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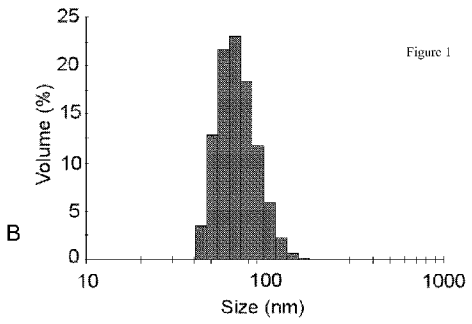

Figure 1

(57) Abstract: Methods of non-invasive imaging of complement-mediated inflammation are provided. Compositions (such as pharmaceutical compositions) comprising CR2-, bacterial- or C4-targeted ultrasmall superparamagnetic nanoparticles or aggregates thereof for use with those methods are also provided

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NON-INVASIVE DETECTION OF COMPLEMENT-MEDIATED INFLAMMATION USING CR2-TARGETED NANOPARTICLES

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/149,975, filed February 4, 2009, which is incorporated herein by reference in its entirety and for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with Government support under Grant Number R01 CA53615 by the National Institutes of Health. The Government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0003] This application pertains to methods and compositions for non-invasive detection of complement-mediated inflammation using CR2-targeted nanoparticles.

[0004] Complement is the collective term for a series of blood proteins that constitute a major effector mechanism of the immune system. Complement plays an important role in the pathology of many autoimmune, inflammatory, and ischemic diseases. Inappropriate complement activation and its deposition on host cells can lead to complement-mediated cell lysis of target structures, as well as tissue destruction due to the generation of powerful mediators of inflammation.

[0005] Complement can be activated by one of the three pathways, the classical, lectin, and alternative pathways. The classical pathway is activated through the binding of the complement system protein C1q to antigen-antibody complexes, pentraxins, or apoptotic cells. The pentraxins include C-reactive protein and serum amyloid P component. The lectin pathway is initiated by binding of microbial saccharides to the mannose-binding lectin. The alternative pathway is activated on surfaces of pathogens that have neutral or positive charge characteristics and do not express or contain complement inhibitors. This results from the process termed "tickover" of C3 that occurs spontaneously, involving the interaction of conformationally altered C3 with factor B, and results in the fixation of active C3b on pathogens or other surfaces. The alternative pathway can also be initiated when certain antibodies block endogenous regulatory mechanisms, by IgA-containing immune complexes,

or when expression of complement regulatory proteins is decreased. In addition, the alternative pathway is activated by a mechanism called the "amplification loop" when C3b that is deposited onto targets via the classical or lectin pathway then binds factor B. *See* e.g., H.J. Müller-Eberhard, 1988, *Ann. Rev. Biochem.* **57**:321. For example, Holers and colleagues have shown that the alternative pathway is amplified at sites of local injury when inflammatory cells are recruited following initial complement activation. *See* e.g., Girardi et al., 2003, *J. Clin. Invest.* **112**:1644. Dramatic complement amplification through the alternative pathway then occurs through a mechanism that involves either the additional generation of injured cells that fix complement, local synthesis of alternative pathway components, or more likely because infiltrating inflammatory cells that carry preformed C3, and properdin greatly increase activation specifically at that site.

[0006] Alternative pathway activation is initiated when circulating factor B binds to activated C3. This complex is then cleaved by circulating factor D to yield an enzymatically active fragment, C3bBb. C3bBb cleaves C3 generating C3b, which drives inflammation and also further amplifies the activation process, generating a positive feedback loop. Factor H is a key regulator (inhibitor) of the alternative complement pathway that competes with factor B for binding to C3b. Binding of C3b to Factor H also leads to degradation of C3b by factor I to the inactive form C3bi (also designated iC3b), thus exerting a further check on complement activation. Factor H regulates complement in the fluid phase, circulating at a plasma concentration of approximately 500 μg/ml, but its binding to cells is a regulated phenomenon enhanced by the presence of a negatively charged surface as well as fixed C3b, iC3b, or C3d. *See* e.g., Jozsi et al., 2004, *Histopathol.* **19**:251-258.

[0007] Complement activation and complement-mediated inflammation are involved in the etiology and progression of numerous diseases. The down-regulation of complement activation has been shown to be effective in treating several diseases in animal models and in *ex vivo* studies, including, for example, systemic lupus erythematosus and glomerulonephritis (Y. Wang et al., 1996, *Proc. Nat'l Acad. Sci. USA* **93**:8563-8568), rheumatoid arthritis (Y. Wang et al., 1995, *Proc. Nat'l Acad. Sci. USA* **92**:8955-8959), cardiopulmonary bypass and hemodialysis (C. S. Rinder, 1995, *J. Clin. Invest.* **96**:1564-1572), hyperacute rejection in organ transplantation (T. J. Kroshus et al., 1995, *Transplantation* **60**:1194-1202), myocardial infarction (J. W. Homeister et al., 1993, *J. Immunol.* **150**:1055-1064; H. F. Weisman et al., 1990, *Science* **249**:146-151), ischemia/reperfusion injury (E. A. Amsterdam et al., 1995, *Am. J. Physiol.* **268**:H448-H457), antibody-mediated allograft rejection, for example, in the kidneys (J. B. Colvin, 2007, *J. Am. Soc. Nephrol.* **18**(4):1046-56), and adult respiratory

distress syndrome (R. Rabinovici et al., 1992, *J. Immunol.* **149**:1744-1750). Moreover, other inflammatory conditions and autoimmune/immune complex diseases are also closely associated with complement activation (B. P. Morgan.1994, *Eur. J. Clin. Invest.* **24**:219-228), including, but not limited to, thermal injury, severe asthma, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, multiple sclerosis, myasthenia gravis, myocarditis, membranoproliferative glomerulonephritis, atypical hemolytic uremic syndrome, Sjögren's syndrome, renal and pulmonary ischemia/reperfusion, and other organ-specific inflammatory disorders.

[0008] A variety of disorders are associated with inflammation, however, so definitive diagnosis of complement-mediated inflammation typically requires confirmation via immuno-staining or other *in vitro* analysis performed on tissue samples retrieved by biopsy. While biopsies are in many respects routine, they have their limitations and are not risk-free. Because commonly used needle or punch biopsies sample only a small portion of the target organ, there is a risk of sample error leading to an incorrect diagnosis. Furthermore, although biopsy is a generally safe procedure, major complications such as internal bleeding may occur in a significant number of cases.

[0009] In some cases, because of the difficulties in diagnosing disease or monitoring disease progression, for example, in patients with systemic lupus erythematosus or lupus nephritis, repeat renal biopsies are therefore frequently necessary to assess the response to therapy or to diagnose disease relapse. *See* e.g., S. Bajaj et al., 2000, *J. Rheumatol.* **27**:2822-2826. Although renal biopsy is generally a safe procedure, complications may occur in 6% or more of biopsies and intra-renal bleeding and hematuria are common. Patients requiring repeat biopsies are at concomitantly greater risk of complications. *See* e.g., W.L. Whittier et al., 2004, *J. Am. Soc. Nephrol.* **15**:142-147; D.C. Mendelssohn et al., 1995, *Am. J. Kidney Dis.* **26**:580-585. Thus, a non-invasive method of detecting or accurately assessing the presence, degree and/or extent of complement-mediated inflammation would be of significant value in diagnosing disease, formulating treatment strategies and monitoring their efficacy for many inflammatory diseases, including lupus nephritis.

[0010] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE INVENTION

[0011] In one aspect, provided herein are methods of detecting complement-mediated inflammation in an individual comprising: (a) administering to the individual an effective

amount a composition comprising an effective amount of CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof; and (b) taking a magnetic resonance image of the individual. In certain embodiments, the CR2-targeted USPIO nanoparticles comprise a targeting moiety comprising an antibody or fragment thereof directed to C3 or a portion thereof, for example but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, a mouse, or a rat. In certain embodiments, the composition is administered by injection. In certain embodiments, the injection is parenteral, intravenous, subcutaneous, or intramuscular.

[0012] In certain embodiments, the composition comprises an effective amount of CR2-targeted USPIO nanoparticle aggregates, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 75 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 150 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid is amine-functionalized. In certain embodiments, the PEGylated, amine-functionalized phospholipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG2000.

[0013] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticle aggregates comprise a targeting moiety comprising an antibody or fragment thereof directed to C3 or a portion thereof, for example C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticle aggregates further comprise a targeting

moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0014] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticle aggregates further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0015] In any of the embodiments described herein, the complement-mediated inflammation is associated with tissue damage resulting from ischemia reperfusion injury, inflammatory disorders, transplant rejection, pregnancy-related diseases, adverse drug reactions, cancer and autoimmune or immune complex disorders. In any of the embodiments described herein, the tissue damage resulting from ischemia reperfusion injury is associated with a disorder selected from the group consisting of myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock, intestinal ischemia, spinal cord injury and traumatic brain injury. In any of the embodiments described herein, the inflammatory disorder is selected from the group consisting of burns, endotoxemia, septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis, anaphylactic shock, asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis. In any of the embodiments described herein, the transplant rejection is hyperacute xenograft rejection. In any of the embodiments described herein, the pregnancy-related disease is selected from the group consisting of recurrent fetal loss and pre-eclampsia. In any of the embodiments described herein, the adverse drug reaction is selected from the group consisting of drug

allergy and IL-2 induced vascular leakage syndrome. In any of the embodiments described herein, the autoimmune or immune complex disorder is selected from the group consisting of myasthenia gravis, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, lupus nephritis, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndromes, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjögren's syndrome, Takayasu's arteritis, autoimmune glomerulonephritis, membranoproliferative glomerulonephritis type II, hemolytic uremic syndrome, and atypical hemolytic uremic syndrome. In any of the embodiments described herein, the autoimmune glomerulonephritis is selected from the group consisting of immunoglobulin A nephropathy and membranoproliferative glomerulonephritis type I.

[0016] Also provided herein are compositions for detecting complement-mediated inflammation in an individual, the compositions comprising an effective amount of CR2-targeted ultrasmall super paramagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, mouse, or rat. In certain embodiments, the compositions comprise an effective amount of CR2-targeted USPIO nanoparticle aggregates, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 75 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 150 nm in diameter, and the USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid. In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid is amine-functionalized. In certain embodiments, the PEGylated, amine-functionalized phospholipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG2000.

[0017] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticle aggregates further comprise a targeting moiety comprising a fusion protein

comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0018] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0019] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles further comprise a targeting moiety comprising an antibody or fragment thereof directed to C3 or a portion thereof, for example but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d.

[0020] In another aspect, provided herein are compositions for detecting complement-mediated inflammation in an individual, the composition comprising an effective amount of CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof, wherein the USPIO nanoparticles or aggregates thereof are coated with dextran or encapsulated with phospholipid. In some embodiments, the composition comprises an antibody or fragment thereof directed to C3 or a portion thereof, for example

but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d. In some embodiments, the compositions further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, mouse, or rat. In certain embodiments, the composition comprises an effective amount of CR2-targeted USPIO nanoparticle aggregates. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 75 nm in diameter. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 150 nm in diameter.

[0021] In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid is amine-functionalized. In certain embodiments, the PEGylated, amine-functionalized phospholipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG2000. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are phospholipid-encapsulated and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the CR2-targeted USPIO nanoparticles are dextran-coated and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular

domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0022] In another aspect, provided herein is the use of any of the compositions as described herein in connection with the methods as described herein, unless otherwise noted or as is clear from the specific context. Any of the compositions as described herein may also be used in the preparation of a medicament for use in the methods as described herein.

[0023] In another aspect, provided herein are articles of manufacture or kits containing pharmaceutical compositions comprising an effective amount of any of the bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof described herein, and instructions for their use in the non-invasive imaging methods described herein. Thus, in certain embodiments, the article of manufacture comprises instructions for the use of pharmaceutical compositions comprising an effective amount of bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof in any of the methods of detecting complement-mediated inflammation in an individual as described herein. In certain embodiments, the composition comprises an effective amount of CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof, wherein the USPIO nanoparticles or aggregates thereof are coated with dextran or encapsulated with phospholipid and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the USPIO nanoparticles or aggregates thereof are coated with dextran or encapsulated with phospholipid and further comprise an antibody or fragment thereof directed to C3 or a portion thereof, for example but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, mouse or rat. In certain embodiments, the composition comprises an effective amount of CR2-targeted USPIO nanoparticle aggregates. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter. In certain

embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 75 nm in diameter. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 150 nm in diameter.

[0024] In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid is amine-functionalized. In certain embodiments, the PEGylated, amine-functionalized phospholipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG2000. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are phospholipid-encapsulated and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the CR2-targeted USPIO nanoparticles are dextran-coated and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] Figures 1A-B depict the appearance and size distribution of ultrasmall superparamagnetic ("USPIO") nanoparticles generated as described in the Materials and Methods section of Example 1. Figure 1A shows a transmission electron micrograph demonstrating the appearance of the as-synthesized USPIO nanoparticles. Figure 1B shows

the size distribution by volume percentage of the phospholipid-encapsulated USPIO nanoparticle aggregates measured by dynamic light scattering. The distribution peak is 74.52 nm with a polydispersity index of 0.034.

[0026] Figures 2A-C depict data which confirms the successful conjugation of CR2-Fc to the surface of USPIO nanoparticles. Figure 2A shows fluorescence-activated cell sorting (FACS) analysis using a monoclonal antibody to the C3d binding region of CR2 confirming successful conjugation of the protein to the nanoparticle surface (solid line). No staining was seen on unconjugated particles (dashed line). Figure 2B shows FACS analysis demonstrating that incubation of CHO cells with 10% mouse serum (solid line) opsonized the cells with C3, whereas no C3 was detectable on unmanipulated cells (dashed line). Figure 2C shows CR2-targeted USPIO nanoparticles bound to opsonized CHO cells (solid line) compared to binding to untargeted USPIO nanoparticles (striped curve) which was similar to isotype staining of CHO cells (dotted line).

[0027] Figures 3A-I depict periodic acid-Schiff (PAS) staining demonstrating the normal glomerulus (arrowhead) of a wild-type mouse (Figure 3A), proliferative changes in a 16-week old MRL/lpr mouse (Figure 3B), and glomerulosclerosis in an 18-week old MRL/lpr mouse (Figure 3C). Figures 3E-I also show immunofluorescence microscopy staining for complement protein C3 in 16-week old MRL/lpr mice demonstrating deposition of C3 in glomeruli (Figure 3D), in tubules of the outer medulla (Figure 3E), and in the inner medulla (Figure 3F). Sparse deposits of C3 were present in the glomeruli (Figure 3G), outer medulla (Figure 3H) and inner medulla (Figure 3I) of wild-type mice. Original magnification ×400.

[0028] Figures 4A-B depict abdominal MRI analyses of MRL/lpr mice used as a model system for lupus nephritis (lower panels) and wild-type mice (upper panels). Figure 4A shows proton density (PD)-weighted rapid acquisition with relaxation enhancement (RARE) images, and Figure 4B shows T_2-weighted multiple slice multiple echo (MSME) images demonstrating systemic pathology in the MRL/lpr mice, including significant lymphadenopathy (arrows) and enlarged kidneys (arrowheads). At baseline, the kidneys of MRL/lpr mice display higher T_2-weighted signal intensity than in wild-type controls. *See* also Table 1.

[0029] Figure 5 depicts relative changes in T_2 values after injection with CR2-targeted and untargeted USPIO nanoparticles in MRL/lpr mice. T_2-weighted signal intensity was measured at various time-points in the cortex, inner and outer medulla as well as in the muscle (as an unchanged comparison) of MRL/lpr mice. After injection with untargeted

USPIO nanoparticles, no changes in T_2-signal intensity were seen in the cortex, outer or inner medulla or in the muscle. In contrast, after injection of CR2-targeted USPIO nanoparticles, significant decreases in the T_2-relaxation time were seen in the cortex, outer and inner medulla. The changes were observed as early as 4 hrs after injection and persisted up to 72 hours after injection. Data are given as mean ± S.D. (n=5). For evaluation of kidneys, 10 repetitions were performed since both the left and right kidneys of each animal were evaluated. * $P < 0.05$ versus pre-injection baseline.

[0030] Figures 6A-B depict T_2-weighted MSME MR images (TR = 2,650 ms) of a wild-type (Figure 6A) and an MRL/lpr mouse (Figure 6B) 48 hours after injection of CR2-conjugated SPIOs. Images at echo times (TE) of 10, 30, 50, and 70 ms are shown. A decrease in T_2 signal intensity in the kidney of the MRL/lpr mouse is visible in all regions of the kidney, particularly at TE=70 ms (arrowhead).

[0031] Figures 7A-D show that iron can be detected in the glomeruli and renal tubules of MRL/lpr mice injected with CR2-targeted USPIO nanoparticles. MRL/lpr and wild-type mice were sacrificed at the completion of the study. Kidney sections were stained with Perl's Prussian Blue to detect iron deposits in the tissue. In the MRL/lpr mice, iron could be detected in the glomeruli (Figure 7A) and in renal tubules (Figure 7B). In wild-type mice, iron was not seen in any glomeruli (Figure 7C) or renal tubules (Figure 7D). Original magnification ×400.

DETAILED DESCRIPTION OF THE INVENTION

[0032] The complement system is activated in a number of inflammatory and autoimmune diseases. M.J. Walport, 2001, *N. Engl. J. Med.* **344**:1058-1066; M.J. Walport, 2001, *N. Engl. J. Med.* **344**:1140-1144. Complement activation is central to the pathogenesis of many diseases, contributing to tissue injury in numerous inflammatory and autoimmune disorders. Moreover, products of complement activation also serve as robust markers of ongoing tissue inflammation, since activation of the complement system leads to rapid deposition of C3b on cell surfaces. Classical pathway activation by immunoglobulin, for example, can deposit more than 240 molecules of C3b to the surface for every molecule of C1q engaged by the antibody. *See* e.g., M.W. Ollert et al., 1994, *J. Immunol.* **153**:2213-2221. Other markers of classical pathway activation include cell-bound C4b, iC4d, and C4d, indirect markers of an antibody response. The complement protein C4 is also cleaved during classical pathway activation. Like C3, C4 undergoes several proteolytic steps generating fragments (C4b, iC4b, and C4d) that remain covalently bound to tissue. The detection of C4 fragments such as C4d

demonstrates that the complement activation involves the classical pathway. This is useful for diagnosing some antibody-mediated diseases, such as antibody mediated transplant rejection. Immuno-staining for deposited C3 and C4 is central to the diagnosis of autoimmune disease, and is routinely performed on kidney biopsy samples when autoimmune disease is suspected. For example, the presence of glomerular C3 deposits helps support the diagnosis of glomerulonephritis. Thus, identification of C3 and/or C4 deposits is integral to the diagnosis and assessment of autoimmune and inflammatory diseases.

[0033] Complement protein C3 is a zymogen. Intact C3 circulates at high concentrations (1-2 mg/ml). *See* e.g., M. Janzi et al., 2005, *Mol. Cell. Proteomics* **4**(12):1942-1947. During complement activation, whole C3 is cleaved to form C3b which becomes covalently bound to target surfaces. Endogenous complement regulatory proteins inactivate tissue-bound C3b to form iC3b and eventually the 35 kilodalton ("kD") C3d fragment. The C3d fragment remains fixed to tissues and serves as a durable marker of complement-mediated inflammation. *See* e.g., I. Leivo et al., 1986, *J. Cell. Biol.* **103**:1091-1100.

[0034] CR2 (CD21) is a ~145 kD transmembrane protein expressed on B cells, T cells, and dendritic cells. *See* e.g., J. Hannan et al., 2002, *Biochem. Soc. Trans.* **30**:983-989; K.A. Young et al., 2007, *J. Biol. Chem.* **282**(50):36614-36625. CR2 protein does not bind intact C3 protein, but binds its breakdown products, including the C3b, iC3b, and C3d cleavage fragments, via a binding site located within the first two amino-terminal short consensus repeats ("SCRs 1 and 2") of the CR2 protein. Consequently, the SCR1-2 domain of CR2 discriminates between cleaved (*i.e.*, activated) forms of C3 and intact circulating C3. As a targeting group, SCRs 1 and 2 of CR2 are therefore able to discriminate between circulating C3 and the C3 fragments generated during complement activation. Although the affinity of CR2 for C3d is only 620-658 nM (J. Hannan et al., 2002, *Biochem. Soc. Trans.* **30**:983-989; J.M. Guthridge et al., 2001, *Biochem.* **40**:5931-5941), the avidity of CR2 for clustered C3d makes it an effective method of targeting molecules to sites of complement activation.

[0035] As shown in the Examples, in the murine MRL/lpr model of lupus nephritis, injection of mice with the CR2-targeted USPIO nanoparticle aggregates caused a significant reduction in T_2-weighted MRI signal in nephritic kidneys, while injection of the CR2-targeted USPIO nanoparticle aggregates into healthy control mice did not change the MRI signal of the kidneys. Moreover, injection of untargeted USPIO nanoparticle aggregates did not alter the T_2-weighted signal of the kidneys in MRL/lpr mice.

[0036] Thus, CR2-targeted nanoparticle aggregates can be used as a contrast agent specifically targeted to sites of complement activation, and provide a novel reagent useful in non-invasive methods of detecting active inflammation in immune-complex glomerulonephritis, lupus nephritis, cancer and other diseases characterized by complement-mediated inflammation. Furthermore, the methods described herein provide a more comprehensive assessment of disease activity than that afforded by biopsy. Magnetic resonance images obtained after injection with CR2-conjugated USPIO nanoparticle aggregates can specifically detect complement activation throughout both kidneys and distinguish renal C3 activation from that occurring at extra-renal locations. In patients known to have lupus nephritis whose clinical parameters have changed, this technique enables the clinician to distinguish active inflammation from increases in proteinuria or decreases in renal function caused by scarring. Currently distinguishing active inflammation from damage caused by scarring requires a biopsy. Moreover, non-invasive assessment of disease etiology and progression by the methods described herein can offer important insight into the rate at which active inflammatory disease responds to therapy and resolves.

Definitions

[0037] General reference to "the composition" or "compositions" includes and is applicable to compositions of the invention.

[0038] As used herein, the singular form of the articles "a", "an", and "the" includes plural references unless indicated otherwise. For example, "a" biologically-active CR2 fragment includes one or more biologically-active CR2 fragments.

[0039] Reference to "about" a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X."

[0040] It is understood that aspects and embodiments of the invention described herein include "consisting" and/or "consisting essentially of" aspects and embodiments.

[0041] As used herein, the term "individual" refers to a vertebrate, preferably a mammal, more preferably a human. Mammals include, but are not limited to, farm animals, sport animals, pets, primates, mice and rats. In some embodiments, the individual is human. In some embodiments, the individual is an individual other than a human. In some embodiments, the individual is an animal model for the study of a disease in which the alternative complement pathway is implicated. Individuals amenable to non-invasive imaging via the methods described herein include those who are suffering from a disease

characterized by complement-mediated inflammation, and those who are suffering from a disease characterized by alternative complement-mediated inflammation.

[0042] As used herein, the term "cancer" refers to all types of cancer, neoplasm or malignant tumors found in mammals, including leukemia, carcinomas and sarcomas. Exemplary cancers include, but are not limited to, cancer of the brain, breast, cervix, colon, head & neck, liver, kidney, lung, non-small cell lung, melanoma, mesothelioma, ovary, sarcoma, stomach, uterus and Medulloblastoma. Additional examples include, Hodgkin's Disease, Non-Hodgkin's Lymphoma, multiple myeloma, neuroblastoma, ovarian cancer, rhabdomyosarcoma, primary thrombocytosis, primary macroglobulinemia, primary brain tumors, cancer, malignant pancreatic insulanoma, malignant carcinoid, urinary bladder cancer, premalignant skin lesions, testicular cancer, lymphomas, thyroid cancer, neuroblastoma, esophageal cancer, genitourinary tract cancer, malignant hypercalcemia, endometrial cancer, adrenal cortical cancer, neoplasms of the endocrine and exocrine pancreas, and prostate cancer.

[0043] Provided herein are compositions (such as pharmaceutical compositions) comprising CR2-targeted nanoparticles or aggregates thereof and non-invasive methods of detecting complement-mediated inflammation using such compositions. The CR2-targeting portion is responsible for delivering the nanoparticles to the sites of complement activation and complement-mediated inflammation, and the nanoparticle portion is responsible for enhancing contrast in a non-invasive image (such as a magnetic resonance image) of the site of inflammation.

Targeted Nanoparticle Compositions for Imaging Applications

[0044] Delivery of ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles, USPIO nanoparticle aggregates, superparamagnetic iron oxide ("SPIO") nanoparticles, SPIO nanoparticle aggregates or other nanoparticle contrast agents to the sites of active inflammation via CR2-targeting to sites of complement activation permits non-invasive magnetic resonance imaging of such inflammation, enabling the specific detection of complement activation throughout the body, and distinguishing complement-mediated inflammation from other types of inflammation. Accordingly, in one aspect, the invention provides compositions comprising CR2-targeted nanoparticle contrast agents for non-invasive medical or diagnostic imaging applications. In certain embodiments, the CR2-targeted nanoparticle contrast agent compositions comprise USPIO nanoparticles or aggregates thereof. In certain embodiments, the CR2-targeted nanoparticle contrast agent

compositions comprise CR2-targeted liposomes or other CR2-targeted delivery vehicles containing Gadolinium chelate ("Gd-chelate") molecules.

[0045] At least two physicochemical characteristics of ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof vary with the size of the individual nanoparticles or nanoparticle aggregates. First, the ability of USPIO nanoparticle preparations to enhance contrast in MRI imaging and the degree of contrast enhancement both vary with nanoparticle diameter, because the magnetic moment of individual USPIO nanoparticles also varies with particle diameter. Iron oxide nanoparticles with diameters up to approximately 15 nm remain superparamagnetic, but larger iron oxide nanoparticles lose their superparamagnetic properties. Thus, there is an upper limit to the diameter of USPIO nanoparticles suitable for use as MRI contrast reagents. This limitation can be overcome by use of multiparticle aggregates of smaller individual USPIO nanoparticles. Such USPIO nanoparticle aggregates effectively enhance MRI contrast because the magnetic moments of the individual nanoparticles within each nanoparticle aggregate are additive. Unlike individual iron oxide nanoparticles, aggregates of ultrasmall super paramagnetic iron oxide nanoparticles do not lose their paramagnetic properties with increased size.

[0046] Second, the *in vivo* half-life (*e.g.*, circulating plasma or blood half-life and tissue half-life) and biodistribution of USPIO nanoparticles or aggregates thereof varies with nanoparticle or aggregate size. For example, USPIO nanoparticles ~10 nm or less in diameter have a circulating blood half-life of ~81 minutes (R. Weissleder et al., 1990, *Radiol.* **175**(2):489-493), USPIO nanoparticles ~50 nm in diameter have a circulating half-life of ~30 minutes (D. Pouliquen et al., 1991, *Magnet. Resonance Imag.* **9**(3):275-283), USPIO nanoparticles ~150 nm in diameter are thought to have a circulating half-life of less than ~30 minutes, and USPIO nanoparticles ~80 nm in diameter have a tissue half-life on the order of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days) and a whole body half-life of ~45 days (R. Weissleder et al., 1989, *Am. J. Roentgenol.* **152**(1):167-173). Effective targeted MRI contrast-enhancing reagents must circulate in the vasculature long enough to recognize and bind the desired target (*e.g.*, renal deposits of C3 breakdown products) while still being cleared quickly enough to minimize any potential toxicity. Optimal USPIO nanoparticle or nanoparticle aggregate sizes for generating clinically useful magnetic resonance images vary depending on the organ (*e.g.*, the kidney), tissue, and/or physiological phenomenon (*e.g.*, complement-mediated inflammation) to be imaged.

[0047] The circulating half-life of USPIO nanoparticles or nanoparticle aggregates can also be altered (*i.e.*, reduced or extended) by coating them with different materials. For instance,

USPIO nanoparticles or nanoparticle aggregates can be coated with natural or synthetic polymers, surfactants, or phospholipids, among other materials, any of which may be modified or derivatized to permit attachment of targeting groups, either directly or indirectly via different types of linkers, including peptides, polypeptides, proteins, or other chemical groups. In some cases, the coatings may be further modified to incorporate synthetic polymers, natural polymers or other molecules (*e.g.*, polyvinylpyrrolidone ("PVP"), poly(lactic-*co*-glycolic acid) ("PLGA"), polyethylene glycol ("PEG"), polyvinyl alcohol ("PVA"), polyacrylic acid, and the like) suitable for stabilizing the aggregates or minimizing their susceptibility to extravasation, opsonization, phagocytosis, endocytosis or other modes of physiological clearance. As with USPIO nanoparticle or nanoparticle aggregate size, the particular coating, modification or derivatization suitable for targeting the nanoparticles or nanoparticle aggregates to a desired organ (*e.g.*, the kidney), tissue, and/or physiological phenomenon (*e.g.*, complement-mediated inflammation) may be determined empirically. The present inventors have identified an optimal USPIO nanoparticle aggregate size range and coating type suitable for production of stable targeted USPIO nanoparticle aggregates with a circulating half-life long enough that the aggregates reach their targets, permitting detection of complement-mediated inflammation in particular tissues, while not being cleared so quickly that they cannot find and bind to their targets.

[0048] In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter and phospholipid-encapsulated. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter and phospholipid-encapsulated. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter and phospholipid-encapsulated. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in

diameter and phospholipid-encapsulated. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter and phospholipid-encapsulated. In certain embodiments, the USPIO nanoparticles or aggregates thereof are about 75 nm in diameter and phospholipid-encapsulated. In certain embodiments, the USPIO nanoparticles or aggregates thereof are about 150 nm in diameter and phospholipid-encapsulated. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In any of the embodiments described herein, the phospholipid is PEGylated. The term "PEGylated" refers in the customary sense to conjugation with polyethylene glycol (PEG). In certain embodiments described herein, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a CR2- or bacterial-targeting group. In certain embodiments described herein, the PEGylated phospholipid further comprises a functional group suitable for cross-linking an antibody directed to C3 or fragment thereof, including but not limited to C3b, iC3b, C3dg, C3d and the like. In certain embodiments described herein, the functional group is an amine. In any of the embodiments described herein, the functional group is maleimide. In any of the embodiments described herein, the functional group is a thiol. In any of the embodiments described herein, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In any of the embodiments described herein, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In any of the embodiments described herein, the phospholipid comprises DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises maleimide-functionalized DSPE-PEG2000. In any of the embodiments described herein, the USPIO nanoparticle aggregates are CR2-targeted, phospholipid-encapsulated, have a circulating plasma half-life of between about 20 minutes and about 40 minutes, and have a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0049] In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter and coated with dextran. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in

diameter and coated with dextran. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter and coated with dextran. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter and coated with dextran. In certain embodiments, the USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter and coated with dextran. In certain embodiments, the USPIO nanoparticles or aggregates thereof are about 75 nm in diameter and coated with dextran. In certain embodiments, the USPIO nanoparticles or aggregates thereof are about 150 nm in diameter and coated with dextran.

