

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 June 2007 (28.06.2007)

PCT

(10) International Publication Number
WO 2007/072011 A1

(51) International Patent Classification:

C07K 14/54 (2006.01) **A01K 67/027** (2006.01)
A61K 38/00 (2006.01) **C12N 1/21** (2006.01)
G01N 33/48 (2006.01) **C12N 1/15** (2006.01)
G01N 33/50 (2006.01) **C12N 5/10** (2006.01)
C12Q 1/68 (2006.01)

(21) International Application Number:

PCT/GB2006/004845

(22) International Filing Date:

21 December 2006 (21.12.2006)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

0526000.5 21 December 2005 (21.12.2005) GB

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(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,
GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS,
JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS,
LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY,
MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS,
RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report*
- *before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments*

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: INTERLEUKIN- 9 LIKE RECEPTOR PROTEIN

(57) Abstract: The invention is based on the discovery that the human protein referred to herein as INSP221 protein is a member of the cytokine receptor-type I family (hematopoietin receptor superfamily). Preferably, INSP221 functions as an IL-9 receptor or an IL-9 receptor-like protein.



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INTERLEUKIN- 9 LIKE RECEPTOR PROTEIN

This invention relates to a novel protein (termed INSP221) herein identified as a splice variant of a member of the cytokine receptor-type 1 (Hematopoietin receptor) superfamily and to the use of this protein and nucleic acid sequences from the encoding gene in the
5 diagnosis, prevention and treatment of disease.

All publications, patents and patent applications cited herein are incorporated in full by reference.

BACKGROUND

The process of drug discovery is presently undergoing a fundamental revolution as the era
10 of functional genomics comes of age. The term "functional genomics" applies to an approach utilising bioinformatics tools to ascribe function to protein sequences of interest. Such tools are becoming increasingly necessary as the speed of generation of sequence data is rapidly outpacing the ability of research laboratories to assign functions to these protein sequences.

15 As bioinformatics tools increase in potency and in accuracy, these tools are rapidly replacing the conventional techniques of biochemical characterisation. Indeed, the advanced bioinformatics tools used in identifying the present invention are now capable of outputting results in which a high degree of confidence can be placed.

Various institutions and commercial organisations are examining sequence data as they
20 become available and significant discoveries are being made on an on-going basis. However, there remains a continuing need to identify and characterise further genes and the polypeptides that they encode, as targets for research and for drug discovery.

Cytokines are a family of growth factors secreted primarily from leukocytes, and are messenger proteins that act as potent regulators capable of effecting cellular processes at
25 sub-nanomolar concentrations. Interleukins, neurotrophins, growth factors, interferons and chemokines all define cytokine families that work in conjunction with cellular receptors to regulate cell proliferation and differentiation. Their size allows cytokines to be quickly transported around the body and degraded when required. Their role in controlling a wide range of cellular functions, especially the immune response and cell growth has been

revealed by extensive research over the last twenty years (Boppana, S.B (1996) Indian. J. Pediatr. 63(4):447-52).

All cytokine communication systems show both pleiotropy (one messenger producing multiple effects) and redundancy (each effect is produced by more than one messenger (Tringali, G. et al (2000) Therapie. 55(1):171-5; Tessarollo, L. (1998) Cytokine Growth Factor Rev. 9(2):125-137). An individual cytokine's effects on a cell can also be dependent on its concentration, the concentration of other cytokines, the temporal sequence of cytokines, and the internal state of the cell (cell cycle, presence of neighbouring cells, cancerous).

- 10 Although cytokines are typically small (under 200 amino acids) proteins they are often formed from larger precursors which are post-translationally spliced. This, in addition to mRNA alternative splicing pathways, give a wide spectrum of variants of each cytokine each of which may differ substantially in biological effect. Membrane and extracellular matrix associated forms of many cytokines have also been isolated (Okada-Ban, M. et al 15 (2000) Int. J. Biochem. Cell Biol. 32(3):263-267; Atamas, S.P. (1997) Life Sci. 61(12):1105-1112).

Cytokines can be grouped into families, though most are unrelated. Categorisation is usually based on secondary structure composition, as sequence similarity is often very low. The families are named after the archetypal member e.g. IFN-like, IL2-like, IL1-like, IL-6 20 like and TNF-like (Zlotnik, A. *et al.*, (2000) Immunity. 12(2):121-127).

