods of treating a disease by administering a therapeutically effective amount of any of the compositions disclosed herein to a subject in need thereof, wherein the disease is selected from the group consisting of a disease associated with Type III hypersensitivity reactions, a disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer.

In some embodiments, the disease associated with Type III hypersensitivity reactions is selected from the group consisting of nephritis, vasculitis, and some forms of arthritis.

In some embodiments, the disease associated with excessive apoptotic debris and inflammation or the autoimmune disease associated with pathogenic IgG autoantibodies, is selected from the group consisting of SLE, scleroderma, rheumatoid arthritis, atherosclerosis, and ischemia.

Blood cancers include, but are not limited to, leukemia, and B and T cell lymphomas.

DIAGNOSTIC METHODS

In another embodiment, the presently disclosed subject matter is directed to a method for predicting the likelihood of developing nephritis in a subject with SLE, the method comprising the steps of: a) obtaining a sample from the subject; b) measuring levels of protective IgM antibodies and of pathogenic IgG in the sample; and c) determining the ratio of protective IgM antibodies to pathogenic IgG in the sample; wherein a higher level of protective IgM antibodies as compared the level of pathogenic IgG is indicative of a lower likelihood of developing nephritis in the subject with SLE.

Within the method for predicting the likelihood of developing nephritis in a subject with SLE, protective antibodies may be defined as having the following characteristics: 1) being of the IgM type; 2) having the ability to bind antinuclear and/or cytoplasmic antigens; and 3) having the ability to bind apoptotic cells and stimulate cytokine secretion by mesangial cells.

Within the method for predicting the likelihood of developing nephritis in a subject with SLE, pathogenic antibodies may be defined by the following characteristics: 1) being of the IgG subtype; and 2) binding nuclear and/or certain cytoplasmic antigens.

Antibodies may be measured either using immunofluorescence techniques with antinuclear antigen assays, mesangial cell cultures, apoptotic binding assays, a specific antigen binding assay such as ELISA, and/or heavy chain immunoglobulin variable region family determination.

While certain embodiments have been described, these embodiments have been presented by way of example only, and are not intended to limit the scope of the inventions. Indeed, the novel methods, devices, and systems described herein may be embodied in a variety of other forms. Furthermore, various omissions, substitutions, and changes in the form of the methods, devices, and systems described herein may be made without departing from the spirit of the inventions. The accompanying claims and their equivalents are intended to cover such forms or modifications as would fall within the scope and spirit of the inventions.

All publications, patent applications, patents, and other references mentioned in the specification are indicative of the level of those skilled in the art to which the presently disclosed subject matter pertains. All publications, patent applications, patents, and other references are herein incorporated by reference to the same extent as if each individual publication, patent application, patent, and other reference was specifically and individually indicated to be incorporated by reference. It will be understood that, although a number of patent applications, patents, and other references are referred to herein, such reference does not constitute an admission that any of these documents forms part of the common general knowledge in the art.

EXAMPLES

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter. The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

Autoreactive B cells play a critical role in the pathogenesis of systemic lupus erythematosus (SLE) (Van *et al.* (1991) *J. Exp. Med.* 173:461; Winker *et al.* (1992) *J. Immunol.* 1992;22:1719;

Jiang *et al.* (2007) *J. Immunol.* 178:7422; Manheimer-Lory *et al.* (1997) *J. Clin. Invest.* 100:2538; Jenks *et al.* (2009) *Autoimmun Rev.* 8:209). SLE is characterized by the circulation of autoantibodies and the deposition of immune complexes in various tissues, particularly the kidneys, leading to glomerulonephritis (Oates *et al.* (2002) *Curr. Opin. Rheumatol.* 14:498). Hallmark SLE autoantibodies include antibodies against double-stranded DNA (antidsDNA), histones and ribonucleoproteins (Yung *et al.* (2008) *Autoimmun Rev.* 7:317). These pathogenic autoantibodies form immune complexes that trigger inflammatory responses, leading to organ damage. Factors that have been implicated in SLE and in lupus-like syndromes in mice include defective apoptosis, Toll receptor signaling, defects in B and T cell tolerance, complement activation, cytokine regulation, and defects in endothelial cell function.(Peng *et al.* (1996) *Mol. Biol. Rep.* 23:247; Pickering *et al.* (2000) *Adv. Immunol.* 76:227; Li *et al.* (2002) *J. Exp. Med.* 196:1543, and others).

Strong evidence suggests an antibody independent contribution of B cells to autoimmunity (Chan *et al.* (1999) *J. Exp. Med.* 189:1639). Lupus-prone mice with B cells unable to secrete antibodies were found to develop a milder form of lupus nephritis, while mice without B cells did not. The evidence suggests that B cells contribute to autoimmunity by activating autoreactive T cells, likely as antigenpresenting cells.

MRL-Faslpr/lpr (MRL/lpr) mice develop an auto-immune syndrome similar to SLE (Andrews *et al.* (1978) *J. Exp Med.* 148:1198; Theofilopoulos & Dixon (1985) *Adv. Immunol.* 37:269). MRL/lpr mice display high levels of autoantibodies to dsDNA and the development of lupus nephritis through immune complex deposition, glomerular disease, and tissue infiltration by inflammatory cells. Many loci contribute to autoimmunity in MRL/lpr mice, leading to defects in cytokine regulation, lymphocyte tolerance, and apoptosis . We generated MRL/lpr mice deficient in activation-induced deaminase (AID) (Jiang *et al.* (2007) *J. Immunol.* 178:7422), a molecule required for isotype switching and immunoglobulin hypermutation (Muramatsu *et al.* (2000) *Cell* 102:553). Heterozygotes experienced a delay in the onset of lupus nephritis that correlated with delayed accumulation of high-affinity anti-dsDNA antibodies (Jiang *et al.* (2009) *Immunology* 126:102). Homozygous AID-deficient MRL/lpr mice lacked IgG anti-dsDNA, but had high levels of autoreactive IgM. Despite this, AID-deficient MRL/lpr mice failed to develop significant kidney disease and experienced survival levels that exceeded those in mice with B cells but lacking secreted antibodies. This prompted us to speculate that, in addition to the lack of pathogenic IgG,

another factor contributed to improved survival. Our report indicates that, in mice, this factor appears to be anti-dsDNA IgM antibodies.

Materials and Methods

Mice: AID–/– MRL/lpr mice were developed by backcrossing C57BL/6 AID-deficient mice onto the MRL/lpr background, using the MRL/MpJ-Faslpr/2J mice (stock no. 006825; The Jackson Laboratory), for 6 generations. They were then backcrossed an additional 9 generations into cryorecovered MRL/lpr mouse strain MRL/MpJ-Faslpr/J (stock no. 000485; The Jackson Laboratory). Mouse strain B6;129S4-Igh-6tm1Che/J with a mutation in the IgM secretory exon (The Jackson Laboratory) has been described previously (Boes et al., (2000) Proc Natl Acad Sci USA, 97:1184). B6;129S4-Igh-6tm1Che/J mice were backcrossed with cryorecovered MRL/lpr mice for 4 generations and with the fifteenth generation–backcrossed AID–/– MRL/lpr mice for an additional 4 generations to generate S–/– AID–/– MRL/lpr mice. These are AID-deficient MRL/lpr mice lacking the secretory exon of IgM. Because B cells from these mice cannot secrete IgM or undergo class-switch recombination, they lack secreted antibodies.

