Title of the Invention:

Bi-Specific Monovalent Fc Diabodies That Are Capable Of Binding CD32B And CD79b And Uses Thereof

Cross-Reference to Related Applications:

[0001] This application claims priority to United States Patent Applications No. 61/864,217 (filed on August 9, 2013; Pending); 61/866,416 (filed on August 15, 2013; Pending); 61/869,519 (filed on August 23, 2013; Pending); and 61/907,525 (filed on November 22, 2013; Pending), each of which applications is herein incorporated by reference in its entirety.

Reference to Sequence Listing:

[0002] This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 *et seq.*, which are disclosed in both paper and computer-readable media, and which paper and computer-readable disclosures are herein incorporated by reference in their entirety.

Background of the Invention:

Field of the Invention:

[0003] The present invention is directed to bi-specific monovalent diabodies that comprise an immunoglobulin Fc Domain ("bi-specific monovalent Fc diabodies") and are composed of three polypeptide chains and which possess at least one binding site specific for an epitope of CD32B and one binding site specific for an epitope of CD79b (*i.e.*, a "CD32B x CD79b Fc diabody"). The bi-specific monovalent Fc diabodies of the present invention are capable of simultaneous binding to CD32B and CD79b. The invention is directed to such compositions, to pharmaceutical compositions that contain such bi-specific monovalent Fc diabodies and to methods for their use in the treatment of inflammatory diseases or conditions, and in particular, systemic lupus erythematosus (SLE) and graft vs. host disease.

Description of Related Art:

I. The Fcγ Receptors and CD32B

[0004] The interaction of antibody-antigen complexes with cells of the immune system results in a wide array of responses, ranging from effector functions such as antibody-dependent cytotoxicity, mast cell degranulation, and phagocytosis to immunomodulatory signals such as regulating lymphocyte proliferation and antibody secretion. All these interactions are initiated through the binding of the Fc Domain of antibodies or immune complexes to specialized cell-surface receptors on hematopoietic cells. The diversity of cellular responses triggered by antibodies and immune complexes results from the structural heterogeneity of Fc receptors. Fc receptors share structurally related ligand binding domains which presumably mediate intracellular signaling.

[0005] The Fc receptors are members of the immunoglobulin gene superfamily of proteins. They are surface glycoproteins that can bind the Fc portion of immunoglobulin molecules. Each member of the family recognizes immunoglobulins of one or more isotypes through a recognition domain on the α chain of the Fc receptor.

[0006] Fc receptors are defined by their specificity for immunoglobulin subtypes (see, Ravetch J.V. *et al.* (1991) "*Fc Receptors*," Annu. Rev. Immunol. 9:457-92; Gerber J.S. *et al.* (2001) "*Stimulatory And Inhibitory Signals Originating From The Macrophage Fcγ Receptors*," Microbes and Infection, 3:131-139; Billadeau D.D. *et al.* (2002) "*ITAMs Versus ITIMs: Striking A Balance During Cell Regulation*," J. Clin. Invest. 2(109):161-168l; Ravetch J.V. *et al.* (2000) "*Immune Inhibitory Receptors*," Science 290:84-89; Ravetch J.V. *et al.* (2001) "*IgG Fc Receptors*," Annu. Rev. Immunol. 19:275-90; Ravetch J.V. (1994) "*Fc Receptors: Rubor Redux*," *Cell*, 78(4): 553-60).

[0007] Fc receptors that are capable of binding to IgG antibodies are termed "FcγRs." Each member of this family is an integral membrane glycoprotein, possessing extracellular domains related to a C2-set of immunoglobulin-related domains, a single membrane spanning domain and an intracytoplasmic domain of

variable length. There are three known FcγRs, designated FcγRI(CD64), FcγRII(CD32), and FcγRIII(CD16). The three receptors are encoded by distinct genes; however, the extensive homologies between the three family members suggest they arose from a common progenitor perhaps by gene duplication.

[0008] FcγRII(CD32) proteins are 40KDa integral membrane glycoproteins which bind only the complexed IgG due to a low affinity for monomeric Ig (10^6 M^{-1}). This receptor is the most widely expressed FcγR, present on all hematopoietic cells, including monocytes, macrophages, B cells, NK cells, neutrophils, mast cells, and platelets. FcγRII has only two immunoglobulin-like regions in its immunoglobulin binding chain and hence a much lower affinity for IgG than FcγRI. There are three human FcγRII genes (FcγRIIA(CD32A), FcγRIIB(CD32B), FcγRIIC(CD32C)), all of which bind IgG in aggregates or immune complexes.

[0009] Distinct differences within the cytoplasmic domains of the FcγRIIA and FcγRIIB create two functionally heterogenous responses to receptor ligation. The fundamental difference is that, upon binding to an IgG Fc region, the FcγRIIA isoform initiates intracellular signaling leading to immune system activation (*e.g.*, phagocytosis, respiratory burst, *etc.*), whereas, upon binding to an IgG Fc region, the FcγRIIB isoform initiates signals that lead to the dampening or inhibition of the immune system (*e.g.*, inhibiting B cell activation, *etc.*).

[0010] Such activating and inhibitory signals are both transduced through the FcγRs following ligation to an IgG Fc region. These diametrically opposing functions result from structural differences among the different receptor isoforms. Two distinct domains within the cytoplasmic signaling domains of the receptor called Immunoreceptor Tyrosine-based Activation Motifs (ITAMs) or Immunoreceptor Tyrosine-Based Inhibitory Motifs (ITIMS) account for the different responses. The recruitment of different cytoplasmic enzymes to these structures dictates the outcome of the FcγR-mediated cellular responses. ITAM-containing FcγR complexes include FcγRI, FcγRIIA, FcγRIIIA, whereas ITIM-containing complexes only include FcγRIIB.

[0011] Human neutrophils express the FcγRIIA gene. FcγRIIA clustering via immune complexes or specific antibody cross-linking serves to aggregate ITAMs along with receptor-associated kinases which facilitate ITAM phosphorylation. ITAM phosphorylation serves as a docking site for Syk kinase, activation of which results in activation of downstream substrates (*e.g.*, PI_3K). Cellular activation leads to release of pro-inflammatory mediators.

[0012] The FcγRIIB gene is expressed on B lymphocytes; its extracellular domain is 96% identical to FcγRIIA and binds IgG complexes in an indistinguishable manner. The presence of an ITIM in the cytoplasmic domain of FcγRIIB defines this inhibitory subclass of FcγR. The molecular basis of this inhibition has been established. When FcγRIIB becomes co-ligated to an activating receptor by way of the Fc regions of the IgG immunoglobulins of an immune complex, the FcγRIIB ITIM becomes phosphorylated and attracts the SH2 domain of the inositol polyphosphate 5'-phosphatase (SHIP), which hydrolyzes phosphoinositol messengers released as a consequence of ITAM-containing, FcγR-mediated tyrosine kinase activation, consequently preventing the influx of intracellular Ca^{++}. Thus such cross-linking of FcγRIIB and an activating receptor dampens the activity of the activating receptor, and thus inhibits cellular responsiveness. Thus, on B-cells, B-cell activation, B-cell proliferation and antibody secretion is dampened or aborted. Thus, at the onset of antigen detection, monomeric IgG-antigen bonding occurs, and the Fc regions of bound antibodies bind to ITAMs of the activating FcγRs to mediate activation of the immune system. As the host's response progresses, multimeric IgG-antigen immune complexes form that are capable of binding to FcγRIIB (thus co-ligating such complexes with an activating receptor), leading to the dampening and ultimate cessation of the immune response (see, *e.g.*, United States Patents No. 8,445,645; 8,217,147; 8,216,579; 8,216,574; 8,193,318; ,192,737; 8,187,593; 8,133,982; 8,044,180; 8,003,774; 7,960,512; 7,786,270; 7,632,497; 7,521,542; 7,425,619; 7,355,008 and United States Patent Publications No.: 2012/0276094; 2012/0269811; 2012/0263711; 2012/0219551; 2012/0213781; 2012/0141476; 2011/0305714; 2011/0243941; 2010/0322924; 2010/0254985; 2010/0196362; 2010/0174053; 2009/0202537; 2009/0191195; 2009/0092610; 2009/0076251; 2009/0074771; 2009/0060910; 2009/0053218; 2009/0017027; 2009/0017026; 2009/0017023;

2008/0138349; 2008/0138344; 2008/0131435; 2008/0112961; 2008/0044429; 2008/0044417; 2007/0077246; 2007/0036799; 2007/0014795; 2007/0004909; 2005/0260213; 2005/0215767; 2005/0064514; 2005/0037000; 2004/0185045).

II. The B-Cell Receptor and CD79b

[0013] B cells are immune system cells that are responsible for producing antibodies. The B-cell response to antigen is an essential component of the normal immune system. B-cells possess specialized cell-surface receptors (B-cell receptors; "BCR"). If a B-cell encounters an antigen capable of binding to that cell's BCR, the B-cell will be stimulated to proliferate and produce antibodies specific for the bound antigen. To generate an efficient response to antigens, BCR-associated proteins and T-cell assistance are also required. The antigen/BCR complex is internalized, and the antigen is proteolytically processed. A small part of the antigen remains complexed with major histocompatability complex-II ("MHC-II") molecules on the surface of the B cells where the complex can be recognized by T-cells. T-cells activated by such antigen presentation secrete a variety of lymphokines that induce B-cell maturation.

[0014] Signaling through the BCR plays an important role in the generation of antibodies, in autoimmunity, and in the establishment of immunological tolerance (Gauld, S.B. *et al.* (2002) "*B Cell Antigen Receptor Signaling: Roles In Cell Development And Disease*," Science 296(5573):1641-1642). Immature B cells that bind self-antigens while still in the bone marrow are eliminated by apoptosis. In contrast, antigen binding on mature B cells results in activation, proliferation, anergy and apoptosis. The particular functional response observed depends upon whether the B-cell receives co-stimulatory signals through other surface receptors and the specific signal transduction pathways that are activated.

[0015] The BCR is composed of a membrane immunoglobulin which, together with non-covalently associated α and β subunits of CD79 ("CD79a" and "CD79b," respectively), forms the BCR complex. CD79a and CD79b are signal transducing subunits that contain a conserved immunoreceptor tyrosine-based activation motif ("ITAM") required for signal transduction (Dylke, J. *et al.* (2007) "*Role of the extracellular and transmembrane domain of Ig-alpha/beta in assembly of the B cell*

antigen receptor (BCR)," Immunol. Lett. 112(1):47-57; Cambier, J.C. (1995) "*New Nomenclature For The Reth Motif (or ARH1/TAM/ARAM/YXXL)*," Immunol. Today 16:110). Aggregation of the BCR complex by multivalent antigen initiates transphosphorylation of the CD79a and CD79b ITAMs and activation of receptor-associated kinases (DeFranco, A.L. (1997) "*The Complexity Of Signaling Pathways Activated By The BCR*," Curr. Opin. Immunol. 9:296-308; Kurosaki, T. (1997) "*Molecular Mechanisms In B-Cell Antigen Receptor Signaling*," Curr. Opin. Immunol. 9:309-318; Kim, K.M. *et al.* (1993) "*Signalling Function Of The B-Cell Antigen Receptors*," Immun. Rev. 132:125-146). Phosphorylated ITAMs recruit additional effectors such as PI_3K, PLC-γ and members of the Ras/MAPK pathway. These signaling events are responsible for both the B cell proliferation and increased expression of activation markers (such as MHC-II and CD86) that are required to prime B cells for their subsequent interactions with T-helper ("T_h") cells.

III. Inflammatory Diseases or Conditions

[0016] Inflammation is a process by which the body's white blood cells and chemicals protect our bodies from infection by foreign substances, such as bacteria and viruses. It is usually characterized by pain, swelling, warmth and redness of the affected area. Chemicals known as cytokines and prostaglandins control this process, and are released in an ordered and self-limiting cascade into the blood or affected tissues. This release of chemicals increases the blood flow to the area of injury or infection, and may result in the redness and warmth. Some of the chemicals cause a leak of fluid into the tissues, resulting in swelling. This protective process may stimulate nerves and cause pain. These changes, when occurring for a limited period in the relevant area, work to the benefit of the body.

