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

PUBLICACIÓN: Gaceta 647 de 29 de Junio de 2012, N° de publicación 824.

PRIORIDAD: US 61/174,711 DE 01/05/2009;

SOLICITANTE: ABBOTT LABORATORIES

APODERADO: ALICIA LLOREDA RICAURTE

TRÁMITE: SUSTENTACIÓN 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 27 de Septiembre de 2012, en los siguientes términos:

I. RATIFICACIÓN

Comendidamente me permito insistir en los argumentos planteados en la oposición respectiva, los cuales, como se dijo en su momento, demuestran que la solicitud de la referencia incumple los requisitos de patentabilidad establecidos por los artículos 14 y 18 de la Decisión 486 de 2000, y que por ello no merece recibir el beneficio patentario.

La mencionada solicitud colombiana en trámite en su capítulo reivindicatorio revela como eje central de sus reclamaciones, una proteína con dos dominios provenientes de dos orígenes los cuales pueden corresponder al mismo dominio, ya que permite unirse a la misma proteína de unión (p.e. reivindicación 2, la proteína se une a epítope de EGFR y EGFR, lo cual incluye aquellas moléculas comprendidas en anticuerpos monoclonales tales como Cetuximab).

Como menciono en mi memorial de oposición, lo reclamado por la solicitud en estudio obedece a una fórmula general ya conocida en el estado de la técnica y que presenta una selección o escogencia de grupos funcionales posibles en la misma, dependiendo de combinaciones arbitrarias que no cumplen los mínimos requisitos para ser objeto de patente.

Así, al ser reclamaciones de funcionalidad, los anticuerpos no están definidos de forma clara y precisa así como tampoco podrían tener sustento en la descripción, adicionalmente, porque la extrapolación realizada al tipo de moléculas abarca una amplitud que excede lo ejemplificado por la solicitud. También se cuestionó la unidad de invención y la concisión en las reivindicaciones.

Esto en consecuencia, implica que las composiciones que contienen anticuerpos duales, que pueden ser reivindicadas por la solicitud anti—EGFR o cualquier otro dominio de unión a determinado receptor, no presentarían tampoco novedad y/o nivel inventivo.

En mi memorial de oposición presenté el documento del estado de la técnica:

US 2007/0071675 “*Dual variable domain immunoglobulin and uses thereof*” que de ahora en adelante se referirá como Wu et al., el cual describe anticipadamente todo lo descrito por la solicitud en estudio, mencionando los dominios de variable dual, las sustituciones en los aminoácidos, incluso la misma fórmula general de la proteína presentada.

Dicho documento publicado por Wu et al., demuestra la falta de novedad y nivel inventivo de la solicitud colombiana en trámite al divulgar que la proteína de unión es capaz de unirse a un par seleccionado del grupo que consiste de: EGFR y CD-3; EGFR e IGF1R; EGFR y RON; EGFR y HGF; VEGF y EGFR; EGFR y ErbB3,

EGFR y DLL-4, EGFR y PLGF, EGFR y EGFR, EGFR y RGMa, EGFR y toxoide de tétanos; VEGF y toxoide de tétanos; toxoide de tétanos y toxoide de tétanos (ver párrafo 0040).

Por su parte, el documento de Adair et al., (US 2003/0039645 "HUMANISED ANTIBODIES") fue mostrado como prueba ya que describe un dominio variable que es 100% homólogo a la SEQ ID No. 27 (ver SEQ ID No. 30).

Este documento afecta los requisitos de patentabilidad de la presente solicitud dado que describe moléculas de anticuerpo con especificidad para determinado receptor (como por ejemplo para TNF alfa), pero con el diseño general de los anticuerpos en las variaciones de las CDRs necesarias para el desarrollo de nuevas secuencias por el simple cambio de residuos de aminoácido comunes. En ésta anterioridad se menciona la técnica para realizar anticuerpos quiméricos a partir de librerías de CDRs, expresión y construcción de genes, secuenciación, expresión en células CHO, y toda la mecánica ya conocida de diseño de anticuerpos según la afinidad a un receptor.

II. COMPLEMENTO DE INFORMACION

Adicional a los dos documentos mencionados anteriormente, se pone de presente el documento:

- WO 2005/120557 de Pereira et al., publicado el 22 de diciembre de 2005.

El cual describe métodos para tratar tumores en mamíferos que comprende la administración de anticuerpos específicos; la molécula objetivo en el caso de dicha anterioridad es la proteína receptora de estimulación de Macrófagos (MSP-R o RON).

Pereira et al., además describe la forma de realizar anticuerpos duales que formen un diacuerpo conocido como un dímero bivalente, esto es descrito en el párrafo 29 de dicha anterioridad (observar el siguiente aparte):

[29] Two single chain antibodies can be combined to form a diabody, also known as a bivalent dimer. Diabodies have two chains and two binding sites, and can be monospecific or bispecific. Each chain of the diabody includes a V_H domain connected to a V_L domain. The domains are connected with linkers that are short enough to prevent

pairing between domains on the same chain, thus driving the pairing between complementary domains on different chains to recreate the two antigen-binding sites.

Adicionalmente, dicha anterioridad enseña un dominio variable que es 100% homólogo a la secuencia SEQ ID No 29 (SEQ ID NO 7) (observar página 80 de la solicitud internacional –equivalente a la solicitud colombiana en trámite- y comparar con la SEQ ID NO 7 de la anterioridad en la figura 2), las cuales corresponden a la secuencia de aminoácidos de la región variable de cadena pesada obedeciendo a una de las cadenas de mayor importancia en la actividad del anticuerpo.

Solicitud SEC ID No 29:

EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISY
DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARFSGWPNNYY
YYGMDVWGQGTTVTVSS

Anterioridad Pereira et al SEC ID NO 7

EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISY
DGSNKYYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARFSGWPNNYY
YYGMDVWGQGTTVTVSS

Dado que la secuencia SEQ ID No 29 se ha reclamado en la reivindicación 1 de la presente solicitud como miembro de un anticuerpo y como única parte caracterizante del mismo, la solicitud no cumple con los requisitos de novedad y nivel inventivo.

En este sentido, y en la medida en que se seleccione otra secuencia del grupo reivindicado, también sería obvio para un técnico medio versado en la materia, emplear las secuencias de Adair o la secuencia pesada del documento de Pereira et al, en un anticuerpo dual con la fórmula ya descrita por el documento de Wu et al., con el fin de obtener un anticuerpo dual que se una a la molécula deseada.

La selección de dichas secuencias debería tener un criterio unificado y no la escogencia arbitraria de compuestos del estado de la técnica; Así como las secuencias fueron reclamadas inicialmente de forma arbitraria en la solicitud, tampoco la eliminación arbitraria de alguna secuencia para cumplir con los requisitos de novedad debería ser aceptable a menos que en la descripción hubiera sido explicado esta discriminación (ya que esto obedecería a un disclaimer).

Ahora, para enfatizar aún más sobre lo divulgado por el estado de la técnica, el documento de Wu et al., no solo enseña la misma materia en términos de anticuerpos duales y su estructura sino que también presenta las misma fórmula bajo las mismas premisas reclamadas por la solicitud en trámite como se observa en el párrafo 0015:

[0015] In a preferred embodiment the binding protein disclosed above comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C-(X2)_n, wherein VD1 is a first light chain variable domain, VD2 is a second light chain variable domain, C is a light chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 does not comprise an Fc region.

Con relación a las modalidades particulares del objeto solicitado, el documento de Wu et al., enseña una proteína de unión que es capaz de unirse a un par seleccionado del grupo que consiste de EGFR y CD-3; EGFR e IGFIR; EGFR y RON; EGFR y HGF; VEGF y EGFR; EGFR y ErbB3, EGFR y DLL-4, EGFR y PLGF, EGFR y EGFR, EGFR y RGMa, EGFR y toxoide tetánico, VEGF y toxoide tetánico, y toxoide tetánico y toxoide tetánico.

[0040] In another embodiment the binding proteins of the invention are capable of binding one or more targets selected from the group consisting of ABCF1; ACVR1; ACVR1B; ACVR2; ACVR2B; ACVRL1; ADORA2A; Aggrecan; AGR2; AICDA; AIF1; AIG1; AKAP1; AKAP2; AMH; AMHR2; ANGPT1; ANGPT2; ANGPTL3; ANGPTL4; ANPEP; APC; APOC1; AR; AZGP1 (zinc- α -glycoprotein); B7.1; B7.2; BAD; BAFF; BAG1; BAI1; BCL2; BCL6; BDNF; BLNK; BLR1 (MDR15); BlyS; BMP1; BMP2; BMP3B (GDF10); BMP4; BMP6; BMP8; BMPR1A; BMPR1B; BMPR2; BPAG1 (plectin); BRCA1; C19orf10 (IL27w); C3; C4A; C5; C5R1; CANT1; CASP1; CASP4; CAV1; CCBP2 (D6/JAB61); CCL1 (1-309); CCL11 (eotaxin); CCL13 (MCP-4); CCL15 (MIP-1d); CCL16 (HCC-4); CCL17 (TARC); CCL18 (PARC); CCL19 (MIP-

...

(ver la continuación de este párrafo en el documento)

Particularmente también el documento de Wu et al., enseña que dichas primeras porciones de unión al antígeno, y dichas segundas porciones de unión al antígeno son seleccionadas del grupo que consiste de anticuerpos humanos, anticuerpos con CDRs seleccionadas, anticuerpos humanizados, etc. (párrafo 0012, 0067-0068, 0073)

En vista de todo lo anterior, es necesario enfatizar que en el estado de la técnica ya se revelaban las características técnicas esenciales de lo reclamado por el solicitante, de manera tal que las reivindicaciones reclaman una fórmula ya conocida en el estado de la técnica para obtener anticuerpos duales seleccionando los sitios de unión de los mismos que se han venido trabajando usualmente y en consecuencia la solicitud en trámite no cumple con el criterio de Novedad y mucho menos puede atribuírsele Nivel Inventivo.

Para terminar, la solicitud se basa en resultados hipotéticos sin tener una clara definición de la estructura de las composiciones o las mejoras existentes definiendo una fórmula general y seleccionando un grupo de receptores a los cuales se les puede unir la molécula, pero sin definir su estructura.