[0050] As used herein, the terms "CR2-targeting group," "CR2-targeted group" and the like refer to a chemical moiety such as a peptide, polypeptide, or protein, attached to a superparamagnetic nanoparticle or aggregates thereof, or to the coating of a superparamagnetic nanoparticle or aggregates thereof that selectively binds to or specifically binds to another molecule of interest, such as, for example, a cell-bound breakdown product of complement protein C3 that serves as a durable marker of complement-mediated inflammation, such as, for example, C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), and C3d (SEQ ID NO:10). Exemplary targeting groups may be, for example but not limited to, a full-length CR2 protein or biologically-active fragment thereof. In some embodiments, the CR2-targeting group is an antibody directed to C3 or a portion thereof, for example but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d. Accordingly, in some embodiments, the CR2-targeted ultrasmall superparamagnetic iron oxide nanoparticles contemplated herein comprise an antibody or fragment thereof directed to C3 or a portion thereof, for example but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody or antibody fragment is a polyclonal antibody. In some embodiments, the antibody or antibody fragment is a monoclonal antibody. In some embodiments, the antibody or antibody fragment is covalently linked to the nanoparticle. In other embodiments, the antibody or antibody fragment is non-covalently linked (e.g. via biotin-streptavidin functional groups or equivalents thereof).

[0051] As used herein, the term "bacterial-targeting group" refers to a chemical moiety such as a peptide, polypeptide, or protein derived from a bacterial source, attached to a superparamagnetic nanoparticle or aggregate thereof or to the coating of a superparamagnetic

nanoparticle or aggregate thereof that selectively binds to or specifically binds to another molecule of interest such as, for example, a cell-bound breakdown product of complement protein C3 that serves as a durable marker of complement-mediated inflammation such as, for example, C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), and C3d (SEQ ID NO:10), or a cell-bound breakdown product of complement protein C4 that serves as a marker of an antibody response such as, for example, C4d (SEQ ID NO:). Exemplary bacterial targeting groups may be, for example, full-length *Staphylococcus aureus* immune subversion protein or biologically-active fragments thereof, or any other bacterium-derived protein that binds to cell-bound breakdown products of complement protein C3, such as, for example, C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), and C3d (SEQ ID NO:10).

[0052] *S. aureus* is a common commensal bacterium found in humans that can cause illnesses ranging from superficial skin infections to serious invasive infections such as septic arthritis, osteomyelitis, and endocarditis. *See* e.g., A. Upadhyay et al., 2008, *J. Biol. Chem.* **283**(32):22113-22120. The broad spectrum of diseases caused by *S. aureus* reflects the bacterium's ability to circumvent various components of the immune system, including the complement cascade. The immune subversion protein (Sbi) is a secreted 436-amino acid protein (SEQ ID NO:20) found in many *S. aureus* strains that comprises four small N-terminal domains extending up to amino acid residue 266, followed by eight copies of a PXXXX repeat motif (where "X" specifies any naturally-occurring amino acid) with a high concentration of glutamine, lysine, aspartate, valine, and isoleucine, followed by a C-terminal tyrosine-rich domain of 130-amino acids. The first two N-terminal domains of Sbi (Sbi-I and Sbi-II) specifically or selectively bind to IgG, while the second two N-terminal domains (Sbi-III and Sbi-IV) specifically or selectively bind to proteolytic fragments of complement protein C3, including C3dg and C3a. *Id.* Thus, biologically-active Sbi fragments comprising Sbi-I-II-III-IV (amino acids 28-266; SEQ ID NO:21) and Sbi-III-IV (amino acids 150-266; SEQ ID NO:22) bind proteolytic fragments of complement protein C3, and are useful for targeted delivery of superparamagnetic nanoparticles to sites of complement-mediated inflammation.

[0053] As used herein, the term "C4-targeting group" refers to a chemical moiety such as a peptide, polypeptide, or protein, attached to a superparamagnetic nanoparticle or aggregates thereof, or to the coating of a superparamagnetic nanoparticle or aggregates thereof that selectively binds to or specifically binds to another molecule of interest, such as, for example, a cell-bound breakdown product of complement protein C4 that serves as a durable marker of

antibody-mediated inflammation, such as, for example, C4b (SEQ ID NO:26), iC4b (SEQ ID NO:25), and C4d (SEQ ID NO:27). Exemplary targeting groups may be, for example, a full-length CR1 protein (SEQ ID NO:28) or biologically-active fragment thereof, or a full-length C4b-binding protein (C4bp) (SEQ ID NOS:29 and 30) or biologically-active fragment thereof.

[0054] As used herein, the term "complement receptor 1," "CR1," or "CD35" refers to a human gene encoding a protein of 2039 amino acids, with a predicted molecular weight of 220 kilodaltons ("kD"), including homologues and fragments thereof. The gene is expressed principally on erythrocytes, monocytes, neutrophils, and B cells, but is also present on some T lymphocytes, mast cells, and glomerular podocytes. CR1 protein is typically expressed at between 100 and 1000 copies per cell. The full-length CR1 protein comprises a 42 amino acid signal peptide, an extracellular domain of 1930 amino acids, a 25 amino acid transmembrane domain, and a 43 amino acid C-terminal cytoplasmic domain. The extracellular domain of CR1 has 25 potential N-glycosylation signal sequences, and comprises 30 short consensus ("SCR") domains, also known as complement control protein (CCP) repeats, or sushi domains, each 60 to 70 amino acids long. The sequence homology between SCRs ranges between 60 to 99 percent. The 30 SCR domains are further grouped into four longer regions termed long homologous repeats ("LHRs"), each encoding approximately 45 kD segments of the CR1 protein, designated LHR-A, -B, -C, and -D. The first three comprise seven SCR domains each, while LHR-D comprises 9 SCR domains. The active sites on the extracellular domain of CR1 protein include a C4b-binding site with lower affinity for C3b in SCRs 1 to 4 comprising amino acids 42-295, a C3b-binding site with lower affinity for C4b in SCRs 8 to 11 comprising amino acids 490-745, a C3b-binding site with lower affinity for C4b in SCRs 15-18 comprising amino acids 940-1196, and a C1q-binding site in SCRs 22-28 comprising amino acids 1394-1842.

[0055] SEQ ID NO:28 represents the full-length human CR1 amino acid sequence (*see, e.g.*, UniProtKB/Swiss-Prot. Accession No. P17927). Amino acids 1-41 correspond to the signal peptide, amino acids 42-2039 correspond to the mature protein, comprising amino acids 42-1971, corresponding to the extracellular domain, amino acids 1972-1996, corresponding to the transmembrane domain, and amino acids 1997-2039, corresponding to the cytoplasmic domain. In the extracellular domain, amino acids 42-101 correspond to SCR 1, 102-163 correspond to SCR2, amino acids 164-234 correspond to SCR3, amino acids 236-295 correspond to SCR4, amino acids 295-355 correspond to SCR5, amino acids 356-418 correspond to SCR6, amino acids 419-489 correspond to SCR7, amino acids 491-551

correspond to SCR8, amino acids 552-613 correspond to SCR9, amino acids 614-684 correspond to SCR10, amino acids 686-745 correspond to SCR11, amino acids 745-805 correspond to SCR12, amino acids 806-868 correspond to SCR13, amino acids 869-939 correspond to SCR14, amino acids 941-1001 correspond to SCR15, amino acids 1002-1063 correspond to SCR16, amino acids 1064-1134 correspond to SCR17, amino acids 1136-1195 correspond to SCR18, amino acids 1195-1255 correspond to SCR 19, amino acids 1256-1318 correspond to SCR 20, amino acids 1319-1389 correspond to SCR 21, amino acids 1394-1454 correspond to SCR 22, amino acids 1455-1516 correspond to SCR 23, amino acids 1517-1587 correspond to SCR 24, amino acids 1589-1648 correspond to SCR 25, amino acids 1648-1708 correspond to SCR 26, amino acids 1709-1771 correspond to SCR 27, amino acids 1772-1842 correspond to SCR 28, amino acids 1846-1906 correspond to SCR 29, amino acids 1907-1967 correspond to SCR 30. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that CR1 protein or biologically-active fragments thereof encompasses all species and strain variations. As used herein, the term "biologically-active" fragment of CR1 protein refers to refers to any soluble fragment of CR1 lacking the transmembrane domain and the cytoplasmic domain, including fragments comprising, consisting essentially of or consisting of 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, or 30 SCR domains, including any fragments of the full-length CR1 protein having some or all the complement inhibitory activity of the full-length CR1 protein, and retaining the ability to bind C4, C4b, iC4b, or C4d.

[0056] As used herein, the term "C4b-binding protein, alpha chain," "C4bp-alpha," or "C4bp-α" refers to a human gene encoding a protein of 597 amino acids, with a predicted molecular weight of 67 kilodaltons ("kD"), including homologues thereof. SEQ ID NO:29 represents the full-length human C4bp-alpha amino acid sequence (*see, e.g.*, UniProtKB/Swiss-Prot. Accession No. P04003). Amino acids 1-48 correspond to the signal peptide, amino acids 49-597 correspond to the mature protein, comprising amino acids 49-110, corresponding to SCR 1, amino acids 111-172, corresponding to SCR2, amino acids 173-236, corresponding to SCR3, amino acids 237-296, corresponding to SCR4, amino acids 297-362, corresponding to SCR5, amino acids 363-424, corresponding to SCR5, amino acids 425-482, corresponding to SCR7, and amino acids 483-540, corresponding to SCR8. As used herein, the term "C4b-binding protein, beta chain," "C4bp-beta," or "C4bp-β" refers to a human gene encoding a protein of 252 amino acids, with a predicted molecular weight of 28 kilodaltons ("kD"), including homologues thereof. SEQ ID NO:30 represents the full-length

human C4bp-beta amino acid sequence (*see, e.g.*, UniProtKB/Swiss-Prot. Accession No. P20851). Amino acids 1-17 correspond to the signal peptide, amino acids 18-252 correspond to the mature protein, comprising amino acids 21-78, corresponding to SCR 1, amino acids 79-136, corresponding to SCR2, and amino acids 137-193, corresponding to SCR3. As used herein, the term "C4-binding protein" or "C4bp" refers to one of three disulfide-linked complexes comprising one or more C4bp-alpha and/or one or more C4bp-beta polypeptides: a 570 kD complex of seven C4bp-alpha polypeptides and one C4bp-beta polypeptide, a 530 kD complex of seven C4bp-alpha polypeptides, or a 500 kD complex of six C4bp-alpha polypeptides and one C4bp-beta polypeptide. Each C4b polypeptide comprises a binding site for C4b.

[0057] As used herein, the term "biologically-active" fragment of C4bp-alpha protein refers to refers to any soluble fragment of C4bp-alpha, including fragments comprising, consisting essentially of or consisting of 1, 2, 3, 4, 5, 6, 7, or 8 SCR domains, including any fragments of the full-length C4bp-alpha protein having some or all the complement inhibitory activity of the full-length C4bp-alpha protein. As used herein, the term "biologically-active" fragment of C4bp-beta protein refers to refers to any soluble fragment of C4bp-beta, including fragments comprising, consisting essentially of or consisting of 1, 2, or 3 SCR domains, including any fragments of the full-length C4bp-beta protein having some or all the complement inhibitory activity of the full-length C4bp-beta protein. As used herein, the term "biologically active" fragment of C4bp refers to any soluble fragment of either C4bp-alpha or C4bp-beta having some or all of the complement inhibitory activity of the full-length C4bp-alpha or C4bp-beta protein and retaining the ability to bind C4, C4b, iC4b, or C4d.

[0058] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter and comprise a CR2-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter and comprise a CR2-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter and comprise a CR2-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter and comprise a CR2-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO

nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter and comprise a CR2-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter and comprise a CR2-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter and comprise a CR2-targeting group attached to the phospholipid coating. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In any of the embodiments described herein, the phospholipid is PEGylated. In any of the embodiments described herein, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a CR2-targeting group. In any of the embodiments described herein, the functional group is an amine. In any of the embodiments described herein, the functional group is maleimide. In any of the embodiments described herein, the functional group is a thiol. In any of the embodiments described herein, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In any of the embodiments described herein, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In any of the embodiments described herein, the phospholipid comprises DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0059] In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are

about 75 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the CR2-targeted USPIO nanoparticle aggregates are about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the CR2-targeted USPIO nanoparticle aggregates have a circulating plasma half-life of between about 20 minutes and about 40 minutes, and have a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0060] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter and comprise a CR2-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter and comprise a CR2-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter and comprise a CR2-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter and comprise a CR2-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter and comprise a CR2-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter and comprise a CR2-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter and comprise a CR2-targeting group attached to the dextran coating.

[0061] In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter and comprise a bacterial-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter and comprise a bacterial-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are

between about 10 nm and about 100 nm in diameter and comprise a bacterial-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter and comprise a bacterial-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter and comprise a bacterial-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter and comprise a bacterial-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter and comprise a bacterial-targeting group attached to the phospholipid coating. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In any of the embodiments described herein, the phospholipid is PEGylated. In any of the embodiments described herein, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a bacterial-targeting group. In any of the embodiments described herein, the functional group is an amine. In any of the embodiments described herein, the functional group is maleimide. In any of the embodiments described herein, the functional group is a thiol. In any of the embodiments described herein, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In any of the embodiments described herein, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In any of the embodiments described herein, the phospholipid comprises DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises maleimide-functionalized DSPE-PEG2000. In any of the embodiments described herein, the USPIO nanoparticle aggregates are bacterial-targeted, phospholipid-encapsulated, have a circulating plasma half-life of between about 20 minutes and about 40 minutes, and have a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0062] In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter and comprise a bacterial-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter and comprise a bacterial-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter and comprise a bacterial-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter and comprise a bacterial-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter and comprise a bacterial-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter and comprise a bacterial-targeting group attached to the dextran coating. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter and comprise a bacterial-targeting group attached to the dextran coating. In any of the embodiments described herein, the bacterial-targeting group is *S. aureus N315* Sbi protein. In any of the embodiments described herein, the bacterial-targeting group is a biologically-active fragment of *S. aureus N315* Sbi protein comprising the first four N-terminal domains (Sbi-I-II-III-IV) of the full-length Sbi protein or comprising the third and fourth N-terminal domains (Sbi-III-IV) of the full-length Sbi protein.

[0063] In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter and comprise a C4-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter and comprise a C4-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter and comprise a C4-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter and comprise a C4-targeting group attached to the phospholipid coating. In certain

embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter and comprise a C4-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter and comprise a C4-targeting group attached to the phospholipid coating. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter and comprise a C4-targeting group attached to the phospholipid coating. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In any of the embodiments described herein, the phospholipid is PEGylated. In any of the embodiments described herein, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a C4-targeting group. In any of the embodiments described herein, the functional group is an amine. In any of the embodiments described herein, the functional group is maleimide. In any of the embodiments described herein, the functional group is a thiol. In any of the embodiments described herein, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In any of the embodiments described herein, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In any of the embodiments described herein, the phospholipid comprises DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises amine-functionalized DSPE-PEG2000. In any of the embodiments described herein, the phospholipid comprises maleimide-functionalized DSPE-PEG2000. In any of the embodiments described herein, the USPIO nanoparticle aggregates are C4-targeted, phospholipid-encapsulated, have a circulating plasma half-life of between about 20 minutes and about 40 minutes, and have a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0064] In certain embodiments, the C4-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the C4-targeted USPIO

nanoparticle aggregates are between about 65 nm and about 85 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the C4-targeted USPIO nanoparticle aggregates are about 75 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the C4-targeted USPIO nanoparticle aggregates are about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1.

[0065] In certain embodiments, the C4-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the C4-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the C4-targeted USPIO nanoparticle aggregates are about 75 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the C4-targeted USPIO nanoparticle aggregates are about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1.

[0066] As used herein, the term "C4-targeted nanoparticle" refers to a nanoparticle, or to aggregates of nanoparticles, between about 1 nm and about 1000 nm in diameter to which C4-targeting groups such as peptides, polypeptides, or proteins that bind cell-bound breakdown fragments of complement protein C4, such as C4b (SEQ ID NO:26), iC4b (SEQ ID NO:25), and C4d (SEQ ID NO:27) have been attached, either directly or through a

peptide, polypeptide, protein or other linker. Such peptides, polypeptides, or proteins include, for example, full-length CR1 protein or biologically-active fragments thereof,, full-length C4bp protein or biologically-active fragments thereof, and the like. Peptide, polypeptide, or protein linkers can include, for example, antibodies or antibody fragments, receptors or receptor fragments, and the like. The nanoparticles or aggregates thereof may be coated with a wide variety of materials, including natural or synthetic polymers, surfactants, or inorganic materials, any of which may be modified or derivatized to permit attachment of, for example, bacterial-targeting groups such as peptides, polypeptides, or proteins as discussed above, either directly or via a linker of some kind, or uncoated. Possible coatings include synthetic polymers, such as those based on poly(ethylene-*co*-vinyl acetate), polyvinylpyrrolidone ("PVP"), poly(lactic-*co*-glycolic acid) ("PLGA"), polyethylene glycol ("PEG"), polyvinyl alcohol ("PVA"), polyacrylic acid, and the like; natural polymers, such as gelatin, dextran, chitosan, pullulan, and the like; surfactants, such as sodium oleate, dodecylamine, sodium carboxymethylcellulose, and the like; inorganic materials, such as gold or silica; and biological materials, such as phospholipids. The nanoparticles may comprise contrast-enhancing agents for diagnostic or medical imaging, such as USPIO nanoparticles or aggregates thereof, or may comprise liposomes or other delivery vehicles containing smaller contrast-enhancing agents, such as gadolinium chelate ("Gd-chelates") molecules. The C4-targeted nanoparticles described herein can be formulated as pharmaceutical compositions as disclosed herein, and, when so formulated can be used in any of the methods of non-invasive imaging described herein.

[0067] As used herein, the term "bacterial-targeted nanoparticle" refers to a nanoparticle, or to aggregates of nanoparticles, between about 1 nm and about 1000 nm in diameter to which bacterial-targeting groups such as peptides, polypeptides, or proteins that bind cell-bound breakdown fragments of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), and C3d (SEQ ID NO:10) have been attached, either directly or through a peptide, polypeptide, protein or other linker. Such peptides, polypeptides, or proteins include, for example, full-length *S. aureus N315* Sbi protein or biologically-active fragments thereof, and the like. As discussed herein, biologically-active fragments of Sbi protein include, for example, a fragment comprising the first four N-terminal domains (Sbi-I-II-III-IV) of the full-length Sbi protein or a fragment comprising the third and fourth N-terminal domains (Sbi-III-IV) of the full-length Sbi protein. Peptide, polypeptide, or protein linkers can include, for example, antibodies or antibody fragments, receptors or receptor fragments, and the like. The nanoparticles or aggregates thereof may be

coated with a wide variety of materials, including natural or synthetic polymers, surfactants, or inorganic materials, any of which may be modified or derivatized to permit attachment of, for example, bacterial-targeting groups such as peptides, polypeptides, or proteins as discussed above, either directly or via a linker of some kind, or uncoated. Possible coatings include synthetic polymers, such as those based on poly(ethylene-*co*-vinyl acetate), polyvinylpyrrolidone ("PVP"), poly(lactic-*co*-glycolic acid) ("PLGA"), polyethylene glycol ("PEG"), polyvinyl alcohol ("PVA"), polyacrylic acid, and the like; natural polymers, such as gelatin, dextran, chitosan, pullulan, and the like; surfactants, such as sodium oleate, dodecylamine, sodium carboxymethylcellulose, and the like; inorganic materials, such as gold or silica; and biological materials, such as phospholipids. The nanoparticles may comprise contrast-enhancing agents for diagnostic or medical imaging, such as USPIO nanoparticles or aggregates thereof, or may comprise liposomes or other delivery vehicles containing smaller contrast-enhancing agents, such as gadolinium chelate ("Gd-chelates") molecules.

[0068] As used herein, the term "complement receptor 2," "CR2," "CD21," or "CR2/CD21" refers to a transmembrane protein of ~145 kilodaltons ("kD") typically expressed on B cells, follicular dendritic cells, and some T cell subtypes, or fragments thereof (e.g. biologically-active fragments thereof). *See* e.g., J. Hannan et al., 2002, *Biochem. Soc. Trans.* **30**:983-989; K.A. Young et al., 2007, *J. Biol. Chem.* **282**(50):36614-36625. CR2 is a member of the structural family of C3/C4 receptor and regulatory proteins known as the regulators of complement activation ("RCA"). Members of this family are characterized by the presence of short repeating domains of ~70 amino acids known as short consensus repeat ("SCR") modules. Each SCR contains a number of conserved amino acid residues, including four cysteines and an invariant tryptophan residue. The conserved cysteine residues form a pattern of disulfide bridges that connect Cys-1 to Cys-3 and Cys-2 to Cys-4. The modular composition of CR2 is well known, and consists of a 15- or 16-SCR extracellular domain, a 24-amino acid transmembrane domain, and a short 34-amino acid intracellular carboxy-terminal tail. Biologically-active fragments of CR2 include any fragment of CR2 capable of binding a CR2 ligand, such as, for example, the complete extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2, a fragment comprising SCRs 1 to 8 of human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2, or a fragment comprising SCRs 1 to 2 of human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2. All known CR2 ligands bind within the first two amino-terminal SCR domains (SCR 1 and SCR 2) at overlapping but distinguishable binding sites, although an additional glycosylation-dependent interaction with

CD23 also involves SCRs 3 to 5. K.A. Young et al., 2007, *J. Biol. Chem.* **282**(50):36614-36625. A "biologically active" fragment of CR2 protein retains the ability to bind one or more CR2 ligands, for example, such as iC3b, C3dg, and C3d, cell-bound breakdown fragments of complement protein C3 that bind to the two N-terminal SCR domains of CR2 (SCRs 1 and 2), EBV gp350/220, IFNα, and CD23.

[0069] As used herein, the term "CR2 ligand" refers to ligands (e.g. natural ligands) capable of binding to the extracellular domain of CR2. Natural ligands for CR2 include, for example, iC3b, C3dg, and C3d, cell-bound breakdown fragments of complement protein C3 that bind to the two N-terminal SCR domains of CR2 (SCR 1 and 2). *See* e.g., K. Iida et al., 1983, *J. Exp. Med.* **158**:1021-1033. Cleavage of C3 results initially in the generation of C3b and the covalent attachment of this C3b to the activating cell surface. The C3b fragment is involved in the generation of enzymatic complexes that amplify the complement cascade. On a cell surface, C3b (SEQ ID NO:13) is rapidly converted to inactive iC3b (SEQ ID NO:12), particularly when deposited on a host surface containing regulators of complement activation (*i.e.*, most host tissue). Even in the absence of membrane-bound complement regulators, substantial levels of iC3b (SEQ ID NO:12) are formed. iC3b (SEQ ID NO:12) is subsequently digested to the membrane bound fragments C3dg (SEQ ID NO:11) and then C3d (SEQ ID NO:10) by serum proteases, but this process is relatively slow. Thus, the C3 ligands for CR2 are relatively long lived once they are generated and are present in high concentrations at sites of complement activation. CR2 therefore can serve as a potent targeting vehicle for bringing molecules to sites of complement activation.

[0070] Other CR2 ligands include the Epstein-Barr Virus 350 kilodalton surface glycoprotein ("EBV gp350") or its truncated 220 kilodalton form (collectively "EBV gp350/220") (K.A. Young et al., 2007, *J. Biol. Chem.* **282**(50):36614-36625), interferon alpha ("IFN-α") (R. Asokan et al., 2006, *J. Immunol.* **177**(1):383-394), and CD23 (J.P. Aubry et al., 1992, *Nature* **358**:505-507; and J.P. Aubry et al., 1994, *J. Immunol.* **152**:5806-5813). Also known as FcεRII, CD23 is a low affinity receptor for immunoglobulin E ("IgE"), an antibody isotype involved in allergy and resistance to parasites. Unlike many of the antibody Fc receptors, CD23 is a C-type lectin found on mature B cells, activated macrophages, eosinophils, follicular dendritic cells and platelets.

[0071] As used herein, the term "specifically binds to" or "selectively binds to" refers to the specific binding of one protein to another (*e.g.*, an antibody or antigen-binding fragment thereof to an antigen, or a receptor to a ligand), wherein the level of binding, as measured by

any standard assay (*e.g.*, an immunoassay), is statistically significantly higher than the background control for the assay. For example, when performing an immunoassay, controls typically include a reaction well or tube that contains an antibody or antigen-binding fragment thereof alone (*i.e.*, in the absence of antigen), wherein an amount of reactivity (*e.g.*, non-specific binding to the well) by the antibody or antigen-binding fragment thereof in the absence of the antigen is considered to be background. Binding can be measured using a variety of methods standard in the art, including, but not limited to: Western blot, immunoblot, enzyme-linked immunosorbant assay ("ELISA"), radioimmunoassay ("RIA"), immunoprecipitation, surface plasmon resonance, chemiluminescence, fluorescent polarization, phosphorescence, immunohistochemical analysis, matrix-assisted laser desorption/ionization time-of-flight ("MALDI-TOF") mass spectrometry, microcytometry, microarray, microscopy, fluorescence activated cell sorting ("FACS"), and flow cytometry.

[0072] As used herein, the term "CR2-targeted nanoparticle" or "CR2-targeted nanoparticle aggregate" refers to nanoparticles or aggregates thereof between about 1 nm and about 1000 nm in diameter to which CR2-targeting groups such as peptides, polypeptides, or proteins (e.g., antibodies or fragments thereof) that bind C3 or fragments thereof (e.g. functional fragments thereof or cell-bound breakdown fragments of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), and C3d (SEQ ID NO:10)) have been attached, either non-covalently or covalently (e.g. directly or through a peptide, polypeptide, protein or other linker). Such peptides, polypeptides, or proteins include, for example, CR2 proteins or biologically-active fragments thereof, antibodies and antibody fragments, and the like. As discussed herein, biologically-active fragments of CR2 protein include, for example, the complete extracellular domain of CR2, a fragment comprising SCRs 1 to 8 of CR2, or a fragment comprising SCRs 1 to 2 of CR2. SCRs are numbered from the amino- to the carboxy-terminus of the CR2 protein, so a CR2 fragment comprising SCRs 1 and 2 of CR2 contains the first two SCR domains at the amino-terminus of the full-length CR2 protein, while a fragment comprising SCRs 1 to 8 of CR2 contains the first eight SCR domains at the amino-terminus of the full-length CR2 protein. Peptide, polypeptide, or protein linkers can include, for example, antibodies or antibody fragments, receptors or receptor fragments, and the like. The nanoparticles may be coated with a wide variety of materials, including natural or synthetic polymers, surfactants, or inorganic materials, any of which may be modified or derivatized to permit attachment of, for example, CR2-targeting groups such as peptides, polypeptides, or proteins as discussed above, either directly or via a linker of some kind, or uncoated. Possible coatings include synthetic

polymers, such as those based on poly(ethylene-*co*-vinyl acetate), polyvinylpyrrolidone ("PVP"), poly(lactic-*co*-glycolic acid) ("PLGA"), polyethylene glycol ("PEG"), polyvinyl alcohol ("PVA"), polyacrylic acid, and the like; natural polymers, such as gelatin, dextran, chitosan, pullulan, and the like; surfactants, such as sodium oleate, dodecylamine, sodium carboxymethylcellulose, and the like; inorganic materials, such as gold or silica; and biological materials, such as phospholipids. The nanoparticles may comprise contrast-enhancing agents for diagnostic or medical imaging, such as USPIO nanoparticles or aggregates thereof, or may comprise liposomes or other delivery vehicles containing smaller contrast-enhancing agents, such as gadolinium chelate ("Gd-chelates") molecules.

[0073] As used herein, the term "ultrasmall superparamagnetic iron oxide nanoparticle" or "USPIO nanoparticle" refers to superparamagnetic iron oxide particles ranging from 1 to 50 nm in diameter, more typically between 5 and 40 nm in diameter (excluding any coating applied after synthesis). USPIO nanoparticles are commonly made of maghemite (Fe_2O_3) or magnetite (Fe_3O_4) having crystal-containing regions of unpaired spins. Those magnetic domains are disordered in the absence of a magnetic field, but when a field is applied (*i.e.*, while taking an MRI), the magnetic domains align to create a magnetic moment much greater than the sum of the individual unpaired electrons without resulting in residual magnetization of the particles. When injected into the blood stream, USPIO nanoparticles are taken up by macrophages and accumulate in inflamed tissues. Their iron moiety negatively enhances signal attenuation on T_2-weighted images, and their relative concentrations can be assessed by decreased T_2-signal intensity or, more precisely, by decreased spin-spin T_2-relaxation time. The decreased T_2-relaxation time (the transverse relaxation time) can thus be used to detect inflammation. The shortened T_2 relaxation time results in a darkening of the magnetic resonance image where the particles are located, thereby generating "negative contrast." This approach has been successfully utilized to detect renal inflammation in several models. In some cases, USPIO nanoparticles may be aggregated after synthesis to produce aggregates thereof (referred to herein as "ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticle aggregates" or "USPIO nanoparticle aggregates") of 25 nm, 50 nm, 75 nm, 100 nm, or 150 nm, in diameter, or even larger.

[0074] The USPIO nanoparticles or aggregates thereof may be coated with a wide variety of materials, including natural or synthetic polymers, surfactants, phospholipids, or inorganic materials, any of which may be modified or derivatized to permit attachment of targeting groups, either directly or via different types of linkers, including peptides, polypeptides, proteins, or other chemical groups, or uncoated. Possible coatings include synthetic polymers,

such as those based on poly(ethylene-*co*-vinyl acetate), polyvinylpyrrolidone ("PVP"), poly(lactic-*co*-glycolic acid) ("PLGA"), polyethylene glycol ("PEG"), polyvinyl alcohol ("PVA"), polyacrylic acid, and the like; natural polymers, such as gelatin, dextran, chitosan, pullulan, and the like; surfactants, such as sodium oleate, dodecylamine, sodium carboxymethylcellulose, and the like; inorganic materials, such as gold or silica; and biological materials, such as phospholipids.