[0017] Inflammatory diseases or conditions reflect an immune system attack on a body's own cells and tissue (*i.e.*, an "autoimmune" response). There are many different autoimmune disorders which affect the body in different ways. For example, the brain is affected in individuals with multiple sclerosis, the gut is affected in individuals with Crohn's disease, and the synovium, bone and cartilage of various joints are affected in individuals with rheumatoid arthritis. As autoimmune disorders progress destruction of one or more types of body tissues, abnormal growth of an

organ, or changes in organ function may result. The autoimmune disorder may affect only one organ or tissue type or may affect multiple organs and tissues. Organs and tissues commonly affected by autoimmune disorders include red blood cells, blood vessels, connective tissues, endocrine glands (e.g., the thyroid or pancreas), muscles, joints, and skin. Examples of autoimmune disorders include, but are not limited to, Hashimoto's thyroiditis, pernicious anemia, Addison's disease, type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus (SLE), dermatomyositis, Sjogren's syndrome, dermatomyositis, lupus erythematosus, multiple sclerosis, autoimmune inner ear disease myasthenia gravis, Reiter's syndrome, Graves' disease, autoimmune hepatitis, familial adenomatous polyposis and ulcerative colitis.

[0018] Inflammatory diseases or conditions can also arise when the body's normally protective immune system causes damage by attacking foreign cells or tissues whose presence is beneficial to the body (*e.g.*, the rejection of transplants (host vs. host disease)) or from the rejection of the cells of an immunosuppressed host by immunocompetent cells of an introduced transplant graft (graft vs. host disease) (DePaoli, A.M. *et al.* (1992) "*Graft-Versus-Host Disease And Liver Transplantation*," Ann. Intern. Med. 117:170-171; Sudhindran, S. *et al.* (2003) "*Treatment Of Graft-Versus-Host Disease After Liver Transplantation With Basiliximab Followed By Bowel Resection*," Am J Transplant. 3:1024-1029; Pollack, M.S. *et al.* (2005) "*Severe, Late-Onset Graft-Versus-Host Disease In A Liver Transplant Recipient Documented By Chimerism Analysis*," Hum. Immunol. 66:28-31; Perri, R. *et al.* (2007) "*Graft Vs. Host Disease After Liver Transplantation: A New Approach Is Needed*," Liver Transpl. 13:1092-1099; Mawad, R. *et al.* (2009) "*Graft-Versus-Host Disease Presenting With Pancytopenia After En Bloc Multiorgan Transplantation: Case Report And Literature Review*," Transplant Proc. 41:4431-4433; Akbulut, S. *et al.* (2012) "*Graft-Versus-Host Disease After Liver Transplantation: A Comprehensive Literature Review*," World J. Gastroenterol. 18(37): 5240-5248).

[0019] Despite recent advances in the treatment of such diseases or conditions, a need continues to exist for compositions capable of treating or preventing inflammatory diseases or conditions.

IV. Bi-Specific Diabodies

[0020] The ability of an intact, unmodified antibody (*e.g.*, an IgG) to bind an epitope of an antigen depends upon the presence of variable domains on the immunoglobulin light and heavy chains (*i.e.*, the VL and VH Domains, respectively). The design of a diabody is based on the single chain Fv construct (scFv) (see, *e.g.*, Holliger *et al.* (1993) "*'Diabodies': Small Bivalent And Bispecific Antibody Fragments,*" Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Olafsen, T. *et al.* (2004) "*Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications*," Protein Eng Des Sel. 17(1):21-27; Wu, A. *et al.* (2001) "*Multimerization Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange*," Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Region*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944).

[0021] Interaction of an antibody light chain and an antibody heavy chain and, in particular, interaction of its VL and VH Domains forms one of the epitope binding sites of the antibody. In contrast, the scFv construct comprises VL and VH Domains of an antibody contained in a single polypeptide chain wherein the domains are separated by a flexible linker of sufficient length to allow self-assembly of the two domains into a functional epitope binding site. Where self-assembly of the VL and VH Domains is rendered impossible due to a linker of insufficient length (less than about 12 amino acid residues), two of the scFv constructs interact with one another other to form a bivalent molecule in which the VL Domain of one chain associates

with the VH Domain of the other (reviewed in Marvin *et al.* (2005) *"Recombinant Approaches To IgG-Like Bispecific Antibodies,"* Acta Pharmacol. Sin. 26:649-658).

[0022] Natural antibodies are capable of binding to only one epitope species (*i.e.*, mono-specific), although they can bind multiple copies of that species (*i.e.*, exhibiting bi-valency or multi-valency). The art has noted the capability to produce diabodies that differ from such natural antibodies in being capable of binding two or more different epitope species (*i.e.*, exhibiting bi-specificity or multispecificity in addition to bi-valency or multi-valency) (see, *e.g.*, Holliger *et al.* (1993) "*'Diabodies': Small Bivalent And Bispecific Antibody Fragments,*" Proc. Natl. Acad. Sci. (U.S.A.) 90:6444-6448; US 2004/0058400 (Hollinger *et al.*); US 2004/0220388 (Mertens *et al.*); Alt *et al.* (1999) FEBS Lett. 454(1-2):90-94; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672; WO 02/02781 (Mertens *et al.*); Mertens, N. *et al.*, "*New Recombinant Bi- and Trispecific Antibody Derivatives*," In: NOVEL FRONTIERS IN THE PRODUCTION OF COMPOUNDS FOR BIOMEDICAL USE, A. VanBroekhoven *et al.* (Eds.), Kluwer Academic Publishers, Dordrecht, The Netherlands (2001), pages 195-208; Wu, A. *et al.* (2001) "*Multimerization Of A Chimeric Anti-CD20 Single Chain Fv-Fv Fusion Protein Is Mediated Through Variable Domain Exchange*," Protein Engineering 14(2):1025-1033; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Region*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Baeuerle, P.A. *et al.* (2009) "*Bispecific T-Cell Engaging Antibodies For Cancer Therapy*," Cancer Res. 69(12):4941-4944).

[0023] The provision of non-monospecific diabodies provides a significant advantage: the capacity to co-ligate and co-localize cells that express different epitopes. Bivalent diabodies thus have wide-ranging applications including therapy and immunodiagnosis. Bi-valency allows for great flexibility in the design and engineering of the diabody in various applications, providing enhanced avidity to

multimeric antigens, the cross-linking of differing antigens, and directed targeting to specific cell types relying on the presence of both target antigens. Due to their increased valency, low dissociation rates and rapid clearance from the circulation (for diabodies of small size, at or below ~50 kDa), diabody molecules known in the art have also shown particular use in the field of tumor imaging (Fitzgerald *et al.* (1997) *"Improved Tumour Targeting By Disulphide Stabilized Diabodies Expressed In Pichia pastoris,"* Protein Eng. 10:1221). Of particular importance is the co-ligating of differing cells, for example, the cross-linking of cytotoxic T-cells to tumor cells (Staerz *et al.* (1985) *"Hybrid Antibodies Can Target Sites For Attack By T Cells,"* Nature 314:628-631, and Holliger *et al.* (1996) *"Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305).

[0024] Diabody epitope binding domains may also be directed to a surface determinant of any immune effector cell such as CD3, CD16, CD32, or CD64, which are expressed on T lymphocytes, natural killer (NK) cells or other mononuclear cells. In many studies, diabody binding to effector cell determinants, *e.g.*, Fcγ receptors (FcγR), was also found to activate the effector cell (Holliger *et al.* (1996) *"Specific Killing Of Lymphoma Cells By Cytotoxic T-Cells Mediated By A Bispecific Diabody,"* Protein Eng. 9:299-305; Holliger *et al.* (1999) *"Carcinoembryonic Antigen (CEA)-Specific T-cell Activation In Colon Carcinoma Induced By Anti-CD3 x Anti-CEA Bispecific Diabodies And B7 x Anti-CEA Bispecific Fusion Proteins,"* Cancer Res. 59:2909-2916; WO 2006/113665; WO 2008/157379; WO 2010/080538; WO 2012/018687; WO 2012/162068). Normally, effector cell activation is triggered by the binding of an antigen bound antibody to an effector cell via Fc-FcγR interaction; thus, in this regard, diabody molecules of the invention may exhibit Ig-like functionality independent of whether they comprise an Fc Domain (*e.g.,* as assayed in any efferctor function assay known in the art or exemplified herein (*e.g.*, ADCC assay)). By cross-linking tumor and effector cells, the diabody not only brings the effector cell within the proximity of the tumor cells but leads to effective tumor killing (see *e.g.*, Cao *et al.* (2003) *"Bispecific Antibody Conjugates In Therapeutics,"* Adv. Drug. Deliv. Rev. 55:171-197).

[0025] However, the above advantages come at salient cost. The formation of such non-monospecific diabodies requires the successful assembly of two or more distinct and different polypeptides (*i.e.*, such formation requires that the diabodies be formed through the heterodimerization of different polypeptide chain species). This fact is in contrast to mono-specific diabodies, which are formed through the homodimerization of identical polypeptide chains. Because at least two dissimilar polypeptides (*i.e.*, two polypeptide species) must be provided in order to form a non-monospecific diabody, and because homodimerization of such polypeptides leads to inactive molecules (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588), the production of such polypeptides must be accomplished in such a way as to prevent covalent bonding between polypeptides of the same species (Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588). The art has therefore taught the non-covalent association of such polypeptides (see, *e.g.*, Olafsen *et al.* (2004) *"Covalent Disulfide-Linked Anti-CEA Diabody Allows Site-Specific Conjugation And Radiolabeling For Tumor Targeting Applications,"* Prot. Engr. Des. Sel. 17:21-27; Asano *et al.* (2004) "*A Diabody For Cancer Immunotherapy And Its Functional Enhancement By Fusion Of Human Fc Region*," Abstract 3P-683, J. Biochem. 76(8):992; Takemura, S. *et al.* (2000) "*Construction Of A Diabody (Small Recombinant Bispecific Antibody) Using A Refolding System,*" Protein Eng. 13(8):583-588; Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0026] However, the art has recognized that bi-specific diabodies composed of non-covalently associated polypeptides are unstable and readily dissociate into non-functional monomers (see, *e.g.*, Lu, D. *et al.* (2005) "*A Fully Human Recombinant IgG-Like Bispecific Antibody To Both The Epidermal Growth Factor Receptor And The Insulin-Like Growth Factor Receptor For Enhanced Antitumor Activity*," J. Biol. Chem. 280(20):19665-19672).

[0027] In the face of this challenge, the art has succeeded in developing stable, covalently bonded heterodimeric non-monospecific diabodies (see, *e.g.*, WO 2006/113665; WO/2008/157379; WO 2010/080538; WO 2012/018687; WO/2012/162068; Johnson, S. *et al.* (2010) "*Effector Cell Recruitment With Novel Fv-Based Dual-Affinity Re-Targeting Protein Leads To Potent Tumor Cytolysis And In Vivo B-Cell Depletion*," J. Molec. Biol. 399(3):436-449; Veri, M.C. *et al.* (2010) "*Therapeutic Control Of B Cell Activation Via Recruitment Of Fcgamma Receptor IIb (CD32B) Inhibitory Function With A Novel Bispecific Antibody Scaffold*," Arthritis Rheum. 62(7):1933-1943; Moore, P.A. *et al.* (2011) "*Application Of Dual Affinity Retargeting Molecules To Achieve Optimal Redirected T-Cell Killing Of B-Cell Lymphoma*," Blood 117(17):4542-4551). Such approaches involve engineering one or more cysteine residues into each of the employed polypeptide species. For example, the addition of a cysteine residue to the C-terminus of such constructs has been shown to allow disulfide bonding between the polypeptide chains, stabilizing the resulting heterodimer without interfering with the binding characteristics of the bivalent molecule.

[0028] Notwithstanding such success, the production of stable, functional heterodimeric, non-monospecific can be further improved by the careful consideration and placement of the domains employed in the polypeptide chains. The present invention is thus directed to the provision of specific polypeptides that are particularly designed to form, via covalent bonding, heterodimeric Fc diabodies that are capable of simultaneously binding CD32B and CD79b.

