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SUPERINTENDENTE DE INDUSTRIA Y COM

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REF:

EXPEDIENTE 12-081-792

**TÍTULO SOLICITADO: "INMUNOGLOBULINAS CON DOMINIO VARIABLE
DUAL Y USOS DE LAS MISMAS"**

**PUBLICACIÓN: Gaceta 655 de 31 de octubre de 2012, N° de
publicación 1686.**

PRIORIDAD: US 61/255.686 28/10/2009

SOLICITANTE: ABBOTT LABORATORIES.

APODERADO: ALICIA LLOREDA RICAURTE

TRÁMITE: SUSTENTACION DE OPOSICIÓN

JOSÉ LUIS REYES VILLAMIZAR, mayor de edad, identificado como aparece al pie de mi firma, abogado en ejercicio, titular de la tarjeta profesional número 44655 del Consejo Superior de la Judicatura, obrando en calidad de apoderado de la sociedad **TECNOQUIMICAS S.A.**, en ejercicio de lo dispuesto por el artículo 42, inciso 2° de la Decisión 486 de 2000, por medio del presente escrito, procedo a sustentar y complementar la oposición presentada el 30 de Enero de 2013, en los siguientes términos:

I. RATIFICACIÓN

Tal como se anotó en la presentación de la oposición, la solicitud 12-081-792, referida a proteínas de unión multivalentes y multiespecíficas y procedimientos para prepararlas, no cumple los requisitos de claridad, concisión, nivel inventivo, y aún de novedad, que debería reunir para merecer el privilegio solicitado.

En primer lugar, se observa que la solicitud carece de concisión y soporte debido en la descripción, habida cuenta de que, tal como se demostró en la oposición, la reivindicación 1 no define el objeto reclamado en función de sus características estructurales, sino mediante una definición de fórmula general, conocida per se, de anticuerpos duales, cuyas revelaciones resultan insuficientes para delimitar en debida forma el alcance de la materia pretendida, lo que implica una inaceptable reclamación genérica de anticuerpos sin precisión ni divulgación suficientes en el capítulo descriptivo.

Se mencionó en la oposición, en efecto, que las reivindicaciones no hacen alusión a una caracterización técnica de los anticuerpos referidos, pues se han reclamado en función de un “resultado esperado” propio o de las moléculas a las que dichos anticuerpos han de unirse, pero no de acuerdo con sus propias secuencias o características.

Nótese cómo la estructura en cuestión puede ser tan amplia, que entre los anticuerpos reclamados se incluyen compuestos de secuencias presentes en los anticuerpos *Adalimumab* e *Infliximab*, entre otros, todos conocidos ampliamente en el estado de la técnica, e incluso con patentes que se encuentran próximas a expirar.

Se objetó además la solicitud, por presentar graves faltas en materia de unidad de invención, dadas por la existencia de proteínas de funciones diferentes y de estructuras en secuencias de aminoácidos igualmente distintas.

Por otra parte, la solicitud en estudio incluyó materia exceptuada de patentabilidad como claramente se observa en las reivindicaciones 59 – 61, ya que reclaman un método de tratamiento de un sujeto.

En adición a lo expuesto, y aun cuando se estima que la solicitud no cumple siquiera con los requisitos formales para ser valorada de fondo, es necesario recordar que existen documentos del estado de la técnica que ya han reclamado la misma materia bajo la fórmula empleada, por ejemplo, la publicación americana US 2009/0215992 “*Dual variable domain immunoglobulin and uses thereof*” describe una proteína de unión multiespecíficas y multivalentes de la misma forma que la solicitud en estudio la revela, específicamente describe los métodos para

realizarlas, y sus usos en prevención y/o tratamiento de enfermedades inflamatorias crónicas y agudas.

Así mismo, dicha anterioridad menciona que la proteína comprende una cadena de polipéptido que comprende $VD1-(X1)_n-VD2-C-(X2)_n$, donde VD1 es un dominio variable de cadena pesada, VD2 es un segundo dominio variable de cadena pesada, C es un dominio pesado de cadena constante, XI es un enlazante con la condición que no es CHI y X2 es una región fc y n es 0 o 1; dado que las características técnicas de la anterioridad son iguales a aquellas de la solicitud se ratifica que la misma carece de Novedad.

De igual manera, es importante resaltar que la pareja de antígenos TNF y NGF ha sido reclamada en la anterioridad citada (párrafo 0013, 0249), como alternativas para la obtención del diseño de las moléculas de anticuerpo duales, por lo que la selección no puede ser una característica decisiva, en la medida en que no existe ningún efecto sorprendente de esta pareja (y no otra) sobre los anticuerpos de unión duales del estado de la técnica.

Por su parte, la publicación americana US 2007/0071675 "*Dual variable domain immunoglobulin and uses thereof*" revela una proteína de unión que comprende una cadena de polipéptido que comprende dominios de cadena pesada, donde el polipéptido comprende dominios de unión que se ligan de igual forma de que la solicitud tanto TNF como NGF, con lo cual era obvio para una persona versada en la materia caracterizar secuencias que se uniera a TNF y NGF e incluirlas en la fórmula ya conocida de anticuerpos duales enseñada por US 2009/0215992.

II. COMPLEMENTO DE INFORMACIÓN

Ahora bien, a pesar que los documentos citados anteceden lo divulgado por la presente solicitud, existen objeciones adicionales que permiten demostrar aun más profundamente, no solo la falta de novedad y de altura inventiva, sino la carencia de unidad de invención de las reclamaciones.

En este caso, la forma de unir la invención en un solo concepto inventivo se representa por la formula reclamada por la reivindicación 1, mientras que las

parejas posibles de unión a antígenos pertenecerían a modalidades particulares de la invención.

Un examinador técnico determinaría, que en caso de que la fórmula no fuera novedosa -(lo que se ha observado en nuestros escritos)-, para determinar la altura inventiva, las parejas de unión a antígenos deberían tener una forma común que ejerciera un efecto ventajoso y sorprendente sobre aquellas del estado de la técnica, lo cual no se observa en ninguno de los anticuerpos reclamados.

Ahora bien, en el caso en el que alguno de los pares de dominios de unión al antígeno ejerciera un efecto sorprendente sobre los del estado de la técnica, dicha pareja sería la única susceptible de ser reivindicada, en lugar de reclamar diferentes parejas de dominios de unión que no han sido evaluadas y en las cuales no se puede reconocer la misma actividad.

Por ejemplo, en relación con las reivindicaciones 24 y 25 que mencionan:

Una proteína de unión, CARACTERIZADA PORQUE comprende una cadena de polipéptidos, en donde dicha cadena de polipéptidos comprende $VD1-(X1)_n-VD2-C-(X2)_n$, en donde;

VD1 es un primer dominio variable de la cadena pesada;

VD2 es un segundo dominio variable de la cadena pesada;

C es un dominio constante de la cadena pesada;

X1 es un conector con la condición que no sea CH1;

X2 es una región Fc; y

n es 0 o 1;

y que describen diferentes secuencias a las cuales se unen los anticuerpos y/o los nombres de los antígenos, el anticuerpo de la solicitud se ha descrito enfocándose en moléculas que reconocen y tienen actividad sobre TNF y NGF.

Teniendo en cuenta lo anterior, el documento ya mencionado US 2009/0215992 enseña que los antígenos son seleccionados de un grupo que contiene tanto TNF como NGF (ver página 41 tal como ya se observó en mi memorial de oposición); a pesar que dicho documento no divulga específicamente la proteína de unión que se une a la SEQ ID NO. 2 correspondiente al TNF en adición a NGF, se observa que en el estado de la técnica otros documentos ya contemplaban dicha pareja

específica lo que destruye la altura inventiva de modalidades particulares de la solicitud.

Es el caso de la publicación americana US20040219144 publicada por Shelton, *"Anti-NGF antibodies for the treatment of various disorders"* la cual describe métodos de empleo de anticuerpos anti-NGF en el tratamiento de enfermedades relacionadas con dicho receptor, que incluyen asma, artritis y soriasis.

Esta anterioridad menciona las proteínas de unión tales como anticuerpos capaces de unirse a dos antígenos como TNF y NGF con el fin de tratar la enfermedad de Crohn o artritis reumatoide. Esto puede verse en el siguiente aparte, extraído del párrafo 0128.

treatment. For the treatment of rheumatoid arthritis or Crohn's disease, the antibodies of the present invention can be administered in combination with other treatment regimens known for the treatment of these conditions. For example, the anti-NGF antibodies herein can be administered in combination with Remicade® (Infliximab, Centocor), or Enbrel® (Etanercept, Wyeth-Ayerst). The present invention also includes bispecific antibodies targeting these diseases. For example, a bispecific antibody could include an anti-TNF specificity combined with the NGF-binding ability of the antibodies herein.

Dicha divulgación hace obvio cualquier anticuerpo biespecífico anti-TNF y anti-NGF de la solicitud en estudio, con el agravante que no se precisa la estructura específica de un solo anticuerpo que mencione propiedades sorprendentes.

Con el ánimo de demostrar la evidente falta de novedad y nivel inventivo, presento además el documento US2009155275 publicado por Wu et al., *"Multispecific epitope binding proteins and uses thereof"*, el cual describe proteínas de unión a epítopos multiespecíficos para la prevención, tratamiento o diagnóstico de enfermedades crónicas o agudas, lo que indica además la gran cantidad de documentos que componen el estado de la técnica y que desvirtúan la patentabilidad de la solicitud en estudio.

En dicho documento se sugiere el uso de los anticuerpos biespecíficos de forma simultánea para bloquear la función de dos blancos asociados al tumor para el tratamiento de cáncer como se observa en el siguiente aparte:

[0302] The invention also provides methods of using multispecific epitope binding proteins of the invention. The present invention also encompasses the use of multispecific epitope binding proteins of the invention for the prevention, diagnosis, management, treatment or amelioration of one or more symptoms associated with diseases, disorders of diseases or disorders, including but not limited to cancer, inflammatory and autoimmune diseases either alone or in combination with other therapies. The invention also encompasses the use of multispecific epitope binding proteins of the invention conjugated or fused to a moiety (e.g., therapeutic agent or drug) for prevention, management, treatment or amelioration of one or more symptoms associated with diseases, disorders or infections, including but not limited to cancer, inflammatory and autoimmune diseases either alone or in combination with other therapies.

Así entonces, de la divulgación contenida en el estado de la técnica se entiende que los anticuerpos biespecíficos han sido reconocidos ampliamente demostrando que la unión de diferentes dominios de anticuerpos conocidos para unirse a dos antígenos, ha sido una práctica muy importante en el ámbito de medicamentos en los últimos años.

Por otra parte, no existe ninguna ventaja técnica, diferente a la divulgada, que pueda demostrarse por la solicitud y más aún cuando en las reivindicaciones de la misma solo se utiliza una descripción superficial de los anticuerpos biespecíficos dados por su actividad.

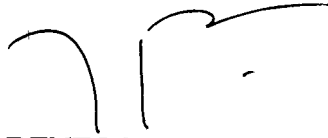
Por lo anterior, los anticuerpos biespecíficos reclamados en la presente solicitud carece de novedad y de un efecto técnico sorprendente que soporte el nivel inventivo de la misma.

III. ANEXOS

- ❖ La publicación americana US20040219144 publicado por Shelton, "*Anti-NGF antibodies for the treatment of various disorders*". Portada, páginas 1, 2, 11, 12, 16 y 17.
- ❖ La publicación americana US2009155275 publicado por Wu et al., "*Multispecific epitope binding proteins and uses thereof*". Portada, páginas 1, 2, 21, 36, 37, 59 – 64.

REYES & REYES
A B O G A D O S

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(54) **ANTI-NGF ANTIBODIES FOR THE
TREATMENT OF VARIOUS DISORDERS**

Related U.S. Application Data

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(57) **ABSTRACT**

The present invention relates generally to methods of using, anti-NGF antibodies in the treatment of various NGF-related disorders, including asthma, arthritis and psoriasis. The methods are effective in treating these disorders in a patient without having a significant adverse effect on the immune system of the patient.

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(86) PCT No.: **PCT/US02/15284**

ANTI-NGF ANTIBODIES FOR THE TREATMENT OF VARIOUS DISORDERS

BACKGROUND OF THE INVENTION

[0001] 1. Field of the Invention

[0002] The present invention relates generally to methods of using anti-NGF antibodies in the treatment of various NGF-related disorders, including asthma, arthritis and psoriasis. The methods are effective in treating these disorders in a patient without having a significant adverse effect on the immune system of the patient.

[0003] 2. Description of the Related Art

Nerve Growth Factor (NGF)

[0004] Nerve growth factor (NGF) was the first neurotrophin to be identified, and its role in the development and survival of both peripheral and central neurons has been well characterized. NGF has been shown to be a critical survival and maintenance factor in the development of peripheral sympathetic and embryonic sensory neurons and of basal forebrain cholinergic neurons (Smeyne et al., *Nature* 368:246-249 (1994); Crowley et al., *Cell* 76:1001-1011 (1994)). NGF upregulates expression of neuropeptides in sensory neurons (Lindsay and Harmer, *Nature* 337:362-364 (1989)) and its activity is mediated through two different membrane-bound receptors. The TrkA tyrosine kinase receptor mediates high affinity binding and the p75 receptor, which is structurally related to other members of the tumor necrosis factor receptor family, mediates low affinity binding (Chao et al., *Science* 232:518-521 (1986)).