All mice were housed in specific pathogen–free facilities at the National Institute of Environmental Health Sciences. All experiments included the same number of males and females among groups. Production of anti-dsDNA monoclonal IgM. IgM antidsDNA– producing hybridomas were generated according to standard protocols. Spleens were collected from nonimmunized AID–/–, AID–/–, and AID–/– MRL/lpr mice (10–12 weeks old; 3–6 mice per group). Splenocytes were fused with murine myeloma NS-1 cells (5:1 ratio) in 50% polyethylene glycol (PEG 1500; Roche). Supernatants were screened for anti-dsDNA specificity by enzyme-linked immunosorbent assay (ELISA). Cell clones secreting IgM anti-dsDNA were injected (2×10^6 cells) into the peritoneal cavity of RAG-1–knockout mice (The Jackson Laboratory) that had been pretreated with 0.2 ml of pristane (Sigma) to induce ascites. Ascites fluid was collected 2–3 weeks after injection and filtered through a small filter on the tip of the serumcontaining syringe (Millex; Millipore). IgM concentrations were determined, and dsDNA specificity was confirmed by ELISA (Bethyl Laboratories). A control non-autoreactive, IgM-producing hybridoma clone was confirmed by antinuclear antibody assay. A mouse B cell line secreting IgM antiphospholipid antibody was a gift from Laurent Verkoczy (Duke University, Durham, NC). An anti-Sm IgM-producing

hybridoma was provided by Barbara Vilen (University of North Carolina at Chapel Hill). These lines were injected into the peritoneal cavity of RAG-1–deficient mice that had been pretreated with pristane. All monoclonal antibodies (mAb) were generated using the same strategy. Passive transfer of serum or mAb. MRL/lpr mice ages 8–10 weeks were injected intraperitoneally with pooled sera collected from older MRL/lpr mice, sera from age-matched AID–/– MRL/lpr mice, or with phosphate buffered saline (PBS) at a dosage of 200 ml per mouse, twice a week for 8 weeks. Wild-type mouse sera contained both IgM and IgG autoantibodies, while AID-deficient sera contained high levels of IgM autoantibodies but no IgG. Urine samples were collected weekly for proteinuria testing. After 8 weeks, mice were killed. In antibody-transfer experiments, MRL/lpr mice were treated with IgM anti-dsDNA mAb at a dosage of 100 microg per mouse, twice weekly for 8–15 weeks, depending on the experiment. For therapeutic experiments, treatment was not started until the mice had moderate proteinuria (50–60 mg/dl). Detection of urinary protein. Urinary protein was tested by dipstick using Multistix 10 SG strips (Siemens Healthcare Diagnostics). Results were scored as described previously (Jiang *et al.* (2007) *J. Immunol.* 178:7422). ELISA. IgM and IgG levels in cell culture supernatants, ascites fluid, and sera were determined with commercial ELISA kits (Bethyl Laboratories). Mouse IgM anti-dsDNA was measured as described previously (Jiang *et al.* (2007) *J. Immunol.* 178:7422). A value that was 3 times higher than the background was set as the cutoff value for positivity.

Sequencing of VH regions from hybridomas and splenic B cells: Anti-dsDNA mAb-producing hybridomas were lysed with TRIzol (Invitrogen) for RNA. Two micrograms of RNA was used to synthesize complementary DNA (cDNA) by SuperScript III First-Strand Synthesis (Invitrogen). Five microliters of cDNA reactions was used to amplify the V regions of immunoglobulins by AccuPrime Pfx DNA polymerase (Invitrogen). Universal primers designed were as follows: universal primer MVH-F (5'- AGGTSMARCTGCAGSAGTCWGG-3') (SEQ ID NO:1) paired with MC-R (5'-CAGGGGGCTCTCGCAGGAGACGAGG-3') (SEQ ID NO:2) was used for in VHDJH-C amplification, whereas MVH-F paired with MC-R (5'-GGACAGGGATCCAGAGTTCC-3') (SEQ ID NO:3) was used for VHDJH-C amplification. The PCR cycle conditions were as follows: 95°C for 2 minutes, with 35 cycles at 95°C for 15 seconds, 58°C for 1 minute, and 68°C for 1 minute. The amplified VH DNA fragments were purified using a

QIAquick Gel Extraction kit (Qiagen). The purified VH fragments were sequenced using a BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems) plus MCμ-R or MCγ-R primer, depending on the VH fragments. For repertoire analysis, spleens were collected from AID wild-type and AID-deficient MRL/lpr mice (3 mice per group, ages 10–14 weeks). CD19+ B cells were purified with anti-CD19 MACS MicroBeads (Miltenyi Biotec) and lysed with TRIzol. The cDNA was synthesized, and the Vh fragments were amplified and sequenced as above.

Immunofluorescence and immunohistochemical staining: Frozen kidney samples were stained for C3. For each animal, 20 randomly selected glomeruli were evaluated to obtain an average score, and were graded on a scale of 0–3, where 0 means no staining; + means weak staining, with limited localization in the glomerulus (50%); ++ means moderate staining, where localization in the glomerulus was more diffuse (50–75%); and +++ means intense staining, with diffuse and homogeneous stain covering most of the glomerulus (>75%). For F4/80 staining, paraffin-fixed kidney sections were deparaffinized, hydrated, and treated with 3% hydrogen peroxide. Slides were placed in a Decloaking Chamber (BioCare Medical). Slides were blocked with 5% rabbit serum (Jackson ImmunoResearch) and avidin from an Avidin/Biotin Blocking kit (Vector). Rat anti-F4/80 mAb BM8 (Santa Cruz Biotechnology) was applied at a 1:25 dilution. Biotinylated rabbit anti-rat IgG (heavy and light chains; Vector) was used at a 1:200 dilution. Slides were incubated with diaminobenzidine chromogen and counterstained with Harris' hematoxylin.

F4/80 staining in glomeruli was scored in 20 randomly selected high-power (400X) microscopic fields per animal, using a scale of 0–3, where 0 = no F4/80-positive cells, + = 1–9 F4/80- positive cells, ++ = 10–20 F4/80-positive cells, and +++ = 20 F4/80-positive cells. Staining for cleaved caspase 3 was performed in a manner similar to that for F4/80 staining, except that rabbit anti–cleaved caspase 3 antibody (Promega) was used at a dilution of 1:500, and biotinylated goat anti-rabbit IgG (Vector) was used at a dilution of 1:1,000. Cleaved caspase 3–positive cells were counted in 10 randomly selected highpower (200X) microscopic fields per animal. The presence of apoptotic cells was graded according to an apoptotic index, which represents the number of cleaved caspase 3–positive cells per high-power microscopic field, where 0 = no positive cells, 1–4 positive cells, 5–10 positive cells, and 10 positive cells.

Histologic assessment. Kidneys were prepared for hematoxylin and eosin staining and for periodic acid–Schiff staining as previously described (23). Glomerulonephritis and lymphocyte

infiltration in the kidneys were examined by a pathologist and graded separately in each animal, using a scale of 1–4, where 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked.

Detection of cytokines. Serum levels of cytokines (interferon-γ, interleukin-1β, IL-12, tumor necrosis factor α IL-6, IL-4, IL-5, and IL-10) were determined with Bio-Plex mouse cytokine assays (Bio-Rad) and mouse Th1/Th2 9-plex ultra-sensitive kit (Meso Scale Discovery). Splenic macrophages were purified with CD11b MicroBeads and magnetic-activated cell sorter separation columns (Miltenyi Biotec), and incubated with lipopolysaccharide (1 μg/ml). A mouse Th1/Th2 9-plex ultra-sensitive kit was used to measure cytokine production. Survival analysis. The μS–/–AID–/– MRL/lpr mice (n = 22) (AID-deficient MRL/lpr mice without secreted antibodies as described above) and AID–/– MRL/lpr mice (n = 26) were monitored for at least 56 weeks to examine mortality in the presence or absence of secreted IgM. Statistical analysis. The Mann-Whitney rank sum test was performed to analyze the data among different treatment groups. For survival analysis, the log rank (Mantel-Cox) test was used to compare survival curves. For repertoire analysis, the likelihood ratio test was used.