Summary of the Invention:

[0029] The invention is directed to CD32B x CD79b bi-specific monovalent diabodies that comprise an immunoglobulin Fc region ("CD32B x CD79b bi-specific monovalent Fc diabodies"). The CD32B x CD79b bi-specific monovalent Fc diabodies of the invention are composed of three polypeptide chains (a "first," "second" and "third" polypeptide chain), wherein the first and second polypeptide chains are covalently bonded to one another and the first and third polypeptide chains are covalently bonded to one another. Such covalent bondings are, for example, by disulfide bonding of cysteine residues located within each polypeptide chain. The

first and second polypeptide chains of the CD32B x CD79b bi-specific monovalent Fc diabodies of the invention associate with one another in a heterodimeric manner to form one binding site specific for an epitope of CD32B and one binding site specific for an epitope of CD79b. The CD32B x CD79b bi-specific monovalent Fc diabodies of the invention are thus monovalent in that they are capable of binding to only one copy of an epitope of CD32B and to only one copy of an epitope of CD79b, but bi-specific in that a single diabody is able to bind simultaneously to the epitope of CD32B and to the epitope of CD79b. The bi-specific monovalent Fc diabodies of the present invention are capable of simultaneous binding to CD32B and CD79b. The invention is directed to such CD32B x CD79b bi-specific monovalent Fc diabodies, and to pharmaceutical compositions that contain such bi-specific monovalent Fc diabodies The invention is additionally directed to methods for the use of such diabodies in the treatment of inflammatory diseases or conditions, and in particular, systemic lupus erythematosus (SLE) and graft vs. host disease.

[0030] In detail, the invention provides a bi-specific monovalent Fc diabody, wherein the bi-specific monovalent Fc diabody is capable of specific binding to an epitope of CD32B and to an epitope of CD79b, and possesses an IgG Fc Domain, wherein the bi-specific monovalent Fc diabody comprises a first polypeptide chain, a second polypeptide chain and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another and the first and third polypeptide chains are covalently bonded to one another, and wherein:

A. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

   i. a Domain 1, comprising:

      (1) a sub-Domain (1A), which comprises a cysteine-containing peptide (especially, a peptide having the sequence of (Peptide 1) **SEQ ID NO:1**); and

      (2) a sub-Domain (1B), which comprises a polypeptide portion of an IgG Fc Domain (most preferably, having CH2 and CH3 domains of an IgG immunoglobulin Fc region);

ii. a Domain 2, comprising:

(1) a sub-Domain (2A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD32B (VL_{CD32B}) (**SEQ ID NO:11**); and

(2) a sub-Domain (2B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD79b (VH_{CD79b}) (**SEQ ID NO:14**),

wherein the sub-Domains (2A) and (2B) are separated from one another by a peptide linker (especially, a peptide linker (Linker 2) having the sequence of **SEQ ID NO:4**);

iii. a Domain 3, wherein the Domain 3 is an E-coil Domain (**SEQ ID NO:7**) or a K-coil Domain (**SEQ ID NO:8**), wherein the Domain 3 is separated from the Domain 2 by a peptide linker (especially, a peptide linker having the sequence of **SEQ ID NO:5**); and

iv. a C-terminal spacer peptide (especially, a spacer peptide having the sequence of **SEQ ID NO:6**);

B. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising:

(1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD79b (VL_{CD79b}) (**SEQ ID NO:13**); and

(2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD32B (VH_{CD32B}) (**SEQ ID NO:12**);

wherein the sub-Domains (1A) and (1B) are separated from one another by a peptide linker (especially, a peptide linker (Linker 2) having the sequence of **SEQ ID NO:4**);

ii. a Domain 2, wherein the Domain 2 is a K-coil Domain (**SEQ ID NO:8**) or an E-coil Domain (**SEQ ID NO:7**), wherein the Domain 2 is separated from the Domain 1 by a peptide linker (especially, a peptide linker having the sequence of **SEQ ID NO:5**); and wherein the

Domain 3 of the first polypeptide chain and the Domain 2 of the second polypeptide chain are not both E-coil Domains or both K-coil Domains; and

C. the third polypeptide chain comprises, in the N-terminal to C-terminal direction, a Domain 1 comprising:

(1) a sub-Domain (1A), which comprises a cysteine-containing peptide (especially, a peptide linker having the sequence of (Peptide 1) **SEQ ID NO:1**); and

(2) a sub-Domain (1B), which comprises a polypeptide portion of an IgG Fc Domain (most preferably, having CH2 and CH3 domains of an IgG immunoglobulin Fc region);

and wherein:

(a) the polypeptide portions of the IgG Fc Domains of the first and third polypeptide chain form the IgG Fc Domain;

(b) the VL Domain of the first polypeptide chain and the VH Domain of the second polypeptide chain form an Antigen-Binding Domain capable of specific binding to an epitope of CD32B; and

(c) the VH Domain of the first polypeptide chain and the VL Domain of the second polypeptide chain form an Antigen-Binding Domain capable of specific binding to an epitope of CD79b.

[0031] The invention additionally provides a bi-specific monovalent Fc diabody, wherein the bi-specific monovalent Fc diabody is capable of specific binding to an epitope of CD32B and to an epitope of CD79b, and possesses an IgG Fc Domain, wherein the bi-specific monovalent Fc diabody comprises a first polypeptide chain, a second polypeptide chain and a third polypeptide chain, wherein the first and second polypeptide chains are covalently bonded to one another and the first and third polypeptide chains are covalently bonded to one another, and wherein:

A. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising:

(1) a sub-Domain (1A), which comprises a cysteine-containing peptide (especially, a peptide linker having the sequence of (Peptide 1) **SEQ ID NO:1**); and

(2) a sub-Domain (1B), which comprises a polypeptide portion of an IgG Fc Domain (most preferably, having CH2 and CH3 domains of an IgG immunoglobulin Fc region);

ii. a Domain 2, comprising:

(1) a sub-Domain (2A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD79b (VL_{CD79b}) (**SEQ ID NO:13**); and

(2) a sub-Domain (2B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD32B (VH_{CD32B}) (**SEQ ID NO:12**);

wherein the sub-Domains (2A) and (2B) are separated from one another by a peptide linker (especially, a peptide linker having the sequence of **SEQ ID NO:4**);

iii. a Domain 3, wherein the Domain 3 is an E-coil Domain (**SEQ ID NO:7**) or a K-coil Domain (**SEQ ID NO:8**), wherein the Domain 3 is separated from the Domain 2 by a peptide (especially, a peptide linker having the sequence of **SEQ ID NO:5**); and

iv. a C-terminal spacer peptide (especially, a spacer peptide having the sequence of **SEQ ID NO:6**);

B. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising:

(1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD32B (VL_{CD32B}) (**SEQ ID NO:11**); and

(2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD79b (VH_{CD79b}) (**SEQ ID NO:14**);

wherein the sub-Domains (1A) and (1B) are separated from one another by a peptide linker (especially, a peptide linker having the sequence of **SEQ ID NO:4**);

ii. a Domain 2, wherein the Domain 2 is a K-coil Domain (**SEQ ID NO:8**) or an E-coil Domain (**SEQ ID NO:7**), wherein the Domain 2 is separated from the Domain 1 by a peptide linker (especially, a peptide linker having the sequence of **SEQ ID NO:5**); and wherein the Domain 3 of the first polypeptide chain and the Domain 2 of the second polypeptide chain are not both E-coil Domains or both K-coil Domains; and

C. the third polypeptide chain comprises, in the N-terminal to C-terminal direction, a Domain 1 comprising:

(1) a sub-Domain (1A), which comprises a cysteine-containing peptide (especially, a peptide linker having the sequence of (Peptide 1) **SEQ ID NO:1**); and

(2) a sub-Domain (1B), which comprises a polypeptide portion of an IgG Fc Domain (most preferably, having CH2 and CH3 domains of an IgG immunoglobulin Fc region);

and wherein:

(a) the polypeptide portions of the Fc Domains of the first and third polypeptide chain form the IgG Fc region;

(b) the VL Domain of the first polypeptide chain and the VH Domain of the second polypeptide chain form an Antigen-Binding Domain capable of specific binding to an epitope of CD79b; and

(c) the VH Domain of the first polypeptide chain and the VL Domain of the second polypeptide chain form an Antigen-Binding Domain capable of specific binding to an epitope of CD32B.

[0032] The invention further concerns the embodiments of all such bi-specific monovalent Fc diabodies, wherein the Domain 1 of the first polypeptide chain comprises a sequence different from that of the Domain 1 of the third polypeptide chain.

[0033] The invention further concerns the embodiments of all such bi-specific monovalent Fc diabodies wherein said sub-Domain (1B) of said first polypeptide chain has the amino acid sequence of **SEQ ID NO:9**, and said sub-Domain (1B) of said third polypeptide chain has the amino acid sequence of **SEQ ID NO:10.**

[0034] The invention further concerns the embodiments of all such bi-specific monovalent Fc diabodies wherein said sub-Domain (1B) of said first polypeptide chain has the amino acid sequence of **SEQ ID NO:10**, and said sub-Domain (1B) of said third polypeptide chain has the amino acid sequence of **SEQ ID NO:9.**

[0035] The invention further concerns the embodiments of all such bi-specific monovalent Fc diabodies, wherein the Domain 1 of the first polypeptide chain and/or the Domain 1 of the third polypeptide chain comprises a variant CH2-CH3 sequence that exhibits altered binding to an Fcγ receptor.

[0036] The invention further concerns the embodiments of all such bi-specific monovalent Fc diabodies wherein the Domain 3 of the first polypeptide chain comprises an E-coil (**SEQ ID NO:7**), and the Domain 2 of the second polypeptide chain comprises a K-coil (**SEQ ID NO:8**).

[0037] The invention further concerns the embodiments of all such bi-specific monovalent Fc diabodies wherein the Domain 3 of the first polypeptide chain comprises a K-coil (**SEQ ID NO:8**), and the Domain 2 of the second polypeptide chain comprises an E-coil (**SEQ ID NO:7**).

[0038] The invention further provides a bi-specific monovalent diabody comprising an IgG immunoglobulin Fc (bi-specific monovalent Fc diabody), wherein the bi-specific monovalent Fc diabody comprises:

(1) a first polypeptide chain having the amino acid sequence of **SEQ ID NO:15**;

(2) a second polypeptide chain having the amino acid sequence of **SEQ ID NO:16**; and

(3) a third polypeptide chain having the amino acid sequence of **SEQ ID NO:17**, wherein amino acid residues 1-10 of said third polypeptide chain are Peptide 1 (**SEQ ID NO:1**), and amino acid residues 11-227 of said third polypeptide

chain are the CH2 and CH3 domains of an IgG antibody Fc region (**SEQ ID NO:10**);

wherein the first and the second polypeptide chains are covalently bonded to one another by a first disulfide bond and the first and third polypeptide chains are covalently bonded to one another by a second disulfide bond.

[0039] The invention further provides a pharmaceutical composition comprising any of the above-described bi-specific monovalent Fc diabodies and a physiologically acceptable carrier.

[0040] The invention further provides for the use of such pharmaceutical composition in the treatment of an inflammatory disease or condition, especially wherein the inflammatory disease or condition is an autoimmune disease, and in particular, wherein the autoimmune disease is systemic lupus erythematosus (SLE).

[0041] The invention further provides for the use of such pharmaceutical composition in the treatment of an inflammatory disease or condition, especially wherein the inflammatory disease or condition is graft vs. host disease (GvHD).

Brief Description of the Drawings:

[0042] **Figure 1** illustrates the three polypeptide chains of a preferred bi-specific monovalent Fc diabody and the structure of the covalently associated chains.

[0043] **Figure 2** illustrates the three polypeptide chains of an alternative bi-specific monovalent Fc diabody and the structure of the covalently associated chains.

[0044] **Figures 3A-3B** show the ability of the preferred CD32B x CD79b Fc diabody and a non-Fc CD32B x CD79b (ABD) diabody to inhibit the proliferation of primary human B cells.

[0045] **Figures 4A-4B** show the ability of the preferred CD32B x CD79b Fc diabody, a non-Fc CD32B x CD79b (ABD) diabody, and a non-Fc CD32B x CD79b diabody to inhibit signaling in naïve (**Figure 4A**) and memory (**Figure 4B**) B cells.

[0046] **Figures 5A-5C** show the ability of the preferred CD32B x CD79b Fc diabody or a non-Fc CD32B x CD79b (ABD) diabody to inhibit the proliferation of SLE cells. Such inhibition was found to be independent of disease status.

[0047] **Figures 6A-6B** show the ability of the preferred CD32B x CD79b Fc diabody or a non-Fc CD32B x CD79b diabody to modulate B cell responses *in vivo*, and demonstrate the unexpected superiority of the preferred CD32B x CD79b Fc diabody.

[0048] **Figure 7** shows the ability of the preferred CD32B x CD79b Fc diabody to decrease xenogeneic GvHD in the mouse.