[0005] In addition to its effects in the nervous system, NGF has been increasingly implicated in processes outside of the nervous system. For example, NGF has been shown to enhance vascular permeability (Otten et al., *Eur. J. Pharmacol.* 106:199-201 (1984)), enhance T- and B-cell immune responses (Otten et al., *Proc. Natl. Acad. Sci. U.S.A.* 86:10059-10063 (1989)), induce lymphocyte differentiation and mast cell proliferation and cause the release of soluble biological signals from mast cells (Matsuda et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:6508-6512 (1988); Pearce et al., *J. Physiol.* 372:379-393 (1986); Bischoff et al., *Blood* 79:2662-2669 (1992); Horigome et al., *J. Biol. Chem.* 268:14881-14887 (1993)).

[0006] NGF is produced by a number of cell types including mast cells (Leon et al., *Proc. Natl. Acad. Sci. U.S.A.* 91:3739-3743 (1994)), B-lymphocytes (Torcia et al., *Cell* 85:345-356 (1996)), keratinocytes (Di Marco et al., *J. Biol. Chem.* 268:22838-22846) and smooth muscle cells (Ueyama et al., *J. Hypertens.* 11:1061-1065 (1993)). NGF receptors have been found on a variety of cell types outside of the nervous system. For example, TrkA has been found on human monocytes, T- and B-lymphocytes and mast cells.

[0007] Consistent with a non-neuronal role for NGF, an association between increased NGF levels and a variety of inflammatory conditions has been observed in human patients as well as in several animal models. These include systemic lupus erythematosus (Bracci-Laudiero et al., *Neuroreport* 4:563-565 (1993)), multiple sclerosis (Bracci-Laudiero et al., *Neurosci. Lett.* 147:9-12 (1992)), psoriasis (Raychaudhuri et al., *Acta Derm. Venereol.* 78:84-86 (1998)), arthritis (Falcini et al., *Ann. Rheum. Dis.* 55:745-748 (1996))

and asthma (Braun et al., *Eur. J. Immunol.* 28:3240-3251 (1998)). Chronic inflammatory conditions such as these are a significant public health problem. For instance, it is estimated that arthritis affects 37.9 million people in the United States alone. Current therapies for treating these conditions are severely limited. An understanding of the role NGF plays in these diseases may provide new methods for treating them.

[0008] A correlation between stress and psoriasis has been observed. Based on this correlation and the symmetry of the cutaneous lesions that accompany the disease, a relationship with the nervous system has been proposed (Raychaudhuri et al., *Acta Derm. Venereol.* 78:84-86 (1998)). In particular, neuropeptides have been suggested to play a role in the pathogenesis of psoriasis. Investigators have reported an increased number of terminal cutaneous nerves along with upregulation of one or more of the neuropeptides, such as substance P (SP), vasoactive intestinal polypeptide (VIP) and CGRP. NGF plays a role in regulating innervation in the skin and also is known to upregulate neuropeptides, suggesting that increased NGF levels may be responsible for the upregulation of neuropeptides and the increased cutaneous innervation seen with psoriasis. In fact, increased expression of NGF has been observed in psoriatic keratinocytes (Raychaudhuri et al., *Acta Derm. Venereol.* 78:84-86 (1998)). It has been suggested that while NGF normally serves as a survival factor for keratinocytes, overexpression of NGF prevents normal cell death, leading to psoriasis (Pincelli et al., *J. Derm. Sci.* 22:71-79 (2000)).

[0009] A number of studies have indicated that neuropeptides such as substance P (SP) and biologically active compounds released from mast cells, such as histamine, also play a role in both naturally occurring arthritis in humans and experimentally induced arthritis in animal models (see e.g. Levine, J., *Science* 226:547-549 (1984)). NGF has been shown to affect mast cell degranulation (Bruni et al., *FEBS Lett.* 138:190-193 (1982)) and substance P release (Donnerer et al., *Neurosci.* 49:693-698 (1992)), implicating it in the pathogenesis of arthritis.

[0010] Consistently, an elevated level of NGF in peripheral tissues is associated with both hyperalgesia and inflammation and has been observed in a number of forms of arthritis. The synovium of patients affected by rheumatoid arthritis expresses high levels of NGF while in non-inflamed synovium NGF has been reported to be undetectable (Aloe et al., *Arch. Rheum.* 35:351-355 (1992)). Similar results were seen in rats with experimentally induced rheumatoid arthritis (Aloe et al., *Clin. Exp. Rheumatol.* 10:203-204 (1992)). Elevated levels of NGF have been reported in transgenic arthritic mice along with an increase in the number of mast cells. (Aloe et al., *Int. J. Tissue Reactions-Exp. Clin. Aspects* 15:139-143 (1993)). However, purified NGF injected into the joint synovium of normal rats does not induce knee joint inflammation, suggesting that NGF does not play a causative role in arthritis (Aloe et al., *Growth Factors* 9:149-155 (1993)).

[0011] High NGF levels have been associated with allergic inflammation and it has been suggested that this is related to mast cell degranulation (Bonini et al., *Proc. Natl. Acad. Sci. U.S.A.* 93:10955-10960 (1996)).

[0012] Elevated NGF levels are also observed in both allergic and non-allergic asthma (Bonini et al., *supra*). Mast

cells, eosinophils and T-lymphocytes have all been proposed to play a role in this inflammatory disease and the correlation between NGF serum levels and total IgE antibody titers suggests that NGF contributes to the inflammatory immune response. Allergen induced airway inflammation has been associated with increased local production of NGF in both mice and humans (Braun et al., *Int. Arch. Allergy Immunol.* 118:163-165 (1999)).

[0013] NGF has been shown to regulate the development of increased airway hyperactive response, a hallmark of bronchial asthma (Braun et al., *Eur. J. Immunol.* 28:3240-3251 (1998)). Indeed, in one study, treatment of allergen-sensitized mice with anti-NGF antibody prevented the development of airway hyperresponsiveness following local allergen challenge (Braun et al., *Int. Arch. Allergy Immunol.* 118:163-165 (1999)).

[0014] Despite the promising results obtained in mice, reported adverse effects of neutralizing anti-NGF antibodies on the immune system have raised serious questions about the feasibility of using anti-NGF antibodies as a therapeutic in the prevention or treatment of asthma or other diseases or disorders in human patients. In particular, Torcia et al., *Cell* 85:345-356 (1996) identified NGF as an autocrine survival factor for memory B lymphocytes, and demonstrated that in vivo administration of neutralizing anti-NGF antibodies caused a depletion of memory B-cells and abolished secondary antigen-specific immune responses in mice. Garaci et al., *Proc. Natl. Acad. Sci. USA* 96:14013-14018 (1999) reported that NGF is an autocrine survival factor that rescues human monocytes/macrophages from the cytopathic effect caused by HIV infection. This report, along with the findings of Torcia et al, supra would suggest that anti-NGF antibodies have the potential of compromising the immune system of the subject treated.

SUMMARY OF THE INVENTION

[0015] The present invention is based on the unexpected finding that in vivo administration of a therapeutically effective amount of an anti-NGF monoclonal antibody (antibody 911) had no adverse effect on the immune system in an experimental mouse model of allergy. Accordingly, this and related antibodies hold great promise in the treatment of NGF-associated disorders, including asthma, in human patients.

[0016] In one aspect, the invention concerns a method of controlling an NGF-related disorder in a human patient by administering to the patient an effective amount of an anti-human NGF (anti-hNGF) monoclonal antibody that is capable of binding hNGF with an affinity in the nanomolar range, and inhibiting the binding of hNGF to human TrkA (hTrkA) in vivo, wherein the antibody has no significant adverse effects on the immune system of the patient.

[0017] In one embodiment the binding affinity of the antibody to hNGF is preferably about 0.10 to about 0.80 nM, more preferably about 0.15 to about 0.75 nM and even more preferably about 0.18 to about 0.72 nM.

[0018] In another embodiment, the antibody binds essentially the same hNGF epitope as an antibody selected from the group consisting of MAb 911, MAb 912 and MAb 938, more preferably the same epitope as MAb 911.

[0019] In yet another embodiment the antibody is able to cross react with murine NGF (muNGF).

[0020] The antibody may also be an antibody fragment, preferably an antibody fragment selected from the group consisting of Fab, Fab', F(ab')₂, Fv fragments, diabodies, single chain antibody molecules and multispecific antibodies formed from antibody fragments, and more preferably a single-chain Fv (scFv) molecule.

[0021] In another embodiment the antibody is chimeric. It may also be humanized or human.

[0022] In yet another embodiment the antibody is bispecific. The bispecific antibody may have an anti-IgE specificity.

[0023] The NGF-related disorder that is controlled is preferably not associated with the effect of NGF on the neuronal system.

[0024] In one embodiment the NGF-related disorder is an inflammatory condition, preferably selected from the group consisting of asthma, arthritis, multiple sclerosis, lupus erythematosus and psoriasis.

[0025] In a preferred embodiment the condition is asthma. In another embodiment the condition is arthritis, preferably rheumatoid arthritis. In yet another embodiment the condition is psoriasis.

[0026] In yet a further embodiment, the antibody is administered in combination with another therapeutic agent for the treatment of an inflammatory condition. Thus the antibody may be administered in combination with another therapeutic agent for the treatment of asthma. In one embodiment the antibody is administered with a corticosteroid, preferably beclomethsone dipropionate (BDP). In another embodiment the antibody is administered with an anti-IgE antibody, such as rhuMAb-E25 or rhuMAb-E26. For the treatment of rheumatoid arthritis, the antibody may be administered in combination with an anti-TNF antibody or an antibody or immunoadhesin specifically binding a TNF receptor.

[0027] In another aspect, the invention concerns a pharmaceutical composition comprising a chimeric, humanized or human anti-human NGF monoclonal antibody capable of binding hNGF with an affinity in the nanomolar range and inhibiting the binding of hNGF to human TrkA in vivo, wherein the antibody has no significant adverse effects on the immune system of a patient, in combination with a pharmaceutically acceptable carrier. The antibody in the pharmaceutical composition may be an antibody fragment, preferably an antibody fragment selected from the group consisting of Fab, Fab', F(ab')₂, Fv fragments, diabodies, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0028] In one embodiment the antibody is a bispecific antibody. The bispecific antibody may be capable of specific binding to native human IgE or native human TNF or a native human TNF receptor.

[0029] In another embodiment the pharmaceutical composition further comprises another pharmaceutically active ingredient, such as an ingredient suitable for the treatment of an inflammatory condition. The inflammatory condition is preferably one selected from the group consisting of asthma, multiple sclerosis, arthritis, lupus erythematosus and psoriasis. In one embodiment the inflammatory condition is asthma. In another embodiment the inflammatory condition

[0117] The active ingredients may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. (1980).

[0118] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g. films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0119] The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably compounds with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide antibodies that bind to a different epitope of NGF or to an NGF receptor in the one formulation. Alternatively, or additionally, the composition may further comprise another biologically active compound, such as an anti-inflammatory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0120] The formulations to be used for in vivo administration must be sterile. This is readily accomplished by, for example, filtration through sterile filtration membranes.

[0121] Therapeutic anti-NGF antibody compositions are generally placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

D). Treatment with anti-NGF Antibodies

[0122] It is contemplated that, according to the present invention, the anti-NGF antibodies may be used to treat various diseases or disorders. Exemplary conditions or disorders include asthma, psoriasis and arthritis. The anti-NGF antibodies may be used to prevent the onset of the active disease state, to treat symptoms that are currently being experienced and to treat the underlying disease itself.

[0123] Despite advances in understanding the cellular and molecular mechanisms that control allergic responses and improved therapies, the incidence of allergic diseases, especially asthma, has increased dramatically in recent years (Beasley et al., *J. Allergy Clin. Immunol.* 105:466-472 (2000); Peat and Li, *J. Allergy Clin. Immunol.* 103:1-10 (1999)). Allergic diseases can be treated, for example, by allergen-based vaccination, in which increasing doses of allergen are given by injection over years. Mild asthma can usually be controlled in most patients by relatively low

doses of inhaled corticosteroids, while moderate asthma is usually managed by the additional administration of inhaled long-acting β -antagonists or leukotriene inhibitors. However, the treatment of severe asthma is still a serious medical problem. Although an anti-IgE antibody currently awaiting FDA approval (rhuMAB-E25, Xolair™, developed in collaboration of Genentech, Inc., Tanox, Inc. and Novartis Pharmaceuticals Corporation) shows promising results for early intervention in the treatment of conditions that lead to symptoms of allergic asthma and seasonal allergic rhinitis, there is need for the development of additional therapeutic strategies and agents to control allergic diseases, such as asthma.

[0124] The anti-NGF antibodies of the present invention can be used for the treatment of asthma and other disorders associated with airway hyperreactivity, typically characterized by episodes of coughs, wheezing, chest tightness, and/or breathing problems.

[0125] The anti-NGF antibodies of the present invention are also useful in the management of other inflammatory conditions, such as multiple sclerosis, colitis, inflammatory bowel disease, bladder cystitis, eczema, contact dermatitis, arthritis, including chronic arthritis and rheumatoid arthritis, Crohn's disease, and psoriasis.