Results

We hypothesized that the increased survival among AID-deficient MRL/lpr mice was due to not only the lack of IgG, but also a protective factor in their serum. Given the high levels of autoreactive IgM, we speculated that the factor might be IgM anti-dsDNA. To test this, we carried out passive transfer experiments using sera or monoclonal IgM anti-dsDNA antibodies from AID-deficient MRL/lpr mice. The results are discussed below.

Improvement in lupus nephritis in mice receiving sera or IgM anti-dsDNA from AID-deficient MRL/lpr mice: To determine whether sera from AID-deficient MRL/lpr mice contained a factor that may contribute to increased survival, we transferred sera from AID-deficient and wild-type MRL/lpr mice into young asymptomatic MRL/lpr mice for at least 8 weeks. These mice were 8–10 weeks of age and did not initially exhibit evidence of proteinuria. There was a significant reduction in levels of proteinuria in mice receiving the sera from AID-deficient MRL/lpr mice compared with PBS or wild-type mouse sera (Jiang *et al.* (2011) *Arth. Rheum.* 63:1086). Accordingly, MRL/lpr mice that received AID-deficient MRL/lpr sera also experienced decreased levels of immune complex deposition in the kidneys, as measured by the amount of C3 complement

immunofluorescence in the glomeruli (Jiang *et al.* (2011) *Arth. Rheum.* 63:1086). The group receiving serum from AID wild-type MRL/lpr mice exhibited a trend toward increased kidney damage, but it was not significantly different from that in the group that received PBS.

We next investigated whether increased levels of autoreactive IgM in the serum of AID-deficient mice contributed to reduced severity of lupus nephritis in mice receiving serum from AID-deficient MRL/lpr mice by generating IgM anti-dsDNA–secreting hybridomas. There was a 7-fold increase in anti-dsDNA clone numbers in hybridomas from AID-deficient MRL/lpr mice. As expected, AID-deficient mice produced only IgM-secreting clones.

Clones secreting IgM anti-dsDNA from AID wild-type and AID-deficient MRL/lpr mice were used in passive transfer experiments. In addition, a nonautoreactive, IgM-secreting clone (as determined by antinuclear antibody assay) was used as a control. Following treatment with IgM anti-dsDNA, asymptomatic MRL/lpr mice experienced a significant delay in the onset of lupus nephritis, as indicated by proteinuria (Figure 1) and IgG immune complex deposition. Accordingly, the kidneys of mice receiving IgM anti-dsDNA treatment were smaller than those from other groups in 3 of the 4 groups receiving IgM anti-dsDNA. In most cases, mice receiving this treatment failed to develop significant kidney disease even after 10 weeks of treatment, a point to which most mice were at least 6 months old and when all of the mice in the group receiving PBS or the group receiving nonautoreactive antibody had developed moderate to severe kidney damage.

To ascertain whether other autoreactive IgM antibodies could also confer protection against lupus nephritis, we repeated these experiments with the inclusion of an IgM antiphospholipid antibody and an IgM anti-Sm antibody. The IgM anti-Sm antibody has been previously described, and it cross-reacts with single-stranded DNA, but not with dsDNA (29). Mice receiving IgM anti-dsDNA fared best compared with all groups, including those treated with anti-Sm and antiphospholipid IgM, which displayed little or no protection. Histopathologic analysis of these groups confirmed that the groups receiving IgM anti-dsDNA experienced decreased kidney damage. There was a trend toward reduced mononuclear cell infiltration, albeit not a statistically significant one, and a significant reduction in glomerulonephritis.

Testing various doses of IgM anti-dsDNA confirmed a positive correlation for better protection with increasing dosages up to 100 μg twice weekly, the dosage used in our studies. There was little gain when using double that amount. After administering 100 μg twice weekly to treat

symptomatic mice (proteinuria of > 60 mg/dl, we found that even in mice with significant proteinuria, IgM anti-dsDNA treatment resulted in a reduction in kidney damage.

Association of IgM anti-dsDNA treatment with reduced macrophage infiltration and reduced levels of apoptotic debris in the kidneys: Electron microscopy of glomeruli revealed the following characteristics seen in most experimental groups, except for mice receiving IgM anti-dsDNA from AID-deficient hybridomas: macrophage and lymphocytic infiltration, electron-dense deposits in glomeruli, and apoptotic cells/debris in the mesangium.

Next, kidney tissues were stained for immunohistochemistry with either F4/80 antibody (macrophage detection) or anti–cleaved caspase 3 antibody. Mice receiving IgM anti-dsDNA treatment displayed lower levels of F4/80 and, in the case of the 12H5 clone, lower levels of cleaved caspase 3, confirming the electron microscopy observations (Jiang *et al.* (2011) *Arth. Rheum.* 63:1086).

As was seen for proteinuria and C3 deposition, treatment with other autoreactive IgM, such as antiphospholipid and anti-Sm antibodies, failed to reduce the areas positive for cleaved caspase 3 or F4/80. Accordingly, lipopolysaccharide-activated splenic B cells and macrophages were examined for cytokine production. There was a trend toward reduced secretion of TNF alpha by cultured macrophages and decreased levels of circulating IgG-containing immune complexes in mice receiving IgM anti-dsDNA; this reached statistical significance for some, but not all, of the IgM anti-dsDNA antibodies tested. There was no correlation between IgM anti-dsDNA treatment and decreased activation of autoreactive T cells by B cells or by an increase in the proportion of regulatory T cells, but activated macrophages from mice receiving the protective IgM secreted less IFN-gamma, and there was a trend of mice receiving the IgM anti-dsDNA to have more of the anti-inflammatory cytokines IL-4 and IL-10 in their sera, although this was not consistent across experiments.

Taken together, these results show that IgM anti-dsDNA blocks the inflammatory cascade that leads to tissue damage and cell death. The variability of the effects of various IgM anti-dsDNA antibodies on correlates of inflammation shows that, while all of them decrease proteinuria and immune complex deposition, their effectiveness and mechanisms of action are different and may be related to their specificity or polyreactivity.

Longer survival of AID-deficient MRL/lpr mice with secreted IgM compared with AID-

deficient, μS-deficient MRL/lpr mice lacking secreted antibodies, despite the presence of minimal proteinuria in both strains: The μS–/– MRL/lpr mice were crossed to the AID–/– MRL/lpr mice to generate MRL/lpr mice with no secreted antibodies; μS–/– MRL/lpr mice lack secreted IgM because of a mutation in the IgM secretory exon (26) and without AID, they are unable to switch to IgG or other isotypes. Comparing these mice to the AID-deficient MRL/lpr mice, which secrete only IgM, enabled two nearly identical strains differing only in whether they secrete IgM to be examined.

Minimal levels of proteinuria were observed in both strains after 52 weeks, showing that IgM antidsDNA protects against lupus nephritis through an IgG-mediated process. This finding is in contrast to that in the parental strain of mice, μS–/– MRL/lpr, which experienced accelerated lupus nephritis since they lacked secreted IgM but secreted all other isotypes (26).

Surprisingly, AID-deficient MRL/lpr mice with IgM experienced significantly lower mortality rates than did AID-deficient MRL/lpr mice without secreted antibodies, which experienced >70% mortality at 56 weeks , despite both groups having little proteinuria. Without being bound by theory, this appears to show an additional mechanism of IgM-mediated protection.