Detailed Description of the Invention:

[0049] The present invention is directed to bi-specific monovalent diabodies that comprise an immunoglobulin Fc Domain ("bi-specific monovalent Fc diabodies") and are composed of three polypeptide chains and which possess at least one binding site specific for an epitope of CD32B and one binding site specific for an epitope of CD79b (*i.e.*, a "CD32B x CD79b Fc diabody"). The bi-specific monovalent Fc diabodies of the present invention are capable of simultaneous binding to CD32B and CD79b. The invention is directed to such compositions, to pharmaceutical compositions that contain such bi-specific monovalent Fc diabodies and to methods for their use in the treatment of inflammatory diseases or conditions, and in particular, systemic lupus erythematosus (SLE) and graft vs. host disease.

[0050] As indicated above, CD79b is expressed by B cells, and is thus expressed on cells that are proliferating in response to antigen recognition. Antibodies capable of immunospecifically binding to CD79b are capable of binding to such B cells. CD32B is an FcγR and is expressed on B cells. Antibodies capable of immunospecifically binding to FcγRIIB(CD32B) and particularly such antibodies that bind to FcγRIIB without substantially interfering or impeding Fc binding are capable of increasing the ability of FcγRIIB to co-ligate with activating receptors of immune complexes. A bi-specific monovalent Fc diabody that is capable of binding to both CD32B and CD79b, has the ability to inhibit or dampen a host's immune system in response to an unwanted B cell activation, B cell proliferation and antibody secretion. Such bi-

specific monovalent Fc diabodies thus have utility in the treatment of inflammatory diseases and disorders.

I. Preferred CD32B x CD79b Fc Diabodies of the Present Invention

[0051] The preferred CD32B x CD79b Fc diabodies of the present invention are termed "Fc" diabodies, because they comprise an Fc Domain. As shown schematically in **Figure 1**, such Fc diabodies are composed of three polypeptide chains, of which the first and second polypeptide chains are covalently bonded to one another and the first and third polypeptide chains are bonded to one another. The VL Domain of the first polypeptide chain interacts with the VH Domain of the second polypeptide chain in order to form a first functional antigen binding site that is specific for the first antigen (*i.e.*, either CD32B or CD79b). Likewise, the VL Domain of the second polypeptide chain interacts with the VH Domain of the first polypeptide chain in order to form a second functional antigen binding site that is specific for the second antigen (*i.e.*, either CD79b or CD32B, depending upon the identity of the first antigen). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains are coordinated, such that the two polypeptide chains collectively comprise VL and VH Domains capable of binding to CD32B and CD79b (*i.e.*, they comprise VL_{CD32B}/VH_{CD32B} and VL_{CD79b}/VH_{CD79b}) (**Figure 1**). Collectively, each such VL and VH Domain, and the intervening Linker that separates them, are referred to as an Antigen-Binding Domain of the molecule.

[0052] The Fc Domain of the Fc diabodies of the present invention may be either a complete Fc region (*e.g.*, a complete IgG Fc region) or only a fragment of a complete Fc region. Although the Fc Domain of the bi-specific monovalent Fc diabodies of the present invention may possess the ability to bind to one or more Fc receptors (*e.g.*, FcγR(s)), more preferably such Fc Domain will cause reduced binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc region) or will substantially eliminate the ability of such Fc Domain to bind to such receptor(s). The Fc Domain of the bi-specific monovalent Fc diabodies of the present invention may include some or all of the CH2 domain and/or some or all of the CH3 domain of a complete Fc region, or may comprise a variant CH2 and/or a variant CH3 sequence

(that may include, for example, one or more insertions and/or one or more deletions with respect to the CH2 or CH3 domains of a complete Fc region). The Fc Domain of the bi-specific monovalent Fc diabodies of the present invention may comprise non-Fc polypeptide portions, or may comprise portions of non-naturally complete Fc regions, or may comprise non-naturally occurring orientations of CH2 and/or CH3 domains (such as, for example, two CH2 domains or two CH3 domains, or in the N-terminal to C-terminal direction, a CH3 domain linked to a CH2 domain, etc.).

[0053] The first polypeptide chain of the preferred CD32B x CD79b bi-specific monovalent Fc diabody comprises (in the N-terminal to C-terminal direction): an amino terminus, a cysteine-containing peptide (Peptide 1), an IgG Fc Domain (preferably, the CH2 and CH3 domains of an antibody Fc region, and most preferably the CH2 and CH3 domains of an antibody Fc region that will cause reduced binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc region) or will substantially eliminate the ability of such Fc Domain to bind to such receptor(s), a first intervening spacer peptide (Linker 1), the VL Domain of a monoclonal antibody capable of binding to either CD32B or CD79b (*i.e.*, either VL_{CD32B} or VL_{CD79b}), a second intervening spacer peptide (Linker 2), a VH Domain of a monoclonal antibody capable of binding to either CD79b (if such first polypeptide chain contains VL_{CD32B}) or CD32B (if such first polypeptide chain contains VL_{CD79b}), a cysteine-containing third intervening spacer peptide (Linker 3), a heterodimer-promoting domain, an optional fourth spacer peptide (Linker 4) to provide improved stabilization to the heterodimer-promoting domain and a C-terminus (**Figure 1**).

[0054] The second polypeptide chain of the preferred CD32B x CD79b bi-specific monovalent Fc diabody comprises (in the N-terminal to C-terminal direction): an amino terminus, a VL Domain of a monoclonal antibody capable of binding to either CD79b or CD32B (*i.e.*, either VL_{CD79b} or VL_{CD32B}, depending upon the VL Domain selected for the first polypeptide chain of the diabody), an intervening linker peptide (Linker 2), a VH Domain of a monoclonal antibody capable of binding to either CD32B (if such second polypeptide chain contains VL_{CD79b}) or CD32B (if such

second polypeptide chain contains VL_{CD32B}), a cysteine-containing spacer peptide (Linker 3), a heterodimer-promoting domain, and a C-terminus (**Figure 1**).

[0055] The third polypeptide chain of the preferred CD32B x CD79b bi-specific monovalent Fc diabody comprises (in the N-terminal to C-terminal direction): an amino terminus, a cysteine-containing peptide (Peptide 1), an IgG Fc Domain (preferably, the CH2 and CH3 domains of an antibody Fc region) having the same isotype as that of the Fc Domain of the first polypeptide chain and a C-terminus. Preferably, the Fc Domain of the third polypeptide chain will cause reduced binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc region) or will substantially eliminate the ability of such Fc Domain to bind to such receptor(s) (**Figure 1**).

[0056] The cysteine-containing peptide (Peptide 1) of the first and third stands may be comprised of the same amino acid sequence or of different amino acid sequences, and will contain 1, 2, 3 or more cysteine residues. A particularly preferred Peptide 1 has the amino acid sequence (**SEQ ID NO:1**): DKTHTCPPCP. The first intervening spacer peptide (Linker 1) comprises the amino acid sequence (**SEQ ID NO:2**): APSSS, and more preferably has the amino acid sequence (**SEQ ID NO:3**): APSSSPME. A preferred second intervening spacer peptide (Linker 2) has the sequence is **SEQ ID NO:4**: GGGSGGGG. The preferred cysteine-containing third intervening spacer peptide (Linker 3) will contain 1, 2, 3 or more cysteines. A preferred cysteine-containing spacer peptide (Linker 3) has the sequence is **SEQ ID NO:5**: GGCGGG. A preferred fourth spacer peptide (Linker 4) has the sequence GGG or is **SEQ ID NO:6**: GGGNS.

[0057] Most preferably, the length of the intervening linker peptide (Linker 2, which separates such VL and VH Domains) is selected to substantially or completely prevent the VL and VH Domains of the polypeptide chain from binding to one another. Thus the VL and VH Domains of the first polypeptide chain are substantially or completely incapable of binding to one another. Likewise, the VL

and VH Domains of the second polypeptide chain are substantially or completely incapable of binding to one another.

[0058] The heterodimer-promoting domains of the first and second polypeptides differ from one another and are designed to associate with one another so as to promote association of the first and second polypeptide chains. Thus, in a preferred embodiment, one of these polypeptide chains will be engineered to contain a heterodimer-promoting "E-coil" Domain (**SEQ ID NO:7**):

EVAAL**E**K**E**VAAL**E**K**E**VAAL**E**K**E**VAAL***E***K

whose residues will form a negative charge at pH 7, while the other of the two polypeptide chains will be engineered to contain a heterodimer-promoting "K-coil" Domain (**SEQ ID NO:8**):

KVAAL**K**E**K**VAAL**K**E**K**VAAL**K**E**K**VAAL**K**E

whose residues will form a positive charge at pH 7. The presence of such charged domains promotes association between the first and second polypeptides, and thus fosters heterodimerization. It is immaterial which coil is provided to which chain, as long as the coils employed on the first and second polypeptide chains differ so as to foster heterodimerization between such chains.

[0059] As indicated above, the CH2 and CH3 domains of the first and third polypeptides are preferably mutated to reduce (relative to a wild-type Fc region) or eliminate binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b). Such mutations are well known in the art and include amino acid substitutions at positions 234 and 235, a substitution at position 265 or a substitution at position 297 (see, for example, US Patent No. 5,624,821, herein incorporated by reference). In a preferred embodiment the CH2 and CH3 domain includes a substitution at position 234 with alanine and 235 with alanine.

[0060] The CH2 and/or CH3 domains of the first and third polypeptides need not be identical, and advantageously are modified to foster complexing between the two polypeptides. For example, an amino acid substitution (preferably a substitution with an amino acid comprising a bulky side group forming a 'knob', *e.g.,* tryptophan) can

be introduced into the CH2 or CH3 domain such that steric interference will prevent interaction with a similarly mutated domain and will obligate the mutated domain to pair with a domain into which a complementary, or accommodating mutation has been engineered, *i.e.*, 'the hole' (*e.g.*, a substitution with glycine). Such sets of mutations can be engineered into any pair of polypeptides comprising the Fc diabody molecule, and further, engineered into any portion of the polypeptides chains of said pair. Methods of protein engineering to favor heterodimerization over homodimerization are well known in the art, in particular with respect to the engineering of immunoglobulin-like molecules, and are encompassed herein (see *e.g.,* Ridgway *et al.* (1996) *"'Knobs-Into-Holes' Engineering Of Antibody CH3 Domains For Heavy Chain Heterodimerization,"* Protein Engr. 9:617-621, Atwell *et al.* (1997) *"Stable Heterodimers From Remodeling The Domain Interface Of A Homodimer Using A Phage Display Library,"* J. Mol. Biol. 270: 26-35, and Xie *et al.* (2005) *"A New Format Of Bispecific Antibody: Highly Efficient Heterodimerization, Expression And Tumor Cell Lysis,"* J. Immunol. Methods 296:95-101; each of which is hereby incorporated herein by reference in its entirety). Preferably the 'knob' is engineered into the CH2-CH3 domains of the first polypeptide chain and the 'hole' is engineered into the CH2-CH3 domains of the third polypeptide chain. Thus, the 'knob' will help in preventing the first polypeptide chain from homodimerizing via its CH2 and/or CH3 domains. As the third polypeptide chain preferably contains the 'hole' substitution it will heterodimerize with the first polypeptide chain as well as homodimerize with itself. A preferred knob is created by modifying a native IgG Fc region to contain the modification T366W. A preferred hole is created by modifying a native IgG Fc region to contain the modification T366S, L368A and Y407V. To aid in purifying the third polypeptide chain homodimer from the final bi-specific monovalent Fc diabody comprising the first, second and third polypeptide chains, the protein A binding site of the CH2 and CH3 domains of the third polypeptide chain is preferably mutated by amino acid substitution at position 435 (H435R). To aid in purifying the third polypeptide chain homodimer from the final bi-specific monovalent Fc diabody comprising the first, second and third polypeptide chains, the protein A binding site of the CH2 and CH3 domains of the third polypeptide chain is preferably mutated by amino acid substitution. Thus the third polypeptide chain

homodimer will not bind to protein A, whereas the bi-specific monovalent Fc diabody will retain its ability to bind protein A via the protein A binding site on the first polypeptide chain.