Studies have shown cytokines are involved in many important reactions in multi-cellular organisms such as immune response regulation (Nishihira, J. (1998) Int. J. Mol. Med. 2(1):17-28), inflammation (Kim, P.K. *et al.*, (2000) Surg. Clin. North. Am. 80(3):885-894), wound healing (Clark, R.A. (1991) J. Cell Biochem. 46(1):1-2), embryogenesis and 25 development, and apoptosis (Flad, H.D. *et al.*, (1999) Pathobiology. 67(5-6):291-293). Pathogenic organisms (both viral and bacterial) such as HIV and Kaposi's sarcoma-associated virus encode anti-cytokine factors as well as cytokine analogues, which allow them to interact with cytokine receptors and control the body's immune response (Sozzani, S. *et al.*, (2000) Pharm. Acta. Helv. 74(2-3):305-312; Aoki, Y. *et al.*, (2000) J. Hematother. 30 Stem Cell Res. 9(2):137-145). Virally encoded cytokines, virokines, have been shown to be required for pathogenicity of viruses due to their ability to mimic and subvert the host

immune system.

The primary function of cytokines is to bind to cell surface receptors, thus promoting a functional association between signalling subunits of the receptors. These receptors are glycoproteins that assemble into oligomeric receptor complexes typically consisting of 2 to 5 4 receptor chains that may be identical or different.

The Hematopoietin receptor superfamily is the largest cytokine receptor superfamily constituted of cytokine receptor subunits except the α -chains of the IL-2 and IL-15 receptors. They share a number of features at the level of primary sequence that determine key elements of their tertiary structures. Receptors of the hematopoietin superfamily 10 possess 1 or 2 conserved regions of approximately 200 residues, called cytokine receptor modules, that consist of 2 β -barrel structural domains (Bagley CJ *et al.* (2001 April) *Int J Hematol* 73(3):299-307).

The IL-9 receptor is a member of the hematopoietin superfamily. Recently, the human IL-9 receptor gene locus has been implicated in determining susceptibility to bronchial 15 hyperresponsiveness and asthma (Grasso L *et al.* J Biol Chem 1998 Sep 11;273(37):24016-24).

The identification of cytokine receptors, and in particular IL-9 receptors or IL-9 receptor-like proteins, is therefore of extreme importance in increasing the understanding of the underlying pathways that lead to the disease states and associated disease states, mentioned 20 above, and in developing more effective gene and/or drug therapies to treat these disorders.

THE INVENTION

The invention is based on the discovery that the human protein referred to herein as INSP221 protein is a splice variant of a protein referred to herein as INSP186, which is a member of the cytokine receptor-type I family (hematopoietin receptor superfamily). 25 Preferably, INSP221 functions as an IL-9 receptor or an IL-9 receptor-like protein. The INSP221 protein does not possess a transmembrane domain and accordingly the INSP221 protein is a potential soluble receptor. It is believed that the INSP221 protein may function as an IL-9 antagonist. Interestingly, the gene encoding the INSP221 protein is located on a different chromosome to the known IL-9 receptor gene.

In a first aspect, therefore, the invention provides a polypeptide, which polypeptide:

- (i) comprises or consists of the amino acid sequence as recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, and/or SEQ ID NO:28;
- (ii) is a fragment thereof having cytokine receptor activity (preferably IL-9 receptor activity or IL-9 receptor-like protein activity) or having an antigenic determinant in common with one or more of the polypeptides of (i); or
- (iii) is a functional equivalent of (i) or (ii).

- 10 Preferably, a polypeptide according to the invention possesses cytokine receptor activity, preferably IL-9 receptor activity or IL-9 receptor-like protein activity.

The polypeptide having the sequence recited in SEQ ID NO:2 is referred to hereafter as "INSP221 protein sequence exon 1". The polypeptide having the sequence recited in SEQ ID NO:4 is referred to hereafter as "INSP221 protein sequence exon 2". The polypeptide
15 having the sequence recited in SEQ ID NO:6 is referred to hereafter as "INSP221 protein sequence exon 3". SEQ ID NO:8 is produced by combining SEQ ID NO: 2, 4, and 6. The polypeptide having the sequence recited in SEQ ID NO:8 is referred to hereafter as "INSP221 full protein sequence".

- It is anticipated that additional amino acids may reside at the N-terminal to those recited in
20 SEQ ID NO:2 and SEQ ID NO:8. Thus, polypeptides of the present invention may include additional N-terminal amino acid residues. The full-length INSP221 polypeptide is included as one aspect of the invention.

- The INSP221 polypeptide may contain one or two substitutions in exon 3 (R103H and R91W), that may represent polymorphisms. The polypeptide having the sequence recited
25 in SEQ ID NO:12 is referred to hereafter as "INSP221 protein sequence exon 3 R103H polymorphism". The polypeptide having the sequence recited in SEQ ID NO:14 is referred to hereafter as "INSP221 full protein sequence R103H polymorphism". The polypeptide having the sequence recited in SEQ ID NO:18 is referred to hereafter as "INSP221 protein sequence exon 3 R91W polymorphism". The polypeptide having the sequence recited in

SEQ ID NO:20 is referred to hereafter as “INSP221 full protein sequence R91W polymorphism”. The polypeptide having the sequence recited in SEQ ID NO:24 is referred to hereafter as “INSP221 protein sequence exon 3 R91W & R103H polymorphisms”. The polypeptide having the sequence recited in SEQ ID NO:26 is referred to hereafter as
5 “INSP221 full protein sequence 3 R91W & R103H polymorphisms”.