[0075] The term "CR2-targeted ultrasmall superparamagnetic iron oxide nanoparticles or aggregates thereof" or "CR2-targeted USPIO nanoparticles or aggregates thereof" refers to USPIO nanoparticles or aggregates thereof to which CR2-targeting groups such as peptides, polypeptides, or proteins (e.g., antibodies or fragments thereof) that bind cell-bound breakdown fragments of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), and C3d (SEQ ID NO:10) have been attached, either non-covalently or covalently (e.g., directly or through a peptide, polypeptide, protein or other linker). Such peptides, polypeptides, or proteins include, for example, CR2 proteins or biologically-active fragments thereof, antibodies and antibody fragments, and the like. As discussed herein, biologically-active fragments of CR2 protein include, for example, the complete extracellular domain of CR2, a fragment comprising SCRs 1 to 8 of CR2, or a fragment comprising SCRs 1 to 2 of CR2. SCRs are numbered from the amino- to the carboxy-terminus of the CR2 protein, so a CR2 fragment comprising SCRs 1 and 2 of CR2 contains the first two SCR domains at the amino-terminus of the full-length CR2 protein, while a fragment comprising SCRs 1 to 8 of CR2 contains the first eight SCR domains at the amino-terminus of the full-length CR2 protein. Peptide, polypeptide, or protein linkers can include, for example, antibodies or antibody fragments, receptors or receptor fragments, and the like.

[0076] The term "bacterial-targeted ultrasmall superparamagnetic iron oxide nanoparticles or aggregates thereof" or "bacterial-targeted USPIO nanoparticles or aggregates thereof" refers to USPIO nanoparticles or aggregates thereof to which bacterial-targeting groups such as peptides, polypeptides, or proteins that bind cell-bound breakdown fragments of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), and C3d (SEQ ID NO:10) have been attached, either directly or through a peptide, polypeptide, protein or other linker. Such peptides, polypeptides, or proteins include, for example, full-length *S. aureus N315* Sbi proteins or biologically-active fragments thereof, and the like. As discussed herein, full-length *S. aureus N315* Sbi protein or biologically-active fragments thereof, and the like. As discussed herein, biologically-active fragments of

S. aureus N315 Sbi protein include, for example, a fragment comprising the first four N-terminal domains (Sbi-I-II-III-IV) of the full-length Sbi protein or a fragment comprising the third and fourth N-terminal domains (Sbi-III-IV) of the full-length Sbi protein. Peptide, polypeptide, or protein linkers can include, for example, antibodies or antibody fragments, receptors or receptor fragments, and the like.

[0077] As used herein, the term "antibody" or "antibody fragment" refers to a class of proteins or fragments thereof with the ability to specifically or selectively bind particular antigens. Antibodies contain immunoglobulin (Ig) domains and are members of the Ig superfamily of proteins. Generally, an antibody molecule comprises two types of chains: a heavy or H chain, and a light or L chain. The light chain contains a variable domain (V_L) and a constant domain (C_L), while the heavy chain contains a variable domain (V_H) and three constant domains (C_H1, C_H2, and C_H3), with the C_H1 and C_H2 domains separated by a hinge region. The distinctive characteristics of each isotype are defined by sequences in the constant domain of the immunoglobulin. Each antibody molecule typically contains two H chains and two L chains. The two H chains are linked together by disulfide bonds and each H chain is linked to an L chain by a disulfide bond. There are only two types of L chains referred to as lambda (λ) and kappa (κ) chains. In contrast, there are five major H chain classes, referred to as isotypes. The five classes include IgM (μ), IgD (δ), IgG (λ), IgA (α), and IgE (or ε). Human immunoglobulin molecules comprise nine isotypes: IgM, IgD, IgE, four subclasses of IgG including IgG_1 (γ_1), IgG_2 (γ_2), IgG_3 (γ_3) and IgG_4 (γ_4), and two subclasses of IgA including IgA_1 (α_1) and IgA_2 (α_2).

[0078] Together, one H chain and one L chain form an arm of an immunoglobulin molecule having an immunoglobulin variable region. A complete immunoglobulin molecule comprises two di-sulfide linked arms. Thus, each arm of a whole immunoglobulin comprises a V_{H+L} region, and a C_{H+L} region. As used herein, the "variable region" or "V region" refers to a V_{H+L} region (also known as an Fv fragment), a V_L region, or a V_H region of an Ig protein. Also as used herein, the term "constant region" or "C region" refers to a C_{H+L} region, a C_L region or a C_H region.

[0079] Limited digestion of an Ig protein with different proteases produces a number of fragments, only some of which retain the capacity to bind antigen. The antigen-binding fragments are referred to as Fab, Fab', or $F(ab')_2$ fragments. A fragment lacking the ability to bind to antigen is referred to as an Fc fragment. An Fab fragment comprises one arm of an immunoglobulin molecule containing an L chain (V_L+C_L domains) paired with the V_H region

and the C_H1 region. An Fab' fragment corresponds to an Fab fragment with part of the hinge region attached to the C_H1 domain. An $F(ab')_2$ fragment corresponds to two Fab' fragments that are normally covalently linked to each other through a disulfide bond, typically in the hinge region.

[0080] Antibodies or antibody fragments may also be humanized antibodies. Humanized antibodies are molecules having an antigen-binding site derived from an immunoglobulin from a non-human species, the remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin, in order to reduce immunogenicity of the protein. The antigen-binding site may comprise either complete variable regions fused onto human constant domains or only the complementarity determining regions (CDRs) grafted onto appropriate human framework regions in the variable domains. Humanized antibodies can be produced, for example, by modeling the antibody variable domains and producing the antibodies using genetic engineering techniques, such as CDR grafting. A description of various techniques for the production of humanized antibodies is found, for example, in Morrison et al., 1984, *Proc. Nat'l Acad. Sci. USA* **81**:6851-55; Whittle et al., 1987, *Prot. Eng.* **1**:499-505; Co et al., 1990, *J. Immunol.* **148**:1149-1154; Co et al., 1992, *Proc. Nat'l Acad. Sci. USA* **88**:2869-2873; Carter et al., 1992, *Proc. Nat'l Acad. Sci. USA* **89**:4285-4289; Routledge et al., 1991, *Eur. J. Immunol.* **21**:2717-2725 and PCT Patent Publication Nos. WO 91/09967; WO 91/09968 and WO 92/113831.

[0081] Whole antibodies can be polyclonal or monoclonal. Alternatively, functional equivalents of whole antibodies, such as antigen-binding fragments in which one or more antibody domains are truncated or absent (*e.g.,* Fv, Fab, Fab', or $F(ab)_2$ fragments), as well as genetically-engineered antibodies or antigen-binding fragments thereof, including single chain antibodies, humanized antibodies (discussed above), antibodies that can bind to more than one epitope (*e.g.*, bi-specific antibodies), or antibodies that can bind to one or more different antigens (*e.g.*, bi- or multi-specific antibodies), may also be used as targeting groups.

[0082] Methods of producing polyclonal antibodies that specifically or selectively bind to a particular antigen are known in the art. Generally, in the production of an antibody, a suitable experimental animal, such as, for example, but not limited to, a rabbit, a sheep, a hamster, a guinea pig, a mouse, a rat, or a chicken, is exposed to an antigen against which an antibody is desired. Typically, an animal is immunized with an effective amount of antigen that is injected into the animal. An effective amount of antigen refers to an amount needed to induce antibody production by the animal. The animal's immune system is then allowed to respond

over a pre-determined period of time. The immunization process can be repeated until the immune system is found to be producing antibodies to the antigen. In order to obtain polyclonal antibodies specific for the antigen, serum is collected from the animal that contains the desired antibodies (or in the case of a chicken, antibody can be collected from the eggs). Such serum is useful as a reagent. Polyclonal antibodies can be further purified from the serum (or eggs) by, for example, treating the serum with ammonium sulfate to precipitate the antibodies.

[0083] Methods of producing monoclonal antibodies that specifically or selectively bind to a particular antigen are known in the art. For example, monoclonal antibodies may be produced according to the methodology of Kohler and Milstein (Kohler & Milstein, 1975, *Nature* **256**:495-497). For example, B lymphocytes are recovered from the spleen (or any suitable tissue) of an immunized animal and then fused with myeloma cells to obtain a population of hybridoma cells capable of continual growth in suitable culture medium. Hybridomas producing the desired antibody are selected by testing the ability of the antibody produced by the hybridoma to bind to the desired antigen, for example in an enzyme-linked immunosorbent assay or other routine method known in the art.

[0084] In certain embodiments described herein, the targeting group is selected from the group consisting of full-length human (SEQ ID NO:1) or mouse (SEQ ID NO:2) CR2 protein, the extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2 protein, the CR2 fragment comprising SCRs 1 to 8 of the full-length human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2 protein, and the CR2 fragment comprising SCRs 1 and 2 of the full-length human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein. In certain embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first two amino-terminal SCR domains of human or mouse CR2, including for example at least any of the first 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 SCR domains of CR2. In certain embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first two amino-terminal SCR domains of human or mouse CR2, including for example at least any of the first 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 SCR domains of CR2, fused to the Fc domain of a human or mouse Ig protein. In certain embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first two amino-terminal SCR domains of human or mouse CR2, including for example at least any of the first 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 SCR domains of CR2, fused to the Fc domain of human or mouse IgG_1 protein. In certain

embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first two amino-terminal SCR domains of human or mouse CR2, including for example at least any of the first 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 SCR domains of CR2, fused to the Fc domain of mouse IgG_1 protein. In certain embodiments described herein, the targeting group comprises an antibody directed to C3 or fragment thereof, including but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d.

[0085] As discussed above, CR2 may contain an extracellular portion having 15 or 16 repeating units known as short consensus repeats, or SCR domains. The SCR domains have a framework of highly conserved residues including four cysteines, two prolines, one tryptophan and several other partially conserved glycines and hydrophobic residues. SEQ ID NO:1 represents the full-length human CR2 protein sequence. In human CR2 protein (SEQ ID NO:1), amino acids 1-20 comprise the leader peptide, amino acids 23-82 comprise SCR1, amino acids 91-146 comprise SCR2, amino acids 154-210 comprise SCR3, amino acids 215-271 comprise SCR4. The C3d binding site is located in SCRs1 and 2 (the first two N-terminal SCR domains). The SCR domains are separated by short sequences of variable length that serve as spacers. The full-length mouse CR2 protein sequence is represented herein by SEQ ID NO:2. In mouse CR2 protein (SEQ ID NO:2), amino acids 14-73 comprise SCR1 and amino acids 82-138 comprise SCR2. Human and mouse CR2 are approximately 66% identical over the full length amino acid sequences represented by SEQ ID NO:1 and SEQ ID NO:2, and approximately 61% identical over the SCR1-SCR2 regions of SEQ ID NO:1 and SEQ ID NO:2. Both mouse and human CR2 bind to C3 in the C3d region. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that the CR2 protein or biologically-active fragments thereof described herein encompass all species and strain variations.

[0086] In some embodiments, the CR2 portion disclosed herein refers to a polypeptide that contains some or all of the ligand binding sites of the CR2 protein, and includes, but is not limited to, full-length CR2 proteins (such as human CR2 as shown in SEQ ID NO:1 or mouse CR2 as shown in SEQ ID NO:2), soluble CR2 proteins (such as a CR2 fragment comprising the complete extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2 protein), other biologically-active fragments of CR2 protein, such as a CR2 fragment comprising SCRs 1 to 8 of human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2 protein, a

CR2 fragment comprising SCR1 and SCR2 of human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein, or any homologue of a naturally occurring CR2 protein or biologically-active fragment thereof, as described in detail below. In some embodiments, the CR2 portion has one of the following properties of CR2: (1) binding to C3d (SEQ ID NO:10), (2) binding to iC3b (SEQ ID NO:12), (3) binding to C3dg (SEQ ID NO:11), and (4) binding to cell-bound breakdown fragment(s) of complement protein C3 that bind to the two N-terminal SCR domains of CR2 (SCRs 1 and 2).

[0087] A homologue of a CR2 protein or a fragment thereof includes proteins which differ from a naturally occurring CR2 protein (or a biologically-active fragment of a CR2 protein) in that at least one or a few amino acids have been deleted (*e.g.*, a truncated version of the protein, such as a peptide, polypeptide or other fragment), inserted, inverted, substituted and/or derivatized (*e.g.*, by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition of glycosylphosphatidyl inositol). In some embodiments, a CR2 homologue has an amino acid sequence that is at least about 70% identical to the amino acid sequence of a naturally occurring CR2 protein (*e.g.*, SEQ ID NO:1 or SEQ ID NO:2), for example at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a naturally occurring CR2 protein (*e.g.*, SEQ ID NO:1, or SEQ ID NO:2). A CR2 homologue or a biologically-active fragment thereof preferably retains the ability to bind to a naturally occurring ligand of CR2 protein (*e.g.*, C3d or other C3 fragments with CR2-binding ability). For example, the CR2 homologue (or biologically-active fragment thereof) may have a binding affinity for C3d that is at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of that of full-length CR2 (or a biologically-active fragment thereof).

[0088] In certain embodiments, the CR2 portion comprises at least the first two N-terminal SCR domains of a human CR2 protein (SEQ ID NO:7), such as a CR2 portion having an amino acid sequence containing at least amino acids 23 through 146 of the human CR2 protein. In certain embodiments, the CR2 portion comprises at least the first two SCR domains of human CR2 protein (SEQ ID NO:7) having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to amino acids 23 through 146 of the full-length human CR2 protein (SEQ ID NO:1).

[0089] An amino acid sequence that is at least about, for example, 95% identical to a reference sequence (such as full-length human CR2 protein, *i.e.*, SEQ ID NO:1) is identical to the reference sequence except that the amino acid sequence may include up to five point alterations per each 100 amino acids of the reference sequence. These up to five point alterations may be deletions, substitutions, or additions, and may occur anywhere in the sequence, interspersed either individually among amino acids in the reference sequence or in one or more continuous groups within the reference sequence.

[0090] In certain embodiments, the CR2 portion comprises part or all of the ligand-binding sites of the CR2 protein. In certain embodiments, the CR2 portion further comprises sequences required to maintain the three dimensional structure of the ligand-binding site. Ligand-binding sites of CR2 protein can be readily determined based on the crystal structure of CR2 protein, such as the human CR2 protein crystal structure disclosed in U.S. Patent No. 6,820,011. For example, in certain embodiments, the CR2 portion comprises the B strand and B-C loop of SCR2 of CR2. In certain embodiments, the CR2 portion comprises a site on strand B and the B-C loop of CR2 SCR comprising the segment G98-G99-Y100-K101-I102-R103-G104-S105-T106-P107-Y108 with respect to SEQ ID NO:1. In certain embodiments, the CR2 portion comprises a site on the B strand of CR2 SCR2 comprising position K119 with respect to SEQ ID NO:1. In certain embodiments, the CR2 portion comprises a segment comprising V149-F150-P151-L152, with respect to SEQ ID NO:1. In certain embodiments, the CR2 portion comprises a segment of CR2 SCR2 comprising T120-N121-F122. In some embodiments, the targeting group has two or more of these sites. For example, in certain embodiments, the CR2 portion comprises a portion comprising G98-G99-Y100-K101-I102-R103-G104-S105-T106-P107-Y108 and K119 with respect to SEQ ID NO:1. Other combinations of these sites are also contemplated herein.

[0091] In certain embodiments described herein, the targeting group is selected from the group consisting of full-length *S. aureus N315* Sbi protein (SEQ ID NO:20), a biologically-active fragment of *S. aureus N315* Sbi protein comprising the first four N-terminal domains (Sbi-I-II-III-IV) of the full-length Sbi protein (SEQ ID NO:21), or a biologically-active fragment of *S. aureus N315* Sbi protein comprising the third and fourth N-terminal domains (Sbi-III-IV) of the full-length Sbi protein (SEQ ID NO:22).

[0092] In certain embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first four amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-I-II-III-IV), including for example at least third and fourth amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-III-IV). In certain

embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first four amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-I-II-III-IV), including for example at least third and fourth amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-III-IV), fused to the Fc domain of a human or mouse Ig protein. In certain embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first four amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-I-II-III-IV), including for example at least third and fourth amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-III-IV), fused to the Fc domain of human or mouse IgG_1 protein. In certain embodiments described herein, the targeting group comprises (and in some embodiments consists of or consists essentially of) at least the first four amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-I-II-III-IV), including for example at least third and fourth amino-terminal domains of *S. aureus N315* Sbi protein (Sbi-III-IV), fused to the Fc domain of mouse IgG_1 protein.

[0093] As discussed above, *S. aureus N315* contains an amino-terminal portion of about 266 amino acids, organized into four conserved structural domains. SEQ ID NO:20 represents the full-length *S. aureus N315* Sbi protein sequence. In *S. aureus N315* Sbi protein (SEQ ID NO:20), amino acids 42-94 comprise the first Sbi domain (Sbi-I), amino acids 92-156 comprise the second Sbi domain (Sbi-II), amino acids 150-205 comprise the third Sbi domain (Sbi-III), and amino acids 198-266 comprise the fourth Sbi domain (Sbi-IV). Thus, a biologically-active fragment of *S. aureus N315* Sbi protein having the first four Sbi domains (Sbi-I-II-III-IV) comprises amino acids 28-266 of SEQ ID NO:20, and a biologically-active fragment of *S. aureus N315* Sbi protein having the third and fourth Sbi domains (Sbi-III-IV) comprises amino acids 150-266 of SEQ ID NO:20. The Sbi-I-II fragment contains binding sites for IgG. The Sbi-III-IV fragment contains binding sites for proteolytic fragments of complement protein C3, such as, for example, C3dg and C3a. It is understood that species and strain variations exist for the disclosed peptides, polypeptides, and proteins, and that the *S. aureus N315* Sbi protein or biologically-active fragments thereof described herein encompass all species and strain variations.

[0094] The *S. aureus N315* Sbi portion disclosed herein refers to a polypeptide that contains some or all of the ligand binding sites of the Sbi protein, and includes, but is not limited to, full-length Sbi proteins (such as *S. aureus N315* Sbi protein as shown in SEQ ID NO:20), or other biologically-active fragments of *S. aureus N315* Sbi protein, such as a fragment of *S. aureus N315* Sbi protein having the first four Sbi domains (Sbi-I-II-III-IV)

comprising amino acids 28-266 of SEQ ID NO:20, or a fragment of *S. aureus N315* Sbi protein having the third and fourth Sbi domains (Sbi-III-IV) comprising amino acids 150-266 of SEQ ID NO:20, or any homologue of a naturally-occurring *S. aureus N315* Sbi protein or biologically-active fragment thereof, as described in detail herein. In some embodiments, the Sbi protein fragment has one of the following properties of CR2: (1) binding to Ig, (2) binding to C3dg, and (3) binding to C3a, and (4) binding to cell-bound breakdown fragment(s) of complement protein C3 that bind to the two N-terminal SCR domains of CR2 (SCRs 1 and 2).

[0095] A homologue of an *S. aureus N315* Sbi protein or a fragment thereof includes proteins which differ from a naturally occurring Sbi protein (or a biologically-active fragment of an Sbi protein) in that at least one or a few amino acids have been deleted (*e.g.*, a truncated version of the protein, such as a peptide, polypeptide or other fragment), inserted, inverted, substituted and/or derivatized (*e.g.*, by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitation, amidation and/or addition of glycosylphosphatidyl inositol). In some embodiments, an *S. aureus N315* Sbi protein homologue has an amino acid sequence that is at least about 70% identical to the amino acid sequence of a naturally occurring *S. aureus N315* Sbi protein (*e.g.*, SEQ ID NO:20), for example at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of a naturally occurring *S. aureus N315* Sbi protein (*e.g.*, SEQ ID NO:20). An *S. aureus N315* Sbi protein homologue or a biologically-active fragment thereof preferably retains the ability to bind to a naturally occurring ligand of Sbi protein (*e.g.*, an IgG protein or proteolytic breakdown products of complement protein C3, such as C3dg, C3a, or other C3 fragments that serve as durable markers of complement activity). For example, the CR2 homologue (or biologically-active fragment thereof) may have a binding affinity for C3dg that is at least about 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of that of full-length *S. aureus N315* Sbi protein (or a biologically-active fragment thereof).

[0096] In certain embodiments, the Sbi protein portion comprises at least the first four N-terminal domains of an *S. aureus N315* Sbi protein (SEQ ID NO:20), such as an Sbi protein portion having an amino acid sequence containing at least amino acids 28 through 266 of the *S. aureus N315* Sbi protein. In certain embodiments, the Sbi protein portion comprises at least the third and fourth N-terminal domains of *S. aureus N315* Sbi protein (SEQ ID NO:20) having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%,

81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identical to amino acids 150 through 266 of the full-length *S. aureus N315* Sbi protein (SEQ ID NO:20).

[0097] An amino acid sequence that is at least about, for example, 95% identical to a reference sequence (such as full-length *S. aureus N315* Sbi protein, *i.e.*, SEQ ID NO:20) is identical to the reference sequence except that the amino acid sequence may include up to five point alterations per each 100 amino acids of the reference sequence. These up to five point alterations may be deletions, substitutions, or additions, and may occur anywhere in the sequence, interspersed either individually among amino acids in the reference sequence or in one or more continuous groups within the reference sequence.

[0098] In another aspect, the invention provides phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof that incorporate a CR2-targeting group that specifically or selectively binds one or more cell-bound breakdown products of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), or C3d (SEQ ID NO:10). In certain embodiments, the CR2-targeting group is selected from the group consisting of full-length human (SEQ ID NO:1) or mouse (SEQ ID NO:2) CR2 protein, the extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2 protein, the CR2 fragment comprising SCRs 1 to 8 of the full-length human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2 protein, or the CR2 fragment comprising SCRs 1 and 2 of the full-length human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein. In certain embodiments, the CR2-targeting group is an antibody directed to C3 or fragment thereof including, but not limited to, C3b, iC3b, C3dg, C3d and the like. In certain embodiments, the CR2-targeting group is modified to incorporate a lipid, glycolipid or phospholipid anchor sufficient to anchor the protein or protein fragment into a lipid mono- or bilayer. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof incorporate a full-length human (SEQ ID NO:1) or mouse (SEQ ID NO:2) CR2 protein modified to incorporate a lipid, glycolipid or phospholipid anchor, an extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2 protein modified to incorporate a lipid, glycolipid or phospholipid anchor, a fragment of human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2 protein comprising SCRs 1 to 8 of the full-length CR2 protein modified to incorporate a lipid, glycolipid or phospholipid anchor, or a fragment of human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein comprising SCRs 1 and 2 of the full-length CR2 protein modified to incorporate a lipid, glycolipid or phospholipid anchor.

[0099] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof incorporate a full-length human (SEQ ID NO:1) or mouse (SEQ ID NO:2) CR2 protein fused to the Fc domain of a mouse IgG_1 protein and modified to incorporate a lipid, glycolipid or phospholipid anchor, an extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2 protein fused to the Fc domain of a mouse IgG_1 protein and modified to incorporate a lipid, glycolipid or phospholipid anchor, a fragment of human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2 protein comprising SCRs 1 to 8 of the full-length CR2 protein fused to the Fc domain of a mouse IgG_1 protein and modified to incorporate a lipid, glycolipid or phospholipid anchor, or a fragment of human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein comprising SCRs 1 and 2 of the full-length CR2 protein fused to the Fc domain of a mouse IgG_1 protein and modified to incorporate a lipid, glycolipid or phospholipid anchor.

[0100] In another aspect, the invention provides phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with a CR2-targeting group that specifically binds one or more cell-bound breakdown products of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11) or C3d (SEQ ID NO:10). In certain embodiments, the CR2-targeting group is selected from the group consisting of the full-length human (SEQ ID NO:1) or mouse (SEQ ID NO:2) CR2 protein, the extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2 protein, a fragment of human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2 protein comprising SCRs 1 to 8 of the full-length CR2 protein, or a fragment of human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein comprising SCRs 1 and 2 of the full-length CR2 protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with a CR2 fragment comprising SCRs 1 and 2 of the full-length CR2 protein.

[0101] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles are derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse

IgG_1 protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1.

[0102] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1.

[0103] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1 (SEQ ID NO:9). In any of the embodiments described herein, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof, have a circulating plasma half-life of between about 20 minutes and about 40 minutes, and have a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0104] In another aspect, the invention provides dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with a targeting group that specifically binds

one or more cell-bound breakdown products of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), or C3d (SEQ ID NO:10). In certain embodiments, the CR2-targeting group is selected from the group consisting of the full-length human (SEQ ID NO:1) or mouse (SEQ ID NO:2) CR2 protein, the extracellular domain of human (SEQ ID NO:3) or mouse (SEQ ID NO:4) CR2 protein, a fragment of human (SEQ ID NO:5) or mouse (SEQ ID NO:6) CR2 protein comprising SCRs 1 to 8 of the full-length CR2 protein, or a fragment of human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein comprising SCRs 1 and 2 of the full-length CR2 protein. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with a CR2 fragment comprising SCRs 1 and 2 of the full-length human (SEQ ID NO:7) or mouse (SEQ ID NO:8) CR2 protein.

[0105] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc-domain of human or mouse IgG_1. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1.

[0106] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc-domain of human or mouse IgG_1. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with

CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1.

[0107] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc-domain of human or mouse IgG_1 (SEQ ID NO:9). In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1 (SEQ ID NO:9).

[0108] In another aspect, the invention provides phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof that incorporate a bacterial-targeting group that specifically or selectively binds one or more cell-bound breakdown products of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), or C3d (SEQ ID NO:10). In certain embodiments, the bacterial-targeting group is selected from the group consisting of full-length *S. aureus N315* Sbi protein (SEQ ID NO:20), a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21), or a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22). In any of the embodiments described herein, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof, have a circulating plasma half-life of between about 20 minutes and about 40 minutes, and have a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0109] In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the phospholipid-encapsulated

bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc-domain of mouse IgG_1.

[0110] In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc-domain of mouse IgG_1.

[0111] In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the phospholipid-

encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc-domain of mouse IgG_1.

[0112] In another aspect, the invention provides dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof that incorporate a bacterial-targeting group that specifically or selectively binds one or more cell-bound breakdown products of complement protein C3, such as C3b (SEQ ID NO:13), iC3b (SEQ ID NO:12), C3dg (SEQ ID NO:11), or C3d (SEQ ID NO:10). In certain embodiments, the bacterial-targeting group is selected from the group consisting of full-length *S. aureus N315* Sbi protein (SEQ ID NO:20), a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21), or a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22).

[0113] In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with bacterial-targeting groups comprising full-length *S. aureus N315* Sbi protein (SEQ ID NO:20) fused to the Fc-domain of mouse IgG_1.

[0114] In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc

domain of a human or a mouse IgG protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (SEQ ID NO:21) fused to the Fc-domain of mouse IgG_1.

[0115] In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth domain of Sbi protein (SEQ ID NO:22) fused to the Fc-domain of mouse IgG_1.

[0116] Also encompassed are variants of the CR2-targeting and bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof). A variant of the CR2-targeting or bacterial-targeting groups described herein may be: (i) one in which one or more of the amino acid residues of the CR2 portion or the Sbi portion is substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code; or (ii) one in which one or more of the amino acid residues in the CR2 portion or the Sbi portion includes a substituent group, or (iii) one in which the CR2-targeting group or bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) is fused with another compound, such as a compound to increase the half-life of the CR2-targeting or bacterial-targeting group (for example,

polyethylene glycol), or (iv) one in which additional amino acids are fused to the CR2-targeting or bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof), such as a leader or secretory sequence or a sequence which is employed for purification of the CR2-targeting or Sbi-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof). Such variants are deemed to be within the scope of those skilled in the art from the teachings herein.

[0117] In certain embodiments, the variant of the CR2-targeting or bacterial-targeting group contains conservative amino acid substitutions (defined further below) made at one or more predicted, preferably nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of a protein without altering the biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[0118] Amino acid substitutions in the CR2 portion or the Sbi portion of the CR2-targeting group or the bacterial-targeting group can be introduced to improve the functionality of the molecule. For example, amino acid substitutions can be introduced into the CR2 portion or the Sbi portion of the molecule to increase binding affinity of the CR2 portion or the Sbi portion to its ligand(s), increase binding specificity of the CR2 portion or the Sbi portion to its ligand(s), improve targeting of the CR2-targeted or bacterial-targeted nanoparticles to desired sites, and improve pharmacokinetics of the CR2-targeting or bacterial-targeting group.

[0119] In certain embodiments, the CR2-targeted group or bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) is fused with another compound, such as a compound to increase the half-life of the polypeptide and/or to reduce potential immunogenicity of the polypeptide (for example, polyethylene glycol, "PEG"). The PEG can be used to impart water solubility, size, slow rate of kidney clearance, and reduced immunogenicity of the CR2-targeting group or the bacterial-targeting

group. *See, e.g.*, U.S. Pat. No. 6,214,966. In the case of PEGylations, the fusion of the CR2-targeting group or the bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) to PEG can be accomplished by any means known to one skilled in the art. For example, PEGylation can be accomplished by first introducing a cysteine mutation into the CR2-targeting group or the bacterial-targeting group, followed by site-specific derivatization with PEG-maleimide. The cysteine can be added to the C-terminus of the CR2-targeting group or the bacterial-targeting group. *See, e.g.*, Tsutsumi et al., 2000, *Proc. Nat'l Acad. Sci. USA* **97**(15):8548-8553. Another modification which can be made to the CR2-targeting group or the bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) involves biotinylation. In certain instances, it may be useful to have the CR2-targeting group or bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) biotinylated so that it can readily react with streptavidin. Methods for biotinylation of proteins are well known in the art. Additionally, chondroitin sulfate can be linked with the CR2-targeting group or the bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof).

[0120] The CR2-targeting group or bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) may include the addition of an immunologically active domain, such as an antibody epitope or other tag, to facilitate purification of the polypeptide. The use of 6xHis and GST (glutathione-S-transferase) as tags is well known. Inclusion of a cleavage site at or near the fusion junction will facilitate removal of the extraneous polypeptide after purification. Other amino acid sequences that may be included in the CR2-targeting portion or the bacterial-targeting portion of the molecule include functional domains, such as active sites from enzymes such as a hydrolase, glycosylation domains, and cellular targeting signals.