[0061] A preferred sequence for the CH2 and CH3 domains of an antibody Fc region present in the first polypeptide chain is (**SEQ ID NO:9**):

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG NVFSCSVMHE
ALHNHYTQKS LSLSPGK

[0062] A preferred sequence for the CH2 and CH3 domains of an antibody Fc region present in the third polypeptide chain is (**SEQ ID NO:10**):

APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED PEVKFNWYVD
GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK CKVSNKALPA
PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK GFYPSDIAVE
WESNGQPENN YKTTPPVLDS DGSFFLVSKL TVDKSRWQQG NVFSCSVMHE
ALHNRYTQKS LSLSPGK

[0063] A preferred sequence for the VL Domain of an antibody that binds CD32B ($\mathbf{VL_{CD32B}}$) is (**SEQ ID NO:11**):

DIQMTQSPSS LSASVGDRVT ITCRASQEIS GYLSWLQQKP GKAPRRLIYA
ASTLDSGVPS RFSGSESGTE FTLTISSLQP EDFATYYCLQ YFSYPLTFGG
GTKVEIK

[0064] A preferred sequence for the VH Domain of an antibody that binds CD32B ($\mathbf{VH_{CD32B}}$) is (**SEQ ID NO:12**):

EVQLVESGGG LVQPGGSLRL SCAASGFTFS DAWMDWVRQA PGKGLEWVAE
IRNKAKNHAT YYAESVIGRF TISRDDAKNS LYLQMNSLRA EDTAVYYCGA
LGLDYWGQGT LVTVSS

[0065] A preferred sequence for the VL Domain of an antibody that binds CD79b ($\mathbf{VL_{CD79b}}$) is (**SEQ ID NO:13**):

DVVMTQSPLS LPVTLGQPAS ISCKSSQSLL DSDGKTYLNW FQQRPGQSPN
RLIYLVSKLD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
LTFGGGTKLE IK

[0066] A preferred sequence for the VH Domain of an antibody that binds CD79b ($\mathbf{VH_{CD79b}}$) is (**SEQ ID NO:14**):

QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWMNWVRQA PGQGLEWIGM
IDPSDSETHY NQKFKDRVTM TTDTSTSTAY MELRSLRSDD TAVYYCARAM
GYWGQGTTVT VSS

[0067] Thus, a preferred sequence for the first polypeptide chain has the structure, in the N-terminal to C-terminal direction, of: Peptide 1, a CH2-CH3 domain of an IgG Fc region, Linker 1, a VL Domain of an antibody that binds CD32B ($\mathbf{VL_{CD32B}}$), Linker 2, a VH Domain of an antibody that binds CD79b ($\mathbf{VH_{CD79b}}$), Linker 3, an E-coil Domain, a Linker 4 and a C-terminus. The amino acid sequence of such a preferred polypeptide is (**SEQ ID NO:15**):

DKTHTCPPCP APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLWCLVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLYSKL TVDKSRWQQG
NVFSCSVMHE ALHNHYTQKS LSLSPGKAPS SSPMEDIQMT QSPSSLSASV
GDRVTITCRA SQEISGYLSW LQQKPGKAPR RLIYAASTLD SGVPSRFSGS
ESGTEFTLTI SSLQPEDFAT YYCLQYFSYP LTFGGGTKVE IKGGGSGGGG
QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYWMNWVRQA PGQGLEWIGM
IDPSDSETHY NQKFKDRVTM TTDTSTSTAY MELRSLRSDD TAVYYCARAM
GYWGQGTTVT VSSGGCGGGE VAALEKEVAA LEKEVAALEK EVAALEKGGG
NS

[0068] In **SEQ ID NO:15**, amino acid residues 1-10 are Peptide 1 (**SEQ ID NO:1**), amino acid residues 11-227 are the CH2 and CH3 domains of an IgG antibody Fc region (**SEQ ID NO:9**), amino acid residues 228-235 are Linker 1 (**SEQ ID NO:3**), amino acid residues 236-342 is the VL Domain of an antibody that binds CD32B ($\mathbf{VL_{CD32B}}$) (**SEQ ID NO:11**), amino acid residues 343-350 are Linker 2 (**SEQ ID NO:4**), amino acid residues 351-463 is the VH Domain of an antibody that binds CD79b ($\mathbf{VH_{CD79b}}$) (**SEQ ID NO:14**), amino acid residues 464-469 are Linker 3 (**SEQ ID NO:5**), amino acid residues 470-497 are the heterodimer-promoting E-coil Domain (**SEQ ID NO:7**), and amino acid residues 498-502 are Linker 4 (**SEQ ID NO:6**).

[0069] A preferred polynucleotide that encodes the first polypeptide chain has the sequence (**SEQ ID NO:23**):

gacaaaactcacacatgcccaccgtgcccagcacctgaagccgcgggggaccgt
cagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccc
tgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttc
aactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggagg
agcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccagga
ctggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcc
cccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt
acaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgtggtg
cctggtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatggg
cagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
tcttcctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaacgt
cttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc
ctctccctgtctccgggtaaagccccttccagctcccctatggaagacatccaga
tgacccagtctccatcctccttatctgcctctgtgggagatagagtcaccatcac
ttgtcgggcaagtcaggaaattagtggttacttaagctggctgcagcagaaacca
ggcaaggcccctagacgcctgatctacgccgcatccactttagattctggtgtcc
catccaggttcagtggcagtgagtctgggaccgagttcaccctcaccatcagcag
ccttcagcctgaagattttgcaacctattactgtctacaatattttagttatccg
ctcacgttcggaggggggaccaaggtggaaataaaaggaggcggatccggcggcg
gaggccaggttcagctggtgcagtctggagctgaggtgaagaagcctggcgcctc
agtgaaggtctcctgcaaggcttctggttacacctttaccagctactggatgaac
tgggtgcgacaggcccctggacaagggcttgagtggatcggaatgattgatcctt
cagacagtgaaactcactacaatcaaaagttcaaggacagagtcaccatgaccac
agacacatccacgagcacagcctacatggagctgaggagcctgagatctgacgac
acggccgtgtattactgtgcgagagctatgggctactgggggcaagggaccacgg
tcaccgtctcctccggaggatgtggcggtggagaagtggccgcactggagaaaga
ggttgctgctttggagaaggaggtcgctgcacttgaaaaggaggtcgcagccctg
gagaaaggcggcgggaactct

[0070] A preferred sequence for the second polypeptide chain is (**SEQ ID NO:16**):

DVVMTQSPLS LPVTLGQPAS ISCKSSQSLL DSDGKTYLNW FQQRPGQSPN
RLIYLVSKLD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
LTFGGGTKLE IKGGGSGGGG EVQLVESGGG LVQPGGSLRL SCAASGFTFS
DAWMDWVRQA PGKGLEWVAE IRNKAKNHAT YYAESVIGRF TISRDDAKNS
LYLQMNSLRA EDTAVYYCGA LGLDYWGQGT LVTVSSGGCG GGKVAALKEK
VAALKEKVAA LKEKVAALKE

[0071] In **SEQ ID NO:16**, amino acid residues 1-112 is the VL Domain of an antibody that binds CD79b ($\mathbf{VL_{CD79b}}$) (**SEQ ID NO:13**), amino acid residues 113-120

are Linker 2 (**SEQ ID NO:4**), amino acid residues 121-236 is the VH Domain of an antibody that binds CD32B ($\mathbf{VH_{CD32B}}$) (**SEQ ID NO:12**), amino acid residues 237-242 are Linker 3 (**SEQ ID NO:5**), and amino acid residues 243-270 are the heterodimer-promoting K-coil Domain (**SEQ ID NO:8**).

[0072] A preferred polynucleotide that encodes the second polypeptide chain has the sequence (**SEQ ID NO:24**):

gatgttgtgatgactcagtctccactctccctgcccgtcacccttggacagccgg
cctccatctcctgcaagtcaagtcagagcctcttagatagtgatggaaagacata
tttgaattggtttcagcagaggccaggccaatctccaaaccgcctaatttatctg
gtgtctaaactggactctggggtcccagacagattcagcggcagtgggtcaggca
ctgatttcacactgaaaatcagcagggtggaggctgaggatgttggggtttatta
ctgctggcaaggtacacattttccgctcacgttcggcggagggaccaagcttgag
atcaaaggaggcggatccggcggcggaggcgaagtgcagcttgtggagtctggag
gaggcttggtgcaacctggaggatccctgagactctcttgtgccgcctctggatt
cacttttagtgacgcctggatggactgggtccgtcaggccccaggcaaggggctt
gagtgggttgctgaaattagaaacaaagctaaaaatcatgcaacatactatgctg
agtctgtgatagggaggttcaccatctcaagagatgacgccaaaaacagtctgta
cctgcaaatgaacagcttaagagctgaagacactgccgtgtattactgtggggct
ctgggccttgactactggggccaaggcaccctggtgaccgtctcctccggaggat
gtggcggtggaaaagtggccgcactgaaggagaaagttgctgctttgaaagagaa
ggtcgccgcacttaaggaaaaggtcgcagccctgaaagag

[0073] A preferred sequence for the third polypeptide chain is **SEQ ID NO:17**:

DKTHTCPPCP APEAAGGPSV FLFPPKPKDT LMISRTPEVT CVVVDVSHED
PEVKFNWYVD GVEVHNAKTK PREEQYNSTY RVVSVLTVLH QDWLNGKEYK
CKVSNKALPA PIEKTISKAK GQPREPQVYT LPPSREEMTK NQVSLSCAVK
GFYPSDIAVE WESNGQPENN YKTTPPVLDS DGSFFLVSKL TVDKSRWQQG
NVFSCSVMHE ALHNRYTQKS LSLSPGK

[0074] In **SEQ ID NO:17**, amino acid residues 1-10 are Peptide 1 (**SEQ ID NO:1**), and amino acid residues 11-227 are the CH2 and CH3 domains of an IgG antibody Fc region (**SEQ ID NO:10**).

[0075] A preferred polynucleotide that encodes the third polypeptide chain has the sequence (**SEQ ID NO:25**):

gacaaaactcacacatgcccaccgtgcccagcacctgaagccgcgggggaccgt
cagtcttcctcttcccccaaaacccaaggacaccctcatgatctcccggacccc
tgaggtcacatgcgtggtggtggacgtgagccacgaagaccctgaggtcaagttc
aactggtacgtggacggcgtggaggtgcataatgccaagacaaagccgcgggagg

agcagtacaacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccagga
ctggctgaatggcaaggagtacaagtgcaaggtctccaacaaagccctcccagcc
cccatcgagaaaaccatctccaaagccaaagggcagccccgagaaccacaggtgt
acaccctgcccccatcccgggaggagatgaccaagaaccaggtcagcctgagttg
cgcagtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatggg
cagccggagaacaactacaagaccacgcctcccgtgctggactccgacggctcct
tcttcctcgtcagcaagctcaccgtggacaagagcaggtggcagcaggggaacgt
cttctcatgctccgtgatgcatgaggctctgcacaaccgctacacgcagaagagc
ctctccctgtctccgggtaaa

[0076] As disclosed in WO 2012/018687, in order to improve the *in vivo* pharmacokinetic properties of diabody molecules, the molecules may be modified to contain a polypeptide portion of a serum-binding protein at one or more of the termini of the diabody molecule. Most preferably, such polypeptide portion of a serum-binding protein will be installed at the C-terminus of the diabody molecule. A particularly preferred polypeptide portion of a serum-binding protein for this purpose is the Albumin-Binding Domain (ABD) from streptococcal protein G. The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 is particularly preferred.