[0126] In addition, anti-NGF antibodies are also useful in treating other diseases that may be associated with increased levels of NGF including, for example, lupus erythematosus, shingles, postherpetic neuralgia, hyperalgesia, and chronic pain.

[0127] The anti-NGF antibodies are administered to a mammal, preferably to a human patient, in accord with known methods, such as intravenous administration, e.g., as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, or topical routes. Anti-NGF antibody can also be administered by inhalation. Commercially available nebulizers for liquid formulations, including jet nebulizers and ultrasonic nebulizers are useful for administration. Liquid formulations can be directly nebulized and lyophilized powder can be nebulized after reconstitution. Alternatively, anti-NGF antibody can be aerosolized using a fluorocarbon formulation and a metered dose inhaler, or inhaled as a lyophilized and milled powder. For the treatment of asthma and other conditions characterized by airway hyperreactivity, a preferred route of administration is by inhalation.

[0128] Other therapeutic regimens may be combined with the administration of the anti-NGF antibody. The combined administration includes co-administration, using separate formulations or a single pharmaceutical formulation, and consecutive administration, in any order, wherein preferably there is a time period while both (or all) active agents simultaneously exert their biological activities. For the treatment of asthma, it might be particularly advantageous to use the antibodies herein in combination with anti IgE antibodies, in particular rhuMAB-E25 (Xolair™), or with second-generation antibody molecule rhuMAB-E26 (Genentech, Inc.). The rhuMAB-E25 antibody is a recombinant humanized anti-IgE monoclonal antibody that was developed to interfere early in the allergic process. Combination use also includes the possibility of administering the two antibodies in a single pharmaceutical formulation, or using a bispecific

antibody, with anti-NGF and anti-IgE specificities. In another preferred embodiment, the anti-NGF antibodies herein are administered in combination with inhaled corticosteroids, such as beclomethasone dipropionate (BDP) treatment. For the treatment of rheumatoid arthritis or Crohn's disease, the antibodies of the present invention can be administered in combination with other treatment regimens known for the treatment of these conditions. For example, the anti-NGF antibodies herein can be administered in combination with Remicade® (Infliximab, Centocor), or Enbrel® (Etanercept, Wyeth-Ayerst). The present invention also includes bispecific antibodies targeting these diseases. For example, a bispecific antibody could include an anti-TNF specificity combined with the NGF-binding ability of the antibodies herein.

[0129] Suitable dosages for any of the above co-administered agents are those presently used and may be lowered due to the combined action (synergy) of the agent and anti-NGF antibody.

[0130] For the prevention or treatment of disease, the appropriate dosage of anti-NGF antibody will depend on the anti-NGF antibody employed, the type of disease to be treated, the severity and course of the disease, whether the antibody is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. Typically the clinician will administer the anti-NGF antibody until a dosage is reached that achieves the desired result.

[0131] The anti-NGF antibody is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 2 mg/kg of antibody is an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous or repeated dosing. A typical daily dosage might range from about 1 g/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. A preferred dosing regimen comprises administering an initial dose of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg of the anti-NGF antibody. However, other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the practitioner wishes to achieve. The progress of this therapy is easily monitored by conventional techniques and assays.

E. Articles of Manufacture

[0132] In another embodiment of the invention, an article of manufacture containing materials useful for the treatment of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert(s) on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is effective for treating the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an anti-NGF antibody. The container may

further comprise a second pharmaceutically active agent. Preferably the second agent is suitable for the treatment of an inflammatory disease such as asthma, multiple sclerosis, arthritis, lupus erythematosus and psoriasis.

[0133] The label or package insert indicates that the composition is used for treating the condition of choice, such as an inflammatory condition. In one embodiment, the label or package inserts indicates that the composition comprising the antibody that binds NGF can be used to treat an inflammatory condition selected from the group consisting of asthma, multiple sclerosis, arthritis, lupus erythematosus and psoriasis. In addition, the label or package insert may indicate that the patient to be treated is one having asthma, psoriasis, arthritis or another disease or disorder. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a first antibody which binds NGF and inhibits its biological activity; and (b) a second container with a composition contained therein, wherein the composition comprises a second antibody which binds an NGF receptor and blocks ligand activation. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the first and second compositions can be used to treat asthma, psoriasis, arthritis or another disease or disorder. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0134] Further details of the invention are illustrated in the following non-limiting examples.

EXAMPLES

[0135] Commercially available reagents referred to in the examples were used according to manufacturer's instructions unless otherwise indicated. The source of those cells identified in the following examples, and throughout the specification, by ATCC accession numbers is the American Type Culture Collection, Manassas, VA.

Example 1

Production and Characterization of Anti-NGF Monoclonal Antibodies

[0136] A. Production of anti-NGF Monoclonal Antibodies

[0137] This example illustrates preparation of monoclonal antibodies that can specifically bind human NGF (hNGF). Techniques for producing monoclonal antibodies are well known in the art and are described, for instance, in Kohler and Milstein, *Nature* 256:495-497 (1975). The experiments described in Examples 1 and 2 are further described in Hongo et al., *Hybridoma* 19:215.227.

[0138] A panel of 23 murine monoclonal antibodies to hNGF was developed by a method analogous to that described in Hongo et al., *Hybridoma* 14:253-260. Briefly, Balb/c mice (Charles River Laboratories, Wilmington, Del.) were immunized with human NGF in Ribi adjuvant (Ribi Immunochem Research, Inc., Hamilton, Md.). Splenocytes

and then 1:2 with ALUM, as an adjuvant, for a final concentration of 300 AU/ml). The non-sensitized mice (NS) received an equal volume of Dulbecco's PBS diluted 1:2 with ALUM as a control.

[0175] Mice were then challenged with inhaled dust mite antigen (DMA) on days 21 and 22. Dust mite was diluted to 6000 AU/ml using Dulbecco's PBS plus 0.01% Tween-20 for aerosolization. All inhalation challenges were administered in a Plexiglas pie exposure chamber. DMA was aerosolized using a PARI IS-2 nebulizer driven at 22 PSI. The nebulizer was filled with 3 ml and run to completion (30 min.). Total deposited dose/exposure into the lung was ~6.5 AU DMA.

[0176] Mice were assessed for airway hyperreactivity, cellular infiltration into the broncheolar lavage (BAL) fluid, cytokine levels in BAL, and serum titer against dust mite as well as serum IgE levels. Briefly, on day 24, 48 hours after the last challenge, mice were anesthetized, catheterized in the jugular vein and tracheotomized. Mice were then paralyzed with 0.28 mg/kg pancuronium and loaded into a Plexiglas flow plethysmograph for measurement of thoracic expansion and airway pressure. Mice were ventilated using 100% oxygen at a frequency of 170 bpm and Vt equal to 9 l/g. Breathing mechanics (lung resistance and dynamic compliance) were continuously monitored using the Buxco XA data acquisition program. Mice were given a volume history (5 breaths at 2.5xVt), allowed to stabilize for 2 min before baseline measurement, and then given a one-time 5 second dose of the agonist at a body weight adjusted flow rate using a Harvard syringe pump and syringe application software.

[0177] Blood (serum), BAL and lungs were collected. Serum was assayed for total and specific IgE and IgG. BAL was obtained by injecting the same aliquot of NaCl 3 times and was assayed for total IgE by ELISA. Total white blood cells and cell differentials were obtained from BAL cells.

[0178] Treatment with anti-NGF monoclonal antibody caused a significant drop in airway hyperreactivity (FIG. 13), as well as in inflammation as measured by cellular infiltration into the BAL (FIG. 14). However, there is still a very high proportion of eosinophils in BAL from anti-NGF treated animals (FIG. 15). Anti-NGF antibody treatment also decreased the level of the Th2 cytokine IL-13 in the BAL (FIG. 16).

[0179] Despite its ability to decrease the inflammatory response to allergen, anti-NGF antibody treatment did not decrease the humoral immune response to dust mite, either as measured by the total serum immunoglobulin titer to dust mite (FIG. 17) or by the serum level of IgE (FIG. 18). This indicates that the antibody did block the biological effect of NGF, but did not affect the survival or function of B-lymphocytes.

[0180] To confirm that the anti-NGF antibody 911 was having a functionally significant effect on NGF, a parallel experiment was carried out in which mice were injected subcutaneously in the scruff of the neck with 0.1, 1 or 10 mg/kg of anti-NGF monoclonal antibody 911 on days 1 and 8, or with NGF at 0.1, 1 or 10 mg/kg on days 1, 3, 6 and 8. Animals were sacrificed on day 9 and examined for the level of the neuropeptide CGRP in the trigeminal ganglion. Treatment with 1 or 10 mg/kg of NGF caused an increase in CGRP, verifying that this peptide is regulated by NGF levels

(FIG. 19). Treatment with 1 or 10 mg/kg of anti-NGF monoclonal antibody 911 caused a decrease in the CGRP content of the ganglion (FIG. 19), verifying that this dose produces a functionally significant blockade of endogenous NGF at the time when the immune response was occurring in the experiment described above.

What is claimed is:

1. A method of controlling an NGF-related disorder in a human patient, comprising administering to said patient an effective amount of an anti-human NGF (anti-hNGF) monoclonal antibody capable of binding hNGF with an affinity in the nanomolar range, and inhibiting the binding of hNGF to human TrkA (hTrkA) in vivo, wherein said antibody has no significant adverse effect on the immune system of said patient.
2. The method of claim 1 wherein the binding affinity of said antibody to hNGF is about 0.10 to about 0.80 nM.
3. The method of claim 2 wherein the binding affinity of said antibody to hNGF is about 0.15 to about 0.75 nM.
4. The method of claim 2 wherein the binding affinity of said antibody to hNGF is about 0.18 to about 0.72 nM.
5. The method of claim 1 wherein said antibody binds essentially the same hNGF epitope as an antibody selected from the group consisting of MAb 911, MAb 912, and MAb 938.
6. The method of claim 5 wherein said antibody binds essentially the same hNGF epitope as the antibody MAb 911.
7. The method of claim 1 wherein said antibody is also able to bind murine NGF (muNGF).
8. The method of claim 1 wherein said antibody is an antibody fragment.
9. The method of claim 8 wherein said antibody fragment is selected from the group consisting of Fab, Fab', F(ab')₂, Fv fragments, diabodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.
10. The method of claim 9 wherein said single-chain antibody molecule is a single-chain Fv (scFv) molecule.
11. The method of claim 1 wherein said antibody is chimeric.
12. The method of claim 1 wherein said antibody is humanized.
13. The method of claim 1 wherein said antibody is human.
14. The method of claim 1 wherein said antibody is a bispecific antibody.
15. The method of claim 14 wherein said bispecific antibody has an anti-IgE specificity.
16. The method of claim 1 wherein said NGF-related disorder is other than a disorder associated with the effect of NGF on the neuronal system.
17. The method of claim 16 wherein said NGF-related disorder is an inflammatory condition.
18. The method of claim 17 wherein said inflammatory condition is selected from the group consisting of asthma, multiple sclerosis, arthritis, lupus erythematosus, and psoriasis.
19. The method of claim 18 wherein said condition is asthma.
20. The method of claim 18 wherein said condition is arthritis.
21. The method of claim 20 wherein said arthritis is rheumatoid arthritis.

22. The method of claim 18 wherein said condition is psoriasis.
23. The method of claim 17 wherein said antibody is administered in combination with another therapeutic agent for the treatment of said inflammatory condition.
24. The method of claim 19 wherein said antibody is administered in combination with a corticosteroid.
25. The method of claim 24 wherein said corticosteroid is bectomethasone dipropionate (BDP).
26. The method of claim 19 wherein said antibody is administered in combination with an anti-IgE antibody.
27. The method of claim 26 wherein said anti-IgE antibody is rhuMab-E25 or rhuMab-E26.
28. The method of claim 21 wherein said antibody is administered in combination with another therapeutic agent for the treatment of rheumatoid arthritis.
29. The method of claim 28 wherein said other therapeutic agent is an anti-TNF antibody or an antibody or immunoadhesin specifically binding a TNF receptor.
30. A pharmaceutical composition comprising a chimeric, humanized or human anti-human NGF (anti-hNGF) monoclonal antibody capable of binding hNGF with an affinity in the nanomolar range, and inhibiting the binding of hNGF to human TrkA (hTrkA) in vivo, wherein said antibody has no significant adverse effect on the immune system of a patient, in combination with a pharmaceutically acceptable carrier.
31. The pharmaceutical composition of claim 30 wherein said antibody is an antibody fragment.
32. The pharmaceutical composition of claim 31 wherein said antibody fragment is selected from the group consisting of Fab, Fab', F(ab')₂, Fv fragments, diabodies, single-chain antibody molecules, and multispecific antibodies formed from antibody fragments.
33. The pharmaceutical composition of claim 30 wherein said antibody is a bispecific antibody.
34. The pharmaceutical composition of claim 33 wherein said bispecific antibody is capable of specific binding to native human IgE,
35. The pharmaceutical composition of claim 33 wherein said bispecific antibody is capable of specific binding to native human TNF or a native human TNF receptor.
36. The pharmaceutical composition of claim 30 further comprising another pharmaceutically active ingredient.
37. The pharmaceutical composition of claim 36 wherein said other pharmaceutically active ingredient is suitable for the treatment of an inflammatory condition.
38. The pharmaceutical composition of claim 37 wherein said inflammatory condition is selected from the group consisting of asthma, multiple sclerosis, arthritis, lupus erythematosus and psoriasis.
39. The pharmaceutical composition of claim 38 wherein said inflammatory condition is asthma.
40. The pharmaceutical composition of claim 38 wherein said inflammatory condition is arthritis.
41. The pharmaceutical composition of claim 40 wherein said arthritis is rheumatoid arthritis.
42. The pharmaceutical composition of claim 41 wherein said inflammatory condition is psoriasis.
43. An article of manufacture comprising:
- a container;
- a pharmaceutical composition of claim 30; and
- instructions for using the composition of matter to control an NGF-related disorder in a human patient.
44. The article of manufacture of claim 43 further comprising a second pharmaceutically active ingredient.
45. The article of manufacture of claim 44 wherein said second pharmaceutically active ingredient is suitable for the treatment of an inflammatory condition.
46. The article of manufacture of claim 45 wherein said inflammatory condition is selected from the group consisting of asthma, multiple sclerosis, arthritis, lupus erythematosus and psoriasis.
47. The article of manufacture of claim 43 further comprising a second container with a composition contained therein, wherein the composition comprises a second antibody which binds an NGF receptor and blocks ligand activation
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(54) MULTISPECIFIC EPITOPE BINDING
PROTEINS AND USES THEREOF

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(57) ABSTRACT

The present invention relates to multispecific epitope binding
proteins, methods of making, and uses thereof in the preven-
tion, management, treatment or diagnosis of acute or chronic
diseases.