In one embodiment of the first aspect of the invention, the polypeptide may comprise or consist of the amino acid sequence as recited in: SEQ ID NO:2 and SEQ ID NO:4 combined; or SEQ ID NO:4 and SEQ ID NO:6 combined; or SEQ ID NO:4 and SEQ ID NO:12 combined; or SEQ ID NO:4 and SEQ ID NO:18 combined; or SEQ ID NO:4 and
10 SEQ ID NO:24 combined. The polypeptide may comprise or consist of the amino acid sequence as recited in SEQ ID NO:8, SEQ ID NO:14, SEQ ID NO:20 or SEQ ID NO:26. The polypeptide of the first aspect of the invention may comprise or consist of a full length INSP221 polypeptide.

The polypeptides of the first aspect of the invention as described above may further
15 comprise a histidine tag. Preferably, the histidine tag is found at the C-terminus of the polypeptides. Preferably the histidine tag comprises 1-10 histidine residues (e.g. 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues). More preferably, the histidine tag comprises 6 histidine residues. Preferred polypeptides of this type are therefore those comprising the sequence recited in SEQ ID NO: 10, SEQ ID NO:16, SEQ ID NO:22 or SEQ ID NO:28. Preferably,
20 the polypeptides consist of the sequence recited in SEQ ID NO: 10, SEQ ID NO:16, SEQ ID NO:22 or SEQ ID NO:28.

The polypeptide having the sequence recited in SEQ ID NO:10 is referred to hereafter as “INSP221 full protein sequence and HIS tag”. The polypeptide having the sequence recited in SEQ ID NO:16 is referred to hereafter as “INSP221 full protein sequence R103H
25 polymorphism and HIS tag”. The polypeptide having the sequence recited in SEQ ID NO:22 is referred to hereafter as “INSP221 full protein sequence R91W polymorphism and HIS tag”. The polypeptide having the sequence recited in SEQ ID NO:28 is referred to hereafter as “INSP221 full protein sequence 3 R91W & R103H polymorphism and HIS tag”.

30 The term “IL-9 receptor-like protein” refers to a molecule containing at least one IL-9-like domain.

Preferably, the "IL-9 receptor-like protein" may be a molecule containing a IL-9-like domain detected with an e-value lower than 0.1, 0.01, 0.001, 0.0001, 0.00001, 0.000001 or 0.0000001.

Preferably, a polypeptide according to any one of the above-described aspects of the
5 invention functions as an IL-9 receptor-like protein.

By "functions as an IL-9 receptor-like protein" we refer to polypeptides that comprise amino acid sequence or structural features that can be identified as conserved features within an IL-9 receptor-like proteins, such that a biological activity is shared with an IL-9 receptor.

10 The polypeptides described herein are members of the Hematopoietin receptor superfamily. This superfamily is the largest cytokine receptor superfamily constituted of cytokine receptor subunits except the α -chains of the IL-2 and IL-15 receptors. They share a number of features at the level of primary sequence that determine key elements of their tertiary structures. Receptors of the hematopoietin superfamily possess 1 or 2 conserved
15 regions of approximately 200 residues, called cytokine receptor modules, that consist of 2 β -barrel structural domains (Bagley CJ *et al.* (2001 April) *Int J Hematol* 73(3):299-307).

IL-9 is a multifunctional cytokine that forms part of this superfamily, that is produced by activated TH2 clones *in vitro* and during TH2-like T cell responses *in vivo*. The biological activities of IL-9 are discussed in, for example, Demoulin JB and Renauld JC *Int Rev*
20 *Immunol* 1998;16(3-4):345-64. The biological activities of IL-9 are believed to include roles in hematopoiesis, tumorigenesis and regulation of the immune system. IL-9 is believed to play a role in various diseases including malignancies such as Hodgkin's disease (Demoulin JB and Renauld JC *Int Rev Immunol* 1998;16(3-4):345-64), asthma (Soussi-Gounni A *et al.* *J Allergy Clin Immunol* 2001 Apr;107(4):575-82; Kauppi P *et al.*
25 *J.Eur J Hum Genet* 2000 Oct;8(10):788-92; and Shimbara A *et al.* *J. Allergy Clin Immunol* 2000 Jan;105(1 Pt 1):108-15). In view of the foregoing it will be appreciated that IL-9 antagonists may be useful in the treatment of malignancies and inflammatory disease such as asthma and other diseases in which IL-9 is implicated.