[0121] Variants of the CR2-targeting group or the bacterial-targeting group (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) include polypeptides having an amino acid sequence sufficiently similar to the amino acid sequence of the CR2-targeting group or the bacterial-targeting group. The term "sufficiently similar" means a first amino acid sequence that contains a sufficient or minimum number of identical or equivalent amino acid residues relative to a second amino acid sequence such that the first and second amino acid sequences have a common structural domain and/or common functional activity. For example, amino acid sequences that contain a common structural domain that is at least about 45%, preferably about 75% through 98%, identical are defined

herein as sufficiently similar. Amino acid sequence similarity can be determined in various ways, for example, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGNTM (DNASTAR) software. One skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0122] Variants include CR2-targeting groups or bacterial-targeting groups that differ in amino acid sequence due to mutagenesis. In addition, bioequivalent analogs of the CR2-targeting groups or bacterial-targeting groups (such as CR2 protein, *S. aureus N315* Sbi protein or biologically-active fragments thereof) may also be constructed by making various substitutions on residues or sequences in the CR2 protein, the *S. aureus N315* Sbi protein or biologically-active fragments thereof.

[0123] The CR2-targeting groups, bacterial-targeting groups or homologues thereof described herein may be made by chemical synthesis methods, or by linkage of a polynucleotide encoding the CR2 portion, the Sbi portion and/or a polynucleotide encoding the Fc domain of a human or mouse Ig protein, IgG protein, or IgG_1 protein (with or without a linker sequence), and introducing the resulting polynucleotide into a vector for transfecting host cells that are capable of expressing the molecule. Chemical synthesis, especially solid phase synthesis, is preferred for short peptides or those containing unnatural or unusual amino acids such as D-Tyr, ornithine, and the like. Recombinant procedures are preferred for longer polypeptides. The CR2-targeting or bacterial-targeting groups can be isolated *in vitro* by standard protein purification methods known to those skilled in the art.

[0124] Recombinant DNA techniques for making a CR2-targeting group or a bacterial-targeting group involve, in simplified form, taking a CR2-targeting group-encoding polynucleotide or a bacterial-targeting group-encoding polynucleotide, inserting it into an appropriate vector, inserting the vector into an appropriate host cell, and recovering or isolating the protein produced thereby.

[0125] Provided herein are polynucleotides encoding a CR2-targeting group (*i.e.*, a full-length human or mouse CR2 protein or biologically-active fragment thereof, such as the complete extracellular domain of human or mouse CR2 protein, a fragment of human or mouse CR2 protein comprising SCRs 1 to 8, or a fragment of human or mouse CR2 protein comprising SCRs 1 and 2)(*see, e.g.*, SEQ ID NOs:1 to 8). Also provided herein are polynucleotides encoding a bacterial-targeting group (*i.e.*, full-length S. aureus N315 Sbi protein, a fragment of S. aureus N315 Sbi protein comprising the first four N-terminal

domains, or a fragment of S. aureus N315 Sbi protein comprising the third and fourth N-terminal domains)(*see, e.g.*, SEQ ID NOs:20, 21, and 22). Such polynucleotides may also be used for expression of CR2-targeting groups or bacterial-targeting groups. For example, in certain embodiments, there are provided polynucleotides encoding human or mouse CR2 protein or biologically active fragments thereof, or encoding fusion proteins comprising a CR2 portion comprising full-length CR2 protein or a biologically-active fragment thereof and an Fc portion comprising an Fc domain from a human or mouse Ig protein, a human or mouse IgG protein, a human or mouse IgG_1 protein, or a mouse IgG_1 protein. In certain embodiments, the polynucleotide further comprises a sequence encoding a signal peptide operably linked at the 5' end of the sequence encoding the CR2-targeting group. Exemplary nucleotide sequences of signal peptides known to those skilled in the art are provided in SEQ ID NOs:14, 16, and 18; exemplary amino acid sequences of signal peptides known to those skilled in the art are provided in SEQ ID NOs:15, 17, and 19. In certain embodiments, a linker sequence is used for linking the CR2 portion and the Fc portion. In certain embodiments, the CR2 portion is attached directly to the Fc portion without a linker sequence. In certain embodiments, the polynucleotide encodes SCRs 1 and 2 of full-length human CR2 protein fused to the Fc domain of mouse IgG_1 having an amino acid sequence of SEQ ID NO:9. In some embodiments, the polynucleotide encodes SCRs 1 and 2 of full-length human CR2 protein fused to the Fc domain of mouse IgG_1 having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence SEQ ID NO:9.

[0126] In certain embodiments, there are further provided polynucleotides encoding *S. aureus N315* Sbi protein or biologically active fragments thereof, or encoding fusion proteins comprising an Sbi protein portion comprising full-length *S. aureus N315* protein or a biologically-active fragment thereof and an Fc portion comprising an Fc domain from a human or mouse Ig protein, a human or mouse IgG protein, a human or mouse IgG_1 protein, or a mouse IgG_1 protein. In certain embodiments, the polynucleotide further comprises a sequence encoding a signal peptide operably linked at the 5' end of the sequence encoding the bacterial-targeting group. Exemplary nucleotide sequences of signal peptides known to those skilled in the art are provided in SEQ ID NOs:14, 16, and 18; exemplary amino acid sequences of signal peptides known to those skilled in the art are provided in SEQ ID NOs:15, 17, and 19. In certain embodiments, a linker sequence is used for linking the Sbi protein portion and the Fc portion. In certain embodiments, the Sbi protein portion is attached

directly to the Fc portion without a linker sequence. In certain embodiments, the polynucleotide encodes a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains (Sbi-I-II-III-IV) fused to the Fc domain of mouse IgG_1 having an amino acid sequence of SEQ ID NO:23. In some embodiments, the polynucleotide encodes a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains (Sbi-I-II-III-IV) fused to the Fc domain of mouse IgG_1 having an amino acid sequence that is at least about any of 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence SEQ ID NO:23.

[0127] In another aspect, the invention provides expression vectors comprising any of the polynucleotides described herein for expression of the CR2-targeting or bacterial-targeting groups. The expression vector can be used to direct expression of a CR2-targeting group or a bacterial-targeting group *in vitro* or *in vivo*. The vector may include any element necessary to establish a conventional function of a vector, for example, a transcription promoter, transcription terminator, selectable marker, and origin of replication. The promoter can be constitutive or regulative, and is selected from, for example, promoters of genes for galactokinase (GAL1), uridylyltransferase (GAL7), epimerase (GAL10), phosphoglycerate kinase (PGK), glyceraldehyde-3-phosphate dehydrogenase (GPD), alcohol dehydrogenase (ADH), and the like.

[0128] Many expression vectors are known to those of skill in the art. For example, *E. coli* may be transformed using pBR322, a plasmid derived from an *E. coli* species (Mandel et al., 1970, *J. Mol. Biol.* **53**:154). Plasmid pBR322 contains genes for ampicillin and tetracycline resistance, and thus provides easy means for selection. Other vectors include different features such as different promoters, which are often important in expression. For example, plasmids pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden), pKK233-2 (Clontech, Palo Alto, Calif., USA), and pGEM1 (Promega Biotech, Madison, Wis., USA), are all commercially available. Other vectors that can be used in the present invention include, but are not limited to, pET21a (Studier et al., 1990, *Methods Enzymol.* **185**: 60-89), pR1T5, and pR1T2T (Pharmacia Biotechnology), and pB0475 (Cunningham et al., 1989, *Science* **243**: 1330-1336; U.S. Pat. No. 5,580,723). Mammalian expression vectors may contain non-transcribed elements such as origins of replication, transcription promoters and enhancers, and 5' or 3' nontranslated sequences such as ribosome binding sites, polyadenylation sites, splice donor and acceptor sites, and transcriptional termination sequences. Promoters for use in mammalian expression vectors usually are for example viral promoters such as polyoma,

Adenovirus, HTLV, Simian Virus 40 (SV 40), and human cytomegalovirus (CMV). Vectors can also be constructed using standard techniques by combining the relevant traits of the vectors described above.

[0129] Also provided are host cells (such as isolated cells, transient cell lines, and stable cell lines) for expressing a CR2-targeting group or a bacterial-targeting group. The host cell may be prokaryotic or eukaryotic. Exemplary prokaryotic host cells include *E. coli K12* strain 294 (ATCC No. 31446), *E. coli B*, *E. coli X1776* (ATCC No. 31537), *E. coli W3110* (F-, gamma-, prototrophic/ATCC No. 27325), *bacilli* such as *Bacillus subtilis*, and other *Enterobacteriaceae*, such as *Salmonella typhimurium* or *Serratia marcesans*, and various *Pseudomonas* species. One suitable prokaryotic host cell is *E. coli BL21* (Stratagene), which is deficient in the OmpT and Lon proteases, which may interfere with isolation of intact recombinant proteins, and useful with T7 promoter-driven vectors, such as the pET vectors. Another suitable prokaryote is *E. coli W3110* (ATCC No. 27325). When expressed by prokaryotes the peptides typically contain an N-terminal methionine or a formyl methionine and are not glycosylated. In the case of fusion proteins, the N-terminal methionine or formyl methionine resides on the amino terminus of the fusion protein or the signal sequence of the fusion protein. These examples are, of course, intended to be illustrative rather than limiting.

[0130] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for fusion-protein-encoding vectors. *Saccharomyces cerevisiae* is a commonly used lower eukaryotic host microorganism. Others include *Schizosaccharomyces pombe* (Beach & Nurse, 1981, *Nature* **290**: 140; EP 139,383 published 2 May 1985); *Kluyveromyces* hosts (U.S. Pat. No. 4,943,529; Fleer et al., 1991, *Bio/Technology* **9**:968-975) such as, *e.g.*, *K. lactis* (MW98-8C, CBS683, CBS4574; Louvencourt et al., 1983, *J. Bacteriol.* **154**(2):737-742), *K. fragilis* (ATCC 12,424), *K. bulgaricus* (ATCC No. 16,045), *K. wickeramii* (ATCC No. 24,178), *K. waltii* (ATCC No. 56,500), *K. drosophilarum* (ATCC No. 36,906; Van den Berg et al., 1990, *Bio/Technology* **8**:135), *K. thermotolerans*, and *K. marxianus*; *yarrowia* (EP 402,226); *Pichia pastoris* (EP 183,070; Sreekrishna et al., 1988, *J. Basic Microbiol.* **28**:265-278); *Candida*; *Trichoderma reesia* (EP 244,234); *Neurospora crassa* (Case et al., 1979, *Proc. Nat'l Acad. Sci. USA* **76**:5259-5263); *Schwanniomyces* such as *Schwanniomyces occidentalis* (EP 394,538 published 31 Oct. 1990); and filamentous fungi such as, *e.g.*, *Neurospora*, *Penicillium*, *Tolypocladium* (WO 91/00357 published 10 Jan. 1991), and *Aspergillus* hosts such as *A. nidulans* (Ballance et al., 1983, *Biochem. Biophys. Res. Commun.* **112**:284-289; Tilburn et al., 1983, *Gene* **26**:205-221; Yelton et al., 1984, *Proc. Nat'l Acad. Sci. USA* **81**: 1470-1474) and

A. niger (Kelly & Hynes, 1985, *EMBO J.* **4**:475-479). Methylotropic yeasts are suitable herein and include, but are not limited to, yeast capable of growth on methanol selected from the genera consisting of *Hansenula*, *Candida*, *Kloeckera*, *Pichia*, *Saccharomyces*, *Torulopsis*, and *Rhodotorula*. A list of specific species that are exemplary of this class of yeasts may be found in C. Anthony, THE BIOCHEMISTRY OF METHYLOTROPHS, 269 (1982). Host cells also include insect cells such as *Drosophila S2* and *Spodoptera Sf9*, as well as plant cells.

[0131] Examples of useful mammalian host cell lines include, but are not limited to, HeLa, Chinese hamster ovary (CHO), COS-7, L cells, C127, 3T3, BHK, CHL-1, NSO, HEK293, WI38, BHK, C127 or MDCK cell lines. Another exemplary mammalian cell line is CHL-1. When CHL-1 is used hygromycin is included as a eukaryotic selection marker. CHL-1 cells are derived from RPMI 7032 melanoma cells, a readily available human cell line. Cells suitable for use in this invention are commercially available from the ATCC.

[0132] In certain embodiments, the host cell is a non-human host cell. In certain embodiments, the host cell is a CHO cell. In some embodiments, the host cell is a 293 cell.

[0133] The CR2-targeting groups or bacterial-targeting groups provided herein can be isolated by a variety of methods known in the art. In certain embodiments, when the CR2-targeting group or bacterial-targeting group comprises a secretory signal sequence so that it is secreted into the growth medium, the CR2-targeting group or bacterial-targeting group can be purified directly from the culture medium. If the CR2-targeting group or bacterial-targeting group is not secreted, it can be isolated from cell lysates. Cells can be disrupted or lysed by any conventional method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. The CR2-targeting groups or bacterial-targeting groups can be purified by various methods, including, but not limited to, immunoaffinity chromatography, reverse phase chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, gel filtration chromatography, and HPLC. For example, the CR2-targeting group or bacterial-targeting groups can be purified by immunoaffinity chromatography using an antibody that recognizes the CR2 portion, the Sbi portion or an antibody that recognizes the Fc portion of a human or mouse Ig protein, IgG protein, or IgG_1 protein, or both. In certain embodiments, an antibody recognizing the first two N-terminal SCR domains of CR2 is used for purifying the CR2-targeting group. In certain embodiments, the CR2-targeting group is purified by ion exchange chromatography. In certain embodiments, an antibody recognizing two or more of the first four N-terminal domains of *S. aureus N315* Sbi protein is used for purifying the bacterial-

targeting group. In certain embodiments, the bacterial-targeting group is purified by ion exchange chromatography.

[0134] The CR2-targeting group or bacterial-targeting group peptide may or may not be properly folded when expressed as a fusion protein. A number of factors determine whether the fusion protein must be denatured and refolded, and if so, whether these procedures are employed before or after cleavage. When denaturing and refolding are needed, typically the peptide is treated with a chaotrope, such a guanidine HCl, and is then treated with a redox buffer, containing, for example, reduced and oxidized dithiothreitol or glutathione at the appropriate ratios, pH, and temperature, such that the peptide is refolded to its native structure.

[0135] The CR2-targeting groups or bacterial-targeting groups described herein may also contain a tag (such as a cleavable tag) for purification. This tag can be fused to the C-terminus or N-terminus of the CR2 portion, the Sbi portion or the Fc portion, and can be used to facilitate protein purification.

[0136] In certain embodiments, the CR2-targeting group or the bacterial-targeting portion could be synthesized *de novo* in whole or in part, using chemical methods well known in the art. For example, the component amino acid sequences can be synthesized by solid phase techniques, cleaved from the resin, and purified by preparative high performance liquid chromatography followed by chemical linkage to form a desired polypeptide. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing.

[0137] The CR2-targeting groups or bacterial-targeting groups can be assayed for their desired properties using *in vitro* or *in vivo* assays. For example, binding of a CR2-targeting group or a bacterial-targeting group to a CR2 ligand can be determined by surface plasmon resonance method. By way of example, kinetic analysis of the interaction of the CR2-targeting group or a bacterial-targeting group with C3d-biotin can be performed using surface plasmon resonance (SPR) measurements made on a BIAcore 3000 instrument (Biacore AB, Uppsala, Sweden). Human C3d-biotin can be bound to the surface of BIAcore streptavidin sensor chips by injecting C3d-biotin over the surface of one flow cell of the chip. Binding can be evaluated over a range of CR2-targeting group or bacterial-targeting group concentrations. Association of CR2-targeting group or bacterial-targeting group with the ligand can be monitored for a certain period of time (such as 120 seconds), after which the complex is allowed to dissociate in the presence of buffer only for an additional period of time (such as

120 seconds). Binding of CR2-targeting groups or bacterial-targeting groups to C3d-immobilized flow cells can be corrected for binding to control flow cells. Binding data can be fitted to a 1:1 Langmuir binding model using BIAevaluation Version 3.1 software (BIAcore) and evaluated for best fit. The kinetic dissociation profiles obtained can be used to calculate on and off rates (k_a and k_d) and affinity constants (K_D) using the BIAevaluation Version 3.1 program. Other assay methods for ligand binding are known in the art and can also be used.

Methods of synthesizing and aggregating nanoparticle contrast agents

[0138] USPIO nanoparticles can be synthesized by a variety of methods. In certain embodiments, USPIO nanoparticles are synthesized by coprecipitation. The coprecipitation method synthesizes either Fe_3O_4 or γ-Fe_2O_3 by co-precipitation of Fe^{2+} or Fe^{3+} aqueous salt solutions after addition of a strong base. Control of particle size, shape, and composition depends on the type of salts used (*e.g.*, chlorides, sulfates, nitrates, perchlorates, and the like), the ratio of Fe^{2+} to Fe^{3+}, reaction pH, and the ionic strength of the reaction. Typically, co-precipitation of magnetite proceeds by adding a base to an aqueous mixture of Fe^{2+} and Fe^{3+} chloride at a 1:2 molar ratio. The chemical reaction $Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O$ generally produces magnetite as a black precipitate.

[0139] In certain embodiments, USPIO nanoparticles are synthesized by the reverse micelle method. The reverse micelle method enables the synthesis of USPIO nanoparticles in a narrow size range with comparatively uniform chemical and physical properties. Highly monodispersed USPIO can be synthesized using the aqueous core of aerosol-OT ("AOT")/*n*-hexane reverse micelles (aerosol-OT or AOT is the common name for sodium *bis*(2-ethylhexyl)sulfo-succinate), which have an aqueous core capable of dissolving hydrophilic compounds, including salts and the like. Thus, a deoxygenated aqueous solution of the Fe^{3+} and Fe^{2+} salts in a molar ratio of 2:1 is dissolved into the aqueous core of AOT/*n*-hexane reverse micelles, and USPIO nanoparticles (*i.e.*, magnetite particles) are precipitated by addition of a deoxygenated solution of strong base, such as sodium hydroxide. Smaller and more uniform USPIO particles can be prepared by precipitation of magnetite from such reverse micelles at lower temperatures in the presence of nitrogen gas. Because the size of the aqueous core of the AOT/*n*-hexane reverse micelles is in the nanometer range, this method produces very small magnetite particles (< 15 nm diameter on average) with a fairly narrow size distribution. In fact, the size of the USPIO particles produced by the reverse micelle method can be controlled by varying the size of the aqueous micelle core.

[0140] In certain embodiments, USPIO nanoparticles are synthesized by a solvothermal method as follows. Trioctylamine (98%), iron(III) acetylacetonate ($Fe(Acac)_3$)(97%), heptanoic acid (99%), anhydrous toluene (99.8%) and ethanol were purchased from Sigma-Aldrich (St. Louis, MO). Fe_3O_4 nanocrystallites can be synthesized without a secondary stabilizing agent by heating 20 ml of trioctylamine to reflux (~260°C) under nitrogen gas in a three-necked round bottom flask. The solution is mixed using a magnetic stir bar. On reaching reflux temperature, 0.045 M $Fe(Acac)_3$ is carefully added to the solvent, turning the solution a dark, opaque brown color. The reaction is allowed to proceed for the desired time (*e.g.*, 1, 3, 6, 12, 24, 36, 48, 72, or more hours), then quenched by addition of toluene, producing a clear brown solvent with a black precipitate. In certain embodiments, the solvothermal method is modified to start with a mixture of 18.6 ml of trioctylamine and 1.4 ml of heptanoic acid as a stabilizing agent.

[0141] In certain embodiments, the USPIO nanoparticles synthesized by the solvothermal method are further treated to form multiparticle USPIO aggregates. USPIO nanoparticles synthesized by the solvothermal method described herein using an Iron (III) Acetylacetonate precursor with trioctylamine and heptanoic acid (Sigma-Aldrich, St. Louis, MO) as surfactants have a diameter of ~10 nm with a hydrophobic heptanoic acid surface termination. In certain embodiments, the as-synthesized USPIO nanoparticles are resuspended in tetrahydrofuran (THF) and titrated with a 1% (v/v) solution of acetic acid until the desired level of aggregation is reached. Without wishing to be bound by a particular theory, it is thought that the acetic acid partially displaces the heptanoic acid on the USPIO nanoparticle surface, decreasing steric hindrance at the nanoparticle surface and permitting increased aggregation of USPIO nanoparticles. The aggregates of as-synthesized USPIO nanoparticles are then encapsulated using phospholipids. In certain embodiments, the USPIO nanoparticle aggregates are encapsulated by amine-functionalized, PEGylated phospholipids, such as 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000](ammonium salt). Phospholipids functionalized for use with any desired surface chemistry can be used, and PEGylated with any desired molecular weight of PEG. The USPIO nanoparticle aggregates and PEGylated, functionalized phospholipids are dried with nitrogen and maintained under vacuum for 24 hours. The iron-oxide/phospholipid mixture is resuspended in deionized water by bath sonication at 60°C to yield amine-functionalized, PEGylated phospholipid-encapsulated USPIO nanoparticle aggregates for subsequent protein crosslinking.

[0142] In certain embodiments, the ultrasmall superparamagnetic nanoparticle compositions comprise liposomes or other delivery vehicles containing gadolinium chelate (Gd-chelate) molecules. Gd-chelate nanoparticles enhance MRI contrast via a T_1 relaxation mechanism, in contrast to superparamagnetic iron oxide nanoparticles, which enhance contrast via a T_2 relaxation mechanism. Gd-chelate molecules can be synthesized by a variety of methods. In certain embodiments, the Gd-chelate molecules are synthesized by reacting a solution of gadolinium citrate with a chelator such as diethylenetriaminepentaacetic acid ("DTPA"), or with DTPA derivatized with a linker containing another active group to facilitate addition of the Gd-chelate to another compound, such as a targeting group. For example, DPTA derivatized with *bis*(stearylamide) ("DPTA-SA") can be incorporated into low density lipoproteins. *See* e.g., I.R. Corbin et al., 2006, *Neoplasia* **8**(6):488-498. A solution of gadolinium citrate can be prepared, for example, by adding a solution of $GdCl_3$ in HCl to an excess of sodium citrate, then adjusting the pH of the solution to 7.4 with HCl or NaOH as necessary. The presence of excess citrate prevents the formation of insoluble gadolinium hydroxides. The chelator can then be labeled with Gd(III) by mixing the gadolinium citrate solution with a solution containing DTPA or a DTPA-derivatized compound at a metal:chelator ratio of 1:1. The mixture is then incubated for one hour at room temperature under a nitrogen atmosphere with gentle stirring, filtered and concentrated to the desired volume by ultrafiltration, for example using an Amicon ultrafiltration cell using a filter with an appropriate molecular weight cutoff.

[0143] Nanoparticle size can be measured by a variety of common techniques, including scanning or transmission electron microscopy, atomic force microscopy, dynamic light scattering, x-ray photoelectron spectroscopy, powder x-ray diffraction, Fourier transform infrared spectroscopy, matrix-assisted laser desorption time-of-flight mass spectroscopy, ultraviolet-visible spectroscopy, and the like.

[0144] The various methods of synthesizing paramagnetic nanoparticle compositions for medical or diagnostic imaging applications described in detail by A.K. Gupta and M. Gupta in "Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications," *Biomaterials* (2005) **26**:3995-4021, are hereby incorporated herein by reference. In addition, the methods of synthesizing Gd-DPTA-SA conjugates for imaging applications described in detail by I.R. Corbin and colleagues in "Low-Density Lipoprotein Nanoparticles as Magnetic Resonance Imaging Contrast Agents," *Neoplasia* (2006) **8**(6):488-498, are hereby incorporated herein by reference.

[0145] In certain embodiments, the superparamagnetic nanoparticles disclosed herein are coated with a natural or synthetic polymer or other material, or formulated with a stabilizer such as a surfactant at the time of preparation. Depending on the method by which they are produced, superparamagnetic iron oxide particles, such as the USPIO nanoparticles described herein, can have hydrophobic surfaces with a large surface area to volume ratio. Consequently, without a surface coating, colloidal suspensions of USPIO nanoparticles may agglomerate to form larger clusters, resulting in increased particle size. Such clusters have strong magnetic dipole-dipole attractions between them, and exhibit ferromagnetic behavior, further increasing their aggregation properties. Thus in some cases, for effective stabilization of USPIO nanoparticles, it is desirable to coat the particles, or to include a stabilizer, such as a surfactant, at the time of preparation. In certain embodiments, the USPIO nanoparticles or aggregates thereof disclosed herein are coated with an inorganic or a polymeric material. Polymeric coating materials may be either natural or synthetic. In certain embodiments, the USPIO nanoparticles or aggregates thereof are coated with synthetic polymer materials, such as those based on poly(ethylene-*co*-vinyl acetate), polyvinylpyrrolidone ("PVP"), poly(lactic-*co*-glycolic acid) ("PLGA"), polyethylene glycol ("PEG"), polyvinyl alcohol ("PVA"), polyacrylic acid, and the like. In certain embodiments, the USPIO nanoparticles or aggregates thereof are coated with natural polymer materials, such as gelatin, dextran, chitosan, pullulan, and the like. In certain embodiments, the USPIO nanoparticles or aggregates thereof are formulated in the presence of a surfactant, such as sodium oleate, dodecylamine, sodium carboxymethylcellulose, and the like. In certain embodiments, the USPIO nanoparticles or aggregates thereof are coated with an inorganic material, such as gold or silica. In certain embodiments, the USPIO nanoparticles or aggregates thereof are encapsulated in phospholipids. In certain embodiments, the phospholipids are derivatized to enable the covalent attachment of proteins or protein fragments to the surface of the phospholipid-encapsulated USPIO nanoparticles or aggregates thereof. In certain embodiments, the phospholipids are derivatized with thiol, carboxylate, or amine groups. In certain embodiments, the USPIO nanoparticles or aggregates thereof are coated with dextran. In certain embodiments, the dextran is derivatized to enable the covalent attachment of proteins or protein fragments to the surface of the dextran-coated USPIO nanoparticles or aggregates thereof.

[0146] As used herein, the term "phospholipid" or "phospholipids" refers to a class of compounds comprising one or more phosphate groups and one or more fatty acids covalently joined by an ester linkage to glycerol. Phospholipids are amphipathic in nature, consisting of

a hydrophilic portion and a hydrophobic portion. They are found in all living cells, and are a major component of cell membranes. Exemplary phospholipids include phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, and cardiolipin.

[0147] In certain embodiments, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a CR2- or bacterial-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments, the PEGylated phospholipid comprises PEG at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

Pharmaceutical Compositions for Imaging Applications

[0148] Also provided herein are pharmaceutical compositions comprising targeted nanoparticles and a pharmaceutically acceptable carrier. In certain embodiments, the targeted nanoparticles are CR2-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the targeted nanoparticles are bacterial targeted USPIO nanoparticles or aggregates thereof. The pharmaceutical compositions may be suitable for a variety of modes of administration as described herein, including, for example, systemic or localized administration. The pharmaceutical compositions can be in the form of injectable solutions. The pharmaceutical compositions described herein can be packaged in single unit dosages or in multidosage forms.

[0149] In certain embodiments, the pharmaceutical compositions comprise CR2-targeted nanoparticles or aggregates thereof and a pharmaceutically acceptable carrier suitable for administration to an individual. In certain embodiments, the CR2-targeted nanoparticles or aggregates thereof are CR2-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the CR2-targeted nanoparticles or aggregates thereof are CR2-targeted liposomes or other delivery vehicles containing Gd-chelate molecules. In certain

embodiments, the pharmaceutical compositions comprise CR2-targeted nanoparticles or aggregates thereof and a pharmaceutically acceptable carrier suitable for intravenous injection into an individual. In certain embodiments, the CR2-targeted nanoparticles or aggregates thereof are CR2-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the pharmaceutical compositions are CR2-targeted liposomes or other delivery vehicles containing Gd-chelate molecules. In certain embodiments, the pharmaceutical compositions comprise CR2-targeted nanoparticles or aggregates thereof and a pharmaceutically acceptable carrier suitable for intravenous injection into an individual. In certain embodiments, the CR2-targeted nanoparticles or aggregates thereof are CR2-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the CR2-targeted nanoparticles or aggregates thereof are CR2-targeted liposomes or other delivery vehicles containing Gd-chelate molecules. In certain embodiments, the pharmaceutical compositions comprise CR2-targeted nanoparticles or aggregates thereof and a pharmaceutically acceptable carrier suitable for injection into the arteries (such as renal arteries). In certain embodiments, the CR2-targeted nanoparticles or aggregates thereof are CR2-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the CR2-targeted nanoparticles or aggregates thereof are CR2-targeted liposomes or other delivery vehicles containing Gd-chelate molecules.

[0150] In certain embodiments, the pharmaceutical compositions comprise bacterial-targeted nanoparticles or aggregates thereof and a pharmaceutically acceptable carrier suitable for administration to an individual. In certain embodiments, the bacterial-targeted nanoparticles or aggregates thereof are bacterial-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the bacterial-targeted nanoparticles or aggregates thereof are bacterial-targeted liposomes or other delivery vehicles containing Gd-chelate molecules. In certain embodiments, the pharmaceutical compositions comprise bacterial-targeted nanoparticles or aggregates thereof and a pharmaceutically acceptable carrier suitable for intravenous injection into an individual. In certain embodiments, the bacterial-targeted nanoparticles or aggregates thereof are bacterial-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the bacterial-targeted nanoparticles or aggregates thereof are bacterial-targeted liposomes or other delivery vehicles containing Gd-chelate molecules. In certain embodiments, the pharmaceutical compositions comprise bacterial-targeted nanoparticles or aggregates thereof and a pharmaceutically acceptable carrier suitable for injection into the arteries (such as renal arteries). In certain embodiments, the bacterial-targeted nanoparticles or aggregates thereof are bacterial-targeted USPIO nanoparticles or

aggregates thereof. In certain embodiments, the bacterial-targeted nanoparticles or aggregates thereof are bacterial-targeted liposomes or other delivery vehicles containing Gd-chelate molecules.

[0151] In any of the embodiments described herein, the pharmaceutical compositions can be administered to provide a dose between about 1 mg/kg and about 1000 mg/kg, between about 100 mg/kg and about 1000 mg/kg, between about 250 mg/kg and about 750 mg/kg, and between about 400 mg/kg and 600 mg/kg, or a dose of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg and about 100 mg/kg.