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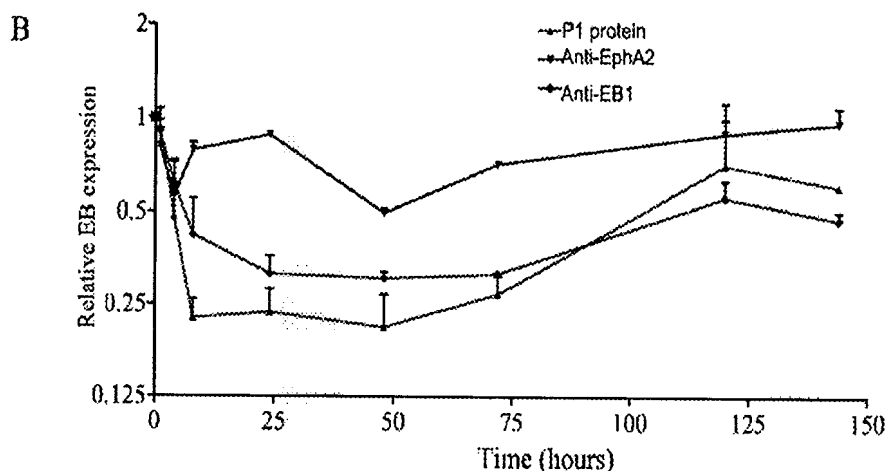
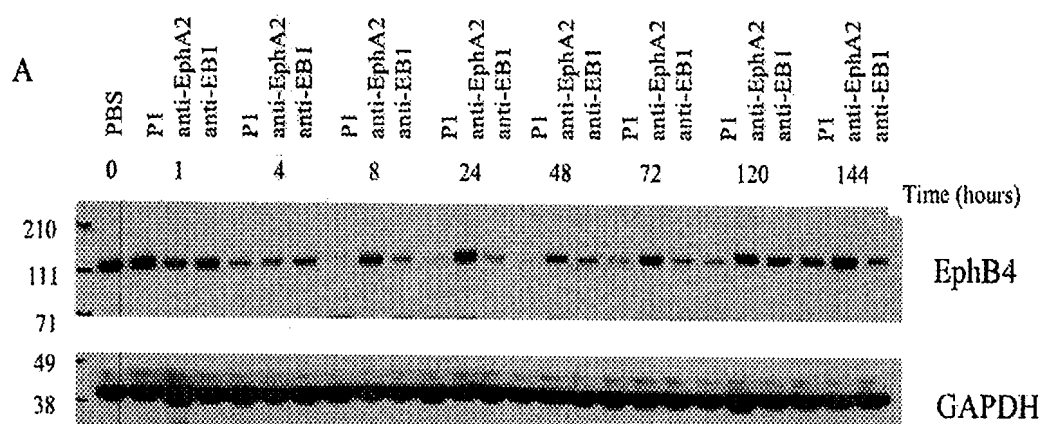
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MULTISPECIFIC EPIOTOPE BINDING PROTEINS AND USES THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit under 35 U.S.C. §119(e) of the following U.S. Provisional Application Nos.: 60/935,199 filed Jul. 31, 2007, 61/012,656 filed Dec. 10, 2007 and 61/074,330 filed Jun. 20, 2008. The priority applications are hereby incorporated by reference herein in their entirety for all purposes.

2. FIELD OF THE INVENTION

[0002] The present invention relates to multispecific epitope binding proteins, methods of making, and uses thereof in the prevention, management, treatment or diagnosis of acute or chronic diseases.

3. BACKGROUND OF THE INVENTION

[0003] Antibody fragments, such as Fabs, scFvs, diabodies, tribodies, and tetrabodies capable of binding one or more antigens may prove to be suitable for a number of clinical applications. These types of epitope binding proteins retain binding specificity to antigens, but lack the functional ability to stimulate an immune response directed against the bound antigen, i.e., they lack effector function.

[0004] Efforts to increase the valency or the number of antigenic determinants that an individual antibody molecule can bind have led to the development of bi-specific antibodies (BsAb) (for examples see Jimenez et al. *Molecular Cancer Therapeutics* 2005:4(3):427-434, Lu et al. *J. of Immun. Methods* 1999:230, 159-171 and U.S. Patent Publication Nos. 20070014794 and 20050100543). BsAbs are immunoglobulin (Ig)-based molecules that bind to two different epitopes on either the same or distinct antigens. The antibodies, for example, could be specific for a tumor cell antigen and an effector cell such as an activated T-cell or a functional agent such as a cytotoxin. Bispecific T-cell Engagers or BiTET™ molecules are one type of BsAbs, which have been shown to be useful for clinical applications (See U.S. Pat. No. 7,112,324 for examples).

[0005] Efforts to increase specificity beyond two distinct antigens have led to the production of a trispecific antibody-like structure composed of three distinct scFv domains flanked by hinge region domains that can be linked via disulfide bonds to circularize the resultant protein (See U.S. Patent Pub No. 20050175606). However, the stability of the antibody-like structures are in question due to the reliance on a single disulfide bond for circularization.

[0006] A major obstacle in the development of multiple epitope binding antibodies such as BsAbs as therapeutics has been difficulty in producing the antibodies in sufficient quantity and quality for clinical studies. In particular, traditional methods, including hybrid hybridoma, in which two distinct hybridomas are fused to create a cell expressing two sets of heavy and light chains, and chemical conjugation (Carter et al. (1995) *J. Hematotherapy* 4:463-70) have been inadequate.

[0007] For instance, coexpression of two different sets of IgG light and heavy chains in a hybrid hybridoma may produce up to 10 light- and heavy-chain pairs, with only one of these pairs forming the functional bispecific heterodimer (Suresh et al. (1986) *Methods Enzymol.* 121:210-28). Purification of the antibodies from the non-functional species,

such as homodimers and mispaired heterodimers of non-cognate Ig light and heavy chains produced by the hybrid hybridoma is cumbersome and inefficient.

[0008] Chemical crosslinking of two IgGs or their fragments is also inefficient and can lead to the loss of antibody activity (Zhu et al. (1994) *Cancer Lett.* 86:127-34). Like the hybrid hybridoma approach, purification of the antibodies from the non-functional species, such as multimeric aggregates resulting from chemical conjugation, is often difficult and the yield is usually low (Cao et al. (1998) *Bioconj. Chem.* 9:635-44).

[0009] To improve efficiency of production of multiple epitope binding antibodies, a variety of recombinant methods have been developed. For example, methods of efficient production of BsAbs have been developed, both as antibody fragments (Carter et al. (1995); Pluckthun et al. (1997) *Immunotechnology* 3:83-105; Todorovska et al. (2001) *J. Immunol. Methods* 248:47-66) and full length IgG formats (Carter (2001) *J. Immunol. Methods* 248:7-15). For example, production of homogeneous full-length IgG-like BsAbs has been achieved by the so-called "knobs-into-holes" engineering for efficient Ig CH3 domain heterodimerization (Ridgway et al. 1996 *Protein Eng.* 9:617-21; Merchant et al. 1998 *Nat. Biotech.* 16:677-81) and by fusing single chain Fvs (scFv) of different specificities onto either the N- or the C-terminus of a full-length IgG molecule (Zhuang et al. *Protein Eng.* 2000 13:361-7; Coloma and Morrison *Nat. Biotechnol.* 1997 15: 159-63). BsAbs have also been constructed by genetically fusing two single chain Fv (scFv) or Fab fragments with or without the use of flexible linkers (Mallender et al. *J. Biol. Chem.* 1994 269:199-206; Mack et al. *Proc. Natl. Acad. Sci. USA.* 1995 92:7021-5; Zapata et al. *Protein Eng.* 1995 8:1057-62), via a dimerization device such as leucine zipper (Kostelny et al. *J. Immunol.* 1992 148:1547-53; de Kruif et al. *J. Biol. Chem.* 1996 271:7630-4) and Ig C α /CH1 domains (Muller et al. *FEBS Lett.* 422:259-64); by diabody (Holliger et al. (1993) *Proc. Nat. Acad. Sci. USA.* 1998 90:6444-8; Zhu et al. *Bio/Technology* (NY) 1996 14:192-6); Fab-scFv fusion (Schoonjans et al. *J. Immunol.* 2000 165:7050-7); and mini-antibody formats (Pack et al. *Biochemistry* 1992 31:1579-84; Pack et al. *Bio/Technology* 1993 11:1271-7). In the majority of the cases, these recombinant approaches result in the production of divalent bispecific antibody molecules that are monovalent to each of their target antigens.

[0010] Antibody alternatives, such as multispecific epitope binding proteins provide many advantages over traditional targeted epitopes for example, but not limited to, access to immunosilent domains, expanded repertoire of targets, new binding specificities, and conjugates to drugs, radionuclides, toxins, enzymes, liposomes and viruses. With these significant advantages, there is a need in the art to construct and efficiently produce functional multivalent and multispecific epitope binding proteins capable of binding at least three or more epitopes with high affinity while retaining the ability to elicit the effector functions of an antibody.

[0011] Citation or discussion of a reference herein shall not be construed as an admission that such is prior art to the present invention.

4. SUMMARY OF THE INVENTION

[0012] The present invention provides novel multispecific epitope binding proteins capable of binding multiple epitopes, and which comprise an Fc region of an antibody constant domain. As used herein the term "Fc region" refers

to a polypeptide comprising the CH3, CH2 and at least a portion of the hinge region of a constant domain of an antibody. Optionally, an Fc region may include a CH4 domain, present in some antibody classes. An Fc region, as used herein may comprise the entire hinge region of a constant domain of an antibody. In one embodiment, multispecific epitope binding proteins of the invention comprise an Fc region and a CH1 region of an antibody. In another embodiment, multispecific epitope binding proteins of the invention comprise an Fc region, a CH1 region and a Ckappa/lambda region from the constant domain of an antibody. In one embodiment, the multispecific epitope binding proteins and/or polypeptide chains of the invention as described herein do not exist in nature or are not native sequence (composition and orientation) Ig molecules. Additionally, in one embodiment, multispecific epitope binding proteins as described herein are not produced in vitro by chemically cross-linking a pair of antibodies or antigen binding fragments thereof.

[0013] The multispecific epitope binding proteins of the invention comprise one, two, three, four, or more polypeptide chains. In specific embodiments the epitope binding proteins of the invention comprise between two to four polypeptide chains. Each chain of the multispecific epitope binding protein of the invention may comprise 1, 2, 3, 4, 5, 6, 7, 8 or more epitope binding domains. The epitope binding domains may be scFvs, single chain diabodies, variable regions of antibodies (e.g., heavy chain and/or light chain variable regions), peptidomimetics, or other epitope binding domains known in the art.

[0014] The Fc region and the epitope binding domains that are comprised within the polypeptide chains may be linked (as used herein throughout "linked" may refer to directly adjacent to, or indirectly linked with intervening sequences or structures, for example an Fc region linked to an epitope binding domain may be directly adjacent to the epitope binding domain, or the Fc region may be linked through intervening sequences to the epitope binding domain) together in many different orientations. In an embodiment, the epitope binding domains of one or more chains are linked to the C-terminus of the Fc region. In other embodiments, the epitope binding domains of one or more chains are linked to the N-terminus of the Fc region. In other embodiments, the epitope binding domains of one or more chains are linked to both the N-terminus and C-terminus of the Fc region.

[0015] The multispecific epitope binding proteins of the invention may be dimers, trimers, tetramers, or higher order multimers and may be homomeric or heteromeric, that is they may comprise multiple polypeptide chains that are the same or different. In one embodiment multispecific epitope binding proteins of the invention may comprise homodimers or heterodimers of two polypeptide chains. In another embodiment, multispecific epitope binding proteins of the invention may comprise homotrimers or heterotrimers of three polypeptide chains. In still another embodiment, multispecific epitope binding proteins of the invention may comprise homotetramers or heterotetramers of four polypeptide chains. The polypeptide chains of multispecific epitope binding proteins of the invention may multimerize (i.e., assemble) through the presence of components of the Fc region, namely the CH3, CH2, the hinge region (or a portion thereof) and/or the CH1 region. The polypeptide chains of multispecific epitope binding proteins of the invention may, alternatively or in addition, multimerize (i.e., assemble) through the interaction of other domains present in the constituent polypeptide chains.