Much of the work on the IL-9 receptor (IL-9R) has been conducted on murine IL-9R. The
30 murine IL-9 receptor contains 468 amino acids including an extracellular domain that

shows the typical features of the hematopoietin superfamily, namely 4 conserved cysteines and a WSEWS motif, located a few residues upstream from the transmembrane domain (Demoulin and Renault JC *supra*). As observed for many members of the hematopoietin receptor superfamily, a murine IL-9R mRNA has been 5 identified that lacks the sequences encoding the transmembrane and cytoplasmic domains, as a result of alternative splicing (Renault JC *et al. Proc Natl Acad Sci U S A* 1992 Jun 15;89(12):5690-4).

Soluble receptor cDNAs, besides their putative physiological role in the regulation of the immune response, could also provide highly specific tools for modulating the activity of 10 their corresponding ligands, either by blocking the active site or by mediating interactions with another cell surface glycoprotein as was described for the IL-6 receptor (Renault JC *et al. Proc Natl Acad Sci U S A* 1992 Jun 15;89(12):5690-4).

Recently, the human IL-9 receptor gene locus has been implicated in determining susceptibility to bronchial hyperresponsiveness and asthma (Grasso L *et al. J Biol Chem* 15 1998 Sep 11;273(37):24016-24).

Cytokine receptors, and more specifically IL-9 receptors and IL-9 receptor-like proteins, may be useful for regulating the immune system (for instance in promoting a response against cancer) and may also be useful in the treatment, prevention and/or diagnosis of medical conditions and diseases which include immune disorders, examples of which 20 include autoimmune disease, rheumatoid arthritis, osteoarthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis, inflammatory disorders, such as allergy, rhinitis, bronchial hyperresponsiveness, conjunctivitis, glomerulonephritis, uveitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, pancreatitis, digestive system inflammation, sepsis, endotoxic shock, septic shock, cachexia, myalgia, ankylosing 25 spondylitis, myasthenia gravis, post-viral fatigue syndrome, pulmonary disease, respiratory distress syndrome, asthma, chronic-obstructive pulmonary disease, airway inflammation, wound healing, endometriosis, dermatological disease, Behcet's disease, neoplastic disorders, such as melanoma, sarcoma, renal tumour, colon tumour, haematological disease, myeloproliferative disorder, Hodgkin's disease, osteoporosis, obesity, diabetes, 30 gout, cardiovascular disorders, reperfusion injury, atherosclerosis, ischaemic heart disease, cardiac failure, stroke, liver disease, AIDS, AIDS related complex, neurological disorders,

male infertility, ageing and infections, including plasmodium infection, bacterial infection and viral infection, particularly human herpesvirus 5 (cytomegalovirus) infection.

The polypeptides of the present invention may modulate a variety of physiological and pathological processes or disorders. Thus, the biological activity or function of these
5 polypeptides can be examined in systems that allow the study of such modulatory activities, using a variety of suitable assays.

Thus, preferably, an IL-9-like protein shows biological activity in at least one of the assays described herein.

An "antigenic determinant" of the present invention may be a part of a polypeptide of the
10 present invention, which binds to an antibody-combining site or to a T-cell receptor (TCR). Alternatively, an "antigenic determinant" may be a site on the surface of a polypeptide of the present invention to which a single antibody molecule binds. Generally an antigen has several or many different antigenic determinants and reacts with antibodies of many different specificities. Preferably, the antibody is immunospecific to a polypeptide of the
15 invention. Preferably, the antibody is immunospecific to a polypeptide of the invention, which is not part of a fusion protein. Preferably, the antibody is immunospecific to INSP221 or a fragment thereof. Antigenic determinants usually consist of chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three dimensional structural characteristics, as well as specific charge
20 characteristics. Preferably, the "antigenic determinant" refers to a particular chemical group on a polypeptide of the present invention that is antigenic, *i.e.* that elicit a specific immune response.

Preferably, there is provided a polypeptide of the first aspect of the invention which is an IL-9 antagonist. To determine if a polypeptide or fragment exhibits IL-9 receptor activity /
25 IL-9 receptor-like protein activity, cells, for example, murine cells, may be transfected with the cDNA encoding said polypeptide and then the cells tested for their proliferative response to human IL-9. Original murine cells are unresponsive to human IL-9 and become responsive (they proliferate) after transfection with cDNA encoding a polypeptide exhibiting IL-9 receptor activity / IL-9 receptor-like protein (see Renauld JC, Druez C,
30 Kermouni A, Houssiau F, Uyttenhove C, Van Roost E, Van Snick J., Expression cloning

of the murine and human interleukin 9 receptor cDNAs. Proc Natl Acad Sci U S A. 1992 Jun 15;89(12):5690-4 (see in particular page 5692 left column and Figure 5)).