IgM repertoire analysis of splenic B cells from AID-deficient and AID wild-type MRL/lpr mice using the same primers did not reveal an increase in VH7183 usage in AID-deficient mice. To examine whether the differences in repertoire and protection correlated with the affinity for dsDNA, the apparent affinities were measured for the antibodies used in the study. The results did not reveal a correlation. For example, IgM anti-dsDNA 4A5 had the highest apparent affinity at 12.96 ml/μg but did not protect better than clones 12H5 and 13D2, with apparent affinities of 0.76 ml/μg and 0.21 ml/μg, respectively. Therefore, having a high level of dsDNA affinity, surprisingly, did not predict better protection.

Discussion

Despite having high levels of autoreactive IgM, AID-deficient MRL/lpr mice experienced a dramatic increase in survival. In the present study, we tested our hypothesis that autoreactive IgM is not only nonpathogenic in lupus nephritis, but may be protective. Treatment with some IgM anti-dsDNA antibodies from AID deficient and wild-type MRL/lpr mouse hybridomas revealed an improvement in all measures of lupus nephritis, such as proteinuria, immune complex deposition, and glomerulonephritis, in MRL/lpr mice. These results show that both factors, high IgM anti-

dsDNA levels and absence of pathogenic IgG, contributed to increased survival of AID-deficient MRL/lpr mice.

In a study by other investigators (Zan *et al.* (2009) *Autoimmunity* 42:89), B cells from MRL/lpr mice were found to express high levels of AID and to secrete highly mutated autoantibodies, showing that the high levels of AID increase the levels of autoreactive antibodies. It was also previously noted that peptides with specificity for dsDNA may be therapeutic in lupus nephritis (Iikuni *et al.* (2009) *Opin. Biol. Ther.* 9:201) and that autoimmune mice lacking secreted IgM but having secreted IgG experienced accelerated autoimmunity (Melamed *et al.* (2000) *J. Immunol.* 165:4353; Shlomchik *et al.* (1990) *J. Exp. Med.* 171:265).

The differential contribution of autoreactive IgM versus IgG to autoimmunity remains a subject of controversy. IgG autoreactive antibodies are thought to be pathogenic in autoimmune disease because of their isotype and their affinity for self antigen. Somatic hypermutation and class-switch recombination tend to co-occur, which means that much of the serum IgG has been fine-tuned to a specific antigen through affinity maturation. In SLE and in MRL/lpr mice, this could mean enhanced affinity for self antigen .

AID heterozygous MRL/lpr mice experienced a delay in lupus nephritis onset that correlated with reduced levels of high-affinity IgG anti-dsDNA antibodies and reduced hypermutation . The IgG subclass is known to influence autoantibody pathogenicity (Takahashi *et al.* (1991) *J. Immunol.* 147:515), and mice deficient in Fc receptor and in Fc receptor type III were shown to experience a reduction in kidney damage (Hazenbos *et al.* (1996) *Immunity* 5:181). It is thus apparent that both class switching and affinity maturation against self antigens through somatic hypermutation contribute to the formation of pathogenic IgG. The role of IgM is less clear, since IgM antibodies are likely to be unmutated. However, the dramatic increase in survival experienced by AID-deficient MRL/lpr mice (which secrete nothing but IgM, much of which is autoreactive) casts serious doubts on the pathogenicity of autoreactive IgM in lupus nephritis.

The data presented herein point to IgM anti-dsDNA as a protective agent. Recently, it was shown that SLE patients whose disease is in remission have high levels of autoreactive naive B cells (Yurasov (2006) *J. Exp. Med.* 203:2255), which, in light of our findings, shows that these cells are secreting protective IgM. Also, a positive correlation was found between a high ratio of IgG to IgM anti-dsDNA in SLE and lupus nephritis patients (Forger *et al.* (2004) *Lupus* 13:36).

Not all autoreactive IgM is protective. Antiphospholipid and anti-Sm IgM did not protect significantly. Furthermore, some autoreactive IgM may contribute to autoimmune disease. Natural IgM antibodies are associated with exacerbation of ischemia-reperfusion injury (Zhang *et al.* (2006) *J. Immunol,* 177: 4727), and Quartier *et al.* ((2004) *Clin. Immunol.* 110: 22) demonstrated an increased incidence of autoimmune cytopenias and other autoimmune disorders with AID deficiency in humans that is almost certainly associated with specific autoreactive natural IgM. However, this is observed not just with AID deficiency, but with most immunodeficiencies, showing that the immune dysregulation associated with immunodeficiency results in increased activation of residual autoreactive lymphocyte populations (Haymore *et al.* (2008) *Autoimmun. Rev,* 7:309).

A recent study, using AID-deficient lpr mice of the C57BL/6 strain, showed a modest increase in proteinuria with AID deficiency (Chen *et al.* (2010) *Clin. Exp. Immunol.* 159:169). There was also increased proliferation of B cells, consistent with a known component of the AID deficiency phenotype . These results seem to argue that increased IgM and AID deficiency actually exacerbates autoimmunity, the opposite of our findings. However, as the available population that experiences hyperproliferation is polyreactive in an autoimmune environment, one might expect that to contribute to autoimmunity. This implies that AID deficiency can enhance autoreactivity by increasing the population of autoreactive B cells, which are known to play an antibody-independent role in enhancing autoimmunity, but ameliorates it by pathogenic IgG deficiency and elevated levels of protective IgM. It is possible that when lupus nephritis is mild in the parental strain, as in C57BL/6 lpr mice, an AID deficiency–mediated increase in polyreactive B cells enhances autoimmunity. However, when the parental strain displays antibody-dependent severe lupus nephritis, the benefits of AID deficiency, such as high levels of protective IgM and absence of pathogenic IgG, outweigh the effects from an increase in polyreactive B cells. It will be interesting to see if mortality rates were increased in the lpr mice of the C57BL/6 background with AID deficiency; in mice of the MRL/lpr background, AID deficiency resulted in a dramatic improvement in survival.

The mechanism by which IgM anti-dsDNA antibodies protect against lupus nephritis is unknown. There was a trend for macrophages from mice receiving IgM anti-dsDNA to secrete lower levels of proinflammatory cytokines, such as TNF α and IFN γ, which is consistent with

decreased macrophages in the kidneys. A correlation between IgG anti-dsDNA antibodies and activation of macrophages to secrete proinflammatory cytokines has previously been demonstrated (Jang *et al.* (2009) *Immunol. Lett.* 124:70); perhaps IgM has the opposite effect. If that is true, it shows that protective IgM helps to create an environment that is less prone to inflammation-induced tissue injury, perhaps by quickly clearing apoptotic debris. However, while all IgM anti-dsDNA tested decreased proteinuria and immune complex deposition, not all correlated with a reduction in markers of inflammation or with apoptotic debris in the kidneys, thus appearing to show variations in the mechanism.

In a previous study, we found similar levels of IgM in the glomeruli of AID-deficient versus wild-type MRL/lpr mice, showing that despite its size, pentameric IgM is able to penetrate the renal glomeruli (Jiang *et al.* (2007) *J. Immunol.* 178:7422). It is therefore possible that IgM also works by blocking the local formation of IgG immune complexes. Indeed, similar to AID-deficient MRL/lpr mice with IgM, AID-deficient MRL/lpr mice that had a defect in secreted IgM (therefore lacking secreted antibodies) experienced a reduction in proteinuria, showing that IgM anti-dsDNA protects against nephritis through an IgG-mediated process.

Of note, while antibody-deficient mice had no signs of lupus nephritis, their AID-deficient but IgM secretion–proficient littermates experienced significantly higher survival rates. Indeed, close to 70% of the antibody-deficient mice had died by 56 weeks, whereas 25% of the IgM-secreting mice had died by that time. Both had survival rates better than those in conventional MRL/lpr mice, which tend to die earlier from lupus nephritis. Given that proteinuria was very low in both groups, these results show additional protective roles for IgM.