[0016] In one embodiment, multispecific epitope binding proteins of the invention are capable of binding multiple epitopes concurrently (for example, in vivo or in vitro). In one embodiment, the epitope binding domains of multispecific epitope binding proteins of the invention are capable of binding at least 1, 2, 3, 4, 5, 6, 7, 8, or more epitopes concurrently (for example, in vivo or in vitro). In another embodiment, each epitope binding domain is specific for the same epitope. In another embodiment, multispecific epitope binding proteins of the invention comprise one or more epitope binding domains that are specific for distinct epitopes.

[0017] In one embodiment, each binding domain within the multispecific epitope binding proteins of the invention are specific for the same or distinct epitopes. In one embodiment, each binding domain within the epitope binding proteins of the invention have different (i.e. a higher or lower) affinity for the antigen compared to the isolated binding domain.

[0018] It is known that the immune system inhibits, reduces, or ablates specific targets through the effector function of antibodies. Antibody Dependent Cell-Mediated Toxicity (ADCC) and Complement Dependent Cytotoxicity (CDC) are two of the effector functions regulated by the Fc region of traditional antibodies. In one embodiment, multispecific epitope binding proteins of the invention retain the ability to efficiently stimulate effector function through the Fc region. In some situations, effector function is not desired, for example, when blocking the interaction between ligand and receptor is sufficient to achieve the desired outcome. Accordingly, in some embodiments, multispecific epitope binding proteins of the invention may not elicit effector function directed at a target.

[0019] The invention also provides methods of producing multispecific epitope binding proteins of the invention. In one embodiment, the polypeptide chains which form multispecific epitope binding proteins of the invention may be expressed from a single vector, or from multiple vectors. The arrangement of the coding regions of the polypeptide chain binding domains within the vector can be varied. For example, the orientation of the coding region for the Fc region (e.g., the CH3, CH2, the hinge region (or a portion thereof) and/or the CH1 region) may be 5' or 3' to the coding region of any of the epitope binding domains contained within the multispecific epitope binding polypeptide chain. Similarly, the orientation of the coding region of any of the epitope binding domains may be 5' and/or 3' to the coding region for the Fc region. In some embodiments, the coding region of an epitope binding domain is present both 5' and 3' to the coding region for the Fc region. In certain embodiments, a CH1 domain may be present 5' to the coding region for the Fc region.

[0020] The multispecific epitope binding proteins of the invention can be produced in many different expression systems. In one embodiment, the multispecific epitope binding proteins of the invention are produced and secreted by mammalian cells. In a specific embodiment, multispecific epitope binding proteins of the invention are produced in and secreted from human cells. In still other embodiments multispecific epitope binding proteins of the invention are produced in and isolated from plant cells.

[0021] The invention also provides methods of using multispecific epitope binding proteins of the invention. For example, many cell types express various common surface antigens and it is the specific combination of antigens that distinguish a specific class of cells. Using proteins of the

[0185] In other specific embodiments, vectors of the invention comprise a promoter, a polynucleotide sequence encoding an antibody light chain variable domain and a Ckappa/lambda arranged N-terminus to C-terminus in the following orientation: VL-Ckappa/lambda, and a poly A tail (see FIG. 4M, inset c and d; FIG. 4O, inset d; and FIG. 4S, inset d). In yet another specific embodiment, vectors of the invention comprise a promoter, a polynucleotide sequence encoding an antibody heavy chain variable domain and a CH1 arranged N-terminus to C-terminus in the following orientation: VH-CH1, and a poly A tail (see FIG. 4O, inset c; FIG. 4Q insets c and d; FIG. 4S inset c; and FIG. 4U, inset b).

[0186] In another specific embodiment, vectors of the invention comprise a promoter, a polynucleotide sequence encoding an antibody light chain variable domain, a Ckappa/lambda, and an Fc region arranged N-terminus to C-terminus in the following orientation: VL-Ckappa/lambda-Fc region, and a poly A tail (see FIG. 4U, inset a).

[0187] In some embodiments, vectors of the invention are not vectors as presented in FIG. 5. of PCT publication WO 01/77342 filed Mar. 20, 2001.

C. Multispecific Epitope Binding Proteins—Two Chains

[0188] Described in the following sections are the assembly and orientation of multispecific epitope binding proteins of the invention comprising two polypeptide chains.

[0189] In some embodiments, the multispecific epitope binding proteins of the invention comprise a first and a second polypeptide chain of the invention, wherein the first and/or second polypeptide chain comprises an Fc region linked to 1, 2, 3, 4, 5, 6, 7, 8, or more epitope binding domains. The polypeptide chains of the invention comprise epitope binding domains which may be scFvs, single chain diabodies, variable regions of antibodies, or other epitope binding domains known in the art. The Fc region and the epitope binding domains may be linked together in many different orientations (See section A, *supra*). In one embodiment, the epitope binding domains are linked to the C-terminus of the Fc region. In other embodiments the epitope binding domains are linked to the N-terminus of the Fc region. In other embodiments, the epitope binding domains are linked to both the N-terminus and C-terminus of the Fc region. In some embodiments, the multispecific epitope binding proteins of the invention comprise a first and a second polypeptide chain of the invention, wherein the first and/or second polypeptide chain comprises 1, 2, 3, 4, 5, 6, 7, 8, or more Fc regions linked to 1, 2, 3, 4, 5, 6, 7, 8, or more epitope binding domains. In one embodiment, the epitope binding domains are linked to the C-terminus of at least one Fc region. In other embodiments the epitope binding domains are linked to the N-terminus of at least one Fc region. In other embodiments, the epitope binding domains are linked to both the N-terminus and C-terminus of at least one Fc region.

[0190] In alternate embodiments, the multispecific epitope binding proteins of the invention do not comprise an Fc region. In such proteins of the invention, the epitope binding domains may be linked N-terminus, C-terminus, or N- and C-terminus to a CH1 domain and/or a Ckappa/lambda domain. It is to be understood that term Fc region may be replaced by CH1 domain and/or Ckappa/lambda in the following sections as to encompass proteins of the invention that do not comprise an Fc region. In some alternate embodiments, the multispecific epitope binding proteins of the inven-

tion comprise a first and a second polypeptide chain of the invention, wherein the first and/or second polypeptide chain comprises 1, 2, 3, 4, 5, 6, 7, 8, or more CH1 and/or Ckappa/lambda regions linked to 1, 2, 3, 4, 5, 6, 7, 8, or more epitope binding domains. In one embodiment, the epitope binding domains are linked to the C-terminus of at least one CH1 and/or Ckappa/lambda region. In other embodiments the epitope binding domains are linked to the N-terminus of at least one CH1 and/or Ckappa/lambda region. In other embodiments, the epitope binding domains are linked to both the N-terminus and C-terminus of at least one CH1 and/or Ckappa/lambda region.

C.1. Multispecific Epitope Binding Proteins with Epitope Binding Domains Linked to the C-Terminus of the Fc Region

[0191] In one embodiment, multispecific epitope binding proteins of the invention comprise a first and a second polypeptide chain wherein the first and/or second polypeptide chain comprises an Fc region. In one embodiment, multispecific epitope binding proteins comprises a first and a second polypeptide chain wherein the first and/or second chain comprises an scFv linked to the C-terminus of the Fc region (see for example FIG. 1B). In another embodiment, the first and/or second polypeptide chain comprises multiple scFvs linked to the C-terminus of the Fc region (see for example FIG. 2B). In another embodiment, epitope binding proteins of the invention comprise a first and/or second chain with at least 1, 2, 3, 4, 5, 6, 7, 8 or more scFvs linked to the C-terminus of the Fc region.

[0192] In one embodiment, the multispecific epitope binding protein comprises a first and a second polypeptide chain wherein the first and/or second chain comprises a single chain diabody linked to the C-terminus of the Fc region (see for example FIG. 3F). In another embodiment, the first and/or second polypeptide chain comprises multiple single chain diabodies linked to the C-terminus of the Fc region. In another embodiment, the epitope binding protein of the invention comprises a first and/or second chain with at least 1, 2, 3, 4, 5, 6, 7, 8 or more single chain diabodies linked to the C-terminus of the Fc region.

[0193] In one embodiment, multispecific epitope binding proteins comprise a first and a second polypeptide chain wherein the first and/or second chain comprises an antibody variable region linked to the C-terminus of the Fc region. In another embodiment, the first and/or second polypeptide chain comprises multiple antibody variable regions linked to the C-terminus of the Fc region. In another embodiment, epitope binding proteins of the invention comprise a first and/or second chain with at least 1, 2, 3, 4, 5, 6, 7, 8 or more antibody variable regions linked to the C-terminus of the Fc region.

[0194] In one embodiment, multispecific epitope binding proteins comprise a first and a second polypeptide chain wherein the first and/or second chain comprise an epitope binding domain known in the art linked to the C-terminus of the Fc region. In another embodiment, the first and/or second polypeptide chain comprises multiple epitope binding domains known in the art linked to the C-terminus of the Fc region. In another embodiment, epitope binding proteins of the invention comprise a first and/or second chain with at least 1, 2, 3, 4, 5, 6, 7, 8 or more epitope binding proteins known in the art wherein the domains are selected from the group consisting of an antibody variable region, an scFv, a single chain diabody, and another epitope binding domain known in the art, linked to the C-terminus of the Fc region.

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proteins of the invention can selectively bind and activate distinct target proteins. In one embodiment, multispecific epitope binding proteins of the invention can selectively bind and activate at least 1, 2, 3, 4, 5, 6, 7, 8, or more distinct target proteins (e.g., in vivo in a mammal (e.g., a human) and/or in vitro). In another embodiment, multispecific epitope binding proteins of the invention can selectively bind distinct target proteins and activate or inhibit said target protein (e.g., in vivo in a mammal (e.g., a human) and/or in vitro). In one embodiment, multispecific epitope binding proteins of the invention can selectively bind and activate or inhibit at least 1, 2, 3, 4, 5, 6, 7, 8, or more distinct target proteins (e.g., in vivo in a mammal (e.g., a human) and/or in vitro). In a further embodiment, multispecific epitope binding proteins of the invention can selectively bind distinct target proteins simultaneously (e.g., in vivo in a mammal (e.g., a human) and/or in vitro).

G. Uses of the Multispecific Epitope Binding Proteins of the Invention and Formulations of the Same

[0302] The invention also provides methods of using multispecific epitope binding proteins of the invention. The present invention also encompasses the use of multispecific epitope binding proteins of the invention for the prevention, diagnosis, management, treatment or amelioration of one or more symptoms associated with diseases, disorders of diseases or disorders, including but not limited to cancer, inflammatory and autoimmune diseases either alone or in combination with other therapies. The invention also encompasses the use of multispecific epitope binding proteins of the invention conjugated or fused to a moiety (e.g., therapeutic agent or drug) for prevention, management, treatment or amelioration of one or more symptoms associated with diseases, disorders or infections, including but not limited to cancer, inflammatory and autoimmune diseases either alone or in combination with other therapies.

[0303] Many cell types express various common cell surface antigens and it is the specific combination of antigens that distinguish a defined subset of cells. Using the multispecific epitope binding proteins of the invention, it is possible to target specific subsets of cells without cross-reacting with other unrelated populations of cells. Further, it is possible that multispecific epitope binding proteins of the invention comprise one to several (two, three, four, five, six, seven, eight, nine, ten, etc.) epitope binding domains that bind cell surface antigens present on non-target cell populations, however, it is the combined avidity of the set of epitope binding domains, which confer an effective level of binding (i.e., a therapeutically effective level of binding) to the target cell population. In other words, several of the epitope binding domains are engaged to facilitate the targeting of a particular cell type, which would not be achieved by binding of an individual isolated domain (e.g., isolated from the multispecific protein) or not achieved by one or more, but not all (i.e., a subset) of epitope binding domains (control peptides) exposed to the same cell, but not as part of a multispecific protein. It is envisioned that the relative avidity contribution of each epitope binding domain may be tailored to only target the specific cell population of interest. Such affinity alterations may be performed by art-accepted techniques, such as affinity maturation, site-specific mutagenesis, and others known in art. It is also contemplated that the relative avidity contribution of each epitope binding domain may also be altered by

changing the relative orientation of said epitope binding domains in the multispecific epitope binding protein.

[0304] Accordingly, provided herein in one embodiment are methods of using multispecific epitope binding proteins of the invention to identify, deplete, modulate (e.g., activate, inhibit) a cell population (e.g., in vivo in a mammal (e.g., a human) and/or in vitro). In one particular embodiment, the multispecific epitope binding proteins of the invention do not significantly bind normal tissue (e.g., non-cancerous tissue or non-diseased tissue, i.e., non-target tissue) when administered to a mammal (e.g., a human). Avoidance of significant binding to non-target tissue may avoid or minimize depletion, modulation (activation, inhibition) of one or more non-targeted cell population. In some embodiments, the increase in avidity exhibited by the multispecific epitope binding proteins of the invention for a target cell population in vitro or when administered to a mammal (e.g., a human) is at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, or at least 25 fold as compared to a "control epitope binding protein" which comprises one or more, but not all (i.e., a subset) of epitope binding domains isolated from said multispecific epitope binding protein. In some embodiments, the increase in avidity exhibited by the multispecific epitope binding proteins of the invention is at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, fold, at least 40%, at least 50%, at least 75%, at least 85%, at least 90%, at least 95%, or at least 150% over said "control epitope binding protein".