An "antigenic determinant" of the present invention may be a part of a polypeptide of the present invention, which binds to an antibody-combining site or to a T-cell receptor (TCR).

5 Alternatively, an "antigenic determinant" may be a site on the surface of a polypeptide of the present invention to which a single antibody molecule binds. Generally an antigen has several or many different antigenic determinants and reacts with antibodies of many different specificities. Preferably, the antibody is immunospecific to a polypeptide of the invention. Preferably, the antibody is immunospecific to a polypeptide of the invention,
10 which is not part of a fusion protein. Preferably, the antibody is immunospecific to the INSP221 polypeptide or to a fragment thereof. Antigenic determinants usually consist of chemically active surface groupings of molecules, such as amino acids or sugar side chains, and can have specific three dimensional structural characteristics, as well as specific charge characteristics. Preferably, the "antigenic determinant" refers to a particular
15 chemical group on a polypeptide of the present invention that is antigenic, i.e. that elicit a specific immune response.

In a second aspect, the invention provides a purified nucleic acid molecule which encodes a polypeptide of the first aspect of the invention.

The term "purified nucleic acid molecule" preferably refers to a nucleic acid molecule of
20 the invention that (1) has been separated from at least about 50 percent of proteins, lipids, carbohydrates, or other materials with which it is naturally found when total nucleic acid is isolated from the source cells, (2) is not linked to all or a portion of a polynucleotide to which the "purified nucleic acid molecule" is linked in nature, (3) is operably linked to a polynucleotide which it is not linked to in nature, or (4) does not occur in nature as part of
25 a larger polynucleotide sequence. Preferably, the isolated nucleic acid molecule of the present invention is substantially free from any other contaminating nucleic acid molecule(s) or other contaminants that are found in its natural environment that would interfere with its use in polypeptide production or its therapeutic, diagnostic, prophylactic or research use. In a preferred embodiment, genomic DNA are specifically excluded from
30 the scope of the invention. Preferably, genomic DNA larger than 10 kbp (kilo base pairs), 50 kbp, 100 kbp, 150 kbp, 200 kbp, 250 kbp or 300 kbp are specifically excluded from the

scope of the invention. Preferably, the “purified nucleic acid molecule” consists of cDNA only.

Preferably, the purified nucleic acid molecule has the nucleic acid sequence as recited in SEQ ID NO:1 (encoding the INSP221 protein sequence exon 1), SEQ ID NO:3 (encoding
5 the INSP221 protein sequence exon 2), SEQ ID NO:5 (encoding the INSP221 protein sequence exon 3), or is a redundant equivalent or fragment of any of these sequences. SEQ ID NO:7 is produced by combining SEQ ID NO: 1, 3 and 5. A purified nucleic acid molecule having the nucleic acid sequence as recited in SEQ ID NO:7 (encoding the INSP221 full protein sequence) is also included in this aspect of the invention. SEQ ID
10 NO:9 (encoding the INSP221 full nucleotide sequence and HIS tag), SEQ ID NO:11 (encoding the INSP221 protein sequence exon 3 R103H polymorphism), SEQ ID NO:13 (encoding the INSP221 full protein sequence R103H polymorphism), SEQ ID NO:15 (encoding the INSP221 full protein sequence R103H polymorphism and HIS tag), SEQ ID NO:17 (encoding the INSP221 protein sequence exon 3 R91W polymorphism), SEQ ID
15 NO:19 (encoding the INSP221 full protein sequence R91W polymorphism), SEQ ID NO:21 (encoding the INSP221 full protein sequence R91W polymorphism and HIS tag), SEQ ID NO:23 (encoding the INSP221 protein sequence exon 3 R91W & R103H polymorphisms), SEQ ID NO:25 (encoding the INSP221 full protein sequence R91W & R103H polymorphisms), SEQ ID NO:27 (encoding the INSP221 full protein sequence
20 R91W & R103H polymorphisms and HIS tag) or is a redundant equivalent or fragment of any of these sequences. Also, the nucleic acid molecules of the invention may also comprise further nucleotides that are positioned 5' to the sequence given in SEQ ID NO:7 which code for the putative additional amino acids mentioned above.

In a third aspect, the invention provides a purified nucleic acid molecule which hybridizes
25 under high stringency conditions with a nucleic acid molecule of the second aspect of the invention. High stringency hybridisation conditions are defined as overnight incubation at 42°C in a solution comprising 50% formamide, 5XSSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5x Denhardts solution, 10% dextran sulphate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the
30 filters in 0.1X SSC at approximately 65°C.

