polypeptide chain and the Light Chain Variable Domain of the fourth polypeptide chain associate to form a fourth Epitope-Binding Site;

wherein two of the formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of PD-1 and two of the formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of LAG-3; and

II. the CH2-CH3 Domains of the first and third polypeptide chains associate to form an Fc Domain.

[0032] The disclosure also concerns the embodiment of such a bi-specific Fc diabody wherein the CH2-CH3 Domains of the first and third polypeptide chains each have the amino acid sequence of **SEQ ID NO:24**.

[0033] The disclosure also concerns the embodiment of such bi-specific Fc diabodies wherein the Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 has the amino acid sequence of **SEQ ID NO:11,** and wherein the Light Chain Variable Domain of an antibody that is immunospecific for LAG-3 has the amino acid sequence of **SEQ ID NO:12**.

[0034] The disclosure also concerns the embodiment of such bi-specific Fc diabodies wherein the Heavy Chain Variable Domain of an antibody that is immunospecific for PD-1 has the amino acid sequence of **SEQ ID NO:2,** and wherein the Light Chain Variable Domain of an antibody that is immunospecific for PD-1 has the amino acid sequence of **SEQ ID NO:3**.

[0035] In one aspect, the invention also concerns a pharmaceutical composition that comprises an effective amount of any one of the above-indicated bi-specific Fc diabodies, and a pharmaceutically acceptable carrier.

[0036] The disclosure also concerns the embodiment of such a pharmaceutical composition wherein the effective amount of the bi-specific Fc diabody is an amount effective to treat cancer in a recipient individual in need of such treatment.

[0041] The disclosure also concerns a method of treating a disease associated with the presence of a pathogen which comprises administering an effective amount of any of the above-indicated pharmaceutical compositions to an individual in need thereof.

[0041a] In one aspect, the invention provides use of any one of the above-indicated bispecific Fc diabodies, or any one of the above-indicated pharmaceutical compositions, in the manufacture of a medicament for treating cancer.

[0041b] In one aspect, the invention provides use of any one of the above-indicated bispecific Fc diabodies, or any one of the above-indicated pharmaceutical compositions, in the manufacture of a medicament for treating a disease associated with the presence of a pathogen, wherein the disease is associated with an undesirably suppressed immune system.

Detailed Description of the Invention:

[0042] The present invention is directed to bi-specific diabodies that comprise two or more polypeptide chains and which possess at least one Epitope-Binding Site that is immunospecific for an epitope of PD-1 and at least one Epitope-Binding Site that is immunospecific for an epitope of LAG-3 (*i.e.*, a "PD-1 x LAG-3 bi-specific diabody"). More preferably, the present invention is directed to bi-specific diabodies that comprise four polypeptide chains and which possess two Epitope-Binding Sites that are immunospecific for one (or two) epitope(s) of PD-1 and two Epitope-Binding Site that are immunospecific for one (or two) epitope(s) of LAG-3 (*i.e.*, a "PD-1 x LAG-3 bi-specific, tetra-valent diabody"). The present invention also is directed to such diabodies that additionally comprise an immunoglobulin Fc Domain ("bi-specific Fc diabodies" and "bi-specific, tetra-valent, Fc diabodies"). The diabodies of the present invention are capable of simultaneously binding to PD-1 and to LAG-3, particularly as such molecules are arrayed on the surfaces of human cells. The invention is directed to pharmaceutical compositions that contain such diabodies, and to methods involving the use of such diabodies in the treatment of cancer and other diseases and conditions.

[0043] The bi-specific diabodies of the present invention are capable of simultaneously binding to PD-1 and to LAG-3, particularly as such molecules are arrayed on the surfaces of human cells. The invention is directed to pharmaceutical compositions that contain such diabodies, and to methods involving the use of such diabodies in the treatment of cancer and other diseases and conditions. In particular, the PD-1 x LAG-3 bi-specific diabodies of the present invention comprise polypeptide chains that are covalently complexed together.