[0305] In some embodiments, multispecific epitope binding proteins of the invention have an increase in avidity for a particular antigen compared to a control epitope binding protein, which comprises one or more, but not all (i.e., a subset) of epitope binding domains isolated from said multispecific epitope binding protein. In some embodiments, the increase in avidity exhibited by the multispecific epitope binding proteins of the invention is at least 2 fold, at least 3 fold, at least 4 fold, at least 5 fold, at least 6 fold, at least 7 fold, at least 8 fold, at least 9 fold, at least 10 fold, at least 15 fold, at least 20 fold, or at least 25 fold over said control epitope binding protein. In some embodiments, the increase in avidity exhibited by the multispecific epitope binding proteins of the invention is at least 5%, at least 10%, at least 15%, at least 20%, at least 30%, fold, at least 40%, at least 50%, at least 75%, at least 85%, at least 90%, at least 95%, or at least 150% over said control epitope binding protein.

[0306] In other embodiments, multispecific epitope binding proteins of the invention comprising X number of epitope binding domains (where X is any positive integer from 1 through 20) exhibit an increase in avidity (in vitro or when administered to a mammal (e.g., a human) for a particular antigen compared to a control epitope binding protein (such as, but not limited to an antibody, another multispecific epitope binding protein, an scFv, or a single chain diabody) wherein said control epitope binding protein comprises X-Y (Where X and Y are any positive integer from 1 through 20 and X is greater than Y) epitope binding domains, wherein at least one epitope binding domain in the control epitope binding domain protein is specific for the same epitope as the epitope binding domain present in the protein of the invention. In other words, the invention provides multispecific epitope binding proteins with increased avidity for a particular target as compared to control epitope binding proteins with greater or fewer epitope binding domains wherein at

least one epitope binding domain is specific for a common antigen with the protein of the invention.

[0307] Such avidity changes in multispecific epitope binding proteins allow for the decrease of toxicity of such therapeutic proteins (for example, combinations of any antibodies listed in Section E. "Specific embodiments of multispecific epitope binding assemblies) in an animal. It is understood that the proteins of the invention may exhibit a "tailor-fit" avidity and/or affinity to decrease toxicity in vivo. As such, the invention also provides multispecific epitope binding proteins of the invention that exhibit a lower toxicity in an animal, than a control epitope binding protein. It is also understood that the invention also provides methods of reducing toxicity of a protein of the invention by the methods described herein.

[0308] It is also appreciated that avidity changes may be evaluated by readily available in vitro methods such as functional assays (including but not limited to cytokine expression/release/binding, gene expression, morphology changes, chemotaxis, calcium flux, and the like), binding measurements determined by BIAcore or KinExa measurements with control epitope binding domain proteins. In some embodiments, control epitope binding proteins may contain a subset of epitope binding domains from the proteins of the invention. In other embodiments, control epitope binding proteins may comprise at least one or more isolated epitope binding domains from the multispecific epitope binding proteins of the invention. For example, for a protein of the invention with 8 epitope binding domains, a control epitope binding protein may comprise 1, 2, 3, 4, 5, 6, or 7 epitope binding domains, with at least one epitope binding domain having specificity for an antigen recognized by both the protein of the invention and the control protein. In other embodiments, the control epitope binding protein comprises an isolated epitope binding domain having specificity for an antigen recognized by both the isolated epitope binding domain and the protein of the invention.

[0309] In one embodiment, the multispecific epitope binding proteins of the invention are used to specifically identify, deplete, activate, inhibit, or target for neutralization cells which are defined by the expression of multiple cell surface antigens. In another embodiment, invention contemplates use of proteins of the invention to purify cells expressing multiple antigens recognized by the multiple epitope binding sites in the protein. As such, methods of the invention include modulating the relative avidity contributions of each individual epitope binding domain within the multispecific epitope binding protein.

[0310] In some embodiments, the methods of the invention used to identify, deplete, activate, inhibit, or target for neutralization cells do not significantly deplete, activate, or inhibit a non-target cell population. In such embodiments, the administration of proteins of the invention does not exhibit a negative outcome that outweighs the benefits achieved from the treatment itself. Such negative outcomes may include, but are not limited to, binding to non-target tissues, increased toxicity, pathological depletion of cells, increased risk of infection, and the like. Negative outcomes presented by therapies by traditional epitope binding domains (including but not limited to antibodies, scFvs, single chain diabodies and the like) may also be minimized by the implementation of therapies employing multispecific epitope binding proteins of the invention.

[0311] Also, it is well understood that cancer cells differentially express cell surface molecules during the tumor pro-

gression. For example, a neoplastic cell may express a cell surface antigen in a benign state, yet down-regulate that particular cell surface antigen upon metastasis. It is understood that this process may occur for many different tumor cell types. As such, it is envisioned that the multispecific-epitope binding proteins of the invention may be designed to comprise epitope binding domains that could target such a tumor cell at many different stages of cancer progression. In other words, the multispecific epitope binding proteins of the invention may be used to target a tumor cell type irrespective of its stage of progression. The multispecific epitope binding proteins of the invention may comprise multiple epitope binding domains that would bind specific tumor cell surface antigens expressed at various stages of tumor progression.

[0312] In one embodiment, multispecific epitope binding proteins of the invention comprise epitope binding domains that are specific for tumor cell surface antigens which may or may not be displayed concurrently. In other embodiments, multispecific epitope binding proteins of the invention comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight epitope binding domains that are specific for tumor cell surface antigens, wherein said antigens may not be displayed on the surface of the tumor cell concurrently. In other embodiments, multispecific epitope binding proteins of the invention comprise at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight epitope binding domains that are specific for tumor cell surface antigens, wherein at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight tumor cell surface antigens are engaged by said protein to elicit a response, such as effector function, internalization, neutralization, and others.

[0313] In other embodiments, the invention provides methods of depleting, killing, neutralizing, and/or sequestering cell types that are defined by the expression of multiple cell surface antigens. Such cell types include, but are not limited to T cells (such as cytotoxic, memory and NK cells), B cells, Mast cells, eosinophils, basophils, neutrophils, stem cells (such as cancer stem cells), and macrophages.

[0314] The multispecific epitope binding proteins of the invention are employed in an amount that is effective for both producing the desired therapeutic effect and for reducing side effects. A cytokine storm is a potentially fatal immune reaction consisting of a positive feedback loop between cytokines and immune cells which is caused for example, when the immune system is fighting pathogens. The cytokine storm (hypercytokinemia) is the systemic expression of a healthy and vigorous immune system resulting in the release of more than 150 inflammatory mediators (cytokines, oxygen free radicals, and coagulation factors). Both pro-inflammatory cytokines (such as Tumor necrosis factor-alpha, Interleukin-1, and Interleukin-6) and anti-inflammatory cytokines (such as interleukin 10, and interleukin 1 receptor antagonist) are elevated in the serum of patients experiencing a cytokine storm. Cytokine storms can occur in a number of infectious and non-infectious diseases including graft versus host disease (GVHD), adult respiratory distress syndrome (ARDS), sepsis, avian influenza, smallpox, stroke, allergic reaction (hypersensitivity), cardiac arrest, toxic shock syndrome, and systemic inflammatory response syndrome (SIRS). A cytokine storm may also be induced as a result of traditional small molecule, or biologic therapy. In some embodiments, multispecific epitope binding proteins of the invention are useful in

Low et al); mannosides (Umezawa et al., (1988) *Biochem. Biophys. Res. Commun.* 153:1038); antibodies (P. G. Bloeman et al. (1995) *FEBS Lett.* 357:140; M. Owais et al. (1995) *Antimicrob. Agents Chemother.* 39:180); surfactant protein A receptor (Briscoe et al. (1995) *Am. J. Physiol.* 1233:134); p 120 (Schreier et al (1994) *J. Biol. Chem.* 269:9090); see also K. Keinänen; M. L. Laukkanen (1994) *FEBS Lett.* 346:123; J. J. Killion; I. J. Fidler (1994) *Immunomethods* 4:273.

[0456] The invention provides protocols for the administration of pharmaceutical composition comprising multispecific epitope binding proteins of the invention alone or in combination with other therapies to a subject in need thereof. The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the present invention can be administered concomitantly or sequentially to a subject. The therapy (e.g., prophylactic or therapeutic agents) of the combination therapies of the present invention can also be cyclically administered. Cycling therapy involves the administration of a first therapy (e.g., a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (e.g., a second prophylactic or therapeutic agent) for a period of time and repeating this sequential administration, i.e., the cycle, in order to reduce the development of resistance to one of the therapies (e.g., agents) to avoid or reduce the side effects of one of the therapies (e.g., agents), and/or to improve, the efficacy of the therapies.

[0457] The therapies (e.g., prophylactic or therapeutic agents) of the combination therapies of the invention can be administered to a subject concurrently. The term "concurrently" is not limited to the administration of therapies (e.g., prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising multispecific epitope binding proteins of the invention are administered to a subject in a sequence and within a time interval such that the multispecific epitope binding proteins of the invention can act together with the other therapy(ies) to provide an increased benefit than if they were administered otherwise. For example, each therapy may be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy can be administered to a subject separately, in any appropriate form and by any suitable route. In various embodiments, the therapies (e.g., prophylactic or therapeutic agents) are administered to a subject less than 15 minutes, less than 30 minutes, less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, 24 hours apart, 48 hours apart, 72 hours apart, or 1 week apart. In other embodiments, two or more therapies (e.g., prophylactic or therapeutic agents) are administered to a within the same patient visit.

[0458] The prophylactic or therapeutic agents of the combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharma-

ceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

T. Kits

[0459] Also within the scope of the invention are kits comprising the compositions (e.g. multispecific epitope binding proteins) of the invention and instructions for use. The kit can further contain a least one additional reagent, or one or more additional multispecific epitope binding proteins of the invention. Kits typically include a label indicating the intended use of the contents of the kit. The term label includes any writing, or recorded material supplied on or with the kit, or which otherwise accompanies the kit.

[0460] Equivalents

[0461] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

[0462] All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

U. Specific Embodiments

[0463] 1. An isolated multispecific epitope binding protein comprising a first and a second polypeptide chain, wherein the first and/or the second chain comprises at least two epitope binding domains (EBDs) and one or more Fc regions.

[0464] 2. The protein of embodiment 1 (or any protein of the invention), wherein said first chain comprises one or more Fc regions linked N-terminal to at least one EBD.

[0465] 3. The protein of embodiment 2 (or any protein of the invention), wherein said first chain comprises domains and Fc regions arranged from N-terminus to C-terminus: one or more Fc regions-EBD-EBD.

[0466] 4. The protein of embodiment 2 (or any protein of the invention), wherein said first chain comprises domains and Fc regions arranged from N-terminus to C-terminus: EBD-one or more Fc regions-EBD.

[0467] 5. The protein of embodiment 1 (or any protein of the invention), wherein said first chain comprises one or more Fc regions linked C-terminal to at least one EBD.

[0468] 6. The protein of embodiment 5 (or any protein of the invention), wherein said first chain comprises domains and Fc regions arranged from N-terminus to C-terminus: EBD-EBD-one or more Fc regions.

[0469] 7. The protein of embodiment 5 (or any protein of the invention), wherein said first chain comprises domains and Fc regions arranged from N-terminus to C-terminus: EBD-one or more Fc regions-EBD-EBD.

[0470] 8. The protein of embodiment 1 (or any protein of the invention), wherein said second chain comprises one or more C κ or C λ regions linked N-terminal to at least one EBD.

[0471] 9. The protein of embodiment 1 (or any protein of the invention), wherein said second chain comprises one or more C κ or C λ regions linked C-terminal to at least one EBD.