What defines a protective antibody in lupus nephritis? Our data show that the IgM isotype and anti-dsDNA specificity are important. In contrast to pathogenic IgG and pathogenicity, high affinity for dsDNA did not enhance the protection by IgM antidsDNA. This shows that dsDNA specificity is a correlate to other self antigens that are important for protection, or that high affinity correlates with reduced polyreactivity and that it is the polyreactivity that is important. Intriguingly, other autoreactive IgM, such as against phospholipids and against Sm (a ribonucleoprotein) did not confer protection against lupus nephritis alone.

The results reported above were for mice treated with a single monoclonal antibody of dsDNA specificity in lupus-prone mice. Also, since high affinity to dsDNA did not correlate with

better protection, it is likely that translating these findings for clinical applications is complicated and will require a mixture of specific autoantibodies defined by several criteria (Figure 2), including selecting specific combinations of autoreactive protective IgM antibodies that mimic the pathogenic IgG spectrum for a particular disease. The basis for this is the fact that immune complex-mediated disorders are defined by the specific IgG autoantibodies that bind and form immune complexes. Therefore for translation to clinical application, an effective treatment will require a mixture of humanized or chimerized IgM autoreactive specificities determined by the range of specificities seen for pathogenic IgG in patients with specific autoimmune disorder,. This shows a variety of antigen-immune complexes and it is therefore unlikely that a single specificity such as anti-dsDNA IgM will work as treatment for SLE or other autoimmune disorders. The same principle can be applied to various autoimmune disorders across organisms. For instance, in dogs, immune complexes are more likely to represent anti-histone specificities than other nuclear antigens and an effective mixture needs to consider this.

In addition, in subsequent experiments, we discovered that additional features correlate with protection such as the antibody's impact on mesangial cells. These features appear to be rare among dsDNA IgM antibodies as only 2 of the anti-dsDNA antibodies tested had this additional property. Therefore these mixtures need to efficiently bind human apoptotic cells and induce phagocytosis of immune complexes by phagocytes such as mesangial cells to prevent inflammation by the migration of inflammatory cells, including macrophages (Figures 3 and 4). Specifically, these mixtures need to cause mesangial cells to secrete various cytokines (Figure 3), proliferate and phagocytose immune complexes (Figure 4), and need to bind over 20% of lymphocytes induced to undergo apoptosis (Figure 5). This is based on two facts: 1) Preliminary data tells us that only a small subset of autoreactive (antinuclear) IgM actually stimulates mesangial cells to secrete or phagocytose immune complexes (Figures 3 and 4) , 2) those specificities were the most efficient at binding apoptotic cells.

These factors can be explored as follows:

1) Testing various ratios of mouse IgM antibodies, modified for human applications of anti-nuclear, anti-cytoplasmic and anti-IgG specificities. The ratios are based on the relative abundance of specificities among autoantigens seen in autoimmune patients. To determine the effectivity of each mixture: (a) the amount of human apoptotic cell binding is measured; (b) the efficient

induction of human mesangial cells to secrete various cytokines and proliferate is assessed; and/or (c) induction to mesangial cells is assessed.

2) Testing effectivity of Igm autoantibody specificities in combination with IvIg for enrichement of IvIg that results in the efficient binding of human immune complexes. Various enrichment ratios are tried from 4-20% IgM.

Finally, evidence shows that a combination of autoreactive specificities of the IgM type that include dsDNA significantly improve outcome in lymphoma-prone mice. The specific IgM combination of antinuclear and anticytoplasmic specificities with the highest lymphoma tumor cell killing for enrichment of IvIg preparations may be determined. A similar approach may be used for other blood cancers.

CLAIMS

What is claimed:

1. A composition comprising a combination of IgM antibodies with different autoreactive specificities, wherein the combination of IgM antibodies comprises IgM antibodies with at least one anti-nuclear specificity and IgM antibodies with at least one anti-cytoplasmic specificity.

2. The composition of claim 1, wherein at least one anti-nuclear specificity is selected from the group consisting of anti-single stranded DNA (anti-ssDNA), anti-double stranded DNA (anti-dsDNA), anti-histone, anti-RNP, anti-Smith (anti-Sm), anti-Ro, anti-La, anti-DNP, anti-Scl-70, and anti-PM/Scl).

3. The composition of any one of claims 1 to 2, wherein at least one anti-cytoplasmic specificity selected from the group consisting of anti-Jo-1, anti-phospholipid, anti-phosphocholine, anti-phosphatidylcholine, and anti-Ribosomal-P.

4. The composition of any one of claims 1 to 3, further comprising IgM antibodies with at least one anti-Fc receptor of IgG specificity.

5. The composition of any one of claims 1 to 3, further comprising at least one IgM antibody capable of activating mesangial cells to induce phagocytosis and at least one IgM antibody capable of activating mesangial cells to secrete cytokines.

6. The composition of any one of claims 1 to 3, further comprising at least one IgM antibody capable of binding apoptotic cells and at least one IgM antibody capable of binding immune complexes.

7. The composition of any one of claims 1 to 3, further comprising at least two autoreactive IgM specificities equivalent to specificities of the pathogenic IgG spectra of a disease, selected from the group consisting of a disease associated with Type III hypersensitivity reactions, a

disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer.

8. The composition of any one of claims 1 to 3, further comprising: a) at least one IgM antibody capable of activating mesangial cells to induce phagocytosis and at least one IgM antibody capable of activating mesangial cells to secrete cytokines; b) at least one IgM antibody capable of binding apoptotic cells and at least one IgM antibody capable of binding immune complexes; and c) at least two autoreactive IgM specificities equivalent to specificities of the pathogenic IgG spectra of a disease , selected from the group consisting of a disease associated with Type III hypersensitivity reactions, a disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer.

9. The composition of any one of claims 1 to 8, further comprising IvIg.

10. The composition of any one of clams 1 to 9, wherein the IgM antibodies are humanized and/or in chimeric form.

11. A method of treating a disease by administering a therapeutically effective amount of the composition of any one of claims 1 to 10 to a subject in need thereof, wherein the disease is selected from the group consisting of a disease associated with Type III hypersensitivity reactions, a disease associated with excessive apoptotic debris and inflammation, an autoimmune disease associated with pathogenic IgG autoantibodies, and a blood cancer.

12. The method of claim 11, wherein the disease associated with Type III hypersensitivity reactions is selected from the group consisting of nephritis, vasculitis, and some forms of arthritis.

13. The method of claim 11, wherein the disease associated with excessive apoptotic debris and inflammation or the autoimmune disease associated with pathogenic IgG autoantibodies

is selected from the group consisting of SLE, scleroderma, rheumatoid arthritis, atherosclerosis, and ischemia.

14. The method of claim 11, wherein the blood cancer is selected from the group consisting of leukemia, B cell lymphoma, and T cell lymphoma.

15. A method for predicting the likelihood of developing nephritis in a subject with SLE, the method comprising the steps of:

a) obtaining a sample from the subject;

b) measuring levels of protective IgM antibodies and of pathogenic IgG in the sample; and

c) determining the ratio of protective IgM antibodies to pathogenic IgG in the sample;

wherein a higher level of protective IgM antibodies as compared the level of pathogenic IgG is indicative of a lower likelihood of developing nephritis in the subject with SLE.

16. The method of claim 15, wherein the protective antibodies have at least three characteristics selected from the group consisting of: 1) being of the IgM type; 2) having the ability to bind antinuclear and/or cytoplasmic antigens; and 3) having the ability to bind apoptotic cells and stimulate cytokine secretion by mesangial cells.