[0044] As discussed above, T-cell activation requires two distinct signals. The first signal is provided by the T-Cell Receptor (TCR) expressed on the surface of a T-cell that has recognized a peptide antigen within the context of human leukocyte antigens (HLA) expressed on an Antigen-Presenting Cell (APCs). The second signal is provided by the interaction of cognate pairs of co-stimulatory ligands: B7-1 and B7-2 expressed on APCs and their corresponding receptors: CD28 and CTLA-4 expressed on T-cells.

polypeptide chain associate to form a second Epitope-Binding Site; and

(2) said Light Chain Variable Domain of said third polypeptide chain and said Heavy Chain Variable Domain of said fourth polypeptide chain associate to form a third Epitope-Binding Site and said Heavy Chain Variable Domain of said third polypeptide chain and said Light Chain Variable Domain of said fourth polypeptide chain associate to form a fourth Epitope-Binding Site;

wherein two of said formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of PD-1 and two of said formed Epitope-Binding Sites are capable of immunospecifically binding to an epitope of LAG-3; and

II. said CH2-CH3 Domains of said first and third polypeptide chains associate to form an Fc Domain.

Claim 2. The bi-specific Fc diabody of claim 1, wherein:

(B) said first and third polypeptide chains of said diabody each comprise, in the N-terminal to C-terminal direction, a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 or LAG-3, a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 or PD-1, a Heterodimer-Promoting Domain and a CH2-CH3 Domain, wherein said Light Chain Variable Domains and said Heavy Chain Variable Domains are incapable of associating to form an Epitope-Binding Site capable of binding an epitope of PD-1 or an epitope of LAG-3; and

(C) said second and said fourth polypeptide chains of said diabody each comprise, in the N-terminal to C-terminal direction, a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 or LAG-3, a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 or PD-1, and a Heterodimer-Promoting Domain, wherein said Light Chain Variable Domains and said Heavy Chain Variable Domains are incapable of associating to form an Epitope-Binding Site capable of binding an epitope of PD-1 or an epitope of LAG-3;

wherein said Heterodimer-Promoting Domain of said first and third polypeptide chains differs from said Heterodimer-Promoting Domain of said second and fourth polypeptide chains.

Claim 3. The bi-specific Fc diabody of claim 2, wherein:

(1) said Heterodimer-Promoting Domain of said first and third polypeptide chains comprises the amino acid sequence of SEQ ID NO:14 and said Heterodimer-Promoting Domain of said second and fourth polypeptide chains comprises the amino acid sequence of SEQ ID NO:15; or

(2) said Heterodimer-Promoting Domain of said first and third polypeptide chains comprises the amino acid sequence of SEQ ID NO:15 and said Heterodimer-Promoting Domain of said second and fourth polypeptide chains comprises the amino acid sequence of SEQ ID NO:14; or

(3) said Heterodimer-Promoting Domain of said first and third polypeptide chains comprises the amino acid sequence of SEQ ID NO:16 and said Heterodimer-Promoting Domain of said second and fourth polypeptide chains comprises the amino acid sequence of SEQ ID NO:17; or

(4) said Heterodimer-Promoting Domain of said first and third polypeptide chains comprises the amino acid sequence of SEQ ID NO:17 and said Heterodimer-Promoting Domain of said second and fourth polypeptide chains comprises the amino acid sequence of SEQ ID NO:16.

Claim 4. The bi-specific Fc diabody of any one of claims 2-3, wherein said Heterodimer-Promoting Domain of said first polypeptide chain comprises SEQ ID NO:14 and said Heterodimer-Promoting Domain of said second polypeptide chain comprises SEQ ID NO:15.

Claim 5. The bi-specific Fc diabody of any one of claims 2-3, wherein said Heterodimer-Promoting Domain of said first polypeptide chain comprises SEQ ID NO:16 and said Heterodimer-Promoting Domain of said second polypeptide chain comprises SEQ ID NO:17.

Claim 6. The bi-specific Fc diabody of any one of claims 2-5, wherein said Heavy Chain Variable Domain is separated from said Heterodimer-Promoting Domain in each of said first, second, third and fourth polypeptide chains by a linker comprising the amino acid sequence of SEQ ID NO:18.

Claim 7. The bi-specific Fc diabody of any one of claims 1-6, wherein said CH2-CH3 Domains of said first and third polypeptide chains each comprise the amino acid sequence of **SEQ ID NO:24**.

Claim 8. The bi-specific Fc diabody of any one of claims 1-7, wherein said Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3 comprises the amino acid sequence of **SEQ ID NO:11,** and wherein said Light Chain Variable Domain of an antibody that is immunospecific for LAG-3 comprises the amino acid sequence of **SEQ ID NO:12**.