En conclusión, ya que los documentos observados en el presente escrito mencionan todas las características técnicas esenciales de los anticuerpos duales,

e incluso las secuencias reclamadas, la solicitud no cumple con los criterios de Novedad o Nivel inventivo

III. ANEXOS

- ❖ La patente americana US 2007/0071675 (Wu et al.) publicada el 29 de marzo de 2007. Portada, páginas 2, 4, 17, 42, 44, 48, 121-124
- ❖ La publicación internacional WO 2005120557 (Pereira et al.) publicado el 22 de diciembre de 2005. Portada, y página 53.
- ❖ La patente americana US 2003/0039645 (Adair et al.) publicada el 27 de febrero de 2003. Portada y página 54.

Señor Superintendente,



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(54) **DUAL VARIABLE DOMAIN
IMMUNOGLOBULIN AND USES THEREOF**

Publication Classification

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435/254.21; 435/348; 536/23.53

(21) Appl. No.: **11/507,050**

(22) Filed: **Aug. 18, 2006**

(57) **ABSTRACT**

Related U.S. Application Data

(60) Provisional application No. 60/709,911, filed on Aug.
19, 2005. Provisional application No. 60/732,892,
filed on Nov. 2, 2005.

The present invention relates to engineered multivalent and
multispecific binding proteins, methods of making, and
specifically to their uses in the prevention and/or treatment
of acute and chronic inflammatory and other diseases.

a single cell inactive homodimers can be produced together with active heterodimers. This necessitates the implementation of additional purification steps in order to obtain homogenous preparations of bispecific diabodies. One approach to force the generation of bispecific diabodies is the production of knob-into-hole diabodies (see Holliger, P., T. Prospero, and G. Winter, *Proc Natl Acad Sci USA*, 1993. 90(14): p. 6444-8.18). This was demonstrated for a bispecific diabody directed against HER2 and CD3. A large knob was introduced in the VH domain by exchanging Val37 with Phe and Leu45 with Trp and a complementary hole was produced in the VL domain by mutating Phe98 to Met and Tyr87 to Ala, either in the anti-HER2 or the anti-CD3 variable domains. By using this approach the production of bispecific diabodies could be increased from 72% by the parental diabody to over 90% by the knob-into-hole diabody. Importantly, production yields did only slightly decrease as a result of these mutations. However, a reduction in antigen-binding activity was observed for several analyzed constructs. Thus, this rather elaborate approach requires the analysis of various constructs in order to identify those mutations that produce heterodimeric molecule with unaltered binding activity. In addition, such approach requires mutational modification of the immunoglobulin sequence at the constant region, thus creating non-native and non-natural form of the antibody sequence, which may result in increased immunogenicity, poor in vivo stability, as well as undesirable pharmacokinetics.

[0008] Single-chain diabodies (scDb) represent an alternative strategy to improve the formation of bispecific diabody-like molecules (see Holliger, P. and G. Winter, *Cancer Immunol Immunother*, 1997. 45(3-4): p. 128-30; Wu, A. M., et al., *Immunotechnology*, 1996. 2(1): p. 21-36). Bispecific single-chain diabodies are produced by connecting the two diabody-forming polypeptide chains with an additional middle linker with a length of approximately 15 amino acid residues. Consequently, all molecules with a molecular weight corresponding to monomeric single-chain diabodies (50-60 kDa) are bispecific. Several studies have demonstrated that bispecific single chain diabodies are expressed in bacteria in soluble and active form with the majority of purified molecules present as monomers (see Holliger, P. and G. Winter, *Cancer Immunol Immunother*, 1997. 45(34): p. 128-30; Wu, A. M., et al., *Immunotechnology*, 1996. 2(1): p. 21-36; Pluckthun, A. and P. Pack, *Immunotechnology*, 1997. 3(2): p. 83-105; Ridgway, J. B., et al., *Protein Eng*, 1996. 9(7): p. 617-21). Thus, single-chain diabodies combine the advantages of tandem scFvs (all monomers are bispecific) and diabodies (soluble expression in bacteria).

[0009] More recently diabody have been fused to Fc to generate more Ig-like molecules, named di-diabody (see Lu, D., et al., *J Biol Chem*, 2004. 279(4): p. 2856-65). In addition, multivalent antibody construct comprising two Fab repeats in the heavy chain of an IgG and capable of binding four antigen molecules has been described (see WO 0177342A1, and Miller, K., et al., *J Immunol*, 2003. 170(9): p. 4854-61).

[0010] There is a need in the art for improved multivalent binding proteins capable of binding two or more antigens. The present invention provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

SUMMARY OF THE INVENTION

[0011] This invention pertains to multivalent binding proteins capable of binding two or more antigens. The present invention provides a novel family of binding proteins capable of binding two or more antigens with high affinity.

[0012] In one embodiment the invention provides a binding protein comprising a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C(X2)_n, wherein VD1 is a first variable domain, VD2 is a second variable domain, C is a constant domain, X1 represents an amino acid or polypeptide, X2 represents an Fc region and n is 0 or 1. In a preferred embodiment the VD1 and VD2 in the binding protein are heavy chain variable domains. More preferably the heavy chain variable domain is selected from the group consisting of a murine heavy chain variable domain, a human heavy chain variable domain, a CDR grafted heavy chain variable domain, and a humanized heavy chain variable domain. In a preferred embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. Preferably C is a heavy chain constant domain. More preferably X1 is a linker with the proviso that X1 is not CH1. Most preferably X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTP; AKTTPPSVTPLAP; AKTTAP; AKTTAPSVYPLAP; ASTKGP; ASTKGPSVFPLAP; GGGGSGGGSGGGGS; GENKVEYAPALMALS; GPAKELTPLKEAKVS; and GHEAAAVMQVQYPAS. Preferably X2 is an Fc region. More preferably X2 is a variant Fc region.

[0013] In a preferred embodiment the binding protein disclosed above comprises a polypeptide chain, wherein said polypeptide chain comprises VD1-(X1)_n-VD2-C(X2)_n, wherein VD1 is a first heavy chain variable domain, VD2 is a second heavy chain variable domain, C is a heavy chain constant domain, X1 is a linker with the proviso that it is not CH1, and X2 is an Fc region.

[0014] In another embodiment VD1 and VD2 in the binding protein are light chain variable domains. Preferably the light chain variable domain is selected from the group consisting of a murine light chain variable domain, a human light chain variable domain, a CDR grafted light chain variable domain, and a humanized light chain variable domain. In one embodiment VD1 and VD2 are capable of binding the same antigen. In another embodiment VD1 and VD2 are capable of binding different antigens. Preferably C is a light chain constant domain. More preferably X1 is a linker with the proviso that X1 is not CL1. Preferably X1 is a linker selected from the group consisting of AKTTPKLEEGEFSEAR; AKTTPKLEEGEFSEARV; AKTTPKLGG; SAKTTPKLGG; AKTTPKLEEGEFSEARV; SAKTTP; SAKTTPKLGG; RADAAP; RADAAPTVS; RADAAAAGGPGS; RADAAA(G₄S)₄; SAKTTP; SAKTTPKLGG; SAKTTPKLEEGEFSEARV; ADAAP; ADAAPTVSIFPP; TVAAP; TVAAPSVFIFPP; QPKAAP; QPKAAPSVTLFPP; AKTTP; AKTTPPSVTPLAP; AKTTAP; AKTTAPSVYPLAP; ASTKGP; and ASTKGPSVFPLAP. Preferably the binding protein does not comprise X2.

1a), CCL4 (MIP-1b), CCL5 (RANTES), CCL7 (MCP-3), CCL8 (mcp-2), CCL11 (eotaxin), CCL13 (MCP-4), CCL15 (MIP-1d), CCL16 (HCC-4), CCL17 (TARC), CCL18 (PARC), CCL19 (MIP-3b), CCL20 (MIP-3a), CCL21 (SLC/exodus-2), CCL22 (MDC/STC-1), CCL23 (MPIF-1), CCL24 (MPIF-2/eotaxin-2), CCL25 (TECK), CCL26 (eotaxin-3), CCL27 (CTACK/ILC), CCL28, CXCL1(GRO1), CXCL2 (GRO2), CXCL3 (GRO3), CXCL5 (ENA-78), CXCL6 (GCP-2), CXCL9 (MIG), CXCL10 (IP 10), CXCL11 (I-TAC), CXCL12 (SDF1), CXCL13, CXCL14, CXCL16, PF4 (CXCL4), PPBP (CXCL7), CX3CL1 (SCYD1), SCYE1, XCL1 (lymphotactin), XCL2 (SCM-1b), BLR1 (MDR15), CCBP2 (D6/JAB61), CCR1 (CKR1/HM145), CCR2 (mcp-1RB/RA), CCR3 (CKR3/CMKBR3), CCR4, CCR5 (CMKBR5/ChemR13), CCR6 (CMKBR6/CKR-L3/STRL22/DRY6), CCR7 (CKR7/EBI1), CCR8 (CMKBR8/TER1/CKR-L1), CCR9 (GPR-9-6), CCRL1 (VSHK1), CCRL2 (L-CCR), XCR1 (GPR5/CCXCR1), CMKLR1, CMKOR1 (RDC1), CX3CR1 (V28), CXCR4, GPR2 (CCR10), GPR31, GPR81 (FKSG80), CXCR3 (GPR9/CKR-L2), CXCR6 (TYMSTR/STRL33/Bonzo), HM74, IL8RA (IL8Ra), IL8RB (IL8Rb), LTB4R (GPR16), TCP10, CKLFSF2, CKLFSF3, CKLFSF4, CKLFSF5, CKLFSF6, CKLFSF7, CKLFSF8, BDNF, CSF1, CSF3, GRCC10 (C10), EPO, FY (DARC), GDF5, HIF1A, IL8, PRL, RGS3, RGS13, SDF2, SLIT2, TLR2, TLR4, TREM1, TREM2, and VHL. The binding protein of the invention is capable of binding cell surface protein selected from the group consisting of integrins. The binding protein of the invention is capable of binding enzyme selected from the group consisting of kinases and proteases. The binding protein of the invention is capable of binding receptor selected from the group consisting of lymphokine receptor, monokine receptor, and polypeptide hormone receptor.