17. The method of any one of claims 15 to 16, wherein the pathogenic antibodies are of the IgG subtype and bind nuclear and/or certain cytoplasmic antigens.

18. The method of any one of claims 15 to 17, wherein the protective antibodies and the pathogenic antibodies are measured by a method selected from the group consisting of immunofluorescence techniques with antinuclear antigen assays, mesangial cell cultures, apoptotic binding assays, a specific antigen binding assay, and heavy chain immunoglobulin variable region family determination.

19. The method of claim 18, wherein the specific antigen binding assay is ELISA.

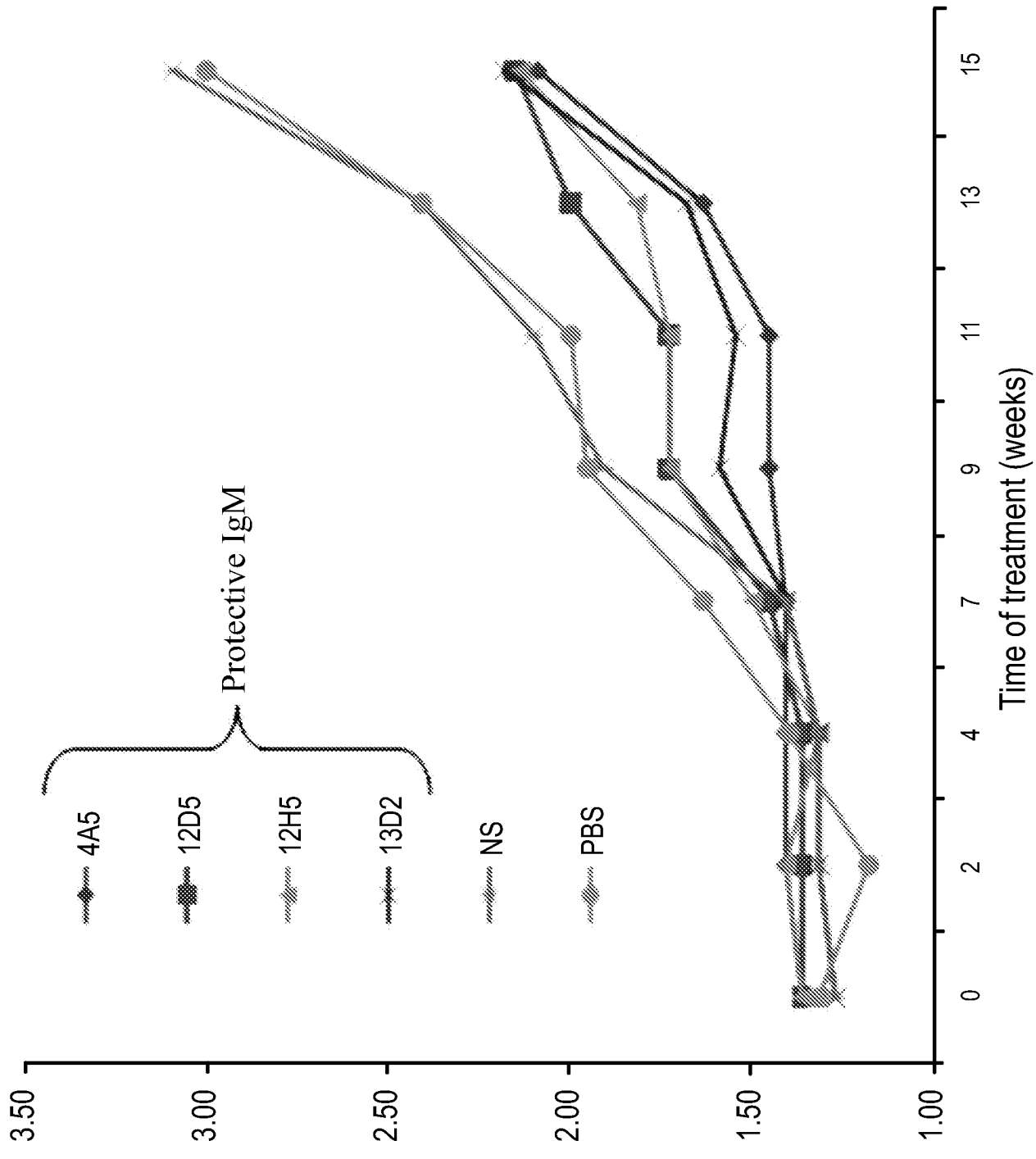
Urine protein score
3.50
3.00
2.50
2.00
1.50
1.00
0
2
4
7
9
11
13
15
Time of treatment (weeks)
Protective IgM
4A5
12D5
12H5
13D2
NS
PBS

FIG. 1

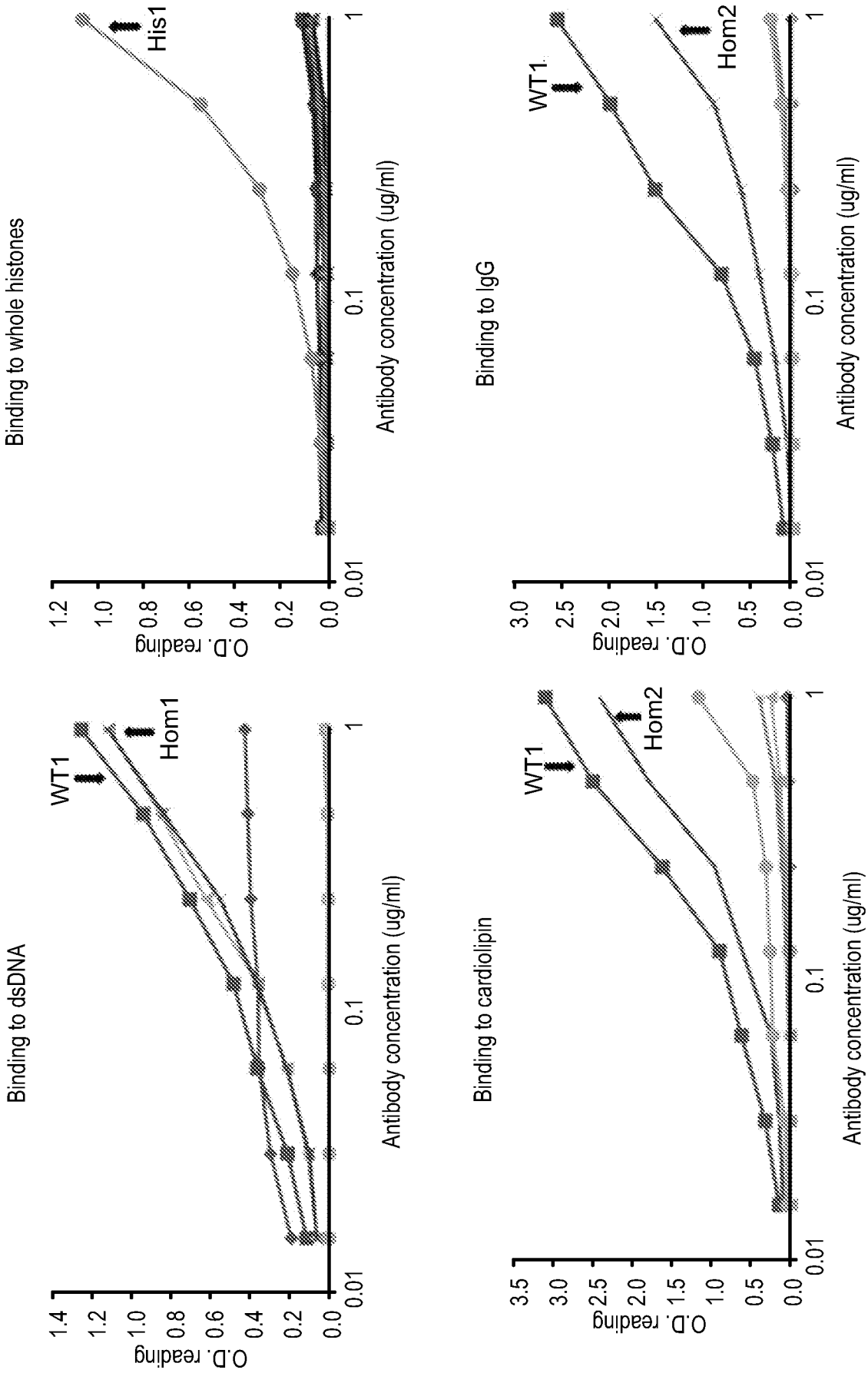
Binding to whole histones
His1
O.D. reading
Antibody concentration (ug/ml)
Binding to IgG
WT1
Hom2
Binding to dsDNA
WT1
Hom1
Binding to cardiolipin
WT1
Hom2