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- [0472] 10. The protein of embodiment 1 (or any protein of the invention), wherein the first and/or the second chain comprises at least three EBDs.
- [0473] 11. The protein of embodiment 10 (or any protein of the invention), wherein the first and/or the second chain comprises at least four epitope binding domains EBDs.
- [0474] 12. The protein of embodiment 10 or 11 (or any protein of the invention), wherein each Fc region is linked N-terminal to at least three EBDs.
- [0475] 13. The protein of embodiment 12 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: one or more Fc regions-EBD-EBD-EBD.
- [0476] 14. The protein of embodiment 12 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: one or more Fc regions-EBD-EBD-EBD-EBD.
- [0477] 15. The protein of embodiment 10 or 11 (or any protein of the invention), wherein each Fc region is linked C-terminal to at least one EBD.
- [0478] 16. The protein of embodiment 15 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: EBD-EBD-EBD-one or more Fc regions.
- [0479] 17. The protein of embodiment 16 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus EBD-EBD-EBD-EBD-one or more Fc regions.
- [0480] 18. The protein of embodiment 10 or 11 (or any protein of the invention), wherein each Fc region is linked N-terminal to at least one EBD and C-terminal to at least one EBD.
- [0481] 19. The protein of embodiment 18 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: at least one EBD-one or more Fc regions-at least one EBD.
- [0482] 20. The protein of embodiment 18 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: EBD-EBD-one or more Fc regions-EBD.
- [0483] 21. The protein of embodiment 18 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: EBD-EBD-one or more Fc regions-EBD-EBD-EBD.
- [0484] 22. The protein of embodiment 18 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: EBD-EBD-EBD-one or more Fc regions-EBD.
- [0485] 23. The protein of embodiment 18 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: EBD-EBD-one or more Fc regions-EBD-EBD.
- [0486] 24. The protein of embodiment 18 (or any protein of the invention), wherein said domains and Fc region are arranged from N-terminus to C-terminus: EBD-one or more Fc regions-EBD-EBD-EBD.
- [0487] 25. The protein of embodiment 10 (or any protein of the invention), wherein said first polypeptide chain comprises 3 scFvs and an Fc region arranged N-terminus to C-terminus: scFv-scFv-Fc region-scFv and wherein said second polypeptide chain comprises 3 scFvs and an Fc region arranged N-terminus to C-terminus: scFv-scFv-Fc region-scFv.
- [0488] 26. The protein of embodiment 11 (or any protein of the invention), wherein said first polypeptide chain comprises 4 scFvs and an Fc region arranged N-terminus to C-terminus: scFv-scFv-Fc region-scFv-scFv and wherein said second polypeptide chain comprises 4 scFvs and an Fc region arranged N-terminus to C-terminus: scFv-scFv-Fc region-scFv-scFv.
- [0489] 27. The protein of embodiment 1 (or any protein of the invention), wherein said protein comprises a first and a second chain;
 - [0490] a. said first chain comprising an scFv, an antibody variable region, and an Fc region arranged N-terminus to C-terminus:scFv-antibody variable region-Fc region; and
 - [0491] b. said second chain comprising an antibody variable region and a Ckappa/lambda region arranged N-terminus to C-terminus:antibody variable region—Ck or Cλ.
- [0492] 28. The protein of embodiment 1 (or any protein of the invention), wherein said protein comprises a first and a second chain;
 - [0493] a. said first chain comprising an antibody variable region, and an Fc region arranged N-terminus to C-terminus: antibody variable region-Fc region; and
 - [0494] b. said second chain comprising an scFv, antibody variable region, and a Ckappa/lambda region arranged N-terminus to C-terminus:scFv-antibody variable region—Ck or Cλ.
- [0495] 29. The protein of embodiment 1 (or any protein of the invention), wherein said protein comprises a first and a second chain;
 - [0496] a. said first chain comprising an scFv, an antibody variable region, and an Fc region arranged N-terminus to C-terminus:scFv-antibody variable region-Fc region; and
 - [0497] b. said second chain comprising an scFv, an antibody variable region, and a Ckappa/lambda region arranged N-terminus to C-terminus:scFv-antibody variable region—Ck or Cλ.
- [0498] 30. The protein of any of embodiments 27-29 (or any protein of the invention), wherein at least two EBDs are linked C-terminal to at least one Fc region.
- [0499] 31. The protein of any of embodiments 27-30 (or any protein of the invention), wherein said protein comprises an antibody heavy chain and an antibody light chain.
- [0500] 32. A multispecific epitope binding protein comprising a first and a second polypeptide chain wherein the first and/or the second chain comprises at least two EBDs and one or more CH1 domains.
- [0501] 33. The protein of embodiment 32 (or any protein of the invention), wherein said protein further comprises a Ckappa/lambda domain.
- [0502] 34. The protein of embodiment 33 (or any protein of the invention), wherein said first chain comprises two scFvs linked to a CH1 domain arranged N-terminus to C-terminus:scFv-CH1-scFv, said second chain comprises two scFvs linked to a Ckappa/lambda domain arranged N-terminus to C-terminus:scFv-Ckappa/lambda-scFv.
- [0503] 35. The protein of embodiment 34 (or any protein of the invention), wherein said first and/or second chain comprises all or a portion of an antibody hinge domain.
- [0504] 36. The protein of embodiment 35 (or any protein of the invention), wherein said first and second polypeptide chains are linked by a disulfide bond.

- [0505] 37. A multispecific epitope binding protein comprising at least a first and a second chain, said first chain comprises two variable domains and a C κ or a C λ domain and said second chain comprises two variable domains, a CH1, a Hinge, a CH2, and a CH3 domain, wherein said variable domains in said first chain associate with the variable domains in said second chain to form at least two distinct epitope binding sites.
- [0506] 38. The protein of embodiment 37 (or any protein of the invention), wherein said first chain comprises variable domains arranged N-terminal to C-terminal that are specific to a first epitope and second epitope and said second chain comprises variable domains arranged N-terminal to C-terminal that are specific to said first and said second epitopes.
- [0507] 39. A multispecific epitope binding protein comprising a first and a second chain, wherein said first chain comprises at least 2 antibody variable domains and at least one CH1 and/or C κ or C λ domains, wherein said variable domains in said first chain associate with the variable domains in said second chain to form at least two different epitope binding sites.
- [0508] 40. The protein of embodiment 39 (or any protein of the invention), wherein said first chain comprises an antibody heavy chain variable domain (VH), a CH1 domain, an antibody light chain variable domain (VL), and a C κ or C λ domain.
- [0509] 41. The protein of embodiment 40 (or any protein of the invention), wherein said first chain is organized N-terminus to C-terminus: VH-CH1-VL-C κ or C λ .
- [0510] 42. The protein of embodiment 40 (or any protein of the invention), wherein said first chain is organized N-terminus to C-terminus: VL-C κ or C λ -VH-CH1.
- [0511] 43. The protein of embodiment 39 (or any protein of the invention), wherein said first chain comprises 2 antibody heavy chain variable domains (VH), and 2 CH1 domains.
- [0512] 44. The protein of embodiment 43 (or any protein of the invention), wherein said first chain is organized N-terminus to C-terminus: VH-CH1-VH-CH1.
- [0513] 45. The protein of embodiment 39 (or any protein of the invention), wherein said first chain comprises 2 antibody light chain variable domains (VL), and 2 C κ or C λ domains.
- [0514] 46. The protein of embodiment 45 (or any protein of the invention), wherein said first chain is organized N-terminus to C-terminus: VL-C κ or C λ -VL-C κ or C λ .
- [0515] 47. The protein of any of embodiments 39-46 (or any protein of the invention), wherein said first chain further comprises an Fc region.
- [0516] 48. The protein of any of embodiments 39-46 (or any protein of the invention), wherein said second chain does not comprise an Fc region.
- [0517] 49. An inverted antibody protein comprising at least a first and a second polypeptide chain wherein said first chain comprises at least one antibody heavy chain variable region (VH) linked to a CH1 domain and said second chain comprises an antibody light chain variable region (VL) linked to a C κ or C λ domain, said C κ or C λ domain further linked to an Fc region, wherein said variable region in said first chain associate with the variable region in said second chain to form an epitope binding site.
- [0518] 50. The protein of embodiment 49 (or any protein of the invention), wherein said first chain is disulfide linked to said second chain.
- [0519] 51. A multispecific epitope binding protein comprising an antibody-like light chain and an antibody-like heavy chain wherein said light chain comprises two variable domains and a C κ or a C λ domain and said heavy chain comprises two variable domains, a CH1, a Hinge, a CH2, and a CH3 domain, wherein said variable domains in said light chain associate with the variable domains in said heavy chain to form at least two distinct epitope binding sites.
- [0520] 52. The protein of embodiment 51 (or any protein of the invention), wherein said light chain comprises variable domains arranged N-terminal to C-terminal are specific to a first epitope and second epitope and said heavy chain comprises variable domains arranged N-terminal to C-terminal are specific to said first and said second epitopes.
- [0521] 53. The protein of embodiment 52 (or any protein of the invention), wherein said protein further comprises at least 2 linked EBDs.
- [0522] 54. The protein of embodiment 53 (or any protein of the invention), wherein at least one EBD is linked to the C-terminus of said heavy chain.
- [0523] 55. The protein of embodiment 53 (or any protein of the invention), wherein at least one EBD is linked to the N-terminus of said light chain.
- [0524] 56. The protein of embodiment 53 (or any protein of the invention), wherein at least one EBD is linked to the N-terminus of said heavy chain.
- [0525] 57. A multispecific epitope binding protein comprising an antibody light chain and an antibody heavy chain, wherein said heavy chain further comprises at least 2 linked EBDs.
- [0526] 58. The protein of embodiment 57 (or any protein of the invention), wherein at least one EBD is linked to the C-terminus of said heavy chain.
- [0527] 59. The protein of embodiment 57 (or any protein of the invention), wherein at least one EBDs is linked to the N-terminus of said heavy chain.
- [0528] 60. The protein of embodiment 57 (or any protein of the invention), wherein at least one EBD is linked to the N-terminus and to the C-terminus of said heavy chain.
- [0529] 61. A multispecific epitope binding protein comprising a first and a second polypeptide chain wherein the first and/or the second chain comprises at least three EBDs and said first chain comprises a C κ or a C λ domain and said second chain comprises a CH1 domain.
- [0530] 62. A multispecific epitope binding protein comprising a structural format presented in any of the FIGS. 1-5 (or any protein of the invention).
- [0531] 63. The protein of any one of embodiments 1-24, 30-36, or 53-61 (or any protein of the invention), wherein each EBD is specific for the same epitope.
- [0532] 64. The protein of any one of embodiments 1-24, 30-36, or 53-61 (or any protein of the invention), wherein at least two EBDs are specific for different epitopes.
- [0533] 65. The protein of embodiment 63 or 64 (or any protein of the invention), wherein each epitope is located on the same antigen.
- [0534] 66. The protein of embodiment 65 (or any protein of the invention), wherein each epitope is located on different antigens.

- [0535] 67. The protein of embodiment any one of embodiments 1-24, 30-36, or 53-61 (or any protein of the invention), wherein two or more EBDs are specific for the same epitope.
- [0536] 68. The protein of any one of embodiments 1-67 (or any protein of the invention), wherein the protein is capable of binding at least two, at least three, at least four, at least five, at least six epitopes concurrently upon administration to a mammal or in an in vitro.
- [0537] 69. The protein of any of any one of embodiments 1-24, 30-36, or 53-61 (or any protein of the invention), wherein at least one EBD specifically binds an epitope with an affinity less than the identical isolated functional EBD.
- [0538] 70. The protein of embodiment 69 (or any protein of the invention), wherein said EBD is selected from the group consisting of the most N-terminal EBD, the second most N-terminal EBD, and the third most N-terminal EBD.
- [0539] 71. The protein of any one of embodiments 1-24, 30-36, 53-61, 63-67, and 69-70 (or any protein of the invention) wherein at least one EBD is selected from the group consisting of an scFv, a single chain single chain diabody, an antibody mimetic, and an antibody variable domain.
- [0540] 72. The protein of embodiment 71 (or any protein of the invention), wherein said antibody mimetic is selected from the group consisting of a minibody, a maxybody, an avimer, an Fn3 based protein scaffold, an ankrin repeat, a VASP polypeptide, an avian pancreatic polypeptide (aPP), a Tetranectin, an affililin, a knottin, an SH3, a PDZ domain, a protein A domain, a lipocalin, a transferrin, and a kunitz domains.
- [0541] 73. The protein of any of embodiments 1-72 (or any protein of the invention), wherein said protein specifically binds distinct cell surface receptors and inhibits and/or neutralizes said cell surface receptors.
- [0542] 74. The protein of embodiment 73 (or any protein of the invention), wherein said cell surface receptors are identical receptors.
- [0543] 75. The protein of embodiment 74 (or any protein of the invention), wherein said cell surface receptors are not identical receptors.
- [0544] 76. The protein of any of embodiments 1-75 (or any protein of the invention), wherein said protein specifically binds distinct soluble ligands and inhibits and/or neutralizes said ligands.
- [0545] 77. The protein of embodiment 76 (or any protein of the invention), wherein said soluble ligands are identical.
- [0546] 78. The protein of embodiment 76 (or any protein of the invention), wherein said soluble ligands are not identical.
- [0547] 79. The protein of any of embodiments 1-78 (or any protein of the invention), wherein said protein specifically binds distinct target proteins and inhibits and/or neutralizes said target proteins.
- [0548] 80. The protein of embodiment 79 (or any protein of the invention), wherein said target proteins are identical.
- [0549] 81. The protein of embodiment 79 (or any protein of the invention), wherein said target proteins are not identical.
- [0550] 82. The protein of any of embodiments 1-24, 30-36, 53-61, 63-67, and 69-70 (or any protein of the invention), wherein at least one EBD retains the functional activity of the identical EBD isolated from said protein.
- [0551] 83. The protein of any of embodiments 1-24, 30-36, 53-61, 63-67, and 69-70 (or any protein of the invention), wherein said protein has at least equivalent functional activity as a composition comprising each EBD isolated from said protein.
- [0552] 84. The protein of any of embodiments 1-24, 30-36, 53-61, 63-67, and 69-70 (or any protein of the invention), wherein said protein has greater functional activity as a composition comprising each EBD isolated from said protein.
- [0553] 85. The protein of any of embodiments 1-24, 30-36, 53-61, 63-67, and 69-70 (or any protein of the invention), wherein said protein has 50% less functional activity as compared to a composition comprising each EBD isolated from said protein.
- [0554] 86. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has the functional activity of depleting a cell population selected from the group consisting of: T cells, B cells, mast cells, eosinophils, basophils, neutrophils, dendritic cells, monocytes, macrophages, and tumor cells.
- [0555] 87. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has the functional activity of inhibiting or reducing proliferation of cells selected from the group consisting of: T cells, B cells, mast cells, eosinophils, basophils, neutrophils, dendritic cells, monocytes, macrophages, and tumor cells.
- [0556] 88. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has the functional activity of inhibiting or reducing secretion of inflammatory mediators from cells selected from the group consisting of: T cells, B cells, mast cells, eosinophils, basophils, neutrophils, dendritic cells, monocytes, macrophages, and tumor cells.
- [0557] 89. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has the functional activity of inhibiting or reducing secretion of cytoplasmic granules from cells selected from the group consisting of: T cells, mast cells, eosinophils, basophils, neutrophils, monocytes, and macrophages.
- [0558] 90. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has the functional activity of inhibiting or reducing the response to an activating stimuli in cells selected from the group consisting of: T cells, B cells, mast cells, eosinophils, basophils, neutrophils, dendritic cells, monocytes, macrophages, and tumor cells.
- [0559] 91. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has the functional activity activating a protein through binding of one or more epitopes.
- [0560] 92. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has the functional activity of deactivating a protein through binding of one or more epitopes.
- [0561] 93. The protein of any of embodiments 1-85 (or any protein of the invention), wherein said protein has effector function when administered to a mammal or in vitro.
- [0562] 94. The protein of embodiment 93 (or any protein of the invention), wherein said effector function is antibody dependent cellular cytotoxicity.
- [0563] 95. The protein of embodiment 93 (or any protein of the invention), wherein said effector function is complement dependent cytotoxicity.