[0023] In a preferred embodiment the binding protein is multivalent. More preferably the binding protein is multispecific. The multivalent and or multispecific binding proteins described above have desirable properties particularly from a therapeutic standpoint. For instance, the multivalent and or multispecific binding protein may (1) be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind; (2) be an agonist antibody; and/or (3) induce cell death and/or apoptosis of a cell expressing an antigen which the multivalent antibody is capable of binding to. The "parent antibody" which provides at least one antigen binding specificity of the multivalent and or multispecific binding proteins may be one which is internalized (and/or catabolized) by a cell expressing an antigen to which the antibody binds; and/or may be an agonist, cell death-inducing, and/or apoptosis-inducing antibody, and the multivalent and or multispecific binding protein as described herein may display improvement(s) in one or more of these properties. Moreover, the parent antibody may lack any one or more of these properties, but may be endowed with them when constructed as a multivalent binding protein as hereindescribed.

[0024] In another embodiment the binding protein of the invention has an on rate constant (Kon) to one or more targets selected from the group consisting of: at least about $10^4 \text{M}^{-1} \text{s}^{-1}$; at least about $10^5 \text{M}^{-1} \text{s}^{-1}$; at least about $10^6 \text{M}^{-1} \text{s}^{-1}$; at least about $10^7 \text{M}^{-1} \text{s}^{-1}$; at least about $10^8 \text{M}^{-1} \text{s}^{-1}$; and at least about $10^9 \text{M}^{-1} \text{s}^{-1}$, as measured by surface plasmon resonance. Preferably, the binding protein of the invention has an on rate constant

(Kon) to one or more targets between $10^2 \text{M}^{-1} \text{s}^{-1}$ to $10^3 \text{M}^{-1} \text{s}^{-1}$; between $10^3 \text{M}^{-1} \text{s}^{-1}$ to $10^4 \text{M}^{-1} \text{s}^{-1}$; between $10^4 \text{M}^{-1} \text{s}^{-1}$ to $10^5 \text{M}^{-1} \text{s}^{-1}$; or between $10^5 \text{M}^{-1} \text{s}^{-1}$ to $10^6 \text{M}^{-1} \text{s}^{-1}$, as measured by surface plasmon resonance.

[0025] In another embodiment the binding protein has an off rate constant (Koff) for one or more targets selected from the group consisting of: at most about 10^{-3}s^{-1} ; at most about 10^{-4}s^{-1} ; at most about 10^{-5}s^{-1} ; and at most about 10^{-6}s^{-1} , as measured by surface plasmon resonance. Preferably, the binding protein of the invention has an off rate constant (Koff) to one or more targets of 10^{-3}s^{-1} to 10^{-4}s^{-1} ; of 10^{-4}s^{-1} to 10^{-5}s^{-1} ; or of 10^{-5}s^{-1} to 10^{-6}s^{-1} , as measured by surface plasmon resonance.

[0026] In another embodiment the binding protein has a dissociation constant (K_D) to one or more targets selected from the group consisting of: at most about 10^{-7}M ; at most about 10^{-8}M ; at most about 10^{-9}M ; at most about 10^{-10}M ; at most about 10^{-11}M ; at most about 10^{-12}M ; and at most 10^{-13}M . Preferably, the binding protein of the invention has a dissociation constant (K_D) to IL-12 or IL-23 of 10^{-7}M to 10^{-8}M ; of 10^{-8}M to 10^{-9}M ; of 10^{-9}M to 10^{-10}M ; of 10^{-10}M to 10^{-11}M ; of 10^{-11}M to 10^{-12}M ; or of 10^{-12}M to 10^{-13}M .

[0027] In another embodiment the binding protein described above is a conjugate further comprising an agent selected from the group consisting of; an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent. Preferably the imaging agent is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. More preferably the imaging agent is a radiolabel selected from the group consisting of: ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm . Preferably the therapeutic or cytotoxic agent is selected from the group consisting of; an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

[0028] In another embodiment the binding protein described above is a crystallized binding protein and exists as a crystal. Preferably the crystal is a carrier-free pharmaceutical controlled release crystal. More preferably the crystallized binding protein has a greater half life in vivo than the soluble counterpart of said binding protein. Most preferably the crystallized binding protein retains biological activity.

[0029] In another embodiment the binding protein described above is glycosylated. Preferably the glycosylation is a human glycosylation pattern.

[0030] One aspect of the invention pertains to an isolated nucleic acid encoding any one of the binding protein disclosed above. A further embodiment provides a vector comprising the isolated nucleic acid disclosed above wherein said vector is selected from the group consisting of pcDNA; pTT (Durocher et al., *Nucleic Acids Research* 2002, Vol 30, No. 2); pTT3 (pTT with additional multiple cloning site); pEFBOS (Mizushima, S. and Nagata, S., (1990) *Nucleic acids Research* Vol 18, No. 17); pBV; pJV; pcDNA3.1 TOPO, pEF6 TOPO and pBJ.

[0031] In another aspect a host cell is transformed with the vector disclosed above. Preferably the host cell is a prokaryotic cell. More preferably the host cell is *E. Coli*. In a related embodiment the host cell is an eukaryotic cell. Preferably

to make the parent antibodies include those disclosed in Witttrup, et al. U.S. Pat. No. 6,699,658 incorporated herein by reference.

[0108] The antibodies described above can be further modified to generate CDR grafted and Humanized parent antibodies. CDR-grafted parent antibodies comprise heavy and light chain variable region sequences from a human antibody wherein one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of murine antibodies capable of binding antigen of interest. A framework sequence from any human antibody may serve as the template for CDR grafting. However, straight chain replacement onto such a framework often leads to some loss of binding affinity to the antigen. The more homologous a human antibody is to the original murine antibody, the less likely the possibility that combining the murine CDRs with the human framework will introduce distortions in the CDRs that could reduce affinity. Therefore, it is preferable that the human variable framework that is chosen to replace the murine variable framework apart from the CDRs have at least a 65% sequence identity with the murine antibody variable region framework. It is more preferable that the human and murine variable regions apart from the CDRs have at least 70% sequence identity. It is even more preferable that the human and murine variable regions apart from the CDRs have at least 75% sequence identity. It is most preferable that the human and murine variable regions apart from the CDRs have at least 80% sequence identity. Methods for producing such antibodies are known in the art (see EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,352).

[0109] Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez/query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/about.pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH-05/kuby05.html; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/about.mrc7/mikeimages.html; www.antibodyresource.com/mcb.harvard.edu/BioLinks/Immunology.html; www.immunologylink.com/; pathbox.wustl.edu/about.hcenter/index.html; www.biotech.ufl.edu/about.hcl/; www.pebio.com/pa/340913/340913.html; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.ac.jp/about.yasuhito/-Eli sa.html; www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/lin-ks.html; www.biotech.ufl.edu/about.fccl/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/about.rek/AEP-Start.html; baserv.uci.kun.nl/aboutjraats/linksl.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/pu-blic/INTRO.html; www.ibt.unam.mx/virV_mice.html; imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/

.about.martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/about.honegger/AHOseminar/Slide01.html; www.cryst.bbk.ac.uk/about.ubcg07s/; www.nimr.mrc.ac.uk/CC/caewg/caewg.htm; www.path.cam.ac.uk/about.mrc7/humanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/abo-ut.fmolina/Webpages/Pept/spottech.html; www.jerini.de/frroducts.htm; www.patents.ibm.com/ibm.html. Kabat et al., *Sequences of Proteins of Immunological Interest*, U.S. Dept. Health (1983), each entirely incorporated herein by reference. Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art.

[0110] Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entirety.) Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, such as but not limited to those described in Jones et al., *Nature* 321:522 (1986); Verhoeven et al., *Science* 239:1534 (1988); Sims et al., *J. Immunol.* 151: 2296 (1993); Chothia and Lesk, *J. Mol. Biol.* 196:901 (1987); Carter et al., *Proc. Natl. Acad. Sci. U.S.A.* 89:4285 (1992); Presta et al., *J. Immunol.* 151:2623 (1993); Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994); PCT publication WO 91/09967, PCT: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, EP 592,106; EP 519,596, EP 239,400, U.S. Pat. Nos. 5,565,332, 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5,814,476, 5,763,192, 5,723,323, 5,766,886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; 4,816,567, each entirely incorporated herein by reference, included references cited therein.

[0111] Parent monoclonal antibodies may be selected from various monoclonal antibodies capable of binding specific targets and well known in the art. These include, but are not

subcloned into pcDNA3.1 TOPO mammalian expression vector (Invitrogen) according to the manufacturer's instructions. Table 4 shows the PCR primers' sequences:

TABLE 4

P5: 5' ATG GCT TGG GTG TGG ACC TTG C 3'	SEQ ID NO. 17
P6: 5' GGG CCC TTG GTC GAC GCT GAG GAG 3'	SEQ ID NO. 18
P7: 5' GCG TCG ACC AAG GGC CCA TCG GTC TTC C 3'	SEQ ID NO. 19
P8: 5' TC ATT TAC CCG GAG ACA GGG AGA GGC 3'	SEQ ID NO. 20

[0237] Similarly, chimeric 13F5.G5-VH-C γ l was generated using primers P21/P22 (for VH) and P7/P8 (for hC γ l) and cloned into pcDNA3.1 TOPO vector, and chimeric 13F5.G5-VL-C κ was generated using primers P23/P24 (for VL) and P3/P4 (for hC κ) and cloned into pEF6 TOPO vector. Table 5 shows the PCR primers' sequences:

TABLE 5

P215' ATA GAA TGG AGC TGG GTT TTC CTC 3'	SEQ ID NO. 21
P225' GGG CCC TTG GTC GAC GC TGA GGA GAC GGT GAC TGA 3'	SEQ ID NO. 22
P235' ATG GTC CTC ATG TCC TTG CTG TTC 3'	SEQ ID NO. 23
P245' GC AGC CAC CGT ACG CCG TTT TAT TTC CAG CTT TG 3'	SEQ ID NO. 24

[0238] To express chimeric Abs, 13F5.G5-VL-C κ and 13F5.G5-VH-C γ l were co-expressed in COS using Lipofectamin (Invitrogen) for 72 hr, and the medium collected and IgG purified by Protein A chromatography. Similarly, 13F5.G5-VL-C κ and 13F5.G5-VH-C γ l were co-expressed in COS using Lipofectamin (Invitrogen) for 72 hr, and the medium collected and IgG purified by Protein A chromatography. Both purified chimeric Abs were characterized by Biacore and MRC-5 bioassay to confirm activity. The results showed that these chimeric Abs displayed similar affinity and potency to that of the original murine mAbs.