[0152] The compositions are generally formulated as sterile, substantially isotonic, and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. In any of the embodiments described herein, the compositions are free of pathogen. For injection, the pharmaceutical composition can be in the form of liquid solutions, for example in physiologically compatible buffers such as Hank's Balanced Salt solution ("HBSS")(*e.g.*, 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, and 4.2 mM $NaHCO_3$), Phosphate-Buffered Saline ("PBS")(*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), normal saline (*e.g.*, 0.9% (w/v) NaCl), half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose), Ringer's solution (*e.g.*, 130 mM Na^+, 109 mM Cl^-, 4 mM K^+, and 3 mM Ca^{2+}), or lactated Ringer's solution (*e.g.*, 130 mM Na^+, 109 mM Cl^-, 28 mM lactate, 4 mM K^+, and 3 mM Ca^{2+}). In addition, the CR2-targeted USPIO nanoparticle or nanoparticle aggregate pharmaceutical composition may be provided in a solid form (*e.g.*, lyophilized) and redissolved or suspended immediately prior to use.

[0153] In certain embodiments, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for injection. In certain embodiments, the pharmaceutical compositions provided herein are formulated for intravenous, intraperitoneal, or intraocular injection. Typically, compositions for injection are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit

dosage form, for example, as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0154] The pharmaceutical compositions may further comprise additional ingredients, for example preservatives, buffers, tonicity agents, antioxidants and stabilizers, nonionic wetting or clarifying agents, viscosity-increasing agents, and the like.

[0155] Suitable preservatives for use in a solution include polyquaternium-1, benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, disodium-EDTA, sorbic acid, benzethonium chloride, and the like. Typically (but not necessarily) such preservatives are employed at a level of from 0.001% to 1.0% by weight.

[0156] Suitable buffers include boric acid, sodium and potassium bicarbonate, sodium and potassium borates, phosphate-buffered saline, sodium and potassium carbonate, sodium acetate, sodium biphosphate and the like, in amounts sufficient to maintain the pH at between about pH 6 and pH 8, and preferably, between about pH 7 and pH 7.5.

[0157] Suitable tonicity agents include dextran 40, dextran 70, dextrose, glycerin, potassium chloride, propylene glycol, sodium chloride, and the like, such that the sodium chloride equivalent of the injectable solution is in the range 0.9 plus or minus 0.2%.

[0158] Suitable antioxidants and stabilizers include sodium bisulfite, sodium metabisulfite, sodium thiosulfite, thiourea and the like. Suitable wetting and clarifying agents include polysorbate 80, polysorbate 20, polyoxamer 282 and tyloxapol. Suitable viscosity-increasing agents include dextran 40, dextran 70, gelatin, glycerin, hydroxyethylcellulose, hydroxmethylpropylcellulose, lanolin, methylcellulose, petrolatum, polyethylene glycol, polyvinyl alcohol, polyvinylpyrrolidone, carboxymethylcellulose and the like.

[0159] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising the extracellular domain of

the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0160] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a

human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0161] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1 (SEQ ID NO:9). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile

phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0162] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a CR2-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments,

the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises thiol-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0163] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with CR1 or a biologically-active fragment thereof fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with CR1 or a biologically-active fragment thereof fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with CR1 or a biologically-active fragment thereof fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (e.g., 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (e.g., 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (e.g., 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions

comprising phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0164] In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-sn-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a C4-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises thiol-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0165] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with C4bp

of a biologically-active fragment thereof fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with C4bp of a biologically-active fragment thereof fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with C4bp of a biologically-active fragment thereof fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof derivatized with C4bp of a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (e.g., 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (e.g., 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (e.g., 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0166] In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the

phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-sn-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a C4-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises thiol-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0167] In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof, wherein the CR2-targeting group is an antibody directed to C3 or fragment thereof, including but not limited to C3b, iC3b, C3dg, C3d and the like. In some embodiments, the antibody is a polyclonal antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a monoclonal antibody or fragment thereof directed to C3d. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates

thereof derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein attached to the Fc-domain of human or mouse IgG_1. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising the extracellular domain of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use.

[0168] In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc-domain of human or mouse IgG_1. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 to 8 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl).

In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use.

[0169] In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc-domain of human or mouse IgG_1. In certain embodiments, the pharmaceutical compositions comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof derivatized with CR2 fragments comprising SCRs 1 and 2 of the full-length human CR2 protein fused to the Fc-domain of mouse IgG_1 (SEQ ID NO:9). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising dextran-coated CR2-targeted USPIO nanoparticles or aggregates

thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0170] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter.

[0171] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc-domain of mouse IgG_1 (SEQ ID NO:23). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the

pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0172] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein (Sbi-I-II-III-IV) comprising the first four N-terminal domains of Sbi protein fused to the Fc-domain of mouse IgG_1 (SEQ ID NO:23). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-

buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0173] In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth N-terminal domains of Sbi protein (Sbi-III-IV) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein (Sbi-III-IV) comprising the third and fourth N-terminal domains of Sbi protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein (Sbi-III-IV) comprising the third and fourth N-terminal domains of Sbi protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth N-terminal domains of Sbi protein (Sbi-III-IV) fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4),

sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0174] In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a bacterial-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises thiol-

functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0175] In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with full-length *S. aureus N315* Sbi protein fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use.

[0176] In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated

bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein (Sbi-I-II-III-IV) comprising the first four N-terminal domains of Sbi protein fused to the Fc-domain of mouse IgG_1 (SEQ ID NO:23). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0177] In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth N-terminal domains of Sbi protein (Sbi-III-IV) fused to the Fc domain of a human or a mouse Ig protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein (Sbi-III-IV) comprising the third and fourth N-terminal domains of Sbi protein fused to the Fc domain of a human or a mouse IgG protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein (Sbi-III-IV)

comprising the third and fourth N-terminal domains of Sbi protein fused to the Fc domain of a human or a mouse IgG_1 protein. In certain embodiments, the pharmaceutical compositions comprise dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof derivatized with a biologically-active fragment of full-length *S. aureus N315* Sbi protein comprising the third and fourth N-terminal domains of Sbi protein (Sbi-III-IV) fused to the Fc-domain of mouse IgG_1. In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the pharmaceutical compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the pharmaceutical compositions comprising dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof having a circulating plasma half-life of between about 20 minutes and about 40 minutes and having a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0178] In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 100 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter.

Methods of Detecting Complement-Mediated Inflammation

[0179] Also provided herein are non-invasive methods of detecting complement-mediated inflammation in an individual using the CR2-targeted USPIO nanoparticle or nanoparticle aggregate compositions or the bacterial-targeted USPIO nanoparticle or nanoparticle aggregate compositions provided herein. In certain embodiments, the invention provides non-invasive methods of detecting complement-mediated inflammation in an individual, the methods comprising: (a) administering to the individual a composition comprising an effective amount of CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof; and (2) taking a magnetic resonance image of the individual. In certain embodiments, the invention provides non-invasive methods of detecting complement-mediated inflammation in an individual, the methods comprising: (a) administering to the individual a composition comprising an effective amount of bacterial-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof; and (2) taking a magnetic resonance image of the individual. In any of the embodiments described herein, the complement-mediated inflammation is alternative complement-mediated inflammation.

[0180] In any of the embodiments described herein, the composition administered to the individual is a pharmaceutical composition comprising any of the CR2-targeted USPIO nanoparticle compositions described herein. In any of the embodiments described herein, the composition administered to the individual is a pharmaceutical composition comprising any of the CR2-targeted USPIO nanoparticle aggregate compositions described herein. In any of the embodiments described herein, the composition administered to the individual is a pharmaceutical composition comprising any of the bacterial-targeted USPIO nanoparticle compositions described herein. In any of the embodiments described herein, the composition administered to the individual is a pharmaceutical composition comprising any of the bacterial-targeted USPIO nanoparticle aggregate compositions described herein. In any of the embodiments described herein, the composition administered to the individual is a pharmaceutical composition comprising any of the C4-targeted USPIO nanoparticle compositions described herein. In any of the embodiments described herein, the composition administered to the individual is a pharmaceutical composition comprising any of the C4-targeted USPIO nanoparticle aggregate compositions described herein.

[0181] As used herein, the term "magnetic resonance imaging" or "MRI" refers to a non-invasive medical imaging technique commonly used to visualize the structure and function of the body that provides detailed images of the body in any plane. MRI provides much greater

contrast between the different soft tissues of the body than other non-invasive imaging methods, such as computed tomography (CT), making it especially useful in neurological, musculoskeletal, cardiovascular, and oncological (cancer) imaging. Unlike CT, it does not require ionizing radiation, instead using a powerful magnetic field to align the nuclear magnetization of hydrogen atoms in water in the body. Radiofrequency fields are used to systematically alter the alignment of this magnetization, causing the hydrogen nuclei to produce a rotating magnetic field detectable by the scanner. This signal can be manipulated by additional magnetic fields to build up enough information to reconstruct an image of the body or a portion thereof.

[0182] When an individual lies in a scanner, the hydrogen nuclei (*i.e.*, protons) found in abundance in water molecules throughout the individual's body, align with the strong main magnetic field. A second electromagnetic field, which oscillates at radiofrequencies and is perpendicular to the main field, is then pulsed to push a proportion of the protons out of alignment with the main field. These protons then drift back into alignment with the main field, emitting a detectable radiofrequency signal as they do so. Since protons in different body tissues (*e.g.*, fat vs. muscle) realign at different speeds, different body structures can be imaged. Contrast agents may be injected intravenously to enhance the appearance of blood vessels, organs, tumors or sites of inflammation.

[0183] As used herein, an "effective amount" of a CR2-targeted or bacterial-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticle or nanoparticle aggregate composition (including any of the pharmaceutical compositions described herein) is an amount sufficient to produce a clinically useful magnetic resonance image of complement-mediated inflammation. A clinically useful magnetic resonance image is one containing sufficient detail to enable an experienced clinician to assess the degree and/or extent of inflammation for purposes of diagnosis, monitoring the efficacy of a therapeutic intervention, and the like.

[0184] Complement-mediated inflammation associated with many diseases in which any of the three complement pathways is implicated can be detected by the non-invasive methods of the present invention. Such diseases include, for example: (1) tissue damage due to ischemia-reperfusion following acute myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock intestinal ischemia, spinal cord injury, and traumatic brain injury; (2) inflammatory disorders, *e.g.*, burns, endotoxemia and septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis; anaphylactic shock, severe asthma, angioedema, Crohn's disease, sickle cell anemia,

poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis; (3) transplant rejection, *e.g.*, hyperacute xenograft rejection; (4) pregnancy related diseases such as recurrent fetal loss and pre-eclampsia, and (5) adverse drug reactions, *e.g.*, drug allergy, IL-2 induced vascular leakage syndrome and radiographic contrast media allergy. Complement-mediated inflammation associated with autoimmune disorders including, but not limited to, myasthenia gravis, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndrome, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjögren's syndrome, and Takayasu's arteritis, may also be detected with the non-invasive methods described herein.

[0185] In certain embodiments, the complement-mediated inflammation to be detected by the non-invasive methods provided herein is associated with any of the following disorders: post cardiopulmonary bypass complications; myocardial infarction; ischemia/reperfusion injury; stroke; acute respiratory distress syndrome (ARDS); sepsis; burn injury; inflammation associated with cardiopulmonary bypass and hemodialysis; plasmapheresis; plateletpheresis; leukopheresis; extracorporeal membrane oxygenation (ECMO); heparin-induced extracorporeal LDL precipitation (HELP); radiographic contrast media-induced allergic response; transplant rejection; and other inflammatory conditions and autoimmune/immune complex diseases such as multiple sclerosis, myasthenia gravis, pancreatitis, rheumatoid arthritis, Alzheimer's disease, asthma, thermal injury, anaphylactic shock, bowel inflammation, urticaria, angioedema, vasculitis, glomerulonephritis, and Sjögren's syndrome, systemic lupus erythromatosus and lupus nephritis.

[0186] Membranoproliferative glomerulonephritis type II (MPGN II) is a rare kidney disease leading to persistent proteinuria, hematuria, and nephritic syndrome. FH deficiency and dysfunction in MPGN II have been reported in several cases. For example, mutations in FH have been found in human patients with MPGN II. Pigs of the Norwegian Yorkshire breed have FH defects that are inherited in a recessive pattern. These animals develop MPGN II, show massive complement deposits in the renal glomeruli and die at an early age because of the renal failure. Furthermore, an autoantibody that recognizes FH has been described in a patient with hypocomplementemic MPGN II. Thus, evidence suggests that the alternative complement pathway is involved in the development and progression of MPGN II.

[0187] Hemolytic uremic syndrome (HUS) is a disease characterized by microangiopathic hemolytic anemia and thrombocytopenia, ultimately resulting in acute renal failure, caused by continuous platelet degradation in the periphery and platelet thrombin in the microcirculation of the kidney. *See* e.g., Zipfel, 2001, *Seminars in Thrombosis Hemostasis* **27**(3):191-199. There is now considerable evidence that the nondiarrheal form of HUS (also known as atypical HUS, or aHUS) is associated with alternations and mutations of FH. In addition, autoantibodies to FH have been reported in aHUS patients. Thus, evidence suggests that the alternative complement pathway is involved in the development and progression of HUS and aHUS.

[0188] Rheumatoid arthritis is a chronic disease which can exhibit a variety of systemic manifestations. This disease has an unknown etiology and characteristically exhibits a persistent inflammatory synovitis which usually involves peripheral joints in a symmetric distribution. The most important feature of this incurable condition is complement-mediated inflammation which causes cartilage destruction, bone erosions and, ultimately, joint deformities that are the hallmark of the disease.

[0189] As used herein, the term "ischemia reperfusion (I/R) injury" refers to inflammatory injury to the endothelium and underlying parenchymal tissues following reperfusion of hypoxic tissues. It is a general syndrome that is responsible for both acute and chronic injury to various tissues including, for example, myocardium, central nervous system, hind limb and intestine. Ischemia reperfusion injury can result in necrosis and irreversible cell injury. The complement pathway (including the alternative complement pathway) is a major mediator of I/R injury. The non-invasive methods provided herein are thus useful for detection of complement-mediated inflammation associated with ischemia reperfusion that occurs in any organ or tissue, including, but not limited to, intestinal ischemia-reperfusion injury, renal ischemia-reperfusion injury, cardiac ischemia-reperfusion injury, ischemia-reperfusion injury of other internal organs such as the lung or liver, central nervous system ischemia-reperfusion injury, ischemia-reperfusion injury of the limbs or digits, trauma-induced hypovolemia, or ischemia-reperfusion injury of any transplanted organ or tissue. Ischemia-reperfusion injury can also occur in conjunction with a variety of other conditions including, but not limited to, stroke, spinal cord injury, trauma-induced hypovolemic shock, and autoimmune diseases such as rheumatoid arthritis (*e.g.*, which can be greatly worsened by ischemic injury of the synovium) or a variety of other inflammatory diseases (diseases mediated by inflammation or wherein inflammation is a symptom that may result in or be associated with ischemic events

and reperfusion). Other conditions and diseases in which ischemia-reperfusion injury occurs will be known to those of skill in the art.

[0190] The non-invasive methods provided herein may also be used to detect complement-mediated inflammation in drusen-associated diseases. As used herein, the term "drusen-associated disease" refers to any disease in which formation of drusen or drusen-like extracellular disease plaque takes place, and for which drusen or drusen-like extracellular disease plaque causes or contributes to thereto or represents a sign thereof. For example, age-related macular degeneration (AMD), characterized by the formation of macular drusen, is considered a drusen-associated disease. Non-ocular drusen-related diseases include, but are not limited to, amyloidosis, elastosis, dense deposit disease, and/or atherosclerosis. The term "drusen-related disease" also includes glomerulonephritis, such as MPGN II.

[0191] In certain embodiments, the invention provides non-invasive methods of detecting complement-mediated inflammation in an individual in need thereof comprising: (a) administering to the individual a composition comprising an effective amount of CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof; and (b) taking a magnetic resonance image of the individual. In certain embodiments, the composition is any of the pharmaceutical compositions comprising CR2-targeted USPIO nanoparticles or aggregates thereof described herein. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, a mouse, or a rat. In certain embodiments, the compositions comprising CR2-targeted USPIO nanoparticles or aggregates thereof are administered by injection. In certain embodiments, the injection is parenteral, intravenous, subcutaneous, or intramuscular. In any of the embodiments described herein, the complement-mediated inflammation is alternative complement-mediated inflammation.

[0192] In any of the embodiments described herein, the compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the compositions

are administered to provide a dose between about 1 mg/kg and about 1000 mg/kg, between about 100 mg/kg and about 1000 mg/kg, between about 250 mg/kg and about 750 mg/kg, and between about 400 mg/kg and 600 mg/kg, or a dose of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg and about 100 mg/kg. In any of the embodiments described herein, the compositions have a circulating plasma half-life of between about 20 minutes and about 40 minutes and a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0193] In certain embodiments, the invention provides non-invasive methods of detecting complement-mediated inflammation in an individual in need thereof comprising: (a) administering to the individual a composition comprising an effective amount of bacterial-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof; and (b) taking a magnetic resonance image of the individual. In certain embodiments, the composition is any of the pharmaceutical compositions comprising bacterial-targeted USPIO nanoparticles or aggregates thereof described herein. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, a mouse, or a rat. In certain embodiments, the compositions comprising bacterial-targeted USPIO nanoparticles or aggregates thereof are administered by injection. In certain embodiments, the injection is parenteral, intravenous, subcutaneous, or intramuscular. In any of the embodiments described herein, the complement-mediated inflammation is alternative complement-mediated inflammation.

[0194] In any of the embodiments described herein, the compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the pharmaceutical compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl,

sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the compositions are administered to provide a dose between about 1 mg/kg and about 1000 mg/kg, between about 100 mg/kg and about 1000 mg/kg, between about 250 mg/kg and about 750 mg/kg, and between about 400 mg/kg and 600 mg/kg, or a dose of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg and about 100 mg/kg. In any of the embodiments described herein, the compositions have a circulating plasma half-life of between about 20 minutes and about 40 minutes and a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0195] In certain embodiments, the invention provides non-invasive methods of detecting complement-mediated inflammation in an individual in need thereof comprising: (a) administering to the individual a composition comprising an effective amount of C4-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof; and (b) taking a magnetic resonance image of the individual. In certain embodiments, the composition is any of the pharmaceutical compositions comprising C4-targeted USPIO nanoparticles or aggregates thereof described herein. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, a mouse, or a rat. In certain embodiments, the compositions comprising C4-targeted USPIO nanoparticles or aggregates thereof are administered by injection. In certain embodiments, the injection is parenteral, intravenous, subcutaneous, or intramuscular. In any of the embodiments described herein, the complement-mediated inflammation is alternative complement-mediated inflammation.

[0196] In any of the embodiments described herein, the compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9%

(w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the compositions are administered to provide a dose between about 1 mg/kg and about 1000 mg/kg, between about 100 mg/kg and about 1000 mg/kg, between about 250 mg/kg and about 750 mg/kg, and between about 400 mg/kg and 600 mg/kg, or a dose of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg and about 100 mg/kg. In any of the embodiments described herein, the compositions have a circulating plasma half-life of between about 20 minutes and about 40 minutes and a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0197] The CR2-targeted, bacterial-targeted and C4-targeted USPIO nanoparticle or nanoparticle aggregate compositions described herein can be administered to an individual via any route, including, but not limited to, intravenous (*e.g.*, by infusion pumps), intraperitoneal, intraocular, intraarterial, intravesicular, intramuscular, subcutaneous, intrathecal, transpleural, intraarterial, subcutaneous, intraarticular, intracisternal, intraventricular, intracranial, intraurethral, intrahepatic, and intratumoral. In certain embodiments, the CR2-targeted, bacterial-targeted or C4-targeted USPIO nanoparticle or nanoparticle aggregate compositions are administered systemically (for example, by intravenous injection). In some embodiments, the CR2-targeted, bacterial-targeted or C4-targeted USPIO nanoparticle or nanoparticle aggregate compositions are administered locally (for example, by intraarterial or intraocular injection).

[0198] In certain embodiments, the compositions are administered directly to the eye or the eye tissue. In certain embodiments, the compositions are administered by injection to the eye (intraocular injection) or to the tissues associated with the eye. The CR2-targeted, bacterial-targeted, or C4-targeted USPIO nanoparticle or nanoparticle aggregate compositions can be administered, for example, by intraocular injection, periocular injection, subretinal injection, intravitreal injection, transseptal injection, subscleral injection, intrachoroidal injection, intracameral injection, subconjunctival injection, subtenon injection, retrobulbar injection, or peribulbar injection. These methods are known in the art. For example, exemplary periocular routes for retinal drug delivery are disclosed in "Periocular routes for retinal drug delivery," Raghava et al., 2004, *Exp. Opin. Drug Deliv.* **1**(1):99-114. The CR2-targeted, bacterial-

targeted, or C4-targeted USPIO nanoparticle or nanoparticle aggregate compositions may be administered, for example, to the vitreous humor, aqueous humor, sclera, conjunctiva, the area between the sclera and conjunctiva, the retina, the choroid, the macula, to any other area in or proximate to the eye of an individual.

[0199] In certain embodiments, the CR2-targeted, bacterial-targeted, or C4-targeted compositions are administered intravascularly, such as intravenously (IV) or intraarterially. In certain embodiments (for example for the treatment of renal diseases), the compositions are administered directly into arteries (such as renal arteries).

[0200] In certain embodiments, the complement-mediated inflammation is associated with tissue damage resulting from ischemia reperfusion injury, inflammatory disorders, transplant rejection, pregnancy-related diseases, adverse drug reactions, and autoimmune or immune complex disorders. In certain embodiments, the tissue damage resulting from ischemia reperfusion injury is associated with a disorder selected from the group consisting of myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock, intestinal ischemia, spinal cord injury and traumatic brain injury. In certain embodiments, the inflammatory disorder is selected from the group consisting of burns, endotoxemia, septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis, anaphylactic shock, asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis. In certain embodiments, the transplant rejection is hyperacute xenograft rejection. In certain embodiments, the pregnancy-related disease is selected from the group consisting of recurrent fetal loss and pre-eclampsia. In certain embodiments, the adverse drug reaction is selected from the group consisting of drug allergy and IL-2 induced vascular leakage syndrome. In certain embodiments, the autoimmune or immune complex disorder is selected from the group consisting of myasthenia gravis, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, lupus nephritis, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndromes, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjögren's syndrome, Takayasu's arteritis, autoimmune glomerulonephritis, membranoproliferative glomerulonephritis type II, hemolytic uremic syndrome, and atypical hemolytic uremic syndrome. In certain embodiments, the autoimmune glomerulonephritis is associated with immunoglobulin A nephropathy or membranoproliferative glomerulonephritis type I.

[0201] In certain embodiments, the compositions comprise CR2-targeted USPIO nanoparticles or aggregates thereof coated with dextran or encapsulated with phospholipid. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety selected from the group consisting of complement receptor 2 (CR2) or a biologically-active fragment thereof, and a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety selected from the group consisting of complement receptor 2 (CR2) or a biologically-active fragment thereof and a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof.

[0202] In certain embodiments, the targeting moiety comprises a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0203] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or

aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a CR2-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises thiol-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0204] In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates between about 1 nm and about 1000 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates between about 5 nm and about 500 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates between about 10 nm and about 100 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO

nanoparticle aggregates between about 50 nm and about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates between about 65 nm and about 85 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates about 75 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0205] In certain embodiments, the compositions comprise C4-targeted USPIO nanoparticles or aggregates thereof encapsulated with phospholipid. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety selected from the group consisting of CR1 or a biologically-active fragment thereof, a fusion protein comprising an antibody or fragment thereof fused to CR1 or a biologically-active fragment thereof, C4bp or a biologically-active fragment thereof, and a fusion protein comprising an antibody or fragment thereof fused to C4bp or a biologically-active fragment thereof. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR1 or a biologically-active fragment thereof. In certain embodiments, the phospholipid-encapsulated C4-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to C4bp or a biologically-active fragment thereof.

[0206] In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 1 nm and about 1000 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a

biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 5 nm and about 500 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 10 nm and about 100 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 50 nm and about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 65 nm and about 85 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates about 75 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising CR1 or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1.

[0207] In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 1 nm and about 1000 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates

between about 5 nm and about 500 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 10 nm and about 100 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 50 nm and about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates between about 65 nm and about 85 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates about 75 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises C4-targeted USPIO nanoparticle aggregates about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the C4-targeting group is a fusion protein comprising C4bp or a biologically-active fragment thereof fused to the Fc-domain of mouse IgG_1.

[0208] In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or

aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter.

[0209] In certain embodiments, the compositions comprise bacterial-targeted USPIO nanoparticles or aggregates thereof coated with dextran or encapsulated with phospholipid. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety selected from the group consisting of full-length *S. aureus N315* Sbi protein or a biologically-active fragment thereof, and a fusion protein comprising an antibody or fragment thereof fused to full-length *S. aureus N315* Sbi protein or a biologically-active fragment thereof. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety selected from the group consisting of full-length *S. aureus N315* Sbi protein or a biologically-active fragment thereof and a fusion protein comprising an antibody or fragment thereof fused to full-length *S. aureus N315* Sbi protein or a biologically-active fragment thereof. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to full-length *S. aureus N315* Sbi protein or a biologically-active fragment thereof. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to full-length *S. aureus N315* Sbi protein or a biologically-active fragment thereof.

[0210] In certain embodiments, the targeting moiety comprises a biologically active fragment of full-length *S. aureus N315* Sbi protein. In certain embodiments, the biologically-active fragment of full-length *S. aureus N315* Sbi protein comprises the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) or the third and fourth N-terminal domains of Sbi protein (Sbi-III-IV). In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of full-length *S. aureus N315* Sbi protein. In certain embodiments, the fusion protein comprises the first four N-terminal domains of Sbi protein (Sbi-I-II-III-IV) fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1) or the third and fourth N-terminal domains of Sbi protein (Sbi-III-IV) fused to the Fc-domain of mouse IgG_1.

[0211] In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter.

In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In certain embodiments, the phospholipid-encapsulated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a bacterial-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises thiol-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0212] In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and

about 150 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the dextran-coated bacterial-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter.

Methods of Detecting Complement-Mediated Renal Inflammation Associated with Systemic Lupus Erythematosus

[0213] Also provided herein are non-invasive methods of detecting complement-mediated inflammation associated with systemic lupus erythematosus (SLE), membranous glomerulonephritis, or lupus nephritis in an individual in need thereof using the CR2-targeted USPIO nanoparticle or nanoparticle aggregate compositions provided herein. In certain embodiments, the complement-mediated inflammation is alternative complement-mediated inflammation.

[0214] As used herein, the term "systemic lupus erythematosus" or "lupus" or "SLE" refers to a chronic, occasionally fatal, autoimmune disease. As with other autoimmune diseases, in SLE, the immune system attacks the body's cells and tissue, resulting in inflammation and tissue damage. SLE can affect any part of the body, but 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, or flares, alternating with periods of remission. Diagnosis can be elusive, with patients sometimes suffering unexplained symptoms and untreated SLE for years. Common initial and chronic complaints are fever, malaise, joint pains, myalgias, fatigue and temporary loss of cognitive abilities. In some cases, the disease is accompanied by chronic renal dysfunction, including the development of lupus nephritis.

[0215] As used herein, the term "membranous glomerulonephritis" or "lupus nephritis" refers to an inflammation of the kidney caused by the chronic autoimmune disease SLE. Those afflicted with lupus nephritis may or may not have renal symptoms, but the disease can manifest itself through weight gain, high blood pressure, darker foamy urine or swelling around the eyes, legs, ankles or fingers.

[0216] SLE is a complex autoimmune disease with pleiotropic clinical manifestations. Up to 80% of patients with lupus develop renal abnormalities, but the renal prognosis varies greatly within this population. C. Parikh et al., (2006) "The Long Term Outcome of Glomerular Diseases, in DISEASES OF THE KIDNEY AND Urinary TRACT: CLINICOPATHOLOGIC

FOUNDATIONS OF MEDICINE (R.W. Schrier ed., 8th ed., Lippincott Williams & Wilkins, Philadelphia, PA. Furthermore, in individual patients the disease may transform from one pattern to another. In some cases, the only renal manifestation of the disease is painless hematuria or proteinuria, but in some cases patients develop lupus nephritis, leading to acute or end-stage renal failure. Patients with active proliferative nephritis are usually treated with steroids in combination with cytotoxic agents or mycophenolate mofetil. Waldman, M. et al., 2006, *Kidney Int.* **70**:1403-1412. Because of the significant morbidity associated with these agents, however, careful consideration is necessary to identify patients who require aggressive therapy. The duration and intensity of therapy are also frequently adjusted according to how well a patient is responding. Thus, one of the great challenges to treating SLE patients with lupus nephritis is assessing the activity of the disease, and tailoring pharmacologic therapy to achieve remission while minimizing toxicity.

[0217] The most commonly used system for classifying the different histologic patterns of lupus nephritis was originally developed by the World Health Organization ("WHO"), and is based upon the appearance of glomeruli by light microscopy. J.J. Weening et al., *2004, J. Am. Soc. Nephrol.* **15**:241-250. Proliferative lupus nephritis (WHO class III or IV) has the worst prognosis and most large clinical trials have focused on the response of these patients to therapy. The histologic pattern of disease may change over time or in response to treatment, however, and clinical parameters do not correlate well with disease activity. Serologic studies, such as measurement of perturbations in circulating levels of C3 and C4, are also poor markers of disease activity and are not specific to renal disease activity.

[0218] Histologically, a hallmark of SLE is membranous glomerulonephritis (also referred to as "lupus nephritis") with "wire loop" abnormalities, comprising a glomerular capillary loop with a circumferential, subendothelial immune complex deposit around the loop. The wire loop lesion results from immune complex deposition along the glomerular basement membrane, which leads to a characteristic granular appearance in immunofluorescence images. Thus, the diagnosis of active lupus nephritis is founded upon the presence of mesangial, subendothelial, and/or subepithelial immune-complexes. Complement activation is an essential prerequisite to active immune complex disease such as lupus nephritis.

[0219] Because effective treatment of proliferative lupus nephritis often requires treatment with potent immunosuppressive agents such as cyclophosphamide or mycophenolate mofetil, treatment is usually guided by examination of a renal biopsy. Definitive diagnosis of glomerular diseases such as active lupus nephritis is based upon the examination of renal biopsy tissue by light microscopy, electron microscopy, and immunofluorescence staining for

clinical markers of inflammation, including, but not limited to, IgM, IgA, IgG, C3, C4, and C1q.