[0077] The Albumin-Binding Domain 3 (ABD3) of protein G of Streptococcus strain G148 consists of 46 amino acid residues forming a stable three-helix bundle and has broad albumin binding specificity (Johansson, M.U. *et al.* (2002) "*Structure, Specificity, And Mode Of Interaction For Bacterial Albumin-Binding Module*s," J. Biol. Chem. 277(10):8114-8120). Albumin is the most abundant protein in plasma and has a half-life of 19 days in humans. Albumin possesses several small molecule binding sites that permit it to non-covalently bind to other proteins and thereby extend their serum half-lives. Preferably, a short linker (Linker 5) (such as GGGS (**SEQ ID NO:18**) or GGGNS (**SEQ ID NO:6**) is employed to separate the E-coil (or K-coil) of such polypeptide chain from the Albumin-Binding Domain. A preferred Albumin-Binding Domain (**ABD**) has the amino acid sequence (**SEQ ID NO:19**):

LAEAKVLANR ELDKYGVSDY YKNLIDNAKS AEGVKALID EILAALP

II. Alternative CD32B x CD79b Fc Diabodies of the Present Invention

[0078] An alternative CD32B x CD79b bi-specific monovalent Fc diabody molecule of the present invention is shown schematically in **Figure 2**. Such alternative CD32B x CD79b Fc diabody molecules possess three polypeptide chains, of which the first and second polypeptide chains are covalently bonded to one another and the first and third polypeptide chains are bonded to one another. The alternative CD32B x CD79b bi-specific monovalent Fc diabody molecules differ in the order of its domains relative to the order present in the preferred CD32B x CD79b bi-specific monovalent Fc diabody molecules. However, as in the case of the preferred CD32B x CD79b Fc diabody, the VL Domain of the first polypeptide chain of the alternative CD32B x CD79b bi-specific monovalent Fc diabody interacts with the VH Domain of the second polypeptide chain of the alternative CD32B x CD79b bi-specific monovalent Fc diabody in order to form a first functional antigen binding site that is specific for the first antigen (*i.e.*, either CD32B or CD79b). Likewise, the VL Domain of the second polypeptide chain of the alternative CD32B x CD79b bi-specific monovalent Fc diabody interacts with the VH Domain of the first polypeptide chain of the alternative CD32B x CD79b bi-specific monovalent Fc diabody in order to form a second functional antigen binding site that is specific for the second antigen (*i.e.*, either CD79b or CD32B, depending upon the identity of the first antigen). Thus, the selection of the VL and VH Domains of the first and second polypeptide chains are coordinated, such that the two polypeptide chains collectively comprise VL and VH Domains capable of binding to CD32B and CD79b (*i.e.*, they comprise VL_{CD32B}/VH_{CD32B} and VL_{CD79b}/VH_{CD79b}) (**Figure 2**). Collectively, each such VL and VH Domain, and the intervening Linker that separates them, are referred to as an Antigen-Binding Domain of the molecule.

[0079] The first polypeptide chain of such alternative CD32B x CD79b Fc diabody comprises, in the N-terminal to C-terminal direction, an amino terminus, the VL Domain of a monoclonal antibody capable of binding to either CD32B or CD79b (*i.e.*, either VL_{CD32B} or VL_{CD79b}), an intervening spacer peptide (Linker 2), a VH Domain of a monoclonal antibody capable of binding to either CD79b (if such first polypeptide chain contains VL_{CD32B}) or CD32B (if such first polypeptide chain contains VL_{CD79b}),

a cysteine-containing third intervening spacer peptide (Linker 3), a heterodimer-promoting domain, an optional fourth spacer peptide (Linker 4) to provide improved stabilization to the heterodimer-promoting domain (preferably an E-coil Domain), a cysteine-containing peptide (Peptide 1), an IgG Fc Domain (preferably, the CH2 and CH3 domains of an antibody Fc region, and a C-terminus. Preferably, the Fc Domain of the first polypeptide chain will cause reduced binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc region) or will substantially eliminate the ability of such Fc Domain to bind to such receptor(s) (**Figure 2**).

[0080] The second polypeptide chain of such alternative CD32B x CD79b Fc diabody comprises, in the N-terminal to C-terminal direction, an amino terminus, a VL Domain of a monoclonal antibody capable of binding to either CD79b or CD32B (*i.e.*, either VL_{CD79b} or VL_{CD32B}, depending upon the VL Domain selected for the first polypeptide chain of the diabody), an intervening linker peptide (Linker 2), a VH Domain of a monoclonal antibody capable of binding to either CD32B (if such second polypeptide chain contains VL_{CD79b}) or CD32B (if such second polypeptide chain contains VL_{CD32B}), a cysteine-containing spacer peptide (Linker 3), a heterodimer-promoting domain (preferably a K-coil Domain), and a C-terminus (**Figure 2**).

[0081] The third polypeptide chain of the preferred CD32B x CD79b Fc diabody comprises, in the N-terminal to C-terminal direction, an amino terminus, a cysteine-containing peptide (Peptide 1), an IgG Fc Domain (preferably, the CH2 and CH3 domains of an antibody Fc region) having the same isotype as that of the Fc Domain of the first polypeptide chain and a C-terminus. Preferably, the Fc Domain of the third polypeptide chain will cause reduced binding to FcγRIA (CD64), FcγRIIA (CD32A), FcγRIIB (CD32B), FcγRIIIA (CD16a) or FcγRIIIB (CD16b) (relative to the binding exhibited by a wild-type Fc region) or will substantially eliminate the ability of such Fc Domain to bind to such receptor(s) (**Figure 2**).

III. Pharmaceutical Compositions

[0082] The compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (*e.g.*, impure or non-sterile

compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of the CD32B x CD79b Fc diabodies of the present invention, and in particular any of the CD32B x CD79b Fc diabodies disclosed herein or a combination of such agents and a pharmaceutically acceptable carrier. Preferably, compositions of the invention comprise a prophylactically or therapeutically effective amount of one or more molecules of the invention and a pharmaceutically acceptable carrier.

[0083] The invention also encompasses pharmaceutical compositions comprising such CD32B x CD79b Fc diabodies and a second therapeutic antibody (*e.g.*, autoimmune or inflammatory disease antigen specific monoclonal antibody) that is specific for a particular autoimmune or inflammatory disease antigen, and a pharmaceutically acceptable carrier.

[0084] In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete and incomplete), excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0085] Generally, the ingredients of compositions of the invention 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.

[0086] The compositions of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include, but are not limited to those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, *etc.*, and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, *etc.*

[0087] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with such disclosed CD32B x CD79b Fc diabodies alone or with such pharmaceutically acceptable carrier. Additionally, one or more other prophylactic or therapeutic agents useful for the treatment of a disease can also be included in the pharmaceutical pack or kit. The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

[0088] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises one or more molecules of the invention. In another embodiment, a kit further comprises one or more other prophylactic or therapeutic agents useful for the treatment of an autoimmune or inflammatory disease, in one or more containers. In another embodiment, a kit further comprises one or

more antibodies that bind one or more autoimmune or inflammatory disease antigens associated with autoimmune or inflammatory disease. In certain embodiments, the other prophylactic or therapeutic agent is a chemotherapeutic. In other embodiments, the prophylactic or therapeutic agent is a biological or hormonal therapeutic.

IV. Uses of the Compositions of the Invention

[0089] The CD32B x CD79b Fc diabodies of the present invention have the ability to treat any disease or condition associated with or characterized by the expression of CD79b or having a B cell component to the disease. Thus, without limitation, pharmaceutical compositions comprising such molecules may be employed in the diagnosis or treatment of autoimmune or inflammatory diseases or conditions.

[0090] Thus, the invention may be used to treat, prevent, slow the progression of, and/or ameliorate a symptom of B cell mediated diseases or disorders, including graft rejection, graft-versus-host disease (GvHD) and systemic lupus erythematosis (SLE).

V. Methods of Administration

[0091] The compositions of the present invention may be provided for the treatment, prophylaxis, and amelioration of one or more symptoms associated with a disease, disorder or infection by administering to a subject an effective amount of a pharmaceutical composition of the invention. In a preferred aspect, such compositions are substantially purified (*i.e.*, substantially free from substances that limit its effect or produce undesired side-effects). In a specific embodiment, the subject is an animal, preferably a mammal such as non-primate (*e.g.*, bovine, equine, feline, canine, rodent, *etc.*) or a primate (*e.g.*, monkey such as, a cynomolgous monkey, human, *etc.*). In a preferred embodiment, the subject is a human.

[0092] Various delivery systems are known and can be used to administer the compositions of the invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or fusion protein, receptor-mediated endocytosis (See, *e.g.*, Wu *et al.* (1987) *"Receptor-Mediated In Vitro Gene Transformation By A Soluble DNA Carrier System,"* J. Biol. Chem.

262:4429-4432), construction of a nucleic acid as part of a retroviral or other vector, *etc.*

[0093] Methods of administering a bi-specific monovalent Fc diabody of the invention include, but are not limited to, parenteral administration (*e.g.*, intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural, and mucosal (*e.g.*, intranasal and oral routes). In a specific embodiment, the molecules of the invention are administered intramuscularly, intravenously, or subcutaneously. The compositions may be administered by any convenient route, for example, by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, *etc.*) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Patent Nos. 6,019,968; 5,985, 320; 5,985,309; 5,934,272; 5,874,064; 5,855,913; 5,290,540; and 4,880,078; and PCT Publication Nos. WO 92/19244; WO 97/32572; WO 97/44013; WO 98/31346; and WO 99/66903, each of which is incorporated herein by reference in its entirety.

[0094] The invention also provides that the CD32B x CD79b Fc diabodies of the invention are packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of such molecules. In one embodiment, the CD32B x CD79b Fc diabodies of the invention are supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted, *e.g.*, with water or saline to the appropriate concentration for administration to a subject. Preferably, the CD32B x CD79b Fc diabodies of the invention are supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 μg, more preferably at least 10 μg, at least 15 μg, at least 25 μg, at least 50 μg, at least 100 μg, or at least 200 μg.

[0095] The lyophilized CD32B x CD79b Fc diabodies of the invention should be stored at between 2 and 8°C in their original container and the molecules should be administered within 12 hours, preferably within 6 hours, within 5 hours, within 3

hours, or within 1 hour after being reconstituted. In an alternative embodiment, the CD32B x CD79b Fc diabodies of the invention are supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the molecule, fusion protein, or conjugated molecule. Preferably, the liquid form of the CD32B x CD79b Fc diabodies of the invention is supplied in a hermetically sealed container in which the molecules are present at a concentration of least 1 μg/ml, more preferably at least 2.5 μg/ml, at least 5 μg/ml, at least 10 μg/ml, at least 50 μg/ml, or at least 100 μg/ml.

[0096] The amount of the CD32B x CD79b Fc diabodies of the invention which will be effective in the treatment, prevention or amelioration of one or more symptoms associated with a disorder can be determined by standard clinical techniques. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[0097] For CD32B x CD79b Fc diabodies Fc diabodies encompassed by the invention, the dosage administered to a patient is typically at least about 0.01 μg/kg, at least about 0.05 μg/kg, at least about 0.1 μg/kg, at least about 0.2 μg/kg, at least about 0.5 μg/kg, at least about 1 μg/kg, at least about 2 μg/kg, at least about 5 μg/kg, at least about 10 μg/kg, at least about 20 μg/kg, at least about 50 μg/kg, at least about 0.1 mg/kg, at least about 1 mg/kg, at least about 5 mg/kg, at least about 10 mg/kg, at least about 30 mg/kg, at least about 50 mg/kg, at least about 75 mg/kg, at least about 100 mg/kg, at least about 125 mg/kg, at least about 150 mg/kg or more of the subject's body weight.

[0098] The dosage and frequency of administration of the bi-specific monovalent Fc diabodies of the invention may be reduced or altered by enhancing uptake and tissue penetration of the bi-specific monovalent Fc diabodies by modifications such as, for example, lipidation.

[0099] In one embodiment, the dosage of the CD32B x CD79b Fc diabodies of the invention administered to a patient may be calculated for use as a single agent therapy. In another embodiment the bi-specific monovalent Fc diabodies of the invention are used in combination with other therapeutic compositions and the dosage administered to a patient are lower than when such bi-specific monovalent Fc diabody molecules are used as a single agent therapy.

[00100] In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a molecule of the invention, care must be taken to use materials to which the molecule does not absorb.

[00101] In another embodiment, the compositions can be delivered in a vesicle, in particular a liposome (*See* Langer (1990) *"New Methods Of Drug Delivery,"* Science 249:1527-1533); Treat *et al.*, in LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASE AND CANCER, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 3 17-327; see generally ibid.).