Example 1.3

Construction, Expression, and Purification of IL-1 α / β Dual Variable Domain Immunoglobulin (DVD-Ig)

[0239] The construct used to generate DVD-Ig capable of binding hIL-1 α and hIL-1 β is illustrated in FIG.1B. Briefly, parent mAbs including two high affinity murine Abs, anti-hIL-1 α (clone 3D12.E3) and anti-hIL-1 β (clone 13F5.G5), were obtained by immunizing Balb/c mice with recombinant IL-1 α protein (rhIL-1 α) and recombinant IL-1 β protein (rhIL-1 β), respectively. The VL/VH genes of these two hybridoma clones were isolated by RT-PCR using the mouse Ig Primer Kit (Novagen, Madison, Wis.). The VL/VH genes were first converted into chimeric antibodies (with human constant regions) to confirm activity and potency. To gen-

erate DVD1-Ig, the VH and VL of 13F5.G5 was directly fused to the N-terminus of the VH and VL of 3D12.E3, respectively (as shown in FIG.1B). The DVD2-Ig was constructed similarly, except that it had a linker between the two variable domains in both the light chain (the linker sequence is ADAAP) and the heavy chain (the linker sequence is AKTTPP). These sequences were selected from the N-termini of murine C κ and CH1 sequences. These linker sequences, selected from the N-termini of murine C κ and CH1, are natural extension of the variable domains and exhibit a flexible conformation without significant secondary structures based on the analysis of several Fab crystal structures. The detailed procedures of the PCR cloning is described below:

Example 1.3.A

Molecular Cloning of hIL-1 α /bDVD1-Ig

[0240] 13F5.G5-VH was PCR amplified using primers P21 and P25; meanwhile 3D12.E3-VH-hC γ l was amplified using primers P14 and P8. Both PCR reactions were performed according to standard PCR techniques and procedures. The two PCR products were gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using primers P21 and P8 using standard PCR conditions. The final PCR product, the DVD1-Ig heavy chain hIL-1 α /bDVD1-VH-hC γ l, was subcloned into pcDNA3.1 TOPO mammalian expression vector (Invitrogen) according to the manufacturer's instructions. Table 6 shows the PCR primers' sequences:

TABLE 6

P14: 5' CAG ATC CAG TTG GTG CAG TCT 3'	SEQ ID NO. 25
P25: 5' CAC CAA CTG GAT CTG TGA GGA GAC GGT GAC TGA GG 3'	SEQ ID NO. 26

[0241] To generate hIL-1 α /bDVD1-Ig light chain, 13F5.G5-VL was PCR amplified using primers P23 and P26; meanwhile 3D12.E3-VL-hC κ was amplified using primers P16 and P4. Both PCR reactions were performed according to standard PCR techniques and procedures. The two PCR products were gel-purified, and used together as overlapping template for the subsequent overlapping PCR reaction using primers P23 and P4 using standard PCR conditions. The final PCR product, the hIL-1 α /bDVD1-Ig light chain hIL-1 α /bDVD1-VL-hC κ , was subcloned into pEF6 TOPO mammalian expression vector (Invitrogen) according to the manufacturer's instructions. Table 7 shows the PCR primers' sequences:

TABLE 7

P16: 5' AAT ATC CAG ATG ACA CAG ACT 3'	SEQ ID NO. 27
P26: 5' GTGT CAT CTG GAT ATT CCG TTT TAT TTC CAG CTT TG 3'	SEQ ID NO. 28

Example 1.3.B

Molecular Cloning of hIL-1 α /bDVD2-Ig

[0242] 13F5.G5-VH was PCR amplified using primers P21 and P17; meanwhile 3D12.E3-VH-hC γ l was amplified

TABLE 10-continued

Amino acid sequence of hIL-1α/βDVD1-Ig and hIL-1α/βDVD2-Ig		
Protein	Sequence	Sequence
Protein region	Identifier	12345678901234567890
		KVDNALQSGNSQESVTEQDS KDSTYSLSSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKS FNRGEC
DVD HEAVY VARIABLE hIL-1a/bDVD2-Ig	SEQ ID NO.:37	QVQLQQSGAELVRPGSSVKI SCKASGYAFSSYWNWVKQR PGQGLEWICQIYPGDGDTNY NGKFKGKATLTADKSSSTSY MQLSGLTSEDSAMYFCVRFPP TGNDYYAMDYWGQGTSTVTS SAKTTTPPQIQLVQSGPELKK PGETVKISCKASGYTFRNYG MNWVKQAPGKDLKRMAMINT YTGESTYADDFKGRFAFSL TSASTAYLQINNLIKNEATAT YFCARGIYYGSSYAMDYWG QGTSTVTSS
13F5.G5 VH	SEQ ID NO.:7	QVQLQQSGAELVRPGSSVKI SCKASGYAFSSYWNWVKQR PGQGLEWIGQIYPGDGDTNY NGKFKGKATLTADKSSSTSY MQLSGLTSEDSAMYFCVRFPP TGNDYYAMDYWGQGTSTVTS S
Linker	SEQ ID NO.:38	AKTTPP
3D12.E3 VH	SEQ ID NO.:1	QIQLVQSGPELKKPGETVKI SCKASGYTFPNYGMNWVKQA PGKDLKRMAMTNTYTGESTY ADDFKGRFAFSLSTASTAY LQINNLIKNEATATYFCARGI YYGSSYAMDYWGQGTSTVTS SS
CH	SEQ ID NO.:34	ASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSS GLYSLSSVVTVPSSSLGTQT YICNVNHKPSNTKVDKKVEP KSCDKHTCPCPPAPELGG PSVFLFPPKPKDTLMISRTPE EVTCTVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPREEQYN STYRVVSVLTVQLQDWLNKG EYKCKVSNKALPAPIEKTIIS KAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDI AVEWESNGQPENNYKTTTPPV LDSGGSFPLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYT QKSLSLSPCK
DVD LIGHT VARIABLE hIL- 1a/bDVD2-Ig	SEQ ID NO.:39	NIVLTQSPASLAVSLGQRAT ISCPASESVDSYGNSYMHY QQKPGQPPKLLIYLASNLES GVPARFSGSGSRTDFTLTID PVEADDAATYYCQQNNEDPF TFGSGTKLEIKRADDAAPNIQ MTQTSSLSASLGDRVTIS RASQDISNCLNWKQKPDGT VKLLIYYTSRLHSGVPSRF SGSGTDYSLTISNLEQEDI ATYFCQQGKTLTPYAFGGGK LEINR
13F5.G5 VL	SEQ ID NO.:8	NIVLTQSPASLAVSLGQRAT ISCRASESVDSYGNSYMHY

TABLE 10-continued

Amino acid sequence of hIL-1α/βDVD1-Ig and hIL-1α/βDVD2-Ig		
Protein	Sequence	Sequence
Protein region	Identifier	12345678901234567890
		QQKPGQPPKLLIYLASNLES GVPARFSGSGSRTDFTLTID PVEADDAATYYCQQNNEDPF TFGSGTKLEIKR
Linker	SEQ ID NO.:40	ADAAP
3D12.E3 VL	SEQ ID NO.:2	NIQMTQTSSLSASLGDRVT ISCRASQDISNCLNWKQK DGTVKLLIYYTSRLHSGVPS RFSGSGSGTDYSLTISNLEQ EDIATYFCQQGKTLTPYAFGG GTKLEINR
CL	SEQ ID NO.:36	TVAAPSVPFIFPPSDEQLKSG TASVVCCLNNFYPREAKVQW KVDNALQSGNSQESVTEQDS KDSTYSLSSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKS FNRGEC

Example 1.3.C

Expression and Purification of hIL-1a/bDVD1-Igs

[0245] The heavy and light chain of each construct was subcloned into pcDNA3.1 TOPO and pEF6 TOPO vectors (Invitrogen Inc.), respectively, and sequenced to ensure accuracy. The plasmids encoding the heavy and light chains of each construct were transiently expressed using Lipofectamine 2000 and 293 fectin reagents, respectively in COS cells as well as human embryonic kidney 293 cells (American Type Culture Collection, Manassas, Va.). The cell culture media was harvested 72 hr-post transient transfection and antibodies purified using protein A chromatography (Pierce, Rockford, Ill.) according to manufacturer's instructions. The Abs were analyzed by SDS-PAGE and quantitated by A280 and BCA (Pierce, Rockford, Ill.). Table 11 shows that the expression levels of hIL-1a/bDVD1-Ig and hIL-1a/bDVD2-Ig are comparable to that of the chimeric Abs, indicating that the DVD-Ig can be expressed efficiently in mammalian cells.

TABLE 11

Expression and molecular weight analysis of hIL-1a/bDVD-Ig					
	Expression level (ng/ml)		Molecular mass (Dalton)		
	COS	Freestyle 293	Light Chain	Heavy Chain	Full length
Mock	0	0			
3D12.E3-Ch	2788	3886	23,696	49,914	147,220
13F5.G5-CH	3260	3562	24,084	49,518	147,204
DVD1-Ig	2988	3300	35,797 (35,790)	64,380 (64,371)	200,346 (200,521)

DVD2-Ig against hIL-1 α were also similar, i.e. 3-fold less than that of the chimeric Abs (see Table III). Consistent with its binding affinity for hIL-1 α , the neutralizing activity of DVD2-Ig to hIL-1 β is slightly less than that of the chimeric Ab 13F5.G5, but 3-fold higher than that of DVD1-Ig. Overall there was no significant decrease in the biological activities of DVD-Ig molecules compared to the original mAbs.