[0220] Percutaneous renal biopsy is the gold standard for the definitive diagnosis of lupus nephritis and for monitoring the course of disease. As discussed herein, however, renal biopsies have their limitations and risks. Because a needle biopsy samples only a small portion of the kidney, there is a risk of sample error leading to an incorrect diagnosis. Furthermore, although biopsy is a generally safe procedure, major complications may occur in a significant percentage of biopsies and intra-renal bleeding and hematuria are common. W.L. Whittier et al., 2004, *J. Am. Soc. Nephrol.* **15**:142-147; D.C. Mendelssohn et al., 1995, *Am. J. Kidney Disease* **26**:580-585. Therefore, there is a need to develop accurate, safe, and non-invasive methods to image and diagnose renal inflammation, including lupus nephritis associated with SLE.

[0221] MRI can be used to non-invasively acquire tissue images with high resolution. USPIO nanoparticles or aggregates thereof enhance signal attenuation on T2-weighted magnetic resonance images, and conjugation of such nanoparticles to binding ligands permits the detection of specific molecules at the cellular level. For example, MRI with nanoparticle detection agents can image cell migration (J.W. Bulte et al., 2001, *Nat. Biotechnol.* **19**:1141-1147), apoptosis (M. Zhao et al., 2001, *Nat. Med.* **7**:1241-1244), and can detect small foci of cancer. *See* e.g., Y.W. Jun et al., 2005, *J. Am. Chem. Soc.* **127**:5732-5733; Y.M. Huh et al., 2005, *J. Am. Chem. Soc.* **127**:12387-12391. Contrast-enhanced MRI is well-suited for the dynamic non-invasive imaging of macromolecules or of molecular events, but it requires ligands that specifically bind to the molecule of interest. J.W. Bulte et al., 2004, *NMR Biomed.* **17**:484-499.

[0222] Because complement (*e.g.*, the alternative complement pathway) is known to be involved in etiology and progression of renal inflammation and lupus nephritis associated with SLE, a ligand capable of targeting components of the complement pathway (*e.g.*, the alternative complement pathway) would be useful in targeted delivery of USPIO nanoparticles or aggregates thereof to sites of renal inflammation in SLE patients. For example, a fragment comprising SCRs 1 and 2 of complement receptor 2 ("CR2") binds the C3b, iC3b, and C3d cleavage products of alternative complement protein C3. Phospholipid-encapsulated or dextran-coated USPIO nanoparticles or aggregates thereof can be covalently conjugated to a protein ligand such as the extracellular domain of CR2, SCRs 1 to 8 of CR2, or SCRs 1 and 2 of CR2 by linkage to thiol, amine, or carboxyl groups, either directly or through an antibody or antibody fragment. The labeled protein can then be used to target the

USPIO nanoparticles or aggregates thereof to sites of complement-mediated (*e.g.*, alternative complement-mediated) inflammation.

[0223] Thus, in certain embodiments, the invention provides non-invasive methods of detecting complement-mediated inflammation associated with systemic lupus erythematosus (SLE), membranous glomerulonephritis, or lupus nephritis in an individual in need thereof, the methods comprising: (a) administering to the individual a composition comprising an effective amount of CR2-targeted USPIO nanoparticles or aggregates thereof; and (2) taking a magnetic resonance image of the individual. In certain embodiments, the complement-mediated inflammation is alternative complement-mediated inflammation. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, a mouse, or a rat. In certain embodiments, the compositions comprising CR2-targeted USPIO nanoparticles or aggregates thereof are administered by injection. In certain embodiments, the injection is parenteral, intravenous, subcutaneous, or intramuscular.

[0224] In any of the embodiments described herein, the compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the compositions are administered to provide a dose between about 1 mg/kg and about 1000 mg/kg, between about 100 mg/kg and about 1000 mg/kg, between about 250 mg/kg and about 750 mg/kg, and between about 400 mg/kg and 600 mg/kg, or a dose of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9 mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg and about 100 mg/kg. In any of the embodiments described herein, the compositions have a circulating

plasma half-life of between about 20 minutes and about 40 minutes and a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

[0225] In certain embodiments, the compositions administered as part of the methods described herein comprise CR2-targeted USPIO nanoparticles or aggregates thereof coated with dextran or encapsulated with phospholipid. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety selected from the group consisting of complement receptor 2 (CR2) or a biologically-active fragment thereof, and a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety selected from the group consisting of complement receptor 2 (CR2) or a biologically-active fragment thereof, and a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof.

[0226] In certain embodiments, the targeting moiety comprises a biologically active fragment of CR2. In certain embodiments, the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2. In certain embodiments, the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2. In certain embodiments, the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0227] In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or

aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the phospholipid-encapsulated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter. In any of the embodiments described herein, the phospholipid comprises 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine ("DSPE"). In certain embodiments, the phospholipid is PEGylated. In certain embodiments, the PEGylated phospholipid further comprises a functional group suitable for cross-linking with a CR2-targeting group. In certain embodiments, the functional group is an amine. In certain embodiments, the functional group is maleimide. In certain embodiments, the functional group is a thiol. In certain embodiments, the PEGylated phospholipid comprises polyethylene glycol ("PEG") at a molecular weight ranging from PEG100 to PEG5000, from PEG 500 to PEG5000, from PEG1000 to PEG5000, or from PEG2000 to PEG4000. In certain embodiments, the PEGylated phospholipid comprises PEG100, PEG200, PEG300, PEG400, PEG500, PEG600, PEG700, PEG800, PEG900, PEG1000, PEG1500, PEG2000, PEG2500, PEG3000, PEG3500, PEG4000, PEG4500, or PEG5000. In certain embodiments, the phospholipid comprises DSPE-PEG2000. In certain embodiments, the phospholipid comprises amine-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises thiol-functionalized DSPE-PEG2000. In certain embodiments, the phospholipid comprises maleimide-functionalized DSPE-PEG2000.

[0228] In certain embodiments, the compositions administered as part of the methods described herein comprise CR2-targeted USPIO nanoparticle aggregates between about 50 nm and about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates between about 65 nm and about 85 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates about 75 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and

wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1. In certain embodiments, the composition comprises CR2-targeted USPIO nanoparticle aggregates about 150 nm in diameter and encapsulated with phospholipid, wherein the phospholipid is amine-functionalized DSPE-PEG2000, and wherein the CR2-targeting group is a fusion protein comprising SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

[0229] In certain embodiments, the compositions administered as part of the methods described herein comprise dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 1 nm and about 1000 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 5 nm and about 500 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 10 nm and about 100 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 50 nm and about 150 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are between about 65 nm and about 85 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 75 nm in diameter. In certain embodiments, the dextran-coated CR2-targeted USPIO nanoparticles or aggregates thereof are about 150 nm in diameter.

[0230] In any of the embodiments described herein, the compositions further comprise sterile phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4). In any of the embodiments described herein, the compositions further comprise sterile normal saline (*e.g.*, 0.9% (w/v) NaCl). In any of the embodiments described herein, the compositions further comprise sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose). In any of the embodiments described herein, the compositions are provided as lyophilized compositions, and are resuspended in phosphate-buffered saline ("PBS") (*e.g.*, 1X PBS = 137 mM NaCl, 10 mM Phosphate, 2.7 mM KCl, and a pH between 7.2 and 7.4), sterile normal saline (*e.g.*, 0.9% (w/v) NaCl), or sterile half normal saline (*e.g.*, 0.45% (w/v) NaCl, sometimes also including 5% (w/v) dextrose) before use. In any of the embodiments described herein, the compositions are administered to provide a dose between about 1 mg/kg and about 1000 mg/kg, between about 100 mg/kg and about 1000 mg/kg, between about 250 mg/kg and about 750 mg/kg, and between about 400 mg/kg and 600 mg/kg, or a dose of about 1 mg/kg, about 2 mg/kg, about 3 mg/kg, about 4 mg/kg, about 5 mg/kg, about 6 mg/kg, about 7 mg/kg, about 8 mg/kg, about 9

mg/kg, about 10 mg/kg, about 11 mg/kg, about 12 mg/kg, about 13 mg/kg, about 14 mg/kg, about 15 mg/kg, about 16 mg/kg, about 17 mg/kg, about 18 mg/kg, about 19 mg/kg, about 20 mg/kg, about 25 mg/kg, about 30 mg/kg, about 35 mg/kg, about 40 mg/kg, about 45 mg/kg, about 50 mg/kg, about 60 mg/kg, about 70 mg/kg, about 80 mg/kg, about 90 mg/kg and about 100 mg/kg. In any of the embodiments described herein, the compositions have a circulating plasma half-life of between about 20 minutes and about 40 minutes and a tissue half life of one to several days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, or more days).

Articles of Manufacture Comprising CR2-Targeted Nanoparticles or Aggregates Thereof

[0231] Also provided herein are articles of manufacture (*e.g.*, kits) containing pharmaceutical compositions comprising an effective amount of bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof as described herein, and instructions for their use in the non-invasive imaging methods described herein. Thus, in certain embodiments, the article of manufacture comprises instructions for the use of pharmaceutical compositions comprising an effective amount of bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof in any of the methods of detecting complement-mediated inflammation in an individual as described herein. In certain embodiments, the article of manufacture comprises a lyophilized preparation of any of the pharmaceutical compositions comprising bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof. In certain embodiments, the individual is a mammal. In certain embodiments, the mammal is a human, mouse or rat.

[0232] The article of manufacture further comprises one or more containers. Suitable containers include, for example, bottles, vials (*e.g.*, dual chamber vials), syringes (such as single or dual chamber syringes) and test tubes. The containers may be formed from a variety of materials such as glass or plastic. In certain embodiments, the containers hold a lyophilized preparation of any of the pharmaceutical compositions comprising bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof and optionally sterile solutions or diluents for resuspending or reconstituting a lyophilized pharmaceutical composition to a desired concentration. In certain embodiments, the article of manufacture comprises a single container comprising a frozen preparation of any of the pharmaceutical compositions comprising bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof. The label, which is on or associated with the container, may indicate directions for resuspending, reconstituting and/or using the pharmaceutical composition. The label may further indicate that the lyophilized or frozen preparations comprising pharmaceutical composition comprising bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof are

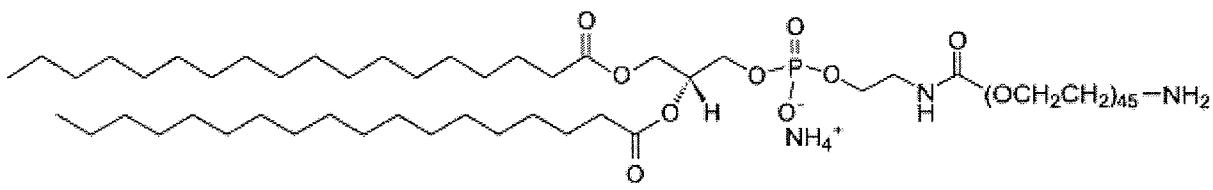
useful or intended for subcutaneous or any other mode of administration disclosed herein. The container holding the formulation may be a single-use vial or a multi-use vial. A multi-use vial allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted pharmaceutical composition. Upon mixing the sterile solution or diluent with the lyophilized pharmaceutical composition, the final concentration of bacterial-, CR2-, or C4-targeted USPIO nanoparticles or aggregates thereof in the resuspended or reconstituted pharmaceutical composition will generally be between at least 0.1 mg/ml and at least 20 mg/ml. The kit may further include other materials desirable from a commercial, therapeutic, and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

EXAMPLES

Example 1. Non-invasive detection of alternative complement-mediated inflammation in MRL/lpr mice, an animal model of lupus nephritis associated with systemic lupus erythematosus.

Materials and Methods

[0233] *Synthesis of iron oxide nanoparticles*. Ultrasmall superparamagnetic iron oxide nanoparticles were generated and functionalized for conjugation to proteins as previously described. *See* e.g., A.J. Barker et al., 2005, *J. Appl. Physics* **98**:063528; B.A. Larsen et al., 2008, *Nanotechnol.* **19**:265102. Briefly, USPIO were synthesized by a solvothermal method using an Iron (III) Acetylacetonate precursor with trioctylamine and heptanoic acid (Sigma-Aldrich, St. Louis, MO) as surfactants, yielding ~10 nm magnetite nanoparticles (Figure 1A) with a hydrophobic heptanoic acid surface termination. The as-synthesized USPIO nanoparticles were resuspended in tetrahydrofuran (THF) and titrated with a 1% (v/v) solution of acetic acid until the desired level of aggregation (~75 nm) was reached. The acetic acid partially displaces the heptanoic acid on the USPIO nanoparticle surface, decreasing steric hindrance at the nanoparticle surface and permitting increased aggregation of USPIO. The aggregates of as-synthesized particles were then encapsulated using amine-functionalized phospholipids (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000](ammonium salt):



(DSPE-PEG2000-Amine) (Avanti Polar Lipids, Alabaster, AL). The resulting micellar USPIO nanoparticle aggregates were ~75 nm in diameter, as measured by dynamic light scattering (Figure 1B). The USPIO aggregates and phospholipids were dried with nitrogen and maintained under vacuum for 24 hours. The iron-oxide/phospholipid mixture was resuspended in deionized water by bath sonication at 60°C to yield amine-functionalized, phospholipid-encapsulated USPIO aggregates for subsequent protein crosslinking. The measured particle diameter was 9.7 ± 2 nm (1151 particles measured by ImageJ particle analysis).

[0234] A recombinant protein containing the first two SCRs of CR2 linked to the Fc portion of a mouse IgG_1 was generated as previously described. *See* e.g., H.E. Gilbert et al., 2006, *J. Mol. Biol.* **356**:397-412. To conjugate CR2-Fc to the USPIO nanoparticle aggregates, 1 mg of the recombinant protein (6.7 nmol) was mixed with 10 mg (1.5 nmol) of the phospholipid-encapsulated USPIO nanoparticle aggregates. Two mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Pierce Biotechnology, Rockford, IL) and 0.2 mg of *N*-hydroxysulfosuccinimide (Sulfo-NHS) (Pierce Biotechnology, Rockford, IL) were added, and the mixture was left at room temperature for two hours while vortexing every 15 minutes. The mixture was then pelleted by centrifugation at 1500 rpm, washed, and resuspended in PBS. The resulting preparation of USPIO nanoparticle aggregates contained CR2-Fc targeting groups attached to the PEGylated head group of the phospholipid via an amide linkage.

[0235] *Flow cytometry*. To confirm conjugation of CR2-Fc to the surface of the USPIO nanoparticle aggregates, 10 μg of conjugated or unconjugated particles were incubated with 1 μg of Cy3-labeled anti- mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) or with biotinylated mouse anti-CR2 (mAb 171; J.M. Guthridge et al., 2001, *J. Immunol.* **167**:5758-5766) for 40 minutes on ice. The biotinylated antibody was then detected with FITC-streptavidin. The particles were washed in PBS and resuspended in 0.5 ml of PBS. Surface staining was detected with a BD Biosciences FACSCalibur machine and analyzed with BD Biosciences CellQuest Pro software (BD Biosciences, San Jose, CA). To test whether the conjugated particles bound to C3 opsonized cells, Chinese hamster ovary cells (CHO cells; ATCC, Manassas, VA) were incubated with 10% normal mouse serum for 1 hour at room temperature. Surface deposition of C3 was confirmed by staining with a FITC-conjugated goat anti-mouse C3 antibody (Cappel/ICN, now MP Biomedicals, Solon, OH) and detection by flow cytometry. The binding of CR2-Fc conjugated USPIO nanoparticle aggregates to CHO cells was performed by incubating opsonized or unmanipulated CHO

cells with conjugated USPIO nanoparticle aggregates for 40 minutes at 4°C. Cy3 conjugated anti-mouse IgG was used to detect the conjugated USPIO, and forward and side scatter were used to identify the CHO cells. Binding of the USPIO nanoparticle aggregates to CHO cells was then identified by examining Cy3 staining in the CHO cell population.

[0236] *MRI image acquisition and imaging parameters*. To evaluate *in vivo* anatomical and molecular abnormalities, 16 week-old MRL/lpr mice (n=5; Jackson Laboratories, Bar Harbor, ME) and C57BL/6 wild-type animals (n=6; Jackson Laboratories, Bar Harbor, ME) were assessed by MRI. The proton density weighted MRI (for anatomical assessment) and series of T2-weighted MRI (for molecular assessment based on T2 values) scans were performed at baseline and 4, 24, 48 and 72 hours after USPIO nanoparticle aggregate injection (untargeted as well as CR2-Fc conjugated). The baseline MRI was performed in the morning prior to the USPIO nanoparticle aggregate injection. The mice were injected with 0.4 mg (10-16 mg/kg) of CR2-Fc conjugated or non-targeted USPIO nanoparticle aggregates via the tail vein in a volume of less than 200 µl. At each time point, animals were weighed, anesthetized by isoflurane (4% for induction, followed by 2-2.5% isoflurane during the scan) and placed into a mouse animal bed. Anesthetized animals were then inserted into a 4.7 Tesla Bruker PharmaScan MRI scanner (Bruker BioSpin Inc., Fremont, CA). A Bruker volume coil (38 mm diameter) tuned to the ^{1}H frequency of 200 MHz was used for radiofrequency (RF) transmission and reception. First, a fast spin echo Rapid Acquisition with Relaxation Enhancement (RARE) tri-pilot scan was performed for anatomical localization for all three dimensions (axial, coronal and sagittal). Then high-resolution RARE proton density (PD)-weighted images were acquired: field of view (FOV)=4.00 cm; slice thickness 1.0 mm; inter-slice distance 1.0 mm (no gap allowed); echo time/repetition time (TE/TR)=31.9/3,000 ms; slice orientation axial; number of slices 16; number of averages 2; matrix size 256x256; total acquisition time 4 min 16 sec. Subsequently, a series of multiple slice multiple echo (MSME) T2-weighted pulses with 16 various echo times was applied for precise T2 mapping and calculation of T2 relaxation times. The scan parameters were as followed: FOV=4.00 cm; slice thickness 1.50 mm; inter-slice distance 1.80 mm; TR=2,650 ms; TE1=10 ms; TE2=20 ms (followed by 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160 ms); slice orientation axial; number of slices 16; number of averages 2; matrix size 128x256; total acquisition time 11 min. After 1 min of pre-contrast images, 0.1 mmol/kg OMNISCAN® was injected using the tail vein catheter. T1-weighted gadolinium-enhanced MRI scans were continuously taken for another 14 minutes.

[0237] *MRI imaging data analysis*. All images were processed using Bruker ParaVision software (version PV3.0.2; Bruker BioSpin Inc., Fremont, CA). For PD-weighted MRI, only visualization of abnormal lymphadenopathy and kidney size was taken into consideration. For the series of T2-weighted images with 16 various echo-time, a low-volume region of interest (ROI) was placed onto muscle, fat, spleen, left and right kidney cortex, left and right outer medulla and left and right inner medulla on a corresponding slice for each set of TE series. From the basic physics of magnetic resonance (MR), the signal intensity of an MR image is proportional to tissue T_1 and T_2 relaxation times, as well as applied repetition and echo times. Thus, for the series of 16 echo times in our MR protocol, a Bruker-based t2vtr-fitting function based on the equations below was applied in order to calculate T_2 relaxation time as a function of signal intensity and TE values of each image: (1) $S=M_0(1-e^{-TR/T1})e^{-TE/T2}$; and (2) $S=C_2(e^{-TE/T2})$, where $C_2=M_0(1-e^{-TR/T1})$ is a constant (which gets fitted). All calculated T_2 times are given in milliseconds (ms).

[0238] *Immunofluorescence microscopy and histology*. For immunofluorescence, sagittal sections of the kidneys were snap-frozen in OCT compound (Sakura Finetek, Torrance, CA). Four μm sections were cut with a cryostat and stored at -70°C. The slides were later fixed with acetone and stained with FITC-conjugated anti-mouse C3 (ICN/Cappel, now MP Biomedicals, Solon, OH), mouse IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA). Sections were counterstained with hematoxylin (Vector Laboratories, Inc., Burlingame, CA). For iron staining, sagittal sections were fixed, embedded in paraffin, and four μm sections were cut and stained with periodic acid-Schiff stain or Perl's Prussian Blue.

[0239] *Statistical analysis*. All numerical data for T2 values are presented as mean ± standard deviation from the replicate experiments. One-way analysis of variance (ANOVA) method was used to determine differences between groups (wild-type vs. lupus mice at a defined time point, as well as differences between various time points in the same group). Tukey's test was used as a post-hoc test in combination with ANOVA to test for significances between groups. The significance level was set at $p<0.05$ for all tests (SigmaPlot®-version 9.01, Systat Software, Point Richmond, CA and SPSS version 14.0, SPSS Inc., Chicago, IL).

Results

[0240] *CR2-conjugated iron oxide nanoparticle aggregates bind to cell-bound C3.* Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles and USPIO nanoparticle aggregates were generated as described above (Figures 1A-B). A recombinant protein

comprising the first two SCRs of human CR2, containing the C3d binding domain, linked to the Fc region of a mouse IgG_1 molecule ("CR2-Fc") (H.E. Gilbert et al., 2006, *J. Mol. Biol.* **356**:397-412) was conjugated to the surface of the lipid-encapsulated USPIO nanoparticle aggregates by cross-linking the carboxyl-terminus of the Fc region to surface amines on the lipid coating. The presence of CR2-Fc on the surface of the USPIO nanoparticle aggregates was confirmed by flow cytometry (Figure 2A). Chinese hamster ovary (CHO) cells were next incubated with normal mouse serum in order to opsonize them with C3 activation fragments (Figure 2B). When CR2-Fc conjugated USPIO nanoparticle aggregates and unconjugated USPIO nanoparticle aggregates were incubated with C3 opsonized CHO cells, the CR2-Fc conjugated USPIO nanoparticle aggregates bound to the CHO cells, but the unconjugated USPIO nanoparticle aggregates did not (Figure 2C). Furthermore, CR2-Fc conjugated USPIO nanoparticle aggregates did not bind to CHO cells that had not been treated with serum. These experiments confirmed that the CR2-Fc conjugated to the surface of the USPIO nanoparticle aggregates mediated binding of those aggregates to cell-bound breakdown fragments of complement protein C3.

[0241] Stability of CR2-targeted USPIO nanoparticle aggregates was tested as follows. CR2-Fc conjugated USPIO nanoparticle aggregates were incubated in normal serum for up to one month. Aliquots were removed at various intervals for analysis by forward and side scatter and flow cytometry as described herein. After one week, the CR2-targeted USPIO nanoparticle aggregates were stable and the surface CR2 was intact. After one month, the appearance of the particles by forward and side scatter remained the same, though the surface CR2 appeared degraded. Thus, the USPIO nanoparticle aggregates were stable for up to one month in normal serum, while the CR2-targeting group was susceptible to degradation, presumably by serum proteases.

[0242] *Immune complexes deposit in the glomeruli of MRL/lpr mice and activate complement.* The MRL/lpr strain of mice is homozygous for the lpr (lymphoproliferation) mutation in the gene for the Fas antigen. *See* e.g., M. Adachi et al., 1993, *Proc. Nat'l Acad. Sci. USA* **90**:1756-1760; R. Watanabe-Fukunaga et al., 1992, *Nature* **356**:314-317. MRL/lpr mice spontaneously develop lupus-like renal disease characterized by glomerular immune-complex deposition and complement activation. *See* e.g., M.H. Foster, 1999, *Semin. Nephrol.* **19**:12-24. This model has been widely utilized to examine the role of complement activation in the pathogenesis of lupus-like renal disease. *See* e.g., C. Atkinson et al., 2008, *J. Immunol.* **180**:1231-1238; L. Bao et al., 2002, *J. Immunol.* **168**:3601-3607; S. Einav et al., 2002, *J. Immunol.* **168**:1036-1041; M.K. Elliott et al., 2004, *Kidney Int.* **65**:129-138; H. Sekine et al.,

2001, *J. Immunol.* **166**:6444-6451; H. Watanabe et al., 2000, *J. Immunol.* **164**:786-794. Examination of the kidneys of 16 week-old mice confirmed that proliferative changes were present in the glomeruli but were not present in wild-type mice (Figures 3A-B). By 18-20 weeks glomerulosclerosis and tubulointerstitial fibrosis were apparent (Figure 3C). Immunofluorescence microscopy confirmed the presence of C3 in the glomeruli of 16 week-old MRL/lpr mice (Figure 3D). In the MRL/lpr mice, C3 was also deposited along the tubular basement membrane in the outer medulla (Figure 3E) and in the inner medulla (Figure 3F). As has been described elsewhere (J.M. Thurman et al., 2003, *J. Immunol.* **170**:1517-1523), small C3 deposits are present along Bowman's capsule and the tubular basement membrane of unmanipulated wild-type mice (Figures 3G, 3H, and 3I).

[0243] Kidneys of MRL/lpr mice have higher baseline T2-values than those of wild -type mice. Various anatomic and morphological abnormalities were present in MR images of 16 week-old MRL/lpr mice. The body weights of MRL/lpr mice (35.1 ± 2.3 g) were higher than those of the wild-type mice (27.6 ± 2.9 g; P=0.006; Table 1). Proton-density-weighted rapid acquisition with relaxation enhancement (RARE) MRI images in the MRL/lpr mice demonstrated multiple areas of lymphadenopathy and hyperplasia of the lymphoid organs (Figure 4A). Multiple slice multiple echo (MSME) T2-weighted MRI series (Figure 4B) showed similar T2 relaxation time values in the muscle, fat and spleens of both groups (Table 1). T2-weighted signal intensity and corresponding T2 relaxation time values in the renal cortex of MRL/lpr mice were significantly higher than in wild-type animals (Table 1). The higher T2 values in the kidneys of MRL/lpr mice likely represent higher water content, possibly the result of tissue inflammation.

Table 1: T2 relaxation time values in various organs at baseline and 4, 24, 48 and 72 hours after injection of CR2-targeted USPIO nanoparticles.

Organ	Baseline		4 hrs post		24 hrs post		48 hrs post		72 hrs post	
	MRL/lpr	WT	MRL/lpr	WT	MRL/lpr	WT	MRL/lpr	WT	MRL/lpr	WT
Cortex	52.6±4.8[A]	47.4±2.6	47.1±3.6[B]	47.6±4.8	46.0±3.3[C]	49.2±4.1	47.5±3.5[C]	50.7±3.7	49.8±5.32	50.0±3.4
O-Medu	68.8±8.4[A]	61.3±4.9	57.4±3.4[B]	63.3±5.8	55.5±3.4[C]	68.9±10.0	54.9±3.2[C]	64.2±4.9	56.4±6.5[C]	62.5±6.3
I-Medu	93.3±10.3	89.7±3.0	77.7±8.3[B]	91.1±8.2	66.3±6.4[C]	92.7±7.4	67.6±7.1[C]	91.9±6.0	77.6±9.6[C]	92.2±6.3
Spleen	41.8±1.0	38.8±4.0	41.5 ±5.4	40.2±7.3	43.2±4.1	38.6±4.2	44.5±2.9	34.5±4.8	44.4±6.5	38.2±7.1
Muscle	33.1±0.8	32.8±2.6	31.0±3.1	37.0±1.4	32.7±3.1	33.0±0.3	35.5±2.8	33.0±1.3	35.4±1.3	33.0±0.4
Fat	103.0±5.1	100.4±7.8	102.0±3.0	100.0±4.1	99.4±6.4	100.2±2.8	102.3±3.1	97.8±3.3	101.7±3.5	98.7±3.9
Body Wt. (g)	35.1±2.3[B]	27.6±2.9	35.1±6.3	27.6±3.1	34.4±2.8	25.5±2.3	34.8±2.2	26.4±2.2	34.6±2.4	26.8±2.6

Abbreviations: *I-Medu*, inner medulla; *O-Medu*, outer medulla; WT, wild-type.

Data are given as means±S.D. n=5 for MRL/lpr and n=3 for wild-type mice for examination of spleen, muscle and fat.

For evaluation of kidneys the number of repetitions were n=10 and n=6, respectively, since left and right kidneys of each animal were evaluated.

[A]P<0.05 MRL/lpr versus wild-type at baseline; [B]P<0.01 MRL/lpr versus wild-type at baseline; [C]P<0.05 post-USPIO injection versus baseline values

[0244] *Untargeted USPIO nanoparticle aggregates do not alter the T2-weighted signal in the kidneys of MRL/lpr mice.* MRL/lpr mice and wild-type mice were injected intravenously with unconjugated USPIO nanoparticle aggregates, and T_2-weighted images were obtained 4, 24, 48, and 72 hours post injection. T_2-relaxation times in the cortex, outer medulla, and inner medulla of the kidneys were determined, and T_2 values in the kidneys of MRL/lpr mice did not significantly decrease after injection of unconjugated USPIO nanoparticle aggregates (Figure 5). In wild-type mice, a small decrease in the signal in the outer medulla of the kidney was observed 48 hours after injection with the unconjugated USPIO nanoparticle aggregates (data not shown). The spleens of wild-type mice also demonstrated slightly decreased T2 values 48 hours after injection of the USPIO nanoparticle aggregates, but decreased T2 values were not seen in the spleens of MRL/lpr mice. No significant decreases in the T2-weighted signal intensity in muscle and fat were observed in either MRL/lpr or wild-type animals at any time-point. No changes in body weights were observed after injection of unconjugated USPIO nanoparticle aggregates at any time point.

[0245] *USPIOs conjugated to CR2-Fc reduce T2 relaxation times in the kidneys of MRL/lpr mice.* After one week the same mice were injected with CR2-targeted USPIO nanoparticle aggregates and T_2-weighted images were again obtained after 4, 24, 48, and 72 hours. T_2-MRI series were then used for precise calculations of the T_2-relaxation times. Unmanipulated wild-type mice have deposited C3 on Bowman's capsule and in the tubulointerstitium (Figures 3G-I). Injection of wild-type mice with the CR2-conjugated USPIO nanoparticle aggregates, however, did not cause a significant reduction in the T_2-weighted signal in any region of the kidneys (Table 1). Signal in the spleen, muscle, and fat of wild-type mice also did not change after injection with CR2-conjugated USPIO nanoparticle aggregates.

[0246] In marked contrast, injection of CR2-targeted USPIO nanoparticle aggregates into MRL/lpr mice caused a significant decrease in the T_2-weighted signal in the kidneys (Figure 5; Table 1). Signal in the cortex was reduced from 4 to 48 hours after injection, and signal in the outer and inner medulla was reduced from 4 to 72 hours after injection (Figure 5). No significant reduction in signal was seen in muscle, fat or spleen tissue of MRL/lpr mice (Table 1). Although the baseline (pre-enhanced) T_2-relaxation times in the cortex were higher in MRL/lpr mice than in wild-type controls, injection of the CR2-targeted USPIO nanoparticle aggregates caused a reduction in the T_2-relaxation times in the kidneys of MRL/lpr below the values of the control mice (Table 1). Darkening in the MRL/lpr kidneys is clearly visible on the T_2-MRI series with increasing echo times (Figure 6B). No changes were

seen in the T_2-relaxation times of kidneys in wild-type mice after injection with the CR2-targeted USPIO nanoparticle aggregates, confirming the specificity of this method.