FIG. 2

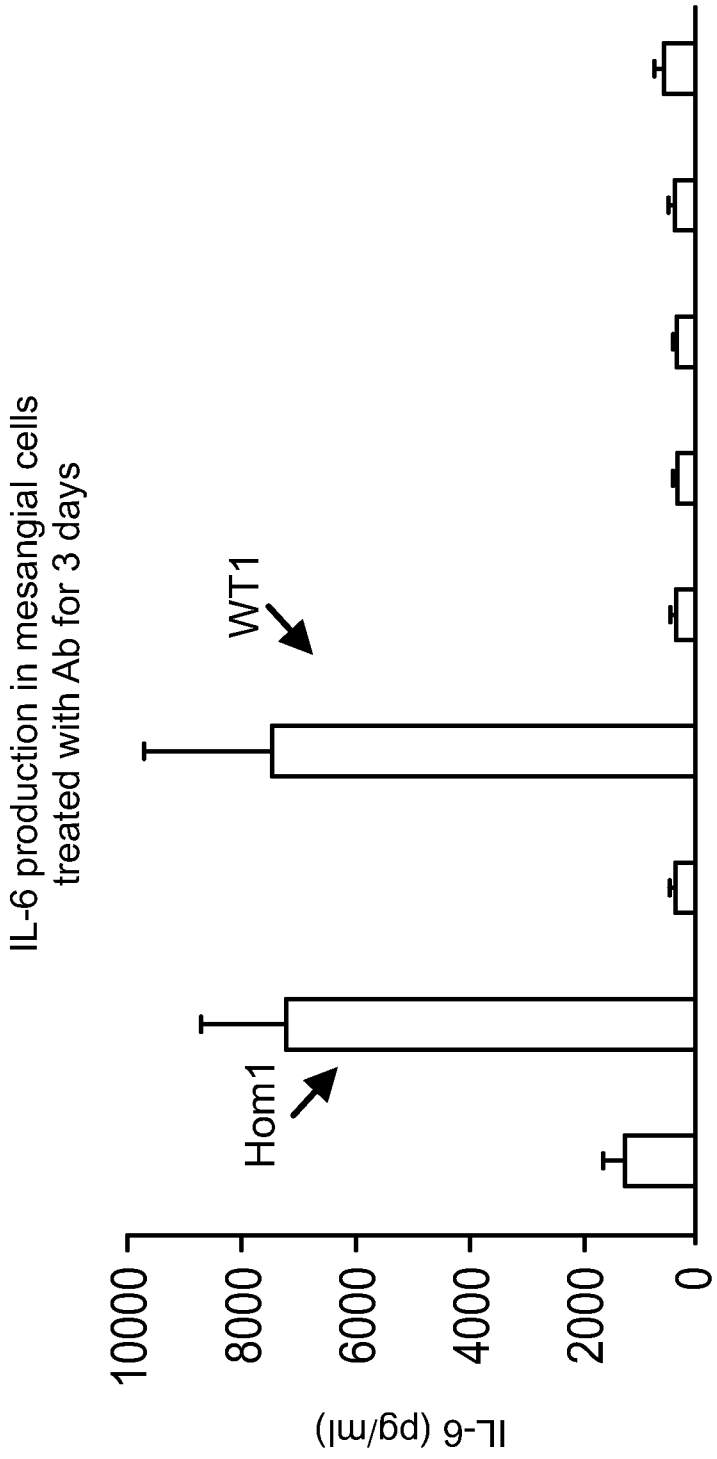
IL-6 production in mesangial cells
treated with Ab for 3 days
IL-6 (pg/ml)
10000
8000
6000
4000
2000
0
Hom1
WT1

FIG. 3

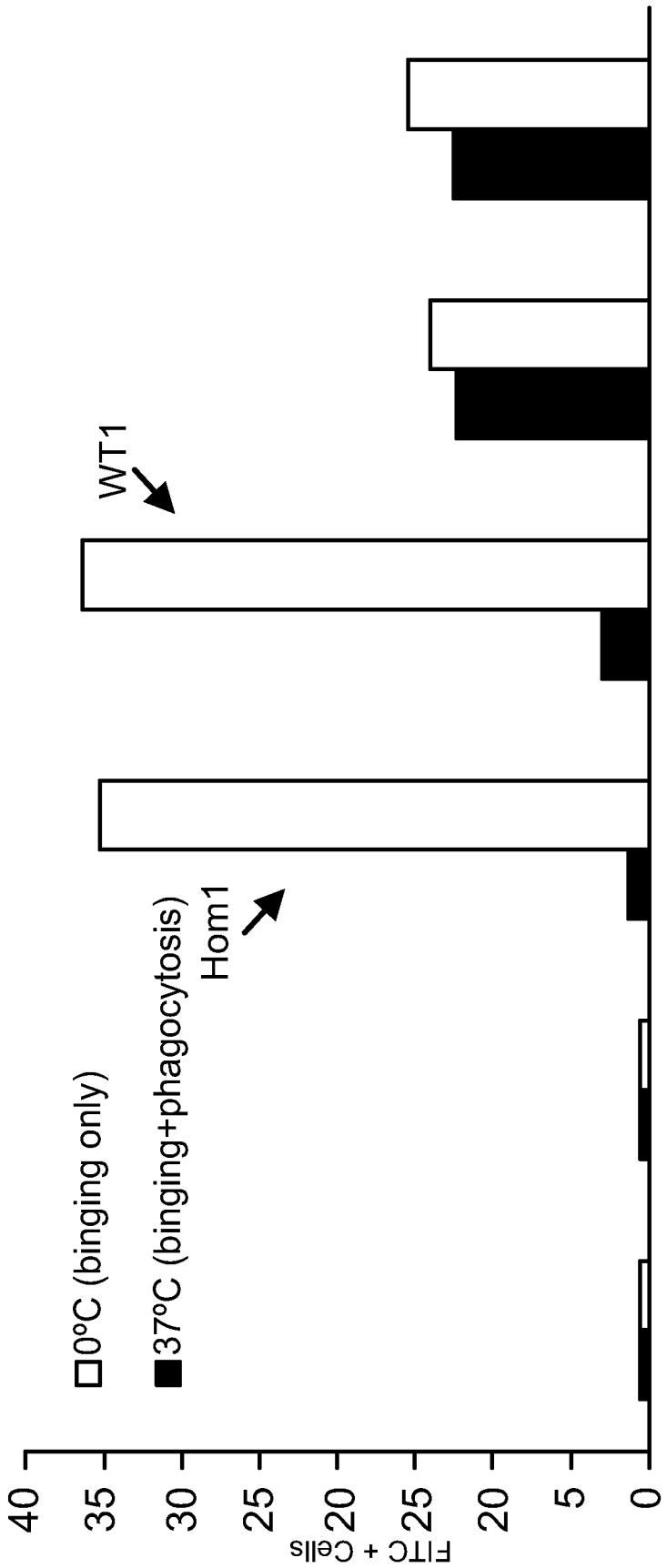
FITC + Cells
0
5
20
25
20
25
30
35
40
□0°C (binging only)
■37°C (binging+phagocytosis)
Hom1
WT1