[0257] To determine if DVD-Ig was able to inhibit IL-8 production in the presence of both IL-1 α and IL-1 β , equal amounts of hIL-1 α and hIL-1 β were added in the same culture system of MRC-5 assay. Both DVD1-Ig and DVD2-Ig were able to inhibit IL-8 synthesis by MRC-5 cells in the presence of both IL-1 α and IL-1 β , with activities similar to that of mono-assays where only one cytokine was present (Table 13). In this assay where both IL-1 α and IL-1 β were present, the dual-inhibition activity of DVD2-Ig (1.2 nM) was higher than that of DVD1-Ig (2.2 nM).

Example 2

Analysis of Linker Size and Variable Domain Orientation in the DVD-Ig Molecule

[0258] Additional DVD-Ig molecules with different parent mAb pairs, as shown in Table 15, were constructed. For each pair of mAbs, four different DVD-Ig constructs were generated: 2 with a short linker and 2 with a long linker, each in two different domain orientations: a-b-C (alpha-beta-constant domain) and b-a-C (beta-alpha-constant domain). The linker sequences, were derived from the N-terminal sequence of human Ck or CH1 domain, as follows:

[0259] Short linker: light chain: TVAAP; heavy chain: ASTKGP

[0260] Long linker: light chain: TVAAPSVFIFPP; heavy chain: ASTKGPSVFPLAP

[0261] All heavy and light chain constructs were subcloned into the pBOS expression vector, and expressed in COS cells or freestyle 293 cells.

[0262] To construct new DVD clones, the variable domains of the two mAbs, both light chain and heavy chain, were first jointed in tandem using overlapping PCR as described for hIL-1abDVD1-Ig and hIL-1abDVD2-Ig. The jointed pieces were then subcloned in pBOS vector using homologous recombination. Briefly, vectors were linearized by restriction digestion (2 ug of pBOS-hCk vector were digested with FspAI and BsiWI in O+ buffer, and 2 ug of pBOS-hC γ z,non a vector was digested with FspAI and SalI in O+ buffer). The digested samples were run on 1% agarose gel and the backbone fragment purified in 50 ul water. For homologous recombination and transformation, DH5 α competent cells were thaw on ice, and mixed with 20-50 ng jointed PCR product and 20-50 ng of linearized vector (in every 50 ul DH5 α cells). The mixture was mixed gently and incubated on ice for 45 minutes, followed by heat shock at 42 $^{\circ}$ C. for 1 minute. Then 100 ul SOC medium were added and incubated at 37 $^{\circ}$ C. for 1 hour. The transformation culture was inoculated on LB/Agar plates containing Ampicillin and incubated at 37 $^{\circ}$ C. for 18-20 hours. The bacterial clones were isolated, from which DNA was purified and subjected to sequencing analysis. The final sequence-verified clones were co-transfected (matching HV and LC of the

same Ab pair) in COS or 293 cells for Ab expression and purification, as previously described.

[0263] Characteristics of the purified DVD-Ig proteins are summarized in Table 16. The left section of the table 16 shows the specificity, binding affinity, and neutralization potency of the 2 pairs of mabs used for the construction of the new hIL-1a/bDVD-Ig molecules. Antibodies 18F4.2C8 and 1B12.4H4 (see example 1.1.D) were used to construct hIL-1a/bDVD3a-Ig, hIL-1a/bDVD4a-Ig, hIL-1a/bDVD3b-Ig, and hIL-1a/bDVD4b-Ig. hIL-1a/bDVD3a-Ig and hIL-1a/bDVD4a-Ig were in a-b-C orientation, with a short and long linker, respectively. hIL-1a/bDVD3b-Ig and hIL-1a/bDVD4b-Ig were in b-a-C orientation, with a short and long linker, respectively. Antibodies 6H3.1A4 and 6B12.4F6 were used to construct hIL-1a/bDVD5a-Ig, hIL-1a/bDVD6a-Ig, hIL-1a/bDVD5b-Ig, and hIL-1a/bDVD6b-Ig. hIL-1a/bDVD5a-Ig and hIL-1a/bDVD6a-Ig were in a-b-C orientation, with a short and long linker, respectively. hIL-1a/bDVD5b-Ig and hIL-1a/bDVD6b-Ig were in b-a-C orientation, with a short and long linker, respectively. The molecular cloning of these additional hIL-1a/bDVD-Igs were performed using the procedure previously described for hIL-1a/bDVD1-Ig (see example 1.3), using overlapping PCR procedures. The amino acid sequences of these additional hIL-1a/bDVD-Igs are disclosed in Table 15.

TABLE 15

Amino acid sequence of heavy chain and light chain of six DVD Ig capable of binding IL-1 α and IL-1 β .		
Protein	Sequence	Sequence
Protein region	Identifier	12345678901234567890
DVD HEAVY VARIABLE	SEQ ID NO.:41	EVQLQQSGAELVKPGASVKLSCTASGLNIKDTYMHWLKQRPEQGLEWIGRIDPANGNAKYDPRFLGKATITADTSSNTAYLQSSLTSEDITAVYYCARGDGNFHFYWGQGTTLTVSS
hIL-1a/b DVD3a-Ig		EVQLQQSGAELVKPGASVKLSCTASGLNIKDTYMHWLKQRPEQGLEWIGRIDPANGNAKYDPRFLGKATITADTSSNTAYLQSSLTSEDITAVYYCARGDGNFHFYWGQGTTLTVSS
18F4.2C8 VH	SEQ ID NO.:3	EVQLQQSGAELVKPGASVKLSCTASGLNIKDTYMHWLKQRPEQGLEWIGRIDPANGNAKYDPRFLGKATITADTSSNTAYLQSSLTSEDITAVYYCARGDGNFHFYWGQGTTLTVSS
LINKER	SEQ ID NO.:42	ASTKGP
1B12.4H4 VH	SEQ ID NO.:9	QVHLKESGPGLVAPSSLSITCTVSGFSLTDYGVSWIRQPGKLEWLGILWGGDTYYNSPLKSRLSIRKDNKSKSQVFLKMNSLQTDITAVYYCAKQRTLWGYDLYGHDYWGQGTSTVTVSS
CH	SEQ ID NO.:34	ASTKGPSVFPLAPSSKSTSGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHCTCPPEPELLGG

HGF; HIF1A; HIP1; histamine and histamine receptors; HLA-A; HLA-DRA; HM74; HMOX1; HUMCYT2A; ICEBERG; ICOSL; ID2; IFN- α ; IFNA1; IFNA2; IFNA4; IFNA5; IFNA6; IFNA7; IFNB1; IFN γ ; IFNW1; IGBP1; IGF1; IGF1R; IGF2; IGFBP2; IGFBP3; IGFBP6; IL-1; IL10; IL10RA; IL10RB; IL11; IL11RA; IL-12; IL12A; IL12B; IL12RB1; IL12RB2; IL13; IL13RA1; IL13RA2; IL14; IL15; IL15RA; IL16; IL17; IL17B; IL17C; IL17R; IL18; IL18BP; IL18R1; IL18RAP; IL19; IL1A; IL1B; IL1F10; IL1F5; IL1F6; IL1F7; IL1F8; IL1F9; IL1HY1; IL1R1; IL1R2; IL1RAP; IL1RAPL1; IL1RAPL2; IL1RL1; IL1RL2; IL1RN; IL2; IL20; IL20RA; IL21R; IL22; IL22R; IL22RA2; IL23; IL24; IL25; IL26; IL27; IL28A; IL28B; IL29; IL2RA; IL2RB; IL2RG; IL3; IL30; IL3RA; IL4; IL4R; IL5; IL5RA; IL6; IL6R; IL6ST (glycoprotein 130); IL7; IL7R; IL8; IL8RA; IL8RB; IL8RB; IL9; IL9R; ILK; INHA; INHBA; INSL3; INSL4; IRAK1; IRAK2; ITGA1; ITGA2; ITGA3; ITGA6 (a6 integrin); ITGAV; ITGB3; ITGB4 (b4 integrin); JAG1; JAK1; JAK3; JUN; K6HF; KAI1; KDR; KITLG; KLF5 (GC Box BP); KLF6; KLF10; KLF12; KLF13; KLF14; KLF15; KLF3; KLF4; KLF5; KLF6; KLF9; KRT1; KRT19 (Keratin 19); KRT2A; KRTHB6 (hair-specific type II keratin); LAMA5; LEP (leptin); Lingo-p75; Lingo-Troy; LPS; LTA (TNF-b); LTB; LTB4R (GPR16); LTB4R2; LTB; MACMARCKS; MAG or Omgp; MAP2K7 (c-Jun); MDK; MIB1; midkine; MIF; MIP-2; MK167 (Ki-67); MMP2; MMP9; MS4A1; MSMB; MT3 (metallothionein-III); MTSS1; MUC1 (mucin); MYC; MYD88; NCK2; neurocan; NFKB1; NFKB2; NGFB (NGF); NGFR; NgR-Lingo; NgR-Nogo66 (Nogo); NgR-p75; NgR-Troy; NME1 (NM23A); NOX5; NPPB; NR0B1; NR0B2; NR1D1; NR1D2; NR1H2; NR1H3; NR1H4; NR1I2; NR1I3; NR2C1; NR2C2; NR2E1; NR2E3; NR2F1; NR2F2; NR2F6; NR3C1; NR3C2; NR4A1; NR4A2; NR4A3; NR5A1; NR5A2; NR6A1; NRP1; NRP2; NTSE; NTN4; OZ1; OPRD1; P2RX7; PAP; PART1; PATE; PAWR; PCA3; PCNA; PDGFA; PDGFB; PECAM1; PF4 (CXCL4); PGF; PGR; phosphacan; PIAS2; PIK3CG; PLAUI (uPA); PLG; PLXDC1; PPBP (CXCL7); PPID; PRI; PRKCQ; PRKD1; PRL; PROC; PROK2; PSAP; PSCA; PTAFR; PTEN; PTGS2 (COX-2); PTN; RAC2 (p21Rac2); RARB; RGS1; RGS13; RGS3; RNF110 (ZNF144); ROBO2; S100A2; SCGB1D2 (lipophilin B); SCGB2A1 (mammaglobin 2); SCGB2A2 (mammaglobin 1); SCYE1 (endothelial Monocyte-activating cytokine); SDF2; SERPINA1; SERPINA3; SERPINB5 (maspin); SERPINE1 (PAI-1); SERPINF1; SHBG; SLA2; SLC2A2; SLC33A1; SLC43A1; SLIT2; SPP1; SPRR1B (Spr1); ST6GAL1; STAB1; STAT6; STEAP2; TB4R2; TBX21; TCP10; TDGF1; TEK; TGFA; TGFB11; TGFB11I; TGFB2; TGFB3; TGFB1; TGFB1R1; TGFB2R2; TGFB3R3; TH1L; THBS1 (thrombospondin-1); THBS2; THBS4; THPO; TIE (Tie-1); TIMP3; tissue factor; TLR10; TLR2; TLR3; TLR4; TLR5; TLR6; TLR7; TLR8; TLR9; TNF; TNF- α ; TNFAIP2 (B94); TNFAIP3; TNFRSF1A; TNFRSF1A; TNFRSF1B; TNFRSF21; TNFRSF5; TNFRSF6 (Fas); TNFRSF7; TNFRSF8; TNFRSF9; TNFSF10 (TRAIL); TNFSF11 (TRANCE); TNFSF12 (APO3L); TNFSF13 (April); TNFSF13B; TNFSF14 (HVEM-L); TNFSF15 (VEG1); TNFSF18; TNFSF4 (OX40 ligand); TNFSF5 (CD40 ligand); TNFSF6 (FasL); TNFSF7 (CD27 ligand); TNFSF8 (CD30 ligand); TNFSF9 (4-1BB ligand); TOLLIP; Toll-like receptors; TOP2A (topoisomerase IIa); TP53; TPM1; TPM2; TRADD; TRAF1; TRAF2; TRAF3; TRAF4; TRAF5;