[0247] *Location of iron and macrophages in the kidneys of mice injected with CR2-targeted USPIO nanoparticles.* Kidneys were obtained from the mice at the end of the study, and stained for iron with Perl's Prussian Blue (Figures 7A-D). Iron was still detectable in some glomeruli and in tubules of MRL/lpr mice injected with the targeted USPIO nanoparticle aggregates, but was not seen in the kidneys of control mice.

Discussion

[0248] USPIO nanoparticles and aggregates thereof have been used as a contrast agent for MR imaging. The experiments described herein used a recombinant form of CR2 protein to target such particles to sites of complement activation. *In vitro* assays confirmed that CR2 linked to the surface of the aggregates mediated binding to cells that were opsonized with C3 activation fragments, and that the particles did not bind to cells in that were not opsonized with C3 fragments.

[0249] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is apparent to those skilled in the art that certain minor changes and modifications will be practiced. Therefore, the description and examples should not be construed as limiting the scope of the invention.

INFORMAL DESCRIPTION OF SEQUENCES

[0250] SEQ ID NO:1 [amino acid sequence of full-length human CR2 protein]: MGAAG
LLGVFLALVAPGVLGISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCI
TKDKVDGTWDKPAPKCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFS
MNGNKSVWCQANNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSV
TYSCESGYLLVGEKIINCLSSGKWSAVPPTCEEARCKSLGRFPNGKVKEPPILRVGVT
ANFFCDEGYRLQGPPSSRCVIAGQGVAWTKMPVCEEIFCPSPPPILNGRHIGNSLANV
SYGSIVTYTCDPDPEEGVNFILIGESTLRCTVDSQKTGTWSGPAPRCELSTSAVQCPHP
QILRGRMVSGQKDRYTYNDTVIFACMFGFTLKGSKQIRCNAQGTWEPSAPVCEKEC
QAPPNILNGQKEDRHMVRFDPGTSIKYSCNPGYVLVGEESIQCTSEGVWTPPVPQCK
VAACEATGRQLLTKPQHQFVRPDVNSSCGEGYKLSGSVYQECQGTIPWFMEIRLCKE
ITCPPPPVIYNGAHTGSSLEDFPYGTTVTYTCNPGPERGVEFSLIGESTIRCTSNDQERG
TWSGPAPLCKLSLLAVQCSHVHIANGYKISGKEAPYFYNDTVTFKCYSGFTLKGSSQI
RCKRDNTWDPEIPVCEKGCQPPPGLHHGRHTGGNTVFFVSGMTVDYTCDPGYLLVG
NKSIHCMPSGNWSPSAPRCEETCQHVRQSLQELPAGSRVELVNTSCQDGYQLTGHA

YQMCQDAENGIWFKKIPLCKVIHCHPPPVIVNGKHTGMMAENFLYGNEVSYECDQG
FYLLGEKNCSAEVILKAWILERAFPQCLRSLCPNPEVKHGYKLNKTHSAYSHNDIVY
VDCNPGFIMNGSRVIRCHTDNTWVPGVPTCIKKAFIGCPPPPKTPNGNHTGGNIARFS
PGMSILYSCDQGYLVVGEPLLLCTHEGTWSQPAPHCKEVNCSSPADMDGIQKGLEPR
KMYQYGAVVTLECEDGYMLEGSPQSQCQSDHQWNPPLAVCRSRSLAPVLCGIAAG
LILLTFLIVITLYVISKHRERNYYTDTSQKEAFHLEAREVYSVDPYNPAS.

[0251] SEQ ID NO:2 [amino acid sequence of full-length mouse CR2 protein]: MLTWF
LFYFSEISCDPPPEVKNARKPYYSLPIVPGTVLRYTCSPSYRLIGEKAIFCISENQVHAT
WDKAPPICESVNKTISCSDPIVPGGFMNKGSKAPFRHGDSVTFTCKANFTMKGSKTV
WCQANEMWGPTALPVCESDFPLECPSLPTIHNGHHTGQHVDQFVAGLSVTYSCEPG
YLLTGKKTIKCLSSGDWDGVIPTCKEAQCEHPGKFPNGQVKEPLSLQVGTTVYFSCN
EGYQLQGQPSSQCVIVEQKAIWTKKPVCKEILCPPPPPVRNGSHTGSFSENVPYGSTV
TYTCDPSPEKGVSFTLIGEKTINCTTGSQKTGIWSGPAPYCVLSTSAVLCLQPKIKRGQ
ILSILKDSYSYNDTVAFSCEPGFTLKGNRSIRCNAHGTWEPPVPVCEKGCQAPPKIING
QKEDSYLLNFDPGTSIRYSCDPGYLLVGEDTIHCTPEGKWTPITPQCTVAECKPVGPH
LFKRPQNQFIRTAVNSSCDEGFQLSESAYQLCQGTIPWFIEIRLCKEITCPPPPVIHNGT
HTWSSSEDVPYGTVVTYMCYPGPEEGVKFKLIGEQTIHCTSDSRGRGSWSSPAPLCK
LSLPAVQCTDVHVENGVKLTDNKAPYFYNDSVMFKCDDGYILSGSSQIRCKANNTW
DPEKPLCKKEGCEPMRVHGLPDDSHIKLVKRTCQNGYQLTGYTYEKCQNAENGTW
FKKIEVCTVILCQPPPKIANGGHTGMMAKHFLYGNEVSYECDEGFYLLGEKSLQCVN
DSKGHGSWSGPPPQCLQSSPLTHCPDPEVKHGYKLNKTHSAFSHNDIVHFVCNQGFI
MNGSHLIRCHTNNTWLPGVPTCIRKASLGCQSPSTIPNGNHTGGSIARFPPGMSVMYS
CYQGFLMAGEARLICTHEGTWSQPPPFCKEVNCSFPEDTNGIQKGFQPGKTYRFGAT
VTLECEDGYTLEGSPQSQCQDDSQWNPPLALCKYRRWSTIPLICGISVGSALIILMSV
GFCMILKHRESNYYTKTRPKEGALHLETREVYSIDPYNPAS.

[0252] SEQ ID NO:3 [amino acid sequence of the extracellular domain of human CR2 protein]: ISCGSPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDG
TWDKPAPKCEYFNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSV
WCQANNMWGPTRLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGY
LLVGEKIINCLSSGKWSAVPPTCEEARCKSLGRFPNGKVKEPPILRVGVTANFFCDEG
YRLQGPPSSRCVIAGQGVAWTKMPVCEEIFCPSPPPILNGRHIGNSLANVSYGSIVTYT
CDPDPEEGVNFILIGESTLRCTVDSQKTGTWSGPAPRCELSTSAVQCPHPQILRGRMV
SGQKDRYTYNDTVIFACMFGFTLKGSKQIRCNAQGTWEPSAPVCEKECQAPPNILNG
QKEDRHMVRFDPGTSIKYSCNPGYVLVGEESIQCTSEGVWTPPVPQCKVAACEATG

RQLLTKPQHQFVRPDVNSSCGEGYKLSGSVYQECQGTIPWFMEIRLCKEITCPPPPVI
YNGAHTGSSLEDFPYGTTVTYTCNPGPERGVEFSLIGESTIRCTSNDQERGTWSGPAP
LCKLSLLAVQCSHVHIANGYKISGKEAPYFYNDTVTFKCYSGFTLKGSSQIRCKADN
TWDPEIPVCEKETCQHVRQSLQELPAGSRVELVNTSCQDGYQLTGHAYQMCQDAEN
GIWFKKIPLCKVIHCHPPPVIVNGKHTGMMAENFLYGNEVSYECDQGFYLLGEKKLQ
CRSDSKGHGSWSGPSPQCLRSPPVTRCPNPEVKHGYKLNKTHSAYSHNDIVYVDCNP
GFIMNGSRVIRCHTDNTWVPGVPTCIKKAFIGCPPPPKTPNGNHTGGNIARFSPGMSIL
YSCDQGYLLVGEALLLCTHEGTWSQPAPHCKEVNCSSPADMDGIQKGLEPRKMYQ
YGAVVTLECEDGYMLEGSPQSQCQSDHQWNPPLAVCRSR.

[0253] SEQ ID NO:4 [amino acid sequence of the extracellular domain of mouse CR2 protein]: ISCDPPPEVKNARKPYYSLPIVPGTVLRYTCSPSYRLIGEKAIFCISENQVHA
TWDKAPPICESVNKTISCSDPIVPGGFMNKGSKAPFRHGDSVTFTCKANFTMKGSKT
VWCQANEMWGPTALPVCESDFPLECPSLPTIHNGHHTGQHVDQFVAGLSVTYSCEP
GYLLTGKKTIKCLSSGDWDGVIPTCKEAQCEHPGKFPNGQVKEPLSLQVGTTVYFSC
NEGYQLQGQPSSQCVIVEQKAIWTKKPVCKEILCPPPPPVRNGSHTGSFSENVPYGST
VTYTCDPSPEKGVSFTLIGEKTINCTTGSQKTGIWSGPAPYCVLSTSAVLCLQPKIKRG
QILSILKDSYSYNDTVAFSCEPGFTLKGNRSIRCNAHGTWEPPVPVCEKGCQAPPKIIN
GQKEDSYLLNFDPGTSIRYSCDPGYLLVGEDTIHCTPEGKWTPITPQCTVAECKPVGP
HLFKRPQNQFIRTAVNSSCDEGFQLSESAYQLCQGTIPWFIEIRLCKEITCPPPPVIHNG
THTWSSSEDVPYGTVVTYMCYPGPEEGVKFKLIGEQTIHCTSDSRGRGSWSSPAPLC
KLSLPAVQCTDVHVENGVKLTDNKAPYFYNDSVMFKCDDGYILSGSSQIRCKANNT
WDPEKPLCKKEGCEPMRVHGLPDDSHIKLVKRTCQNGYQLTGYTYEKCQNAENGT
WFKKIEVCTVILCQPPPKIANGGHTGMMAKHFLYGNEVSYECDEGFYLLGEKSLQC
VNDSKGHGSWSGPPPQCLQSSPLTHCPDPEVKHGYKLNKTHSAFSHNDIVHFVCNQ
GFIMNGSHLIRCHTNNTWLPGVPTCIRKASLGCQSPSTIPNGNHTGGSIARFPPGMSV
MYSCYQGFLMAGEARLICTHEGTWSQPPPFCKEVNCSFPEDTNGIQKGFQPGKTYRF
GATVTLECEDGYTLEGSPQSQCQDDSQWNPPLALCKYRRW.

[0254] SEQ ID NO:5 [amino acid sequence of SCRs 1 to 8 of human CR2 protein]: ISCGS
PPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDKPAPKCEYF
NKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVWCQANNMWGPT
RLPTCVSVFPLECPALPMIHNGHHTSENVGSIAPGLSVTYSCESGYLLVGEKIINCLSS
GKWSAVPPTCEEARCKSLGRFPNGKVKEPPILRVGVTANFFCDEGYRLQGPPSSRCVI
AGQGVAWTKMPVCEEIFCPSPPPILNGRHIGNSLANVSYGSIVTYTCDPDPEEGVNFIL
IGESTLRCTVDSQKTGTWSGPAPRCELSTSAVQCPHPQILRGRMVSGQKDRYTYNDT

VIFACMFGFTLKGSKQIRCNAQGTWEPSAPVCEKECQAPPNILNGQKEDRHMVRFDP GTSIKYSCNPGYVLVGEESIQCTSEGVWTPPVPQCKVAACEATGRQLLTKPQHQFVR PDVNSSCGEGYKLSGSVYQECQGTIPWFMEIRLCKE.

[0255] SEQ ID NO:6 [amino acid sequence of SCRs 1 to 8 of mouse CR2 protein]: ISCD PPPEVKNARKPYYSLPIVPGTVLRYTCSPSYRLIGEKAIFCISENQVHATWDKAPPICE SVNKTISCSDPIVPGGFMNKGSKAPFRHGDSVTFTCKANFTMKGSKTVWCQANEMW GPTALPVCESDFPLECPSLPTIHNGHHTGQHVDQFVAGLSVTYSCEPGYLLTGKKTIK CLSSGDWDGVIPTCKEAQCEHPGKFPNGQVKEPLSLQVGTTVYFSCNEGYQLQGQPS SQCVIVEQKAIWTKKPVCKEILCPPPPPVRNGSHTGSFSENVPYGSTVTYTCDPSPEK GVSFTLIGEKTINCTTGSQKTGIWSGPAPYCVLSTSAVLCLQPKIKRGQILSILKDSYSY NDTVAFSCEPGFTLKGNRSIRCNAHGTWEPPVPVCEKGCQAPPKIINGQKEDSYLLNF DPGTSIRYSCDPGYLLVGEDTIHCTPEGKWTPITPQCTVAECKPVGPHLFKRPQNQFIR TAVNSSCDEGFQLSESAYQLCQGTIPWFIEIRLCKE.

[0256] SEQ ID NO:7 [amino acid sequence of SCRs 1 and 2 of human CR2 protein]: ISCG SPPPILNGRISYYSTPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDKPAPKCEY FNKYSSCPEPIVPGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVWCQANNMWGP TRLPTCVS.

[0257] SEQ ID NO:8 [amino acid sequence of SCRs 1 and 2 of mouse CR2 protein]: ISCD PPPEVKNARKPYYSLPIVPGTVLRYTCSPSYRLIGEKAIFCISENQVHATWDKAPPICE SVNKTISCSDPIVPGGFMNKGSKAPFRHGDSVTFTCKANFTMKGSKTVWCQANEMW GPTALPVCES.

[0258] SEQ ID NO:9 [amino acid sequence of SCRs 1 and 2 of human CR2 protein fused to the Fc domain of mouse IgG_1 (CR2 sequences are underlined)]: ISCGSPPPILNGRISYY STPIAVGTVIRYSCSGTFRLIGEKSLLCITKDKVDGTWDKPAPKCEYFNKYSSCPEPIV PGGYKIRGSTPYRHGDSVTFACKTNFSMNGNKSVWCQANNMWGPTRLPTCVSVFPL EGSIEGRGGSELADPEVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVV VDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFK CRVNSAAFPAPIEKTISKTKGRPKAPQLYTIPPPKEQMAKDKVSLTCMITDFFPEDITV EWQWNGQPAENYKNTQPIMNTNESYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHN HHTEKSLSHSPGK.

[0259] SEQ ID NO:10 [amino acid sequence of human C3d protein]: HLIVTPSGCGEQN MIGMTPTVIAVHYLDETEQWEKFGLEKRQGALELIKKGYTQQLAFRQPSSAFAAFVK RAPSTWLTAYVVKVFSLAVNLIAIDSQVLCGAVKWLILEKQKPDGVFQEDAPVIHQE

MIGGLRNNNEKDMALTAFVLISLQEAKDICEEQVNSLPGSITKAGDFLEANYMNLQR
SYTVAIAGYALAQMGRLKGPLLNKFLTTAKDKNRWEDPGKQLYNVEATSYALLAL
LQLKDFDFVPPVVRWLNEQRYYGGGYGSTQATFMVFQALAQYQKDAPDHQELNLD
VSLQLPSR.

[0260] SEQ ID NO:11 [amino acid sequence of human C3dg protein]: EGVQKEDIPPADL
SDQVPDTESETRILLQGTPVAQMTEDAVDAERLKHLIVTPSGCGEQNMIGMTPTVIA
VHYLDETEQWEKFGLEKRQGALELIKKGYTQQLAFRQPSSAFAAFVKRAPSTWLTA
YVVKVFSLAVNLIAIDSQVLCGAVKWLILEKQKPDGVFQEDAPVIHQEMIGGLRNNN
EKDMALTAFVLISLQEAKDICEEQVNSLPGSITKAGDFLEANYMNLQRSYTVAIAGY
ALAQMGRLKGPLLNKFLTTAKDKNRWEDPGKQLYNVEATSYALLALLQLKDFDFV
PPVVRWLNEQRYYGGGYGSTQATFMVFQALAQYQKDAPDHQELNLDVSLQLPSR.

[0261] SEQ ID NO:12 [amino acid sequence of human iC3b protein]: EGVQKEDIPPADL
SDQVPDTESETRILLQGTPVAQMTEDAVDAERLKHLIVTPSGCGEQNMIGMTPTVIA
VHYLDETEQWEKFGLEKRQGALELIKKGYTQQLAFRQPSSAFAAFVKRAPSTWLTA
YVVKVFSLAVNLIAIDSQVLCGAVKWLILEKQKPDGVFQEDAPVIHQEMIGGLRNNN
EKDMALTAFVLISLQEAKDICEEQVNSLPGSITKAGDFLEANYMNLQRSYTVAIAGY
ALAQMGRLKGPLLNKFLTTAKDKNRWEDPGKQLYNVEATSYALLALLQLKDFDFV
PPVVRWLNEQRYYGGGYGSTQATFMVFQALAQYQKDAPDHQELNLDVSLQLPSR.

[0262] SEQ ID NO:13 [amino acid sequence of human C3b protein]: SNLDEDIIAEEN
IVSRSEFPESWLWNVEDLKEPPKNGISTKLMNIFLKDSITTWEILAVSMSDKKGICVA
DPFEVTVMQDFFIDLRLPYSVVRNEQVEIRAVLYNYRQNQELKVRVELLHNPAFCSL
ATTKRRHQQTVTIPPKSSLSVPYVIVPLKTGLQEVEVKAAVYHHFISDGVRKSLKVVP
EGIRMNKTVAVRTLDPERLGREGVQKEDIPPADLSDQVPDTESETRILLQGTPVAQM
TEDAVDAERLKHLIVTPSGCGEQNMIGMTPTVIAVHYLDETEQWEKFGLEKRQGAL
ELIKKGYTQQLAFRQPSSAFAAFVKRAPSTWLTAYVVKVFSLAVNLIAIDSQVLCGA
VKWLILEKQKPDGVFQEDAPVIHQEMIGGLRNNNEKDMALTAFVLISLQEAKDICEE
QVNSLPGSITKAGDFLEANYMNLQRSYTVAIAGYALAQMGRLKGPLLNKFLTTAKD
KNRWEDPGKQLYNVEATSYALLALLQLKDFDFVPPVVRWLNEQRYYGGGYGSTQA
TFMVFQALAQYQKDAPDHQELNLDVSLQLPSRSSKITHRIHWESASLLRSEETKENE
GFTVTAEGKGQGTLSVVTMYHAKAKDQLTCNKFDLKVTIKPAPETEKRPQDAKNT
MILEICTRYRGDQDATMSILDISMMTGFAPDTDDLKQLANGVDRYISKYELDKAFSD
RNTLIIYLDKVSHSEDDCLAFKVHQYFNVELIQPGAVKVYAYYNLEESCTRFYHPEK
EDGKLNKLCRDELCRCAEENCFIQKSDDKVTLEERLDKACEPGVDYVYKTRLVKVQ

LSNDFDEYIMAIEQTIKSGSDEVQVGQQRTFISPIKCREALKLEEKKHYLMWGLSSDF WGEKPNLSYIIGKDTWVEHWPEEDECQDEENQKQCQDLGAFTESMVVFGCPN.

[0263] SEQ ID NO:14 [cDNA sequence of human CD5 signal peptide]: ATGCCCATG GGGTCTCTGCAACCGCTGGCCACCTTGTACCTGCTGGGGATGCTGGTCGCTTCCT GCCTCGGA.

[0264] SEQ ID NO:15 [amino acid sequence of human CD5 signal peptide]: MPMGSLQPLATLYLLGMLVAS.

[0265] SEQ ID NO:16 [cDNA sequence of human CR2 signal peptide, short version]: ATGGGCGCCGCGGGCCTGCTCGGGGTTTTCTTGGCTCTCGTCGCACCGGGG.

[0266] SEQ ID NO:17 [amino acid sequence of human CR2 signal peptide, long version]: MGAAGLLGVFLALVAPG.

[0267] SEQ ID NO:18 [cDNA sequence of human CR2 signal peptide, long version]: ATGGGAGCCGCTGGTCTGCTCGGCGTGTTCCTCGCCTTGGTGGCACCTGGCGTCC TGGGC.

[0268] SEQ ID NO:19 [amino acid sequence of human CR2 signal peptide, long version]: MGAAGLLGVFLALVAPGVLG.

[0269] SEQ ID NO:20 [amino acid sequence of full-length *S. aureus N315* immune subversion protein (Sbi)]: MKNKYISKLLVGAATITLATMISNGEAKASENTQQTSTKH QTTQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNP DRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKAKERQ NIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDE RVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVKEHLQKQLDALVAQKDAEKK VAPKVEAPQIQSPQIEKPKAESPKVEVPQIQSPKVEVPQSKLLGYYQSLKDSFNYGYK YLTDTYKSYKEKYDTAKYYYNTYYKYKGAIDQTVLTVLGSGSKSYIQPLKVDDKNG YLAKSYAQVRNYVTESINTGKVLYTFYQNPTLVKTAIKAQETASSIKNTLSNLLSFW K.

[0270] SEQ ID NO:21 [amino acid sequence of a fragment of *S. aureus N315* Sbi protein comprising the first four N-terminal domains (Sbi-I-II-III-IV) of the full-length Sbi protein]: KASENTQQTSTKHQTTQNNYVTDQQKAFYQVLHLKGITEEQRNQYIKTLREHPERA QEVFSESLKDSKNPDRRVAQQNAFYNVLKNDNLTEQEKNNYIAQIKENPDRSQQVW VESVQSSKAKERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFV

EKVSIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVKEHLQKQL DALVAQKDAEKKVA.

[0271] SEQ ID NO:22 [amino acid sequence of a fragment of *S. aureus N315* Sbi protein comprising the third and fourth N-terminal domains (Sbi-III-IV) of the full length Sbi protein]: ERQNIENADKAIKDFQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEK VSIEKAIVRHDERVKSANDAISKLNEKDSIENRRLAQREVNKAPMDVKEHLQKQLDA LVAQKDAEKKVA.

[0272] SEQ ID NO:23 [amino acid sequence of a fragment of *S. aureus N315* Sbi protein comprising the first four N-terminal domains (Sbi-I-II-III-IV) fused to the Fc domain of mouse IgG_1 (Sbi protein sequences are underlined)]: KASENTQQTSTKHQTTQNNYVTD QQKAFYQVLHLKGITEEQRNQYIKTLREHPERAQEVFSESLKDSKNPDRRVAQQNAF YNVLKNDNLTEQEKNNYIAQIKENPDRSQQVWVESVQSSKAKERQNIENADKAIKD FQDNKAPHDKSAAYEANSKLPKDLRDKNNRFVEKVSIEKAIVRHDERVKSANDAIS KLNEKDSIENRRLAQREVNKAPMDVKEHLQKQLDALVAQKDAEKKVAGSIEGRGG SELADPEVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPE VQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAF PAPIEKTISKTKGRPKAPQLYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQ PAENYKNTQPIMNTNESYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLS HSPGK.

[0273] SEQ ID NO:24 [amino acid sequence of Fc fragment of mouse IgG_1]: GSIEGRGG SELADPEVPRDCGCKPCICTVPEVSSVFIFPPKPKDVLTITLTPKVTCVVVDISKDDPE VQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSELPIMHQDWLNGKEFKCRVNSAAF PAPIEKTISKTKGRPKAPQLYTIPPPKEQMAKDKVSLTCMITDFFPEDITVEWQWNGQ PAENYKNTQPIMNTNESYFVYSKLNVQKSNWEAGNTFTCSVLHEGLHNHHTEKSLS HSPGK.

[0274] SEQ ID NO:25 [amino acid sequence of human C4 protein]: MRLLWGLIWASSFF TLSLQKPRLLLFSPSVVHLGVPLSVGVQLQDVPRGQVVKGSVFLRNPSRNNVPCSPK VDFTLSSERDFALLSLQVPLKDAKSCGLHQLLRGPEVQLVAHSPWLKDSLSRTTNIQ GINLLFSSRRGHLFLQTDQPIYNPGQRVRYRVFALDQKMRPSTDTITVMVENSHGLR VRKKEVYMPSSIFQDDFVIPDISEPGTWKISARFSDGLESNSSTQFEVKKYVLPNFEVK ITPGKPYILTVPGHLDEMQLDIQARYIYGKPVQGVAYVRFGLLDEDGKKTFFRGLES QTKLVNGQSHISLSKAEFQDALEKLNMGITDLQGLRLYVAAAIIESPGGEMEEAELTS WYFVSSPFSLDLSKTKRHLVPGAPFLLQALVREMSGSPASGIPVKVSATVSSPGSVPE

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VFQAAGLAFSDGDQWTLSRKRLSCPKEKTTRKKRNVNFQKAINEKLGQYASPTAKR
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ANQMRNFLVRASCRLRLEPGKEYLIMGLDGATYDLEGHPQYLLDSNSWIEEMPSER
LCRSTRQRAACAQLNDFLQEYGTQGCQV.

[0275] SEQ ID NO:26 [amino acid sequence of human C4b protein]: ALEILQEEDLIDE
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[0276] SEQ ID NO:27 [amino acid sequence of human C4d protein]: TLEIPGNSDPNMIP
DGDFNSYVRVTASDPLDTLGSEGALSPGGVASLLRLPRGCGEQTMIYLAPTLAASRY
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KVLSLAQEQVGGSPEKLQETSNWLLSQQQADGSFQDPCPVLDRSMQGGLVGNDETV
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AYALSLTKAPVDLLGVAHNNLMAMAQETGDNLYWGSVTGSQSNAVSPTPAPRNPS
DPMPQAPALWIETTAYALLHLLLHEGKAEMADQASAWLTRQGSFQGGFRSTQDTVI
ALDALSAYWIASHTTEERGLNVTLSSTGR.

[0277] SEQ ID NO:28 [amino acid sequence of human complement receptor 1 protein (CR1)]: MGASSPRSPEPVGPPAPGLPFCCGGSLLAVVVLLALPVAWGQCNAPEWLP
FARPTNLTDEFEFPIGTYLNYECRPGYSGRPFSIICLKNSVWTGAKDRCRRKSCRNPP
DPVNGMVHVIKGIQFGSQIKYSCTKGYRLIGSSSATCIISGDTVIWDNETPICDRIPCGL
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WDPPLAKCTSRAHDALIVGTLSGTIFFILLIIFLSWIILKHRKGNNAHENPKEVAIHLHS
QGGSSVHPRTLQTNEENSRVLP.

[0278] SEQ ID NO:29 [amino acid sequence of human C4b-binding protein (C4bp), alpha chain]: MHPPKTPSGALHRKRKMAAWPFSRLWKVSDPILFQMTLIAALLPAVLGNC
GPPPTLSFAAPMDITLTETRFKTGTTLKYTCLPGYVRSHSTQTLTCNSDGEWVYNTFC
IYKRCRHPGELRNGQVEIKTDLSFGSQIEFSCSEGFFLIGSTTSRCEVQDRGVGWSHPL
PQCEIVKCKPPPDIRNGRHSGEENFYAYGFSVTYSCDPRFSLLGHASISCTVENETIGV
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SKWNPSPPACEPNSCINLPDIPHASWETYPRPTKEDVYVVGTVLRYRCHPGYKPTTD
EPTTVICQKNLRWTPYQGCEALCCPEPKLNNGEITQHRKSRPANHCVYFYGDEISFSC
HETSRFSAICQGDGTWSPRTPSCGDICNFPPKIAHGHYKQSSSYSFFKEEIIYECDKGYI
LVGQAKLSCSYSHWSAPAPQCKALCRKPELVNGRLSVDKDQYVEPENVTIQCDSGY
GVVGPQSITCSGNRTWYPEVPKCEWETPEGCEQVLTGKRLMQCLPNPEDVKMALEV
YKLSLEIEQLELQRDSARQSTLDKEL.

[0279] SEQ ID NO:30 [amino acid sequence of human C4b-binding protein (C4bp), beta chain]: MFFWCACCLMVAWRVSASDAEHCPELPPVDNSIFVAKEVEGQILGTYVCIK
GYHLVGKKTLFCNASKEWDNTTTECRLGHCPDPVLVNGEFSSSGPVNVSDKITFMC
NDHYILKGSNRSQCLEDHTWAPPFPICKSRDCDPPGNPVHGYFEGNNFTLGSTISYYC
EDRYYLVGVQEQQCVDGEWSSALPVCKLIQEAPKPECEKALLAFQESKNLCEAMEN
FMQQLKESGMTMEELKYSLELKKAELKAKLL.

WHAT IS CLAIMED IS:

1. A method of detecting complement-mediated inflammation in an individual comprising:

(a) administering to the individual an effective amount a composition comprising an effective amount of CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof; and

(b) taking a magnetic resonance image of the individual.

2. The method of claim 1, wherein said CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof comprise an antibody directed to C3 or fragment thereof.

3. The method of claim 2, wherein said antibody is a monoclonal antibody.

4. The method of any of claim 1 to claim 3, wherein the individual is a mammal.

5. The method of claim 4, wherein the mammal is a human, a mouse or a rat.

6. The method of claim 5, wherein the composition is administered by injection.

7. The method of claim 6, wherein the injection is parenteral, intravenous, subcutaneous, or intramuscular.

8. The method of claim 7, wherein the composition comprises an effective amount of CR2-targeted USPIO nanoparticle aggregates.

9. The method of claim 8, wherein the CR2-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter.

10. The method of claim 9, wherein the CR2-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter.

11. The method of claim 10, wherein the CR2-targeted USPIO nanoparticle aggregates are about 75 nm in diameter.

12. The method of claim 9, wherein the CR2-targeted USPIO nanoparticle aggregates are about 150 nm in diameter.

13. The method of any one of claims 8, 9, 10, 11, or 12 wherein the CR2-targeted USPIO nanoparticle aggregates are coated with dextran or encapsulated with phospholipid.

14. The method of claim 13, wherein the phospholipid is PEGylated.

15. The method of claim 14, wherein the PEGylated phospholipid is amine-functionalized.

16. The method of claim 15, wherein the PEGylated, amine-functionalized phospholipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG2000.

17. The method of claim 13, wherein the phospholipid-encapsulated CR2-targeted USPIO nanoparticle aggregates further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof.

18. The method of claim 13, wherein the dextran-coated CR2-targeted USPIO nanoparticle aggregates further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof.