FIG. 4

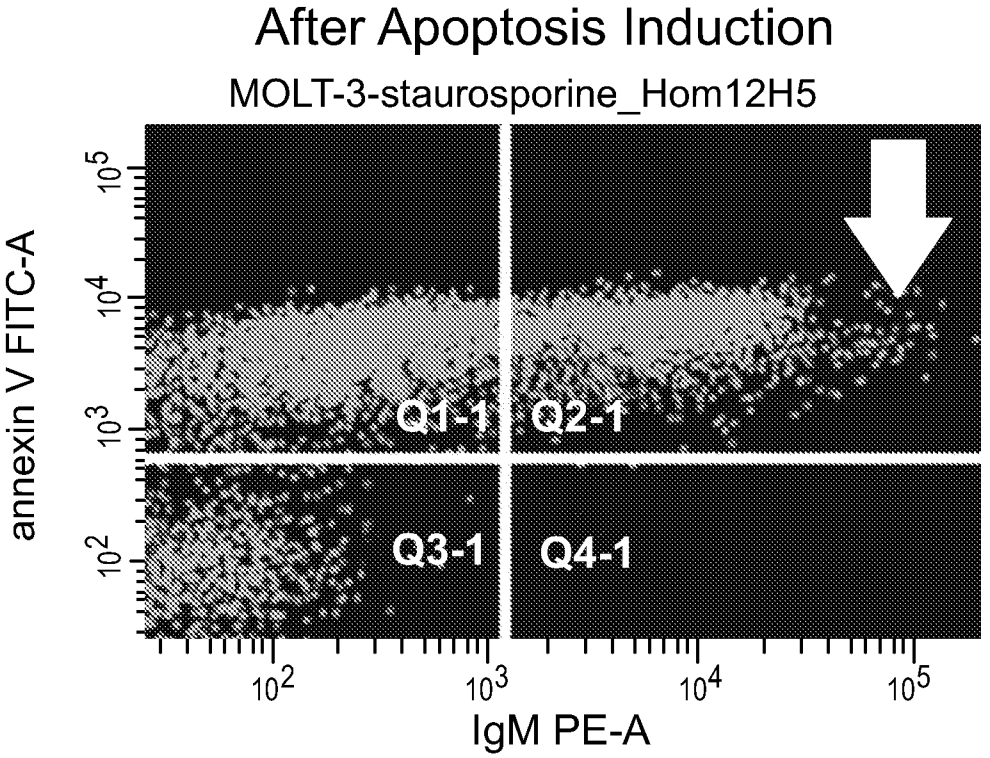
After Apoptosis Induction
MOLT-3-staurosporine_Hom12H5
annexin V FITC-A
IgM PE-A
Q1-1
Q2-1
Q3-1
Q4-1
Protective Ig
MOLT-3-staurosporine_TEPC-183
annexin V FITC-A
IgM PE-A
Q1-1
Q2-1
Q3-1
Q4-1
Non-Protective Ig

FIG. 5

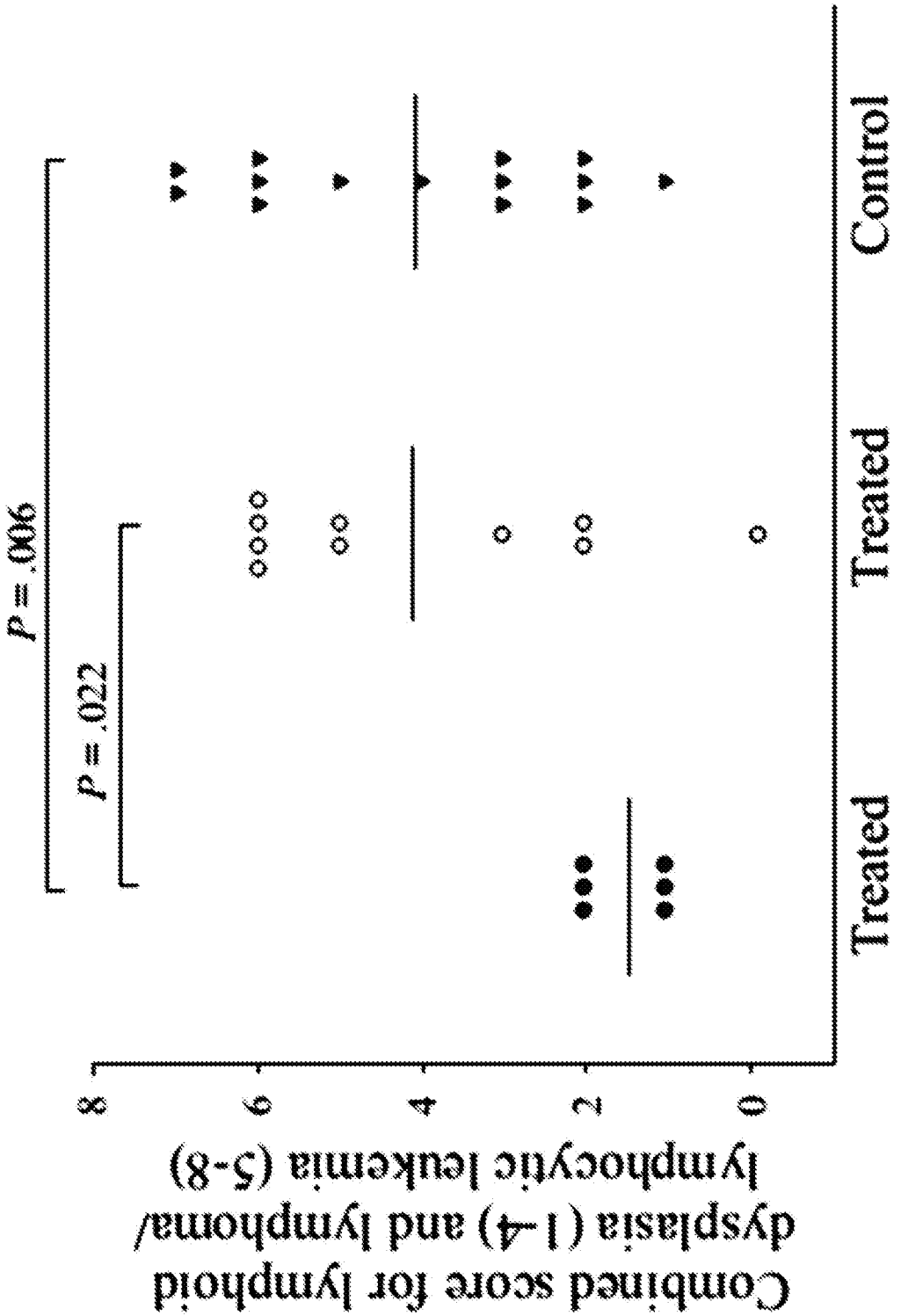
Combined score for lymphoid dysplasia (1–4) and lymphoma/ lymphocytic leukemia (5-8)
8
6
4
2
0
P = .006
P = .022
Treated (high IgM)
Treated (very low IgM)
Control

FIG. 6