TRAF6; TREM1; TREM2; TRPC6; TSLP; TWEAK; VEGF; VEGFB; VEGFC; versican; VHL C5; VLA-4; XCL1 (lymphotactin); XCL2 (SCM-1b); XCR1 (GPR5/CCXCR1); YY1; and ZFPM2.

25. The binding protein according to claim 1, wherein said binding protein is capable of binding a two targets, wherein the two targets are selected from the group consisting of CD138 and CD20; CD138 and CD40; CD20 and CD3; CD38 & CD138; CD38 and CD20; CD38 and CD40; CD40 and CD20; CD19 and CD20; CD-8 and IL-6; PDL-1 and CTLA-4; CTLA-4 and BTNO2; CSPGs and RGM A; IGF1 and IGF2; IGF1/2 and Erb2B; IL-12 and IL-18; IL-12 and TWEAK; IL-13 and ADAM8; IL-13 and CL25; IL-13 and IL-1beta; IL-13 and IL-25; IL-13 and IL-4; IL-13 and IL-5; IL-13 and IL-9; IL-13 and LHR agonist; IL-13 and MDC; IL-13 and MIF; IL-13 and PED2; IL-13 and SPRR2a; IL-13 and SPRR2b; IL-13 and TARC; IL-13 and TGF- β ; IL-1 α and IL-1 β ; MAG and RGM A; NgR and RGM A; NogoA and RGM A; OMGP and RGM A; RGM A and RGM B; Te38 and TNF α ; TNF α and IL-12; TNF α and IL-12p40; TNF α and IL-13; TNF α and IL-15; TNF α and IL-17; TNF α and IL-18; TNF α and IL-1beta; TNF α and IL-23; TNF α and MIF; TNF α and PEG2; TNF α and PGE4; TNF α and VEGF; and VEGFR and EGFR; TNF α and RANK ligand; TNF α and Blys; TNF α and GP130; TNF α and CD-22; and TNF α and CTLA-4.

26. The binding protein according to claim 23, wherein the binding protein is capable of modulating a biological function of one or more targets.

27. The binding protein according to claim 23, wherein the binding protein is capable of neutralizing one or more targets.

28. The binding protein according to any one of claims 23-25, wherein the target is selected from the group consisting of cytokine, chemokine, cell surface protein, enzyme and receptor.

29. The binding protein according to claim 28 wherein the cytokine is selected from the group consisting of lymphokines, monokines, and polypeptide hormones.

30. The binding protein according to claim 29, wherein said cytokines are IL-1 α and IL-1 β .

31. The binding protein according to claim 30, wherein the binding protein comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 33, SEQ ID NO. 37, SEQ ID NO. 41, SEQ ID NO. 45, SEQ ID NO. 47, SEQ ID NO. 51, SEQ ID NO. 53, SEQ ID NO. 55, SEQ ID NO. 57, and SEQ ID NO. 59; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 35, SEQ ID NO. 39, SEQ ID NO. 43, SEQ ID NO. 46, SEQ ID NO. 49, SEQ ID NO. 52, SEQ ID NO. 54, SEQ ID NO. 56, SEQ ID NO. 58, and SEQ ID NO. 60.

32. The binding protein according to claim 29, wherein said cytokines are TNF- α and IL-13.

33. The binding protein according to claim 29, wherein said cytokines are IL-12 and IL-18.

34. The binding protein according to claim 33, wherein the binding protein comprises a DVD heavy chain amino acid sequence selected from the group consisting of SEQ ID NO. 83, SEQ ID NO. 90, SEQ ID NO. 93, SEQ ID NO. 95, and SEQ ID NO. 114; and a DVD light chain amino acid sequence selected from the group consisting of SEQ ID NO. 86, SEQ ID NO. 91, SEQ ID NO. 94, SEQ ID NO. 46, SEQ ID NO. 96, and SEQ ID NO. 116.

35. The binding protein according to claim 28 wherein the chemokine is selected from the group consisting of CCR2, CCR5 and CXCL-13.

36. The binding protein according to claim 28 wherein the cell surface protein is an integrin.

37. The binding protein according to claim 28 wherein the cell surface proteins are CD-20 and CD3.

38. The binding protein according to claim 37, wherein the binding protein comprises a DVD heavy chain amino acid sequence is SEQ ID NO. 97, and a DVD light chain SEQ ID NO. 101.

39. The binding protein according to claim 28 wherein the enzyme is selected from the group consisting of kinases and proteases.

40. The binding protein according to claim 28 wherein the receptor is selected from the group consisting of lymphokine receptor, monokine receptor, and polypeptide hormone receptor.

41. The binding protein according to claim 28, wherein said binding protein has an on rate constant (K_{on}) to said one or more targets selected from the group consisting of: at least about $10^2 M^{-1} s^{-1}$; at least about $10^3 M^{-1} s^{-1}$; at least about $10^4 M^{-1} s^{-1}$; at least about $10^5 M^{-1} s^{-1}$; and at least about $10^6 M^{-1} s^{-1}$, as measured by surface plasmon resonance.

42. The binding protein according to claim 28, wherein said binding protein has an off rate constant (K_{off}) to said one or more targets selected from the group consisting of: at most about $10^{-3} s^{-1}$; at most about $10^{-4} s^{-1}$; at most about $10^{-5} s^{-1}$; and at most about $10^{-6} s^{-1}$, as measured by surface plasmon resonance.

43. The binding protein according to claim 28, wherein said binding protein has a dissociation constant (K_D) to said one or more targets selected from the group consisting of: at most about $10^{-7} M$; at most about $10^{-8} M$; at most about $10^{-9} M$; at most about $10^{-10} M$; at most about $10^{-11} M$; at most about $10^{-12} M$; and at most $10^{-13} M$.

44. A binding protein conjugate comprising a binding protein described in any one of claims 1-43, said binding protein conjugate further comprising an agent selected from the group consisting of; an immunoadhesion molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.

45. The binding protein conjugate according to claim 44, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

46. The binding protein conjugate according to claim 45, wherein said imaging agent is a radiolabel selected from the group consisting of: ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , and ^{153}Sm .

47. The binding protein conjugate according to claim 45, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of; an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

48. The binding protein according to claim 1, wherein said binding protein is a crystallized binding protein.

49. The crystallized binding protein according to claim 48, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

50. The crystallized binding protein according to claim 48, wherein said binding protein has a greater half life in vivo than the soluble counterpart of said binding protein.

51. The crystallized binding protein according to claim 48, wherein said binding protein retains biological activity.

52. An isolated nucleic acid encoding a binding protein amino acid sequence of any one of claims 1-22.

53. A vector comprising an isolated nucleic acid according to claim 52.

54. The vector of claim 53 wherein said vector is selected from the group consisting of pcDNA, pTT, pTT3, pEFBOS, pBV, pJV, pcDNA3.1 TOPO, pEF6 TOPO, and pBJ.

55. A host cell comprising a vector according to claim 53.

56. The host cell according to claim 55, wherein said host cell is a prokaryotic cell.

57. The host cell according to claim 56, wherein said host cell is *E. Coli*.

58. The host cell according to claim 55, wherein said host cell is a eukaryotic cell.

59. The host cell according to claim 58, wherein said eukaryotic cell is selected from the group consisting of protist cell, animal cell, plant cell and fungal cell.

60. The host cell according to claim 58, wherein said eukaryotic cell is an animal cell selected from the group consisting of; a mammalian cell, an avian cell, and an insect cell.

61. The host cell according to claim 58, wherein said host cell is a CHO cell.

62. The host cell according to claim 58, wherein said host cell is COS.

63. The host cell according to claim 58, wherein said host cell is a yeast cell.

64. The host cell according to claim 63, wherein said yeast cell is *Saccharomyces cerevisiae*.

65. The host cell according to claim 58, wherein said host cell is an insect Sf9 cell.

66. A method of producing a binding protein, comprising culturing a host cell described in any one of claims 55-65 in culture medium under conditions sufficient to produce the binding protein.

67. The method of claim 66, wherein 50%-75% of the binding protein produced is a dual specific tetravalent binding protein.

68. The method of claim 66, wherein 75%-90% of the binding protein produced is a dual specific tetravalent binding protein.