In a fourth aspect, the invention provides a vector, such as an expression vector, that contains a nucleic acid molecule of the second or third aspect of the invention.

In a fifth aspect, the invention provides a host cell transformed with a vector of the fourth aspect of the invention.

- 5 In a sixth aspect, the invention provides a ligand which binds specifically to, and which preferably inhibits the cytokine receptor activity of, a polypeptide of the first aspect of the invention.

- In a seventh aspect, the invention provides a compound that is effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the
10 invention or to regulate the activity of a polypeptide of the first aspect of the invention.

- Ligands to a polypeptide according to the invention may come in various forms, including natural or modified substrates, enzymes, receptors, small organic molecules such as small natural or synthetic organic molecules of up to 2000Da, preferably 800Da or less, peptidomimetics, inorganic molecules, peptides, polypeptides, antibodies, structural or
15 functional mimetics of the aforementioned.

Such compounds may be identified using the assays and screening methods disclosed herein.

A compound of the seventh aspect of the invention may either increase (agonise) or decrease (antagonise) the level of expression of the gene or the activity of the polypeptide.

- 20 Importantly, the identification of the function of the INSP221 polypeptides allows for the design of screening methods capable of identifying compounds that are effective in the treatment and/or diagnosis of disease. Ligands and compounds according to the sixth and seventh aspects of the invention may be identified using such methods. These methods are included as aspects of the present invention.
- 25 Another aspect of this invention resides in the use of a INSP221 gene or polypeptide as a target for the screening of candidate drug modulators, particularly candidate drugs active against immune related disorders.

A further aspect of this invention resides in methods of screening of compounds for therapy of immune related disorders, comprising determining the ability of a compound to bind a INSP221 gene or polypeptide, or a fragment thereof.

A further aspect of this invention resides in methods of screening of compounds for
5 therapy of immune related disorders, comprising testing for modulation of the activity of a INSP221 gene or polypeptide, or a fragment thereof.

In an eighth aspect, the invention provides a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the
10 invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, for use in therapy or diagnosis. These molecules (the polypeptides of the first aspect of the invention, a nucleic acid molecule of the second or third aspect of the invention, a vector of the fourth aspect of the invention, a host cell of the fifth aspect of the invention, a ligand of the sixth aspect of the invention, a compound of the seventh
15 aspect of the invention) may also be used in the manufacture of a medicament for the treatment of certain diseases including, but not limited to, immune disorders, such as autoimmune disease, rheumatoid arthritis, osteoarthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis, inflammatory disorders, such as allergy, rhinitis, bronchial hyperresponsiveness, conjunctivitis, glomerulonephritis, uveitis, Crohn's
20 disease, ulcerative colitis, inflammatory bowel disease, pancreatitis, digestive system inflammation, sepsis, endotoxic shock, septic shock, cachexia, myalgia, ankylosing spondylitis, myasthenia gravis, post-viral fatigue syndrome, pulmonary disease, respiratory distress syndrome, asthma, chronic-obstructive pulmonary disease, airway inflammation, wound healing, endometriosis, dermatological disease, Behcet's disease, neoplastic
25 disorders, such as melanoma, sarcoma, renal tumour, colon tumour, haematological disease, myeloproliferative disorder, Hodgkin's disease, osteoporosis, obesity, diabetes, gout, cardiovascular disorders, reperfusion injury, atherosclerosis, ischaemic heart disease, cardiac failure, stroke, liver disease, AIDS, AIDS related complex, neurological disorders, male infertility, ageing and infections, including plasmodium infection, bacterial infection
30 and viral infection, particularly human herpesvirus 5 (cytomegalovirus) infection.

Thus, embodiments of this invention provide for the use of the polypeptides in inflammatory diseases, parasitic infections or malignancies/cancer.

In one embodiment of the invention, the parasitic infection is selected from a nematode infection or trypanosomal disease. Preferably, the nematode infection is toxocariasis.

5 Preferably, the trypanosomal disease is selected from leishmaniasis or trypanosomiasis.

In one embodiment of the invention, the inflammatory disease is selected from allergic diseases, asthma, pulmonary inflammatory diseases or asymptomatic bronchial hyperresponsiveness (BHR). Preferably, the pulmonary inflammatory disease is selected from chronic obstructive pulmonary disease (COPD) or lung fibrosis. Preferably, the
10 allergic disease is allergic rhinitis.

In one embodiment of the invention, the malignancy/cancer is a lymphoma. Preferably, the lymphoma is Hodgkin's disease.