19. The method of claim 17, wherein the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2.

20. The method of claim 18, wherein the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2.

21. The method of claim 19, wherein the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1

(IgG$_1$), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG$_1$, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG$_1$.

22. The method of claim 20, wherein the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G$_1$ (IgG$_1$), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG$_1$, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG$_1$.

23. The method of claim 21, wherein the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG$_1$.

24. The method of claim 22, wherein the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG$_1$.

25. The method of claim 17, wherein the complement-mediated inflammation is associated with tissue damage resulting from cancer, ischemia reperfusion injury, inflammatory disorders, transplant rejection, pregnancy-related diseases, adverse drug reactions, and autoimmune or immune complex disorders.

26. The method of claim 25, wherein the tissue damage resulting from ischemia reperfusion injury is associated with a disorder selected from the group consisting of myocardial infarction, aneurysm, stroke, hemorrhagic shock, crush injury, multiple organ failure, hypovolemic shock, intestinal ischemia, spinal cord injury and traumatic brain injury.

27. The method of claim 25, wherein the inflammatory disorder is selected from the group consisting of burns, endotoxemia, septic shock, adult respiratory distress syndrome, cardiopulmonary bypass, hemodialysis, anaphylactic shock, asthma, angioedema, Crohn's disease, sickle cell anemia, poststreptococcal glomerulonephritis, membranous nephritis, and pancreatitis.

28. The method of claim 25, wherein the transplant rejection is hyperacute xenograft rejection.

29. The method of claim 25, wherein the pregnancy-related disease is selected from the group consisting of recurrent fetal loss and pre-eclampsia.

30. The method of claim 25, wherein the adverse drug reaction is selected from the group consisting of drug allergy and IL-2 induced vascular leakage syndrome.

31. The method of claim 25, wherein the autoimmune or immune complex disorder is selected from the group consisting of myasthenia gravis, Alzheimer's disease, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, lupus nephritis, insulin-dependent diabetes mellitus, acute disseminated encephalomyelitis, Addison's disease, antiphospholipid antibody syndrome, autoimmune hepatitis, Crohn's disease, Goodpasture's syndromes, Graves' disease, Guillain-Barré syndrome, Hashimoto's disease, idiopathic thrombocytopenic purpura, pemphigus, Sjögren's syndrome, Takayasu's arteritis, autoimmune glomerulonephritis, membranoproliferative glomerulonephritis type II, hemolytic uremic syndrome, and atypical hemolytic uremic syndrome.

32. The method of claim 31, wherein the autoimmune glomerulonephritis is selected from the group consisting of immunoglobulin A nephropathy or membranoproliferative glomerularnephritis type I.

33. A composition for detecting complement-mediated inflammation in an individual, the composition comprising an effective amount of CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof, wherein the USPIO nanoparticles or aggregates thereof are coated with dextran or encapsulated with phospholipid and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof.

34. The composition of claim 33, wherein said CR2-targeted ultrasmall superparamagnetic iron oxide ("USPIO") nanoparticles or aggregates thereof comprise an antibody directed to C3 or fragment thereof.

35. The composition of claim 34, wherein said antibody is a monoclonal antibody.

36. The composition of any of claim 33 to claim 35, wherein the individual is a mammal.

37. The composition of claim 36, wherein the mammal is a human, mouse, or rat.

38. The composition of any one of claims 33, 36 or 37, comprising an effective amount of CR2-targeted USPIO nanoparticle aggregates.

39. The composition of claim 38, wherein the CR2-targeted USPIO nanoparticle aggregates are between about 50 nm and about 150 nm in diameter.

40. The composition of claim 39, wherein the CR2-targeted USPIO nanoparticle aggregates are between about 65 nm and about 85 nm in diameter.

41. The composition of claim 40, wherein the CR2-targeted USPIO nanoparticle aggregates are about 75 nm in diameter.

42. The composition of claim 39, wherein the CR2-targeted USPIO nanoparticle aggregates are about 150 nm in diameter.

43. The composition of claim 39, wherein the phospholipid is PEGylated.

44. The composition of claim 43, wherein the PEGylated phospholipid is amine-functionalized.

45. The composition of claim 44, wherein the PEGylated, amine-functionalized phospholipid is 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-PEG2000.

46. The composition of claim 39, wherein the CR2-targeted USPIO nanoparticle aggregates are phospholipid-encapsulated and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof.

47. The composition of claim 39, wherein the CR2-targeted USPIO nanoparticles are dextran-coated and further comprise a targeting moiety comprising a fusion protein comprising an antibody or fragment thereof fused to CR2 or a biologically-active fragment thereof.

48. The composition of claim 46, wherein the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2.

49. The composition of claim 48, wherein the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2.

50. The composition of claim 47, wherein the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

51. The composition of claim 50, wherein the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

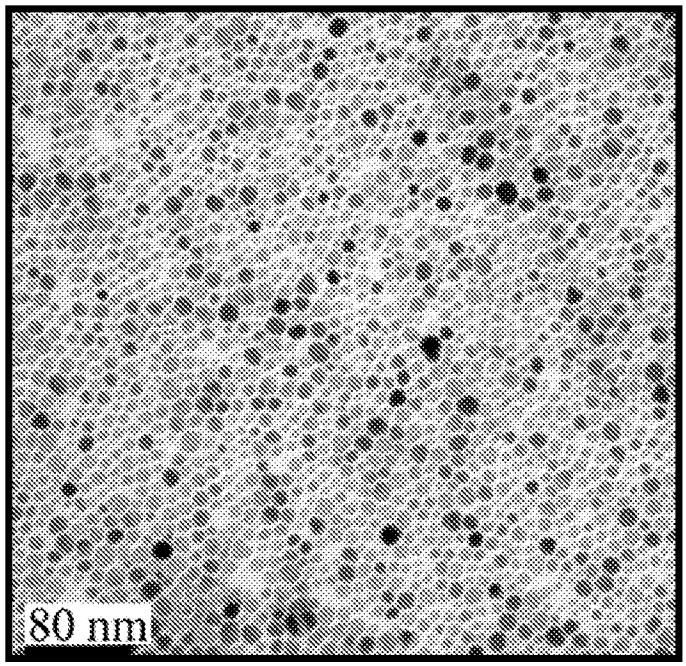
52. The composition of claim 46, wherein the targeting moiety comprises a fusion protein comprising an antibody fragment fused to a biologically active fragment of CR2.

53. The composition of claim 52, wherein the biologically-active fragment of CR2 comprises the extracellular domain of CR2, short consensus repeat sequences (SCRs) 1 to 8 of CR2, or SCRs 1 and 2 of CR2.

54. The composition of claim 53, wherein the fusion protein comprises the extracellular domain of CR2 fused to the Fc-domain of mouse immunoglobulin isotype G_1 (IgG_1), SCRs 1 to 8 of CR2 fused to the Fc-domain of mouse IgG_1, or SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse IgG_1.

55. The composition of claim 54, wherein the fusion protein comprises SCRs 1 and 2 of CR2 fused to the Fc-domain of mouse

A



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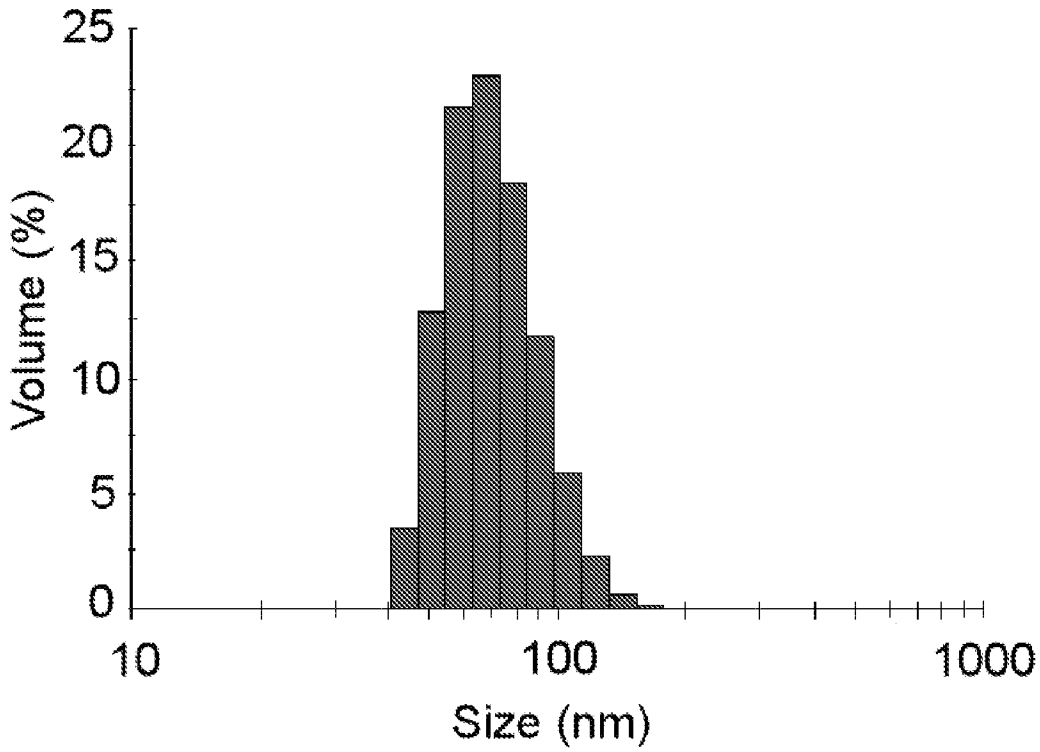


Figure 1

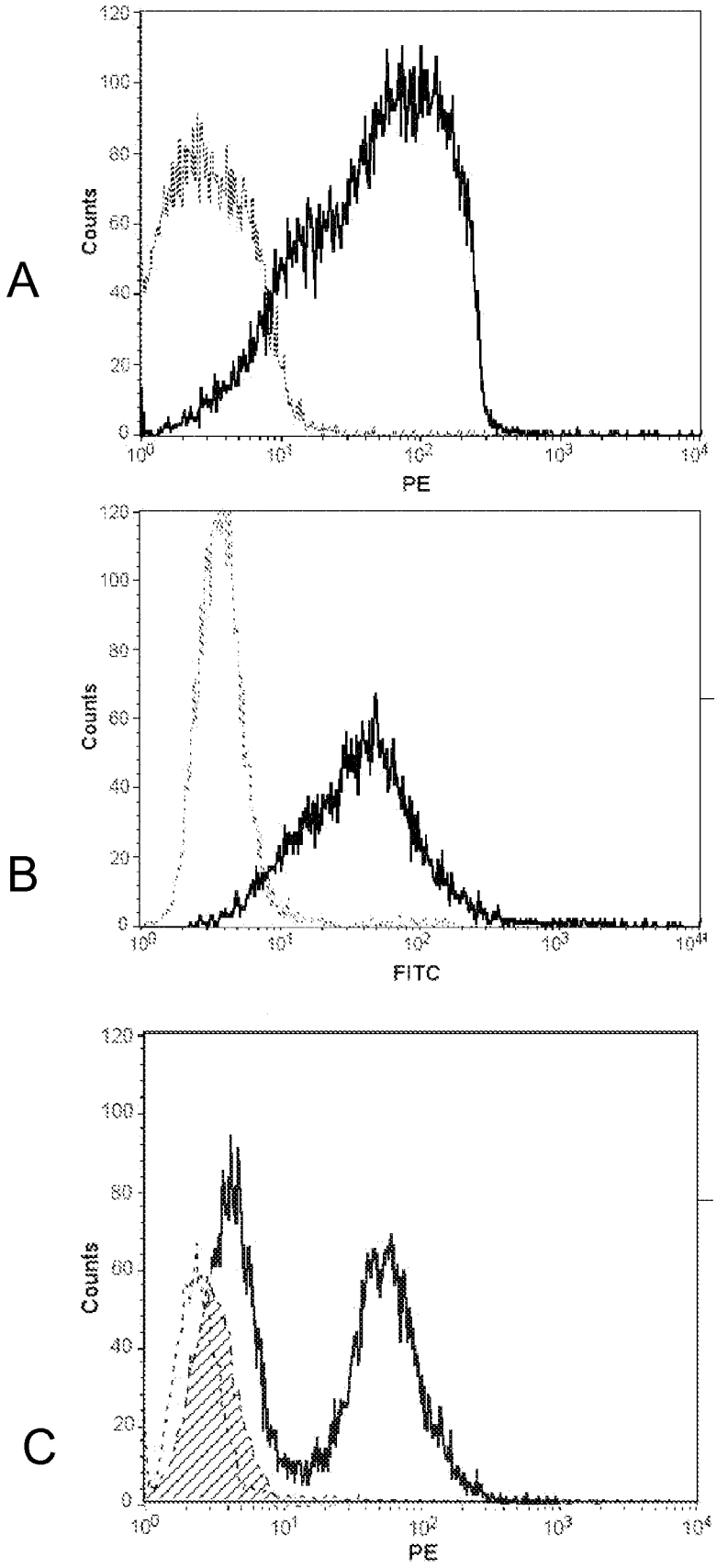


Figure 2

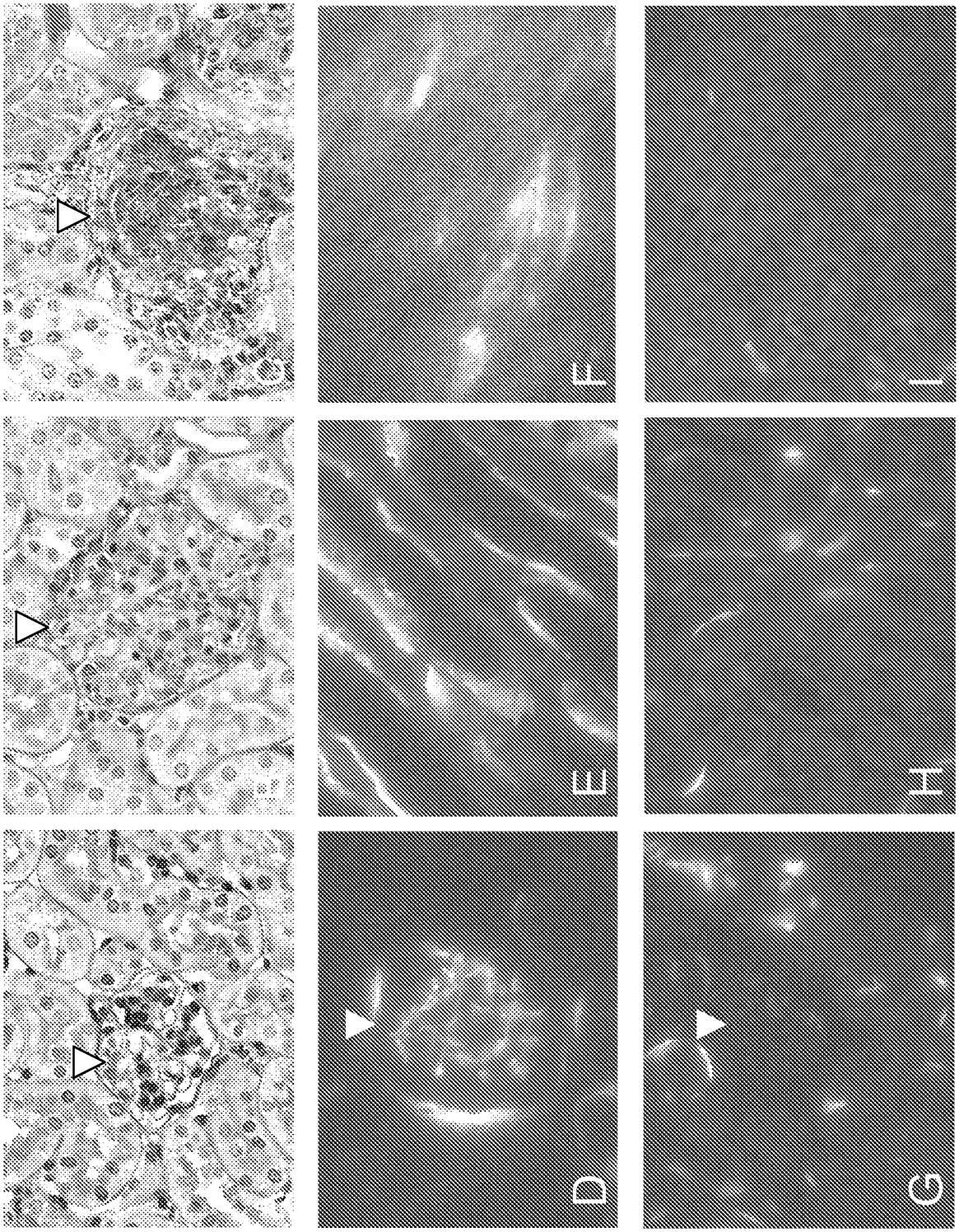
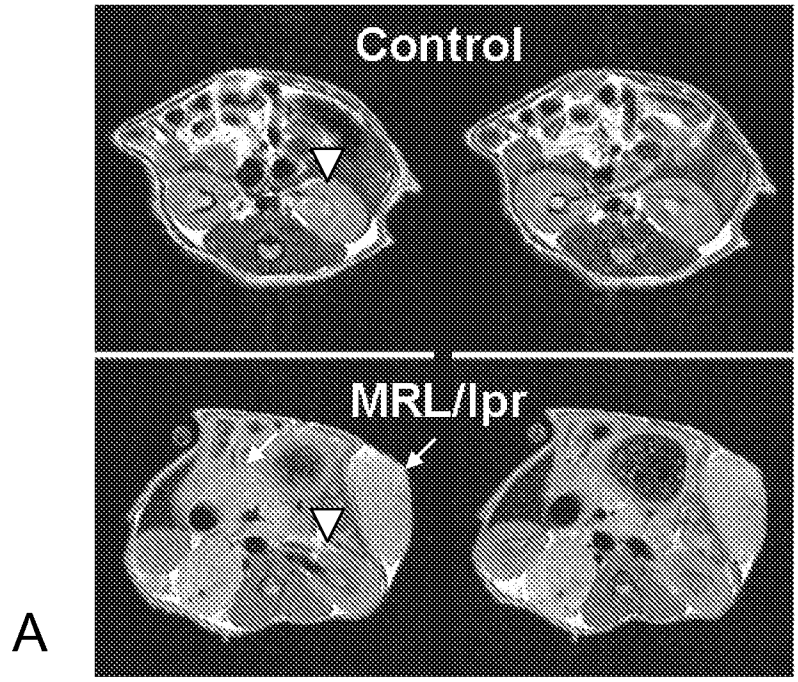


Figure 3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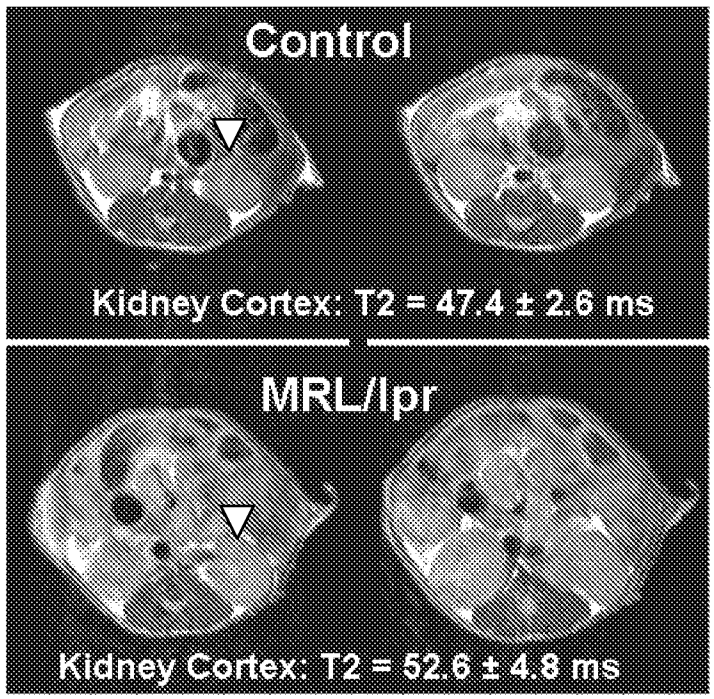
MRL/lpr

A


Control
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MRL/lpr
Kidney Cortex: T2 = 52.6 ± 4.8 ms

B

Figure 4

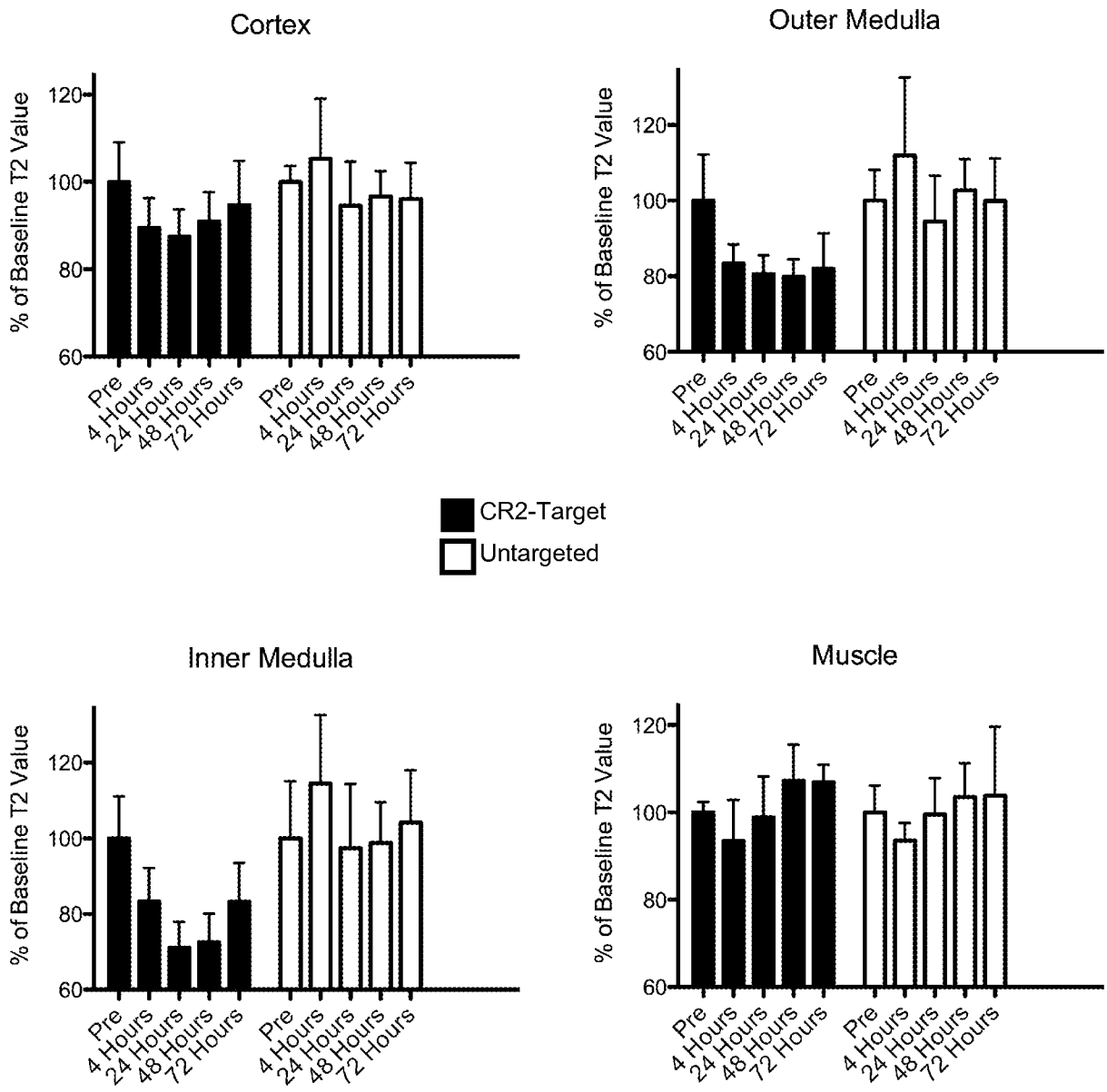
Cortex
Outer Medulla
Inner Medulla
Muscle
% of Baseline T2 Value
120
100
80
60
Pre
4 Hours
24 Hours
48 Hours
72 Hours
CR2-Target
Untargeted

Figure 5

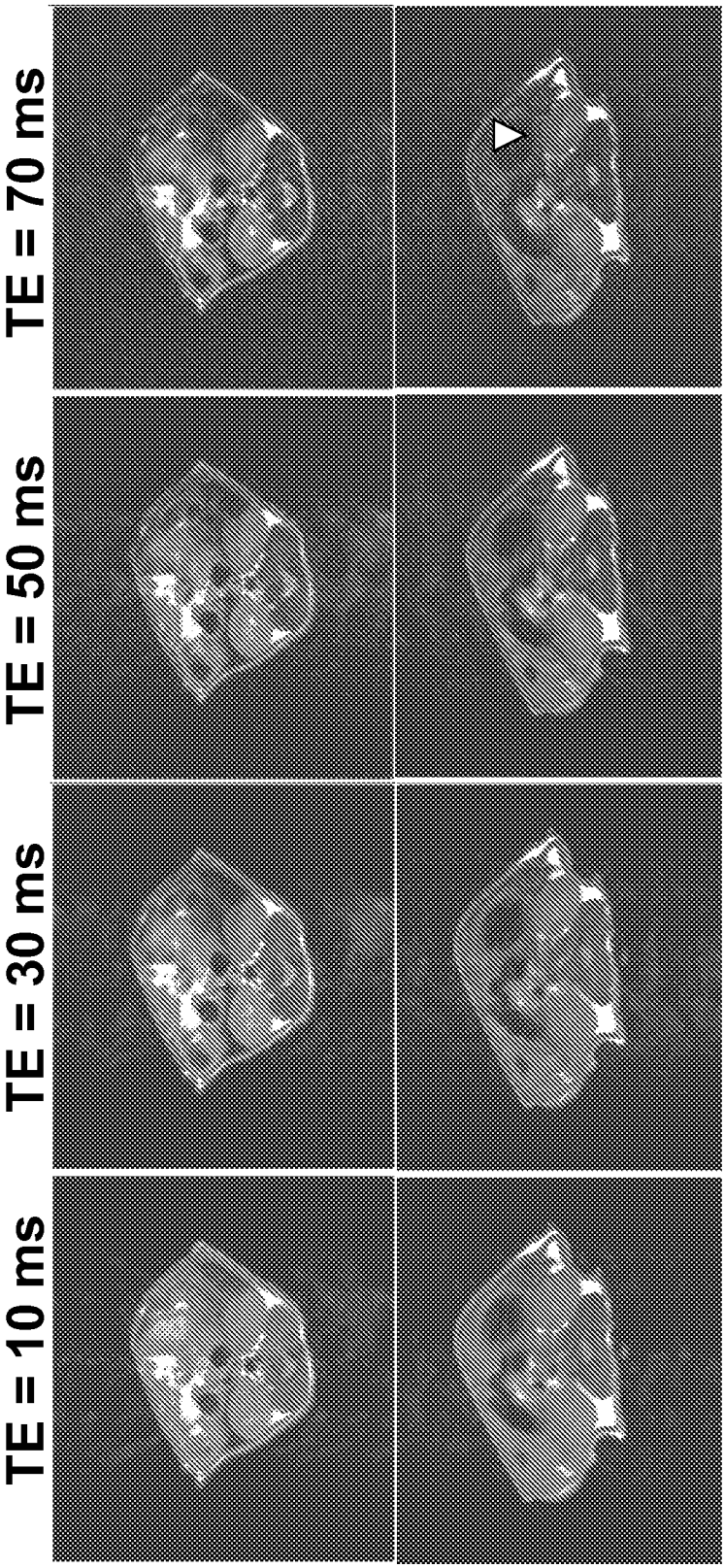

Figure 6

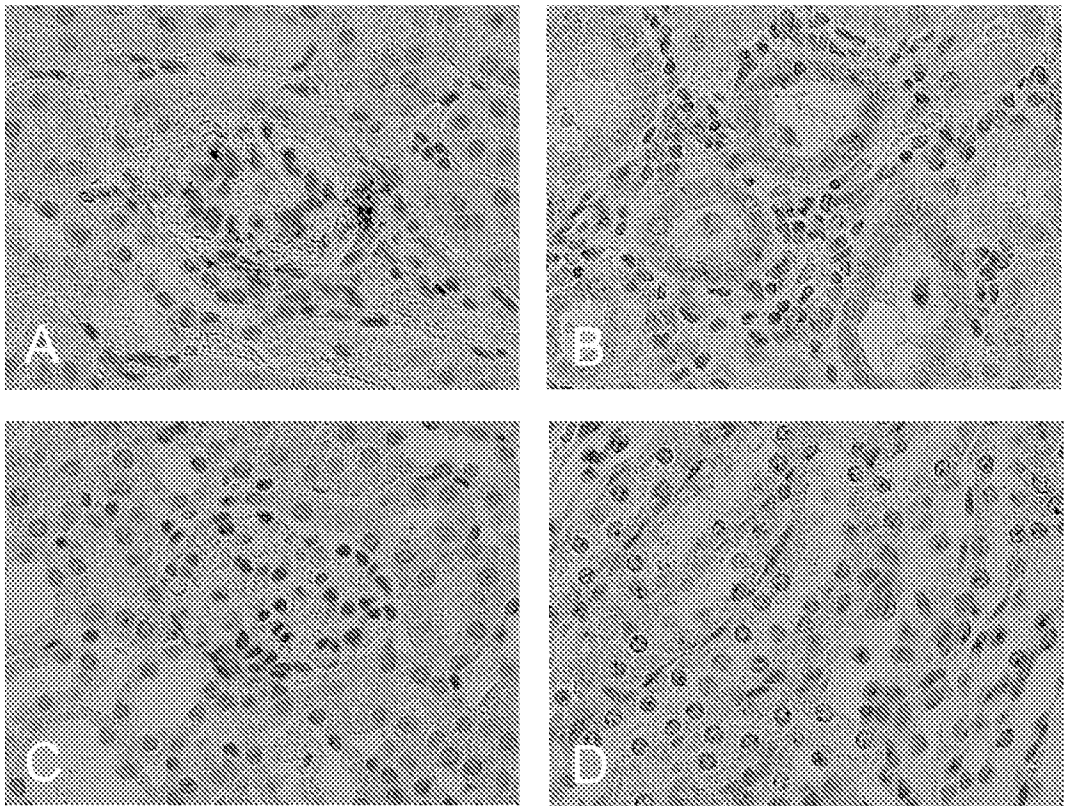

Figure 7