69. The method of claim 66, wherein 90%-95% of the binding protein produced is a dual specific tetravalent binding protein.

70. A protein produced according to the method of claim 66.

71. A pharmaceutical composition comprising the binding protein of any one of claims 1-51 and 70, and a pharmaceutically acceptable carrier.

72. The pharmaceutical composition of claim 71 further comprising at least one additional therapeutic agent.

73. The pharmaceutical composition of claim 72, wherein said additional agent is selected from the group consisting of: Therapeutic agent, imaging agent, cytotoxic agent, angiogenesis inhibitors; kinase inhibitors; co-stimulation molecule blockers; adhesion molecule blockers; anti-cytokine antibody or functional fragment thereof; methotrexate; cyclosporin; rapamycin; FK506; detectable label or reporter; a TNF antagonist; an antirheumatic; a muscle relaxant, a

narcotic, a non-steroid anti-inflammatory drug (NSAID), an analgesic, an anesthetic, a sedative, a local anesthetic, a neuromuscular blocker, an antimicrobial, an antipsoriatic, a corticosteroid, an anabolic steroid, an erythropoietin, an immunization, an immunoglobulin, an immunosuppressive, a growth hormone, a hormone replacement drug, a radiopharmaceutical, an antidepressant, an antipsychotic, a stimulant, an asthma medication, a beta agonist, an inhaled steroid, an epinephrine or analog, a cytokine, and a cytokine antagonist.

74. A method for treating a subject for a disease or a disorder by administering to the subject the binding protein of any one of claims 1-51 and 70 such that treatment is achieved.

75. The method of claim 74, wherein said disorder is selected from the group comprising rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, septic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, *yersinia* and *salmonella* associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune haemolytic anaemia, Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia, myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis, Acquired Immunodeficiency Disease Syndrome, Hepatitis B, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure, fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease, mixed connective tissue disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung

disease, haemosiderosis associated lung disease, drug-induced interstitial lung disease, fibrosis, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease, postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycaemia, type B insulin resistance with acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthritis, primary sclerosing cholangitis, psoriasis type 1, psoriasis type 2, idiopathic leucopaenia, autoimmune neutropaenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, Lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS, sperm autoimmunity, multiple sclerosis (all subtypes), sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Sjögren's syndrome, Takayasu's disease/arteritis, autoimmune thrombocytopaenia, idiopathic thrombocytopaenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis, vitiligo acute liver disease, chronic liver diseases, alcoholic cirrhosis, alcohol-induced liver injury, choleosatatis, idiosyncratic liver disease, Drug-Induced hepatitis, Non-alcoholic Steatohepatitis, allergy and asthma, group B streptococci (GBS) infection, mental disorders (e.g., depression and schizophrenia), Th2 Type and Th1 Type mediated diseases, acute and chronic pain (different forms of pain), and cancers such as lung, breast, stomach, bladder, colon, pancreas, ovarian, prostate and rectal cancer and hematopoietic malignancies (leukemia and lymphoma) Abetalipoproteinemia, Acrocyanosis, acute and chronic parasitic or infectious processes, acute leukemia, acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), acute or chronic bacterial infection, acute pancreatitis, acute renal failure, adenocarcinomas, aerial ectopic beats, AIDS dementia complex, alcohol-induced hepatitis, allergic conjunctivitis, allergic contact dermatitis, allergic rhinitis, allograft rejection, alpha-1-antitrypsin deficiency, amyotrophic lateral sclerosis, anemia, angina pectoris, anterior horn cell degeneration, anti cd3 therapy, antiphospholipid syndrome, anti-receptor hypersensitivity reactions, aortic and peripheral aneurysms, aortic dissection, arterial hypertension, arteriosclerosis, arteriovenous fistula, ataxia, atrial fibrillation (sustained or paroxysmal), atrial flutter, atrioventricular block, B cell lymphoma, bone graft rejection, bone marrow transplant (BMT) rejection, bundle branch block, Burkitt's lymphoma, Burns, cardiac arrhythmias, cardiac stun syndrome, cardiac tumors, cardiomyopathy, cardiopulmonary bypass inflammation response, cartilage transplant rejection, cerebellar cortical degenerations, cerebellar disorders, chaotic or multifocal atrial tachycardia, chemotherapy associated disorders, chronic myelocytic leukemia (CML), chronic alcoholism, chronic inflammatory pathologies, chronic lymphocytic leukemia (CLL), chronic obstructive pulmonary disease (COPD), chronic salicylate intoxication, colorectal carcinoma, congestive heart failure, conjunctivitis, contact dermatitis, cor pulmonale, coronary artery dis-

ease, Creutzfeldt-Jakob disease, culture negative sepsis, cystic fibrosis, cytokine therapy associated disorders, Dementia pugilistica, demyelinating diseases, dengue hemorrhagic fever, dermatitis, dermatologic conditions, diabetes, diabetes mellitus, diabetic atherosclerotic disease, Diffuse Lewy body disease, dilated congestive cardiomyopathy, disorders of the basal ganglia, Down's Syndrome in middle age, drug-induced movement disorders induced by drugs which block CNS dopamine receptors, drug sensitivity, eczema, encephalomyelitis, endocarditis, endocrinopathy, epiglottitis, epstein-barr virus infection, erythromelalgia, extrapyramidal and cerebellar disorders, familial hemaphagocytic lymphohistiocytosis, fetal thymus implant rejection, Friedreich's ataxia, functional peripheral arterial disorders, fungal sepsis, gas gangrene, gastric ulcer, glomerular nephritis, graft rejection of any organ or tissue, gram negative sepsis, gram positive sepsis, granulomas due to intracellular organisms, hairy cell leukemia, Hallerorden-Spatz disease, hashimoto's thyroiditis, hay fever, heart transplant rejection, hemachromatosis, hemodialysis, hemolytic uremic syndrome/thrombolytic thrombocytopenic purpura, hemorrhage, hepatitis (A), His bundle arrhythmias, HIV infection/HIV neuropathy, Hodgkin's disease, hyperkinetic movement disorders, hypersensitivity reactions, hypersensitivity pneumonitis, hypertension, hypokinetic movement disorders, hypothalamic-pituitary-adrenal axis evaluation, idiopathic Addison's disease, idiopathic pulmonary fibrosis, antibody mediated cytotoxicity, Asthenia, infantile spinal muscular atrophy, inflammation of the aorta, influenza a, ionizing radiation exposure, iridocyclitis/uveitis/optic neuritis, ischemia-reperfusion injury, ischemic stroke, juvenile rheumatoid arthritis, juvenile spinal muscular atrophy, Kaposi's sarcoma, kidney transplant rejection, *legionella*, leishmaniasis, leprosy, lesions of the corticospinal system, lipedema, liver transplant rejection, lymphoderma, malaria, malignant Lymphoma, malignant histiocytosis, malignant melanoma, meningitis, meningococchemia, metabolic/idiopathic, migraine headache, mitochondrial multi.system disorder, mixed connective tissue disease, monoclonal gammopathy, multiple myeloma, multiple systems degenerations (Mencel Dejerine-Thomas Shi-Drager and Machado-Joseph), myasthenia gravis, *mycobacterium avium* intracellular, *mycobacterium tuberculosis*, myelodysplastic syndrome, myocardial infarction, myocardial ischemic disorders, nasopharyngeal carcinoma, neonatal chronic lung disease, nephritis, nephrosis, neurodegenerative diseases, neurogenic I muscular atrophies, neutropenic fever, non-hodgkins lymphoma, occlusion of the abdominal aorta and its branches, occulsive arterial disorders, okt3 therapy,

orchitis/epididymitis, orchitis/vasectomy reversal procedures, organomegaly, osteoporosis, pancreas transplant rejection, pancreatic carcinoma, paraneoplastic syndrome/hypercalcemia of malignancy, parathyroid transplant rejection, pelvic inflammatory disease, perennial rhinitis, pericardial disease, peripheral atherosclerotic disease, peripheral vascular disorders, peritonitis, pernicious anemia, *pneumocystis carinii* pneumonia, pneumonia, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes syndrome), post perfusion syndrome, post pump syndrome, post-MI cardiomyopathy, preeclampsia, Progressive supranucleo Palsy, primary pulmonary hypertension, radiation therapy, Raynaud's phenomenon and disease, Raynaud's disease, Refsum's disease, regular narrow QRS tachycardia, renovascular hypertension, reperfusion injury, restrictive cardiomyopathy, sarcomas, scleroderma, senile chorea, Senile Dementia of Lewy body type, seronegative arthropathies, shock, sickle cell anemia, skin allograft rejection, skin changes syndrome, small bowel transplant rejection, solid tumors, specific arrhythmias, spinal ataxia, spinocerebellar degenerations, streptococcal myositis, structural lesions of the cerebellum, Subacute sclerosing panencephalitis, Sympcope, syphilis of the cardiovascular system, systemic anaphalaxis, systemic inflammatory response syndrome, systemic onset juvenile rheumatoid arthritis, T-cell or FAB ALL, Telangiectasia, thromboangitis obliterans, thrombocytopenia, toxicity, transplants, trauma/hemorrhage, type III hypersensitivity reactions, type IV hypersensitivity, unstable angina, uremia, urosepsis, urticaria, valvular heart diseases, varicose veins, vasculitis, venous diseases, venous thrombosis, ventricular fibrillation, viral and fungal infections, vital encephalitis/aseptic meningitis, vital-associated hemaphagocytic syndrome, Wernicke-Korsakoff syndrome, Wilson's disease, xenograft rejection of any organ or tissue.

76. The method according to claim 74, wherein said administering to the subject is by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

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(54) Title: INHIBITION OF MACROPHAGE-STIMULATING PROTEIN RECEPTOR (RON)

(57) Abstract: The present invention relates to methods for treatment of tumors and other diseases in a mammal comprising administration of antibodies specific for Macrophage-Stimulating Protein Receptor ("MSP-R" or "RON"). The present invention further provides for compositions comprising antibodies of antibody fragments specific for Ron, including human antibodies, that inhibit RON activation.