Claim 9. The bi-specific Fc diabody of any one of claims 1-8, wherein said Heavy Chain Variable Domain of an antibody that is immunospecific for PD-1 comprises the amino acid sequence of **SEQ ID NO:2,** and wherein said Light Chain Variable Domain of an antibody that is immunospecific for PD-1 comprises the amino acid sequence of **SEQ ID NO:3**.

Claim 10. The bi-specific Fc diabody of any one of claims 1-9, wherein:

(A) said first and third polypeptide chains each comprise a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 and a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3; and

(B) said second and fourth polypeptide chains each comprise a Light Chain Variable Domain of an antibody that is immunospecific for LAG-3 and a Heavy Chain Variable Domain of an antibody that is immunospecific for PD-1.

Claim 11. The bi-specific Fc diabody of any one of claims 1-9, wherein:

(A) said first and third polypeptide chains each comprise a Light Chain Variable Domain of an antibody that is immunospecific for LAG-3 and a Heavy Chain Variable Domain of an antibody that is immunospecific for PD-1; and

(B) said second and fourth polypeptide chains each comprise a Light Chain Variable Domain of an antibody that is immunospecific for PD-1 and a Heavy Chain Variable Domain of an antibody that is immunospecific for LAG-3.

Claim 12. A pharmaceutical composition that comprises an effective amount of the bi-specific Fc diabody of any one of claims 1-11, and a pharmaceutically acceptable carrier.

Claim 13. The pharmaceutical composition of claim 12, wherein said effective amount of said bi-specific Fc diabody is an amount effective to treat cancer in a recipient individual in need of such treatment.

Claim 14. The pharmaceutical composition of claim 13, wherein said cancer is an adrenal gland cancer, an AIDS-associated cancer, an alveolar soft part sarcoma, an astrocytic tumor, bladder cancer, bone cancer, a brain and spinal cord cancer, a metastatic brain tumor, a breast cancer, a carotid body tumors, a cervical cancer, a chondrosarcoma, a chordoma, a chromophobe renal cell carcinoma, a clear cell carcinoma, a colon cancer, a colorectal cancer, a cutaneous benign fibrous histiocytoma, a desmoplastic small round cell tumor, an ependymoma, a Ewing's tumor, an extraskeletal myxoid chondrosarcoma, a gallbladder or bile duct cancer, gastric cancer, a gestational trophoblastic disease, a germ cell tumor, a head and neck cancer, hepatocellular carcinoma, an islet cell tumor, a Kaposi's sarcoma, a kidney cancer, a leukemia, a lipoma/benign lipomatous tumor, a liposarcoma/malignant lipomatous tumor, a liver cancer, a lymphoma, a lung cancer, a medulloblastoma, a melanoma, a meningioma, a multiple endocrine neoplasia, a multiple myeloma, a myelodysplastic syndrome, a neuroblastoma, a neuroendocrine tumors, an ovarian cancer, a pancreatic cancer, a papillary thyroid carcinoma, a parathyroid tumor, a pediatric cancer, a peripheral nerve sheath tumor, a phaeochromocytoma, a pituitary tumor, a prostate cancer, a posterior uveal melanoma, a renal metastatic cancer, a rhabdoid tumor, a rhabdomysarcoma, a sarcoma, a skin cancer, a soft-tissue sarcoma, a squamous cell cancer, a stomach cancer, a synovial sarcoma, a testicular cancer, a thymic carcinoma, a thymoma, a thyroid metastatic cancer, or a uterine cancer.

Claim 15. The pharmaceutical composition of claim 12, wherein said effective amount of said bi-specific Fc diabody is an amount effective to treat a disease associated with the presence of a pathogen in a recipient individual in need of such treatment, wherein the disease is associated with an undesirably suppressed immune system.

Claim 16. The pharmaceutical composition of claim 15, wherein said pathogen is a bacterium, a fungus, a protozoan, or a virus.

Claim 17. Use of the bispecific Fc diabody of any one of claims 1-11, or of the pharmaceutical composition of any one of claims 13-14, in the manufacture of a medicament for treating cancer.

Claim 18. Use of the bispecific Fc diabody of any one of claims 1-11, or of the pharmaceutical composition of any one of claims 15-16, in the manufacture of a medicament for treating a disease associated with the presence of a pathogen, wherein the disease is associated with an undesirably suppressed immune system.