The moieties of the present invention (*i.e.* the polypeptides of the first aspect of the invention, a nucleic acid molecule of the second or third aspect of the invention, a vector
15 of the fourth aspect of the invention, a host cell of the fifth aspect of the invention, a ligand of the sixth aspect of the invention, a compound of the seventh aspect of the invention) may have particular utility in the therapy or diagnosis of disorders/diseases (the two terms are used interchangeably herein) of the immune system (*e.g.* an autoimmune disease), malignancies, inflammatory disorders, asthma, rheumatoid arthritis, Crohn's disease and
20 MS.

In a ninth aspect, the invention provides a method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide of the first aspect of the invention or the activity of a polypeptide of the first aspect of the invention in tissue from said patient and comparing said level of expression or activity to a
25 control level, wherein a level that is different to said control level is indicative of disease. Such a method will preferably be carried out *in vitro*. Similar methods may be used for monitoring the therapeutic treatment of disease in a patient, wherein altering the level of expression or activity of a polypeptide or nucleic acid molecule over a period of time towards a control level is indicative of regression of disease.

A preferred method for detecting polypeptides of the first aspect of the invention comprises the steps of: (a) contacting a ligand, such as an antibody, of the sixth aspect of the invention with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

- 5 A number of different such methods according to the ninth aspect of the invention exist, as the skilled reader will be aware, such as methods of nucleic acid hybridization with short probes, point mutation analysis, polymerase chain reaction (PCR) amplification and methods using antibodies to detect aberrant protein levels. Similar methods may be used on a short or long term basis to allow therapeutic treatment of a disease to be monitored in
- 10 a patient. The invention also provides kits that are useful in these methods for diagnosing disease.

Preferably, the disease diagnosed by the ninth aspect of the invention is a disease in which a cytokine receptor (and preferably an IL-9 receptor or IL-9 receptor-like protein) is implicated. Preferably, the disease diagnosed by the ninth aspect of the invention is an

15 immune system disorder (*e.g.* an autoimmune disease), a malignancy, an inflammatory disorder, asthma, rheumatoid arthritis, Crohn's disease or MS. In a particularly preferred embodiment the disease is an autoimmune disease.

In a tenth aspect, the invention provides for the use of a polypeptide of the first aspect of the invention as a cytokine receptor, preferably as an IL-9 receptor or an IL-9 receptor-like

20 protein. In one embodiment of the tenth aspect of the invention there is provided the use of a polypeptide of the first aspect of the invention as an IL-9 antagonist.

In an eleventh aspect, the invention provides a pharmaceutical composition comprising a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell

25 of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, in conjunction with a pharmaceutically-acceptable carrier.

In a twelfth aspect, the present invention provides a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a

30 vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the

invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention, for use in the manufacture of a medicament for the diagnosis or treatment of a disease including, but not limited to, immune disorders, such as autoimmune disease, rheumatoid arthritis, osteoarthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis, inflammatory disorders, such as allergy, rhinitis, bronchial hyperresponsiveness, conjunctivitis, glomerulonephritis, uveitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, pancreatitis, digestive system inflammation, sepsis, endotoxic shock, septic shock, cachexia, myalgia, ankylosing spondylitis, myasthenia gravis, post-viral fatigue syndrome, pulmonary disease, respiratory distress syndrome, asthma, chronic-obstructive pulmonary disease, airway inflammation, wound healing, endometriosis, dermatological disease, Behcet's disease, neoplastic disorders, such as melanoma, sarcoma, renal tumour, colon tumour, haematological disease, myeloproliferative disorder, Hodgkin's disease, osteoporosis, obesity, diabetes, gout, cardiovascular disorders, reperfusion injury, atherosclerosis, ischaemic heart disease, cardiac failure, stroke, liver disease, AIDS, AIDS related complex, neurological disorders, male infertility, ageing and infections, including plasmodium infection, bacterial infection and viral infection, particularly human herpesvirus 5 (cytomegalovirus) infection. Preferably, the medicament of the twelfth aspect of the invention is for a disease in which a cytokine receptor (and preferably an IL-9 receptor or IL-9 receptor-like protein) is implicated. Preferably, the disease is an autoimmune disease.

In a thirteenth aspect, the invention provides a method of treating a disease in a patient comprising administering to the patient a polypeptide of the first aspect of the invention, or a nucleic acid molecule of the second or third aspect of the invention, or a vector of the fourth aspect of the invention, or a host cell of the fifth aspect of the invention, or a ligand of the sixth aspect of the invention, or a compound of the seventh aspect of the invention.

For diseases in which the expression of a natural gene encoding a polypeptide of the first aspect of the invention, or in which the activity of a polypeptide of the first aspect of the invention, is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, ligand or compound administered to the patient should be an agonist. Conversely, for diseases in which the expression of the natural gene or activity of the polypeptide is higher in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide,

nucleic acid molecule, ligand or compound administered to the patient should be an antagonist. Examples of such antagonists include antisense nucleic acid molecules, ribozymes and ligands, such as antibodies.