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FIGURE 2

SEQ. ID. No. 1 (CDR1 cDNA sequence of heavy chain of IMC-41A10)

AGCTATGCTATGCAC

SEQ. ID. No. 2 (CDR1 amino acid sequence of heavy chain of IMC-41A10)

SYAMH

SEQ. ID. No. 3 (CDR2 cDNA sequence of heavy chain region of IMC-41A10)

GTTATATCATATGATGGAAGTAATAAATACTACGCAGACTCCGTGAAGGGC

SEQ ID. No. 4 (CDR2 heavy chain amino acid sequence of IMC-41A10)

VISYDGSNKYYADSVKG

SEQ. ID. No. 5 (CDR3 cDNA sequence of heavy chain of IMC-41A10)

TTCAGTGGCTGGCCCAACAACACTACTACTACTACGGTATGGACGTC

SEQ ID. No. 6 (CDR3 amino acid sequence of heavy chain of IMC-41A10)

FSGWPNYYYYGMDV

SEQ ID. NO. 7 (Amino acid sequence of Heavy chain variable region of IMC-41A10)

EVQLVQSGGGLVKPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYY
ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARFSGWPNYYYYGMDVWGQGT
TVSS

SEQ. ID. No. 8 (Heavy chain cDNA sequence of IMC-41A10 (Ig gamma1))

ATGGGATGGTCATGTATCATCCTTTTTCTAGTAGCAACTGCAACTGGAGTACATTCAGAG
GTCCAGCTGGTGCAGTCTGGGGGAGGCTTGCTCAAGCCTGGAGGGTCCCTGAGACTCTCC
TGTGCAGCCTCTGGATTACCTTCAGTAGCTATGCTATGCACTGGGTCCGCCAGGCTCCA
GGCAAGGGGCTGGAGTGGGTGGCAGTTATATCATATGATGGAAGTAATAAATACTACGCA
GACTCCGTGAAGGGCCGATTACCATCTCCAGAGACAAATCCAAGAACACGCTGTATCTG
CAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCGAGGTTCAGTGGC
TGGCCCAACAACACTACTACTACTACGGTATGGACGTCTGGGGCCAAGGGACCACGGTCACC
GTCTCAAGCGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGC
ACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTG
ACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCCGCTGTCTTA
CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGC
ACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAA
GTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTC
CTGGGGGGACCGTCAGTCTTCTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCC
CGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAG
TTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGAGGAG



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435/69.1; 435/328

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(57) **ABSTRACT**

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(22) Filed: **Feb. 28, 2001**

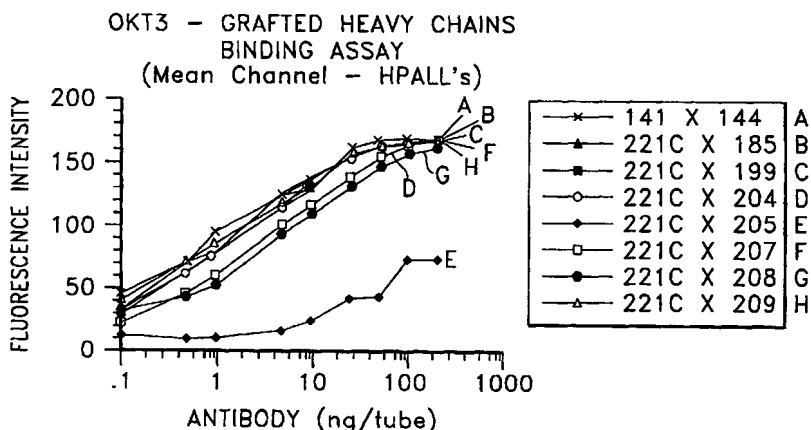
Related U.S. Application Data

(63) Continuation of application No. 08/846,658, filed on May 1, 1997, which is a continuation of application No. 08/303,569, filed on Sep. 7, 1994, now Pat. No. 5,859,205, which is a continuation of application No. 07/743,329, filed on Sep. 17, 1991, now abandoned, filed as 371 of international application No. PCT/GB90/02017, filed on Dec. 21, 1990.

(30) **Foreign Application Priority Data**

Dec. 21, 1989 (GB) 8928874.0

Recombinant, in particular humanised, e.g. humanised chimeric and CDR-grafted humanised, antibody molecules having specificity for human TNF alpha, are provided for use in diagnosis and therapy. In particular the antibody molecules have antigen binding sites derived from murine monoclonal antibodies CB0006, CB010, hTNF3 or 101.4. Preferred CDR-grafted humanised anti-hTNF alpha antibodies comprise variable region domains comprising human acceptor framework and donor antigen binding regions and wherein the frameworks comprise donor residues at specific positions. The antibody molecules may be used for therapeutic treatment of human patients suffering from or at risk of disorders associated with undesirably high levels of TNF, in particular for treatment of immunoregulatory and inflammatory disorders or of septic, endotoxic or cardiovascular shock.



(205)	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,
(208)	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,
(204)	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,
(199)	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,
(207)	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,
(185)	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,
(209)	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,
141 X 144	6, 23, 24, 48, 49, 71, 73, 76, 78, 88, 91,

-continued

Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Phe Thr
85 90 95
Phe Gly Gln Gly Thr Lys Leu Gln Ile Thr Arg
100 105

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 107 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile
1 5 10
Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser Ala Ser
15 20 25
Ser Ser Val Ser Tyr Met Asn Trp Tyr Gln Gln Lys Ser Gly Thr Ser
30 35 40
Pro Lys Arg Trp Ile Tyr Asp Thr Ser Lys Leu Ala Ser Gly Val Pro
45 50 55
Ala His Phe Arg Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
60 65 70
Ser Gly Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp
75 80 85 90
Ser Ser Asn Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Asn
95 100 105

Arg

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 119 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
1 5 10
Pro Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe
15 20 25
Thr Arg Tyr Thr Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
30 35 40 45
Glu Trp Ile Gly Tyr Ile Asn Pro Ser Arg Gly Tyr Thr Asn Tyr Asn
50 55 60
Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Thr Asp Lys Ser Ser Ser
65 70 75
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
80 85 90
Tyr Tyr Cys Ala Arg Tyr Tyr Asp Asp His Tyr Cys Leu Asp Tyr Trp
95 100 105

-continued

Gly Gln Gly Thr Thr Leu Thr Val Ser Ser
110 115

1. A CDR-grafted antibody heavy chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 6, 23 and/or 24, 48 and/or 49, 71 and/or 73, 75 and/or 76 and/or 78 and 88 and/or 91.

2. A CDR-grafted heavy chain according to claim 1 comprising donor residues at positions 23, 24, 49, 71, 73 and 78, or at positions 23, 24 and 49.

3. A CDR-grafted heavy chain according to claim 2 comprising donor residues at positions 2, 4, 6, 25, 36, 37, 39, 47, 48, 93, 94, 103, 104, 106 and 107.

4. A CDR-grafted heavy chain according to claim 2 or 3, comprising donor residues at one, some or all of positions:

1 and 3,

69 (if 48 is different between donor and acceptor),

38 and 46 (if 48 is the donor residue),

67,

82 and IS (if 67 is the donor residue),

91, and

any one or more of 9, 11, 41, 87, 108, 110 and 112.

5. A CDR-grafted heavy chain according to any of the preceding comprising donor CDRs at positions 26-35, 50-65 and 95-100.

6. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 1 and/or 3 and 46 and/or 47.

7. A CDR-grafted light chain according to claim 6 comprising donor residues at positions 46 and 47.

8. A CDR-grafted antibody light chain having a variable region domain comprising acceptor framework and donor antigen binding regions wherein the framework comprises donor residues at at least one of positions 46, 48, 58 and 71.

9. A CDR-grafted light chain according to claim 8 comprising donor residues at positions 46, 48, 58 and 71.

10. A CDR-grafted light chain according to claim 8 or 9, comprising donor residues at positions 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64-69, 85, 87, 98, 99, 101 and 102.

11. A CDR-grafted light chain according to claim 9 or 10, comprising donor residues at one, some or all of positions:

1 and 3,

63,

60 (if 60 and 54 are able to form a potential saltbridge),

70 (if 70 and 24 are able to form a potential saltbridge),

73 and 21 (if 47 is different between donor and acceptor),

37 and 45 (if 47 is different between donor and acceptor), and

any one or more of 10, 12, 40, 83, 103 and 105.

12. A CDR-grafted light chain according to any one of claims 6-11, comprising donor CDRs at positions 24-34, 50-56 and 89-97.

13. A CDR-grafted antibody molecule comprising at least one CDR-grafted heavy chain according to any one of claims 1-5 and at least one CDR-grafted light chain according to any one of claims 6-12.

14. A CDR-grafted antibody molecule according to claim 13, which is a site-specific antibody molecule.

15. A CDR-grafted antibody molecule according to claim 13 which has specificity for an interleukin, hormone or other biologically active compound or a receptor therefor.

16. A CDR-grafted antibody heavy or light chain or molecule according to any one of the preceding claims comprising human acceptor residues and non-human donor residues.

17. A DNA sequence which codes for a CDR-grafted heavy chain according to claim 1 or a CDR-grafted light chain according to claim 6 or claim 8.

18. A cloning or expression vector containing a DNA sequence according to claim 17.

19. A host cell transformed with a DNA sequence according to claim 17.

20. A process for the production of a CDR-grafted antibody sequence according to claim 17 in a transformed host cell.

21. A process for producing a CDR-grafted antibody product comprising:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy chain according to claim 1;

and/or

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light chain according to claim 6 or claim 8;

(c) transfecting a host cell with the or each vector;

and

(d) culturing the transfected cell line to produce the CDR-grafted antibody product.

22. A therapeutic or diagnostic composition comprising a CDR-grafted antibody heavy chain according to claim 1, or a CDR-grafted light chain according to claim 6 or claim 8, or a CDR-grafted antibody molecule according to claim 13 in combination with a pharmaceutically acceptable carrier, diluent or excipient.

23. A method of therapy or diagnosis comprising administering an effective amount of a CDR-grafted heavy chain according to claim 1, or a CDR-grafted light chain according to claim 6 or claim 8, or a CDR-grafted antibody molecule according to claim 13 to a human or animal subject.

* * * * *