[00102] In yet another embodiment, the compositions can be delivered in a controlled-release or sustained-release system. Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more molecules of the invention. *See, e.g.*, U.S. Patent No. 4,526,938; PCT publication WO 91/05548; PCT publication WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody*

For Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety. In one embodiment, a pump may be used in a controlled-release system (*See* Langer, *supra*; Sefton, (1987) *"Implantable Pumps,"* CRC Crit. Rev. Biomed. Eng. 14:201-240; Buchwald *et al.* (1980) *"Long-Term, Continuous Intravenous Heparin Administration By An Implantable Infusion Pump In Ambulatory Patients With Recurrent Venous Thrombosis,"* Surgery 88:507-516; and Saudek *et al.* (1989) *"A Preliminary Trial Of The Programmable Implantable Medication System For Insulin Delivery,"* N. Engl. J. Med. 321:574-579). In another embodiment, polymeric materials can be used to achieve controlled release of antibodies (*see e.g.*, MEDICAL APPLICATIONS OF CONTROLLED RELEASE, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); CONTROLLED DRUG BIOAVAILABILITY, DRUG PRODUCT DESIGN AND PERFORMANCE, Smolen and Ball (eds.), Wiley, New York (1984); Levy *et al.* (1985) *"Inhibition Of Calcification Of Bioprosthetic Heart Valves By Local Controlled-Release Diphosphonate,"* Science 228:190-192; During *et al.* (1989) *"Controlled Release Of Dopamine From A Polymeric Brain Implant: In Vivo Characterization,"* Ann. Neurol. 25:351-356; Howard *et al.* (1989) *"Intracerebral Drug Delivery In Rats With Lesion-Induced Memory Deficits,"* J. Neurosurg. 7(1):105-112); U.S. Patent No. 5,679,377; U.S. Patent No. 5,916,597; U.S. Patent No. 5,912,015; U.S. Patent No. 5,989,463; U.S. Patent No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253). Examples of polymers used in sustained-release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In yet another embodiment, a controlled-release system can be placed in proximity of the therapeutic target (*e.g.*, the lungs), thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in MEDICAL APPLICATIONS OF CONTROLLED RELEASE, *supra*, vol. 2, pp. 115-138 (1984)). In another embodiment, polymeric compositions useful as controlled-release implants are used according to Dunn *et al.* (See U.S. 5,945,155). This particular method is based upon the therapeutic effect of the in situ controlled-

release of the bioactive material from the polymer system. The implantation can generally occur anywhere within the body of the patient in need of therapeutic treatment. In another embodiment, a non-polymeric sustained delivery system is used, whereby a non-polymeric implant in the body of the subject is used as a drug delivery system. Upon implantation in the body, the organic solvent of the implant will dissipate, disperse, or leach from the composition into surrounding tissue fluid, and the non-polymeric material will gradually coagulate or precipitate to form a solid, microporous matrix (See U.S. 5,888,533).

[00103] Controlled-release systems are discussed in the review by Langer (1990, *"New Methods Of Drug Delivery,"* Science 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained-release formulations comprising one or more therapeutic agents of the invention. See, *e.g.*, U.S. Patent No. 4,526,938; International Publication Nos. WO 91/05548 and WO 96/20698; Ning *et al.* (1996) *"Intratumoral Radioimmunotheraphy Of A Human Colon Cancer Xenograft Using A Sustained-Release Gel,"* Radiotherapy & Oncology 39:179-189, Song *et al.* (1995) *"Antibody Mediated Lung Targeting Of Long-Circulating Emulsions,"* PDA Journal of Pharmaceutical Science & Technology 50:372-397; Cleek *et al.* (1997) *"Biodegradable Polymeric Carriers For A bFGF Antibody For Cardiovascular Application,"* Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854; and Lam *et al.* (1997) *"Microencapsulation Of Recombinant Humanized Monoclonal Antibody For Local Delivery,"* Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, each of which is incorporated herein by reference in its entirety.

[00104] In a specific embodiment where the composition of the invention is a nucleic acid encoding a bi-specific monovalent Fc diabody of the invention, the nucleic acid can be administered *in vivo* to promote expression of its encoded bi-specific monovalent Fc diabody, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, *e.g.*, by use of a retroviral vector (*See* U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (*e.g.*, a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (See *e.g.*, Joliot *et al.*

(1991) *"Antennapedia Homeobox Peptide Regulates Neural Morphogenesis,"* Proc. Natl. Acad. Sci. (U.S.A.) 88:1864-1868), *etc.* Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

[00105] Treatment of a subject with a therapeutically or prophylactically effective amount of the CD32B x CD79b Fc diabodies of the invention can include a single treatment or, preferably, can include a series of treatments. In a preferred example, a subject is treated with molecules of the invention one time per week for between about 1 to 10 weeks, preferably between 2 to 8 weeks, more preferably between about 3 to 7 weeks, and even more preferably for about 4, 5, or 6 weeks. In other embodiments, the pharmaceutical compositions of the invention are administered once a day, twice a day, or three times a day. In other embodiments, the pharmaceutical compositions are administered once a week, twice a week, once every two weeks, once a month, once every six weeks, once every two months, twice a year or once per year. It will also be appreciated that the effective dosage of the molecules used for treatment may increase or decrease over the course of a particular treatment.

[00106] Having now generally described the invention, the same will be more readily understood through reference to the following examples, which are provided by way of illustration and are not intended to be limiting of the present invention unless specified.

Example 1
Construction Of CD32B x CD79b Bi-Specific Monovalent Fc Diabodies And Control Diabodies

[00107] Table 1 contains a list of sequences of the polypeptide chains of the preferred CD32B x CD79b Fc diabody that were expressed and purified. Additionally, two control diabodies were produced: one bi-specific monovalent for CD32B and FITC and the second bi-specific monovalent for CD79b and FITC.

Table 1

Preferred CD32B x CD79b Bi-specific Fc Diabody	Substituent Polypeptides (in the N-Terminal to C-Terminal Direction)
First Polypeptide Chain (**SEQ ID NO:15**)	**SEQ ID NO:1** **SEQ ID NO:9** **SEQ ID NO:3** **SEQ ID NO:11** **SEQ ID NO:4** **SEQ ID NO:14** **SEQ ID NO:5** **SEQ ID NO:7** **SEQ ID NO:6**
Second Polypeptide Chain (**SEQ ID NO:16**)	**SEQ ID NO:13** **SEQ ID NO:4** **SEQ ID NO:12** **SEQ ID NO:5** **SEQ ID NO:8**
Third Polypeptide Chain (**SEQ ID NO:17**)	**SEQ ID NO:1** **SEQ ID NO:10**

[00108] The above-described CD32B x CD79b Fc diabody was found to be capable of simultaneously binding to CD32B and to CD79b. The control CD32B x FITC diabody was found to be capable of simultaneously binding to CD32B and to FITC. The control CD79b x FITC diabody was found to be capable of simultaneously binding to CD79b and to FITC. The CD32B x CD79b Fc diabody is a heterotrimer composed of three polypeptide chains (one chain of each recited amino acid sequence). Methods for forming bi-specific monovalent diabodies are provided in

WO 2006/113665, WO 2008/157379, WO 2010/080538, WO 2012/018687, WO 2012/162068 and WO 2012/162067.

[00109] In order to further demonstrate the advantages of such preferred CD32B x CD79b Fc diabody, two non-Fc containing CD32B x CD79b diabodies were also prepared. These diabodies are each composed of two polypeptide chains, and differ in that one of the diabodies (the CD32B x CD79b (ABD) diabody) contains an Albumin-Binding Domain, whereas the other (the CD32B x CD79b diabody) does not:

CD32B x CD79b (ABD) Diabody

[00110] The CD32B x CD79b (ABD) diabody is formed from a first polypeptide chain that comprises, in the N-terminal to C-terminal direction, the VL Domain of an antibody that binds CD32B ($\mathbf{VL_{CD32B}}$), Linker 2, the VH Domain of an antibody that binds CD79b ($\mathbf{VH_{CD79b}}$), Linker 3, the E-coil Domain, Linker 5, an Albumin-Binding Domain and a C-terminus. The second polypeptide chain comprises, in the N-terminal to C-terminal direction, the VL Domain of an antibody that binds CD79b ($\mathbf{VL_{CD79b}}$), Linker 2, the VH Domain of an antibody that binds CD32B ($\mathbf{VH_{CD32B}}$), Linker 3, the K-coil Domain and a C-terminus. The amino acid sequences of such polypeptides are as follows:

Amino Acid Sequence of First Polypeptide Chain (SEQ ID NO:20):

DIQMTQSPSS LSASVGDRVT ITCRASQEIS GYLSWLQQKP GKAPRRLIYA
ASTLDSGVPS RFSGSESGTE FTLTISSLQP EDFATYYCLQ YFSYPLTFGG
GTKVEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTSYWMN
WVRQAPGQGL EWIGMIDPSD SETHYNQKFK DRVTMTTDTS TSTAYMELRS
LRSDDTAVYY CARAMGYWGQ GTTVTVSSGG CGGGEVAALE KEVAALEKEV
AALEKEVAAL EKGGGSLAEA KVLANRELDK YGVSDYYKNL IDNAKSAEGV
KALIDEILAA LP

Amino Acid Sequence of Second Polypeptide Chain (SEQ ID NO:21):

DVVMTQSPLS LPVTLGQPAS ISCKSSQSLL DSDGKTYLNW FQQRPGQSPN
RLIYLVSKLD SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCWQGTHFP
LTFGGGTKLE IKGGGSGGGG EVQLVESGGG LVQPGGSLRL SCAASGFTFS
DAWMDWVRQA PGKGLEWVAE IRNKAKNHAT YYAESVIGRF TISRDDAKNS

LYLQMNSLRA EDTAVYYCGA LGLDYWGQGT LVTVSSGGCG GGKVAALKEK VAALKEKVAA LKEKVAALKE

CD32B x CD79b Diabody

[00111] The CD32B x CD79b diabody differs from the CD32B x CD79b (ABD) diabody in not having an Albumin-Binding Domain. Thus, such diabody is formed from a first polypeptide chain that comprises, in the N-terminal to C-terminal direction, the VL Domain of an antibody that binds CD32B ($\mathbf{VL_{CD32B}}$), Linker 2, the VH Domain of an antibody that binds CD79b ($\mathbf{VH_{CD79b}}$), Linker 3, the E-coil Domain, and a C-terminus. The second polypeptide chain comprises, in the N-terminal to C-terminal direction, the VL Domain of an antibody that binds CD79b ($\mathbf{VL_{CD79b}}$), Linker 2, the VH Domain of an antibody that binds CD32B ($\mathbf{VH_{CD32B}}$), Linker 3, the K-coil Domain and a C-terminus. The amino acid sequence of the first such first polypeptide chain of this diabody is (**SEQ ID NO:22**):

DIQMTQSPSS LSASVGDRVT ITCRASQEIS GYLSWLQQKP GKAPRRLIYA ASTLDSGVPS RFSGSESGTE FTLTISSLQP EDFATYYCLQ YFSYPLTFGG GTKVEIKGGG SGGGGQVQLV QSGAEVKKPG ASVKVSCKAS GYTFTSYWMN WVRQAPGQGL EWIGMIDPSD SETHYNQKFK DRVTMTTDTS TSTAYMELRS LRSDDTAVYY CARAMGYWGQ GTTVTVSSGG CGGGEVAALE KEVAALEKEV AALEKEVAAL EK

The amino acid sequence of the second polypeptide chain of this diabody is (**SEQ ID NO:21**, which is presented above.

Example 2
CD32B x CD79b Bi-Specific Monovalent Fc Diabodies Inhibit Human Primary B Cell Proliferation

[00112] In order to further demonstrate the ability of the CD32B x CD79b Fc diabodies of the present invention to dampen or inhibit the immune system, the above-described preferred CD32B x CD79b Fc diabody was incubated in the presence of primary human B cells obtained from two donors. Proliferation was monitored by the uptake of ^{3}H-TdR after 48 hours in the presence of goat anti-human IgM Fc μ $F(ab)_2$ (5 μg/ml) and differing concentrations of either CD32B x CD79b Fc diabody or CD32B x CD79b ABD diabody. The results are shown in **Figure 3A** (Donor 1) and **Figure 3B** (Donor 2), and indicate a marked reduction in B cell proliferation in

the presence of the CD32B x CD79b Fc diabody or the CD32B x CD79b (ABD) diabody.

Example 3
CD32B x CD79b Bi-Specific Monovalent Fc Diabodies
Inhibit Signaling in Naïve and Memory B Cells

[00113] In order to further demonstrate the ability of the CD32B x CD79b Fc diabodies of the present invention to dampen or inhibit signaling of the immune system by B cells, purified naïve or memory B cells were incubated for 30 minutes in the presence of goat anti-human IgM Fc μ (anti-μ) (30 μg/ml) alone or in the additional presence of the above-described preferred CD32B x CD79b Fc diabody. As seen in **Figure 4A** (naïve B cells) and **Figure 4B** (memory B cells), the presence of the preferred CD32B x CD79b Fc diabody, the CD32B x CD79b (ABD) diabody, or the CD32B x CD79b diabody all markedly reduced B cell signaling.