The INSP221 polypeptides are IL-9 proteins and thus have roles in many disease states.

- 5 Antagonists of the INSP221 polypeptides are of particular interest as they provide a way of modulating these disease states.

Preferably, the disease is a disease in which a cytokine receptor (and preferably an IL-9 receptor or IL-9 receptor-like protein) is implicated. Preferably, the disease is an autoimmune disease.

- 10 In a fourteenth aspect, the invention provides transgenic or knockout non-human animals that have been transformed to express higher, lower or absent levels of a polypeptide of the first aspect of the invention. Such transgenic animals are very useful models for the study of disease and may also be using in screening regimes for the identification of compounds that are effective in the treatment or diagnosis of such a disease.
- 15 As used herein, "functional equivalent" refers to a protein or nucleic acid molecule that possesses functional or structural characteristics that are substantially similar to a polypeptide or nucleic acid molecule of the present invention. A functional equivalent of a protein may contain modifications depending on the necessity of such modifications for the performance of a specific function. The term "functional equivalent" is intended to
- 20 include the fragments, mutants, hybrids, variants, analogs, and chemical derivatives of a molecule.

Preferably, the "functional equivalent" may be a protein or nucleic acid molecule that exhibits any one or more of the functional activities of the polypeptides of the present invention.

- 25 Preferably, the "functional equivalent" may be a protein or nucleic acid molecule that displays substantially similar activity compared with INSP221 or fragments thereof in a suitable assay for the measurement of biological activity or function. Preferably, the "functional equivalent" may be a protein or nucleic acid molecule that displays identical or higher activity compared with INSP221 or fragments thereof in a suitable assay for the
- 30 measurement of biological activity or function. Preferably, the "functional equivalent" may

be a protein or nucleic acid molecule that displays 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, 100% or more activity compared with INSP221 or fragments thereof in a suitable assay for the measurement of biological activity or function.

Preferably, the "functional equivalent" may be a protein or polypeptide capable of
5 exhibiting a substantially similar *in vivo* or *in vitro* activity as the polypeptides of the invention. Preferably, the "functional equivalent" may be a protein or polypeptide capable of interacting with other cellular or extracellular molecules in a manner substantially similar to the way in which the corresponding portion of the polypeptides of the invention would. For example, a "functional equivalent" would be able, in an immunoassay, to
10 diminish the binding of an antibody to the corresponding peptide (*i.e.*, the peptide the amino acid sequence of which was modified to achieve the "functional equivalent") of the polypeptide of the invention, or to the polypeptide of the invention itself, where the antibody was raised against the corresponding peptide of the polypeptide of the invention. An equimolar concentration of the functional equivalent will diminish the aforesaid
15 binding of the corresponding peptide by at least about 5%, preferably between about 5% and 10%, more preferably between about 10% and 25%, even more preferably between about 25% and 50%, and most preferably between about 40% and 50%.

For example, functional equivalents can be fully functional or can lack function in one or more activities. Thus, in the present invention, variations can affect the function, for
20 example, of ubiquitin binding, ubiquitin recognition, interaction with ubiquitinated substrate protein, such as binding or proteolysis, subunit interaction, particularly within the proteasome, activation or binding by ATP, developmental expression, temporal expression, tissue-specific expression, interacting with cellular components, such as transcriptional regulatory factors, and particularly trans-acting transcriptional regulatory factors,
25 proteolytic cleavage of peptide bonds in polyubiquitin and peptide bonds between ubiquitin or polyubiquitin and substrate protein, and proteolytic cleavage of peptide bonds between ubiquitin or polyubiquitin and a peptide or amino acid.

A summary of standard techniques and procedures which may be employed in order to utilise the invention are given below. It will be understood that this invention is not limited
30 to the particular methodology, protocols, cell lines, vectors and reagents described. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and it is not intended that this terminology should limit the

scope of the present invention. The extent of the invention is limited only by the terms of the appended claims.

Standard abbreviations for nucleotides and amino acids are used in this specification.

The practice of the present invention will employ, unless otherwise indicated, conventional
5 techniques of molecular biology, microbiology, recombinant DNA technology and immunology, which are within the skill of those working in the art.

Such techniques are explained fully in the literature. Examples of particularly suitable texts for consultation include the following: Sambrook Molecular Cloning; A Laboratory Manual, Second Edition (1989); DNA Cloning, Volumes I and II (D.N Glover ed. 1985);
10 Oligonucleotide Synthesis (M.J. Gait ed. 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds. 1984); Transcription and Translation (B.D. Hames & S.J. Higgins eds. 1984); Animal Cell