- [0564] 96. An isolated nucleic acid molecule encoding the protein, or epitope-binding portion thereof, of any one of embodiments 1-95 (or any protein of the invention).
- [0565] 97. An expression vector comprising the nucleic acid molecule of embodiment 96.
- [0566] 98. A host cell comprising the expression vector of embodiment 97.
- [0567] 99. A method of making the protein of any of embodiments 1-95 (or any protein of the invention), wherein said method comprises a scalable process for making said protein wherein said scalable process results in a production efficiency of said protein from about 10 mg/L to about 300 mg/L and said protein retains at least one functional activity.
- [0568] 100. The method of embodiment 99, wherein said protein produced from said process exhibits an aggregation level of no more than 5% by weight of protein as measured by HPSEC.
- [0569] 101. The method of embodiment 99, wherein said protein produced by said process exhibits an aggregation level of no more than 5% by weight of protein as measured by rCGE.
- [0570] 102. The method of embodiment 99, wherein said protein produced by said process exhibits a low level of fragmentation as demonstrated by 80% or higher of the total peak area in the peaks representing the intact said proteins as measured by HPSEC.
- [0571] 103. A liquid formulation comprising the protein of any of embodiments 1-95 (or any protein of the invention), wherein said formulation exhibits an aggregation level of no more than 5% by weight of protein as measured by HPSEC.
- [0572] 104. A liquid formulation comprising the protein of any of embodiments 1-95 (or any protein of the invention), wherein said formulation exhibits an aggregation level of no more than 5% by weight of protein as measured by rCGE.
- [0573] 105. A liquid formulation comprising the protein of any of embodiments 1-95 (or any protein of the invention), wherein said formulation exhibits a low level of fragmentation as demonstrated by 80% or higher of the total peak area in the peaks representing the intact said proteins as measured by HPSEC.
- [0574] 106. A sterile formulation comprising a therapeutically effective amount of the protein of any of embodiments 1-95 (or any protein of the invention) in a pharmaceutically-acceptable excipient.
- [0575] 107. A method of ameliorating, treating or preventing cancer or a symptom thereof by administering the formulation of embodiment 106 to a patient in need thereof.
- [0576] 108. The method of embodiment 107, wherein said cancer is of the head, neck, eye, mouth, throat, esophagus, chest, bone, lung, colon, rectum, colorectal, stomach, spleen, renal, skeletal muscle, subcutaneous tissue, metastatic melanoma, endometrial, prostate, breast, ovaries, testicles, skin, thyroid, blood, lymph nodes, kidney, liver, pancreas, brain or central nervous system.
- [0577] 109. A method of depleting a cell population in a mammal wherein said cell population is selected from the group consisting of eosinophils, basophils, neutrophils, T cells, B cells, mast cells, monocytes and tumor cell comprising contacting said cell with the formulation of embodiment 106.
- [0578] 110. A method of killing or targeting a pathogen comprising contacting the pathogen in a mammal with the formulation of embodiment 106.
- [0579] 111. A method of inactivating, inhibition, or depleting a cytokine comprising contacting said cytokine in a mammal with the formulation of embodiment 106.
- [0580] 112. The method of embodiment 111, wherein said cytokine is C5a.
- [0581] 113. A method of preventing, treating managing or diagnosing an inflammatory or autoimmune disease in a patient in need thereof by administering the formulation of embodiment 106.
- [0582] 114. A method of inhibiting angiogenesis in a patient in need thereof by administering the formulation of embodiment 106.
- [0583] 115. The method of embodiment 113 or 114, wherein said patient has cancer, rheumatoid arthritis, SLE, or Sjogren's syndrome.
- [0584] 116. The protein of any of embodiments 1-95 (or any protein of the invention), wherein said protein identifies and/or depletes a population of cells when administered to a mammal or in vitro, said cells defined by the expression of distinct cell surface epitopes, said distinct cell surface epitopes selectively engaged by at least one epitope binding domain of said protein.
- [0585] 117. The protein of embodiment 116 (or any protein of the invention), wherein said cell population is selected from the group consisting of tumor cells, cancer stem cells, B cells, and T cells.
- [0586] 118. The protein of any of embodiments 1-95 (or any protein of the invention), wherein said protein comprises epitope binding domains specific for cell surface antigens presented on a tumor cell.
- [0587] 119. The protein of embodiment 118 (or any protein of the invention), wherein said cell surface antigens are not displayed concurrently.
- [0588] 120. The protein of embodiment 118 or 119 (or any protein of the invention), wherein said protein elicits antibody effector function when administered to a mammal, directed at the tumor cell when at least one epitope binding domain is engaged.
- [0589] 121. The protein of any of embodiments 1-95 (or any protein of the invention), wherein said protein comprises at least one epitope binding domain, said binding domain specific for a cargo molecule to be delivered to a cell when said protein is administered to a mammal, or in vitro.
- [0590] 122. The protein of embodiment 121 (or any protein of the invention), wherein said cargo molecule is selected from the group consisting of a cytotoxic drug, an anti-metabolite, a toxin, a peptide, a DNA molecule, an RNA molecule, a small molecule, a radioisotope, a fluorophore, an enzyme, an enzyme inhibitor, a prodrug, or a mitochondrial poison.
- [0591] 123. The protein of embodiment 121 or 122 (or any protein of the invention), wherein said protein internalizes when bound to said cell.
- [0592] 124. The protein of any of embodiments 121-123 (or any protein of the invention), wherein said epitope binding domain specific for a cargo molecule exhibits high binding affinity for the cargo molecule at about pH 7.4, and a low binding affinity for the cargo molecule at about pH 6.0.
- [0593] 125. A method of identifying, depleting, activating, or inhibiting a target cell population comprising contacting

the protein of any of embodiments 1-95 (or any protein of the invention) with said target cell population when administered to a mammal or in vitro, wherein said protein does not significantly deplete, activate, or inhibit a non-target cell population.

- [0594] 126. The method of embodiment 125, wherein said protein exhibits increased avidity for the target cell population over a control epitope binding protein, wherein said control epitope binding protein comprises either:
- [0595] a. a subset of epitope binding domains present in the multispecific epitope binding protein; or
- [0596] b. at least one isolated epitope binding domain present in the multispecific epitope binding protein.
- [0597] 127. The method of embodiment 126, wherein said subset comprises at least one, at least two, at least three, at least four, at least five, at least six, at least seven, or at least eight epitope binding domains.
- [0598] 128. The method of embodiments 125-127, wherein said target cell is selected from the group consisting of: a cancerous cell, a cancer stem cell, a T cell, a B cell, a melanoma cell, a lymphoma cell, a tumor cell, a pre-B cell, a pre-T cell, a basophil, a monocyte, and a macrophage.
- [0599] 129. A method of prevention, management, treatment or diagnosis of acute or chronic diseases in a patient using a protein of any of embodiments 1-95 or 116-124 (or any protein of the invention).
- [0600] 130. A method of prevention, management, treatment or diagnosis of acute or chronic diseases in a patient using the method of any of embodiments 107-115 or 125-128.
- [0601] 131. A method of using the protein of any of embodiments 1-95 or 116-124 (or any protein of the invention), in a mammal to reduce toxicity associated with exposure to one or more agents selected from the group consisting of abrin, brucine, cicutoxin, diphtheria toxin, botulism toxin, shiga toxin, endotoxin, tetanus toxin, pertussis toxin, anthrax toxin, cholera toxin, falcarinol, alfa toxin, geldanamycin, gelonin, lotaustralin, ricin, strychnine, snake venom toxin and tetratoxin.
- [0602] 132. A method of detecting and/or purifying at least one soluble compound from a solution using a protein of any of embodiments 1-95 or 116-124 (or any protein of the invention).
- [0603] 133. The method of embodiment 132, wherein said solution is a bodily fluid, cell culture media, fermentation media fluid, biological sample, or potable water.
- [0604] 134. The method of embodiment 133, wherein said bodily fluid is selected from the group consisting of blood, sweat, lymph, urine, tears, bile, saliva, serum, amniotic fluid, cerumen (earwax), Cowper's fluid, semen, chyle, chime, cerebrospinal fluid, stool, stool water, pancreatic juice, synovial fluid, aqueous humor, interstitial fluid, breast milk, mucus, pleural fluid, pus, sebum, and vomit.
- [0605] 135. A method of reducing toxicity of a cytokine storm in a mammal using the protein of any of embodiments 1-95 or 116-124 (or any protein of the invention).
- [0606] 136. A method of reducing therapy-induced toxicity in a mammal using the protein of any of embodiments 1-95 or 116-124 (or any protein of the invention), wherein said therapy is a biological therapy.
- [0607] 137. The method of embodiment 136, wherein said protein comprises at least one epitope binding domain derived from said biological therapy.

[0608] 138. The method of embodiment 136, wherein said proteins comprises at least one epitope binding domain that competes with at least one component of said biological therapy.

[0609] 139. The protein of any of embodiments 1-95 or 116-124 (or any protein of the invention), wherein said protein exhibits a half-life of at least 1 day when administered to a mammal.

[0610] 140. The protein of any of embodiments 1-95 or 116-124 (or any protein of the invention), wherein said Fc region is directly linked to at least one additional domain.

[0611] 141. The protein of any of embodiments 1-95 or 116-124 (or any protein of the invention), wherein said Fc region is indirectly linked to at least one additional domain.

[0612] In addition, the following United States provisional patent applications: 60/935,199 filed Jul. 31, 2007, 61/012,656 filed Dec. 10, 2007 and 61/074,330 filed Jun. 20, 2008 are hereby incorporated by reference herein in their entireties for all purposes.

8. EXAMPLES

[0613] The following examples serve merely to illustrate the invention and are not intended to limit the invention in any way.

Example 1

Expression of Proteins Comprising scFv Domains Fused to an Fc Region

[0614] Purpose: To demonstrate high level expression of epitope binding proteins comprising scFv domains linked to Fc regions.

[0615] Methods: The vectors encoding 3F2-522-Fc region and 522-Fc region are used to transfect 293 F cells using the 293 Fectin™ reagent (Invitrogen Cat. 51-0031) and Freestyle™ media (Invitrogen Cat. 12338)+10% fetal bovine serum following the manufacturer's recommendations. The cells are fed on the third day post transfection and the supernatant are harvested on day six. Purification of the antibodies is accomplished via a protein A column and is followed by dialysis into PBS. The molecules are evaluated in the denatured and non-denatured forms on a protein gel to determine the size and relative purity of the protein.

[0616] Results: Presented in FIG. 7 are the results of a PAGE gel experiment wherein various proteins were subjected to (A) Non-denaturing or (B) denaturing conditions. Lanes 1 and 5 represent 3F2-522-Fc region (an example of 2 scFv domains linked to an Fc region) loaded at 1 µg/well. Lanes 2 and 6 represent 3F2-522-Fc region loaded at 4 µg/well. Lanes 3 and 7 represent an scFv-Fc region protein 522-Fc (an example of one scFv linked to an Fc region) loaded at 1 µg/well. Lanes 4 and 8 represent 522-Fc loaded at 4 µg/well. Lane M represents standard molecular weight markers (SeeBlue 2™). The production and subsequent purification of the proteins yielded at considerably pure product of the predicted size. These results demonstrate that the proteins 3F2-522scFv-Fc and 522-Fc can be made and purified to homogeneity using the above described methods.

Example 2

Multiple Epitope Binding Proteins Exhibit Specificity for Target Antigens

[0617] Purpose: To evaluate the ability of the certain proteins to bind target antigens