Example 4
CD32B x CD79b Bi-Specific Monovalent Fc Diabodies
Inhibit Proliferation of SLE Patient B Cells

[00114] In order to further demonstrate the ability of the CD32B x CD79b Fc diabodies of the present invention to dampen or inhibit signaling of the immune system by B cells, B cells of a patient suffering from systemic lupus erythematosus (SLE) were incubated in the presence of goat anti-human IgM Fc μ (anti-μ) alone or in the additional presence of the above-described preferred CD32B x CD79b Fc diabody. Proliferation was monitored by the uptake of ^{3}H-TdR.

[00115] As shown in **Figure 5A**, the above-described preferred CD32B x CD79b Fc diabody was found to be able to bind to both CD32B and CD79b. **Figure 5B**, demonstrates that the provision of the goat anti-human IgM (GAH anti-μ) caused increased proliferation of the B cells, relative to the control, and that the additional administration of the above-described preferred CD32B x CD79b Fc diabody or the CD32B x CD79b (ABD) diabody markedly inhibit the extent of such proliferation.

[00116] The capability of the above-described preferred CD32B x CD79b Fc diabody or of the CD32B x CD79b (ABD) diabody to decrease the extent of B cell proliferation of individuals suffering from SLE was found to be independent of the

status of the disease. The extent of the reduction of B cell proliferation in patients with active or inactive SLE was approximately 40% relative to the proliferation observed in the presence of only the goat anti-human IgM (GAH anti-μ), and thus was irrespective of disease status (**Figure 5C**). **Figure 5C** further demonstrates that the preferred CD32B x CD79b Fc diabody provided greater inhibition than the CD32B x CD79b (ABD) diabody.

Example 5
CD32B x CD79b Bi-Specific Monovalent Fc Diabodies Modulate B Cell Responses *in vivo*

[00117] In order to further demonstrate the ability of the CD32B x CD79b Fc diabodies of the present invention to dampen or inhibit signaling of the immune system by B cells, human PBMC were injected into immunodeficient NSG mice (Agliano, A. *et al.* (2008) "*Human Acute Leukemia Cells Injected In NOD/Ltsz-Scid/IL-2Rgamma Null Mice Generate A Faster And More Efficient Disease Compared To Other NOD/Scid-Related Strains*," Int. J. Cancer 123(9):2222-2227; Sanchez, P.V. *et al.* (2009) "*A Robust Xenotransplantation* Model *For Acute Myeloid Leukemia*," Leukemia 23(11):2109-2117; Racki, W.J. *et al.* (2010) "*NOD-Scid IL2rgamma(Null) Mouse Model Of Human Skin Transplantation And Allograft Rejection*," Transplantation 89(5):527-536; Choi, B. *et al.* (2011) "*Human B Cell Development And Antibody Production In Humanized NOD/SCID/IL-2Rγ(Null) (NSG) Mice Conditioned By Busulfan*," J. Clin. Immunol. 31(2):253-264; Sartelet, H. *et al.* (2012) "*Description Of A New Xenograft Model Of Metastatic Neuroblastoma Using NOD/SCID/Il2rg Null (NSG) Mice*," In Vivo 26(1):19-29; Spranger, S. *et al.* (2012) "*NOD/scid IL-2Rg(null) Mice: A Preclinical Model System To Evaluate Human Dendritic Cell-Based Vaccine Strategies in vivo*," J. Transl. Med. 10:30; von Bonin, M. *et al.* (2013) "*in vivo Expansion Of Co-Transplanted T Cells Impacts On Tumor Re-Initiating Activity Of Human Acute Myeloid Leukemia In NSG Mice*," PLoS One. 8(4):e60680). Animals were administered a control vehicle (100 μl of phosphate buffered saline (PBS)/animal, q3d x 2 weeks), the above-described preferred CD32B x CD79b Fc diabody (100 μl/animal, q3d x 2 weeks), or a CD32B x CD79b diabody (composed of only two polypeptide strains and containing an albumin-binding domain). Plasma was assayed by ELISA at day 7 and day 14 for the presence of

human IgM (**Figure 6A**) or human IgG (**Figure 6B**), both being indicative of the onset of graft vs. host disease.

[00118] Mice receiving the control vehicle exhibited high levels of human IgM and human IgG. In contrast, such antibodies were essentially not detected in mice that had received the above-described preferred CD32B x CD79b Fc diabody (**Figure 6A** and **Figure 6B**). Mice that had received the CD32B x CD79b diabody exhibited diminished levels of human IgM and human IgG, compared to mice receiving the control vehicle, but such levels were nevertheless substantially higher than those receiving the CD32B x CD79b Fc diabody. These findings demonstrate that bi-specific monovalent CD32B x CD79b diabodies have therapeutic utility and effectiveness, but that the above-described preferred CD32B x CD79b Fc diabody of the present invention is unexpectedly superior to such non-Fc diabodies and possesses even greater therapeutic utility and effectiveness (**Figure 6A** and **Figure 6B**).

Example 6
CD32B x CD79b Bi-Specific Monovalent Fc Diabodies Decrease Xenogeneic GvHD in the Mouse

[00119] In order to further demonstrate the ability of the CD32B x CD79b Fc diabodies of the present invention to dampen or inhibit signaling of the immune system by B cells, human PBMC (5×10^6 cells, intravenously injected) were injected into immunodeficient NOD.scid IL2rγnull NSG mice. Animals were administered a control vehicle (100 μl of phosphate buffered saline (PBS)/animal), the above-described preferred CD32B x CD79b Fc diabody (at either 5 mg/kg or at 10 mg/kg) or an anti-CD20 antibody (rituximab; 5 mg/kg; dosed once). The cumulative survival of the mice was measured over time. As shown in **Figure 7**, animals receiving either dose of the preferred CD32B x CD79b Fc diabody exhibited markedly enhanced survival; relative to mice receiving either the PCS control or rituximab.

[00120] All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference in its entirety. While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further

modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth.

What Is Claimed Is:

Claim 1. A bi-specific monovalent Fc diabody, wherein said bi-specific monovalent Fc diabody is capable of specific binding to an epitope of CD32B and to an epitope of CD79b, and possesses an IgG Fc Domain, wherein the bi-specific monovalent Fc diabody comprises a first polypeptide chain, a second polypeptide chain and a third polypeptide chain, wherein said first and second polypeptide chains are covalently bonded to one another and said first and third polypeptide chains are covalently bonded to one another, and wherein:

A. the first polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising:

(1) a sub-Domain (1A), which comprises a cysteine-containing peptide (**SEQ ID NO:1**); and

(2) a sub-Domain (1B), which comprises a polypeptide portion of an IgG Fc Domain having CH2 and CH3 domains of an IgG immunoglobulin Fc region;

ii. a Domain 2, comprising:

(1) a sub-Domain (2A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD32B (VL_{CD32B}) (**SEQ ID NO:11**); and

(2) a sub-Domain (2B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD79b (VH_{CD79b}) (**SEQ ID NO:14**),

wherein said sub-Domains (2A) and (2B) are separated from one another by a peptide linker (Linker 2) (**SEQ ID NO:4**);

iii. a Domain 3, wherein said Domain 3 is an E-coil Domain (**SEQ ID NO:7**) or a K-coil Domain (**SEQ ID NO:8**), wherein said Domain 3 is separated from said Domain 2 by a peptide linker (**SEQ ID NO:5**); and

iv. a C-terminal spacer peptide (**SEQ ID NO:6**);

B. the second polypeptide chain comprises, in the N-terminal to C-terminal direction:

i. a Domain 1, comprising:

(1) a sub-Domain (1A), which comprises a VL Domain of a monoclonal antibody capable of binding to CD79b (VL_{CD79b}) (**SEQ ID NO:13**); and

(2) a sub-Domain (1B), which comprises a VH Domain of a monoclonal antibody capable of binding to CD32B (VH_{CD32B}) (**SEQ ID NO:12**);

wherein said sub-Domains (1A) and (1B) are separated from one another by a peptide linker (Linker 2) (**SEQ ID NO:4**);

ii. a Domain 2, wherein said Domain 2 is a K-coil Domain (**SEQ ID NO:8**) or an E-coil Domain (**SEQ ID NO:7**), wherein said Domain 2 is separated from said Domain 1 by a peptide linker (**SEQ ID NO:5**); and wherein said Domain 3 of said first polypeptide chain and said Domain 2 of said second polypeptide chain are not both E-coil Domains or both K-coil Domains; and

C. the third polypeptide chain comprises, in the N-terminal to C-terminal direction, a Domain 1 comprising:

(1) a sub-Domain (1A), which comprises a cysteine-containing peptide (**SEQ ID NO:1**); and

(2) a sub-Domain (1B), which comprises a polypeptide portion of an IgG Fc Domain having CH2 and CH3 domains of an IgG immunoglobulin Fc region;

and wherein:

(a) said polypeptide portions of the IgG Fc Domains of said first and third polypeptide chain form said IgG Fc Domain;

(b) said VL Domain of said first polypeptide chain and said VH Domain of said second polypeptide chain form an Antigen-Binding Domain capable of specific binding to an epitope of CD32B; and

(c) said VH Domain of said first polypeptide chain and said VL Domain of said second polypeptide chain form an Antigen-Binding Domain capable of specific binding to an epitope of CD79b.

Claim 2. The bi-specific monovalent Fc diabody of claim 1, wherein said sub-Domain (1B) of said first polypeptide chain comprises a sequence different from that of said sub-Domain (1B) of said third polypeptide chain.

Claim 3. The bi-specific monovalent Fc diabody of claim 1, wherein said sub-Domain (1B) of said first polypeptide chain has the amino acid sequence of **SEQ ID NO:9**, and said sub-Domain (1B) of said third polypeptide chain has the amino acid sequence of **SEQ ID NO:10.**

Claim 4. The of claim 1, wherein said sub-Domain (1B) of said first polypeptide chain has the amino acid sequence of **SEQ ID NO:10**, and said sub-Domain (1B) of said third polypeptide chain has the amino acid sequence of **SEQ ID NO:9.**

Claim 5. The bi-specific monovalent Fc diabody of any of claims 1-2, wherein said Domain 1 of said first polypeptide chain and/or said Domain 1 of said third polypeptide chain comprises a variant CH2-CH3 sequence that exhibits altered binding to an Fcγ receptor.

Claim 6. The bi-specific monovalent Fc diabody of any of claims 1-5, wherein said Domain 3 of said first polypeptide chain comprises an E-coil (**SEQ ID NO:7**), and said Domain 2 of said second polypeptide chain comprises a K-coil (**SEQ ID NO:8**).

Claim 7. The bi-specific monovalent Fc diabody of any of claims 1-5, wherein said Domain 3 of said first polypeptide chain comprises a K-coil (**SEQ ID NO:8**), and said Domain 2 of said second polypeptide chain comprises an E-coil (**SEQ ID NO:7**).

Claim 8. A bi-specific monovalent Fc diabody, wherein said bi-specific monovalent Fc diabody is capable of specific binding to an epitope of CD32B and to an epitope of CD79b, and possesses an IgG Fc Domain, wherein said bi-specific monovalent Fc diabody comprises:

(1) a first polypeptide chain having the amino acid sequence of **SEQ ID NO:15**;

(2) a second polypeptide chain having the amino acid sequence of **SEQ ID NO:16**; and

(3) a third polypeptide chain having the amino acid sequence of **SEQ ID NO:17,** wherein amino acid residues 1-10 of said third polypeptide chain are Peptide 1 (**SEQ ID NO:1**), and amino acid residues 11-227 of said third polypeptide chain are the CH2 and CH3 domains of an IgG antibody Fc region (**SEQ ID NO:10**);

wherein said first and said second polypeptide chains are covalently bonded to one another by a first disulfide bond and said first and third polypeptide chains are covalently bonded to one another by a second disulfide bond.

Claim 9. A pharmaceutical composition comprising the bi-specific monovalent Fc diabody of any of claims 1-8 and a physiologically acceptable carrier.

Claim 10. Use of the pharmaceutical composition of claim 9 in the treatment of an inflammatory disease or condition.

Claim 11. The use of claim 10, wherein said inflammatory disease or condition is an autoimmune disease.

Claim 12. The use of claim 10, wherein said an autoimmune disease is systemic lupus erythematosus (SLE).

Claim 13. The use of claim 9, wherein said inflammatory disease or condition is graft vs. host disease (GvHD).