[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 disclosure~~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 disclosure~~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 disclosure~~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 disclosure~~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 disclosure~~invention~~ are capable of simultaneous binding to CD32B and CD79b. The disclosure~~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 disclosure~~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 disclosure~~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);

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.

[0030a] In one aspect, there is provided 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.

[0031] The disclosure~~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:

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 disclosure~~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 disclosure~~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 disclosure~~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 disclosure~~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 disclosure~~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 disclosure~~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 disclosure~~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.

[0038a] In one aspect, there is provided 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.

[0039] ~~The invention further provides~~In another aspect, there is provided a pharmaceutical composition comprising any of the above-described bi-specific monovalent Fc diabodies and a physiologically acceptable carrier.

[0039a] In yet another aspect, there is provided use of the above-described pharmaceutical composition in the manufacture of a medicament for treating an inflammatory disease or condition.

[0040] The disclosure~~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 disclosure~~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.

(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 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: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 manufacture of a medicament for treating ~~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~~11, wherein said ~~an~~ autoimmune disease is systemic lupus erythematosus (SLE).

Claim 13. The use of claim ~~9~~10, wherein said inflammatory disease or condition is graft vs. host disease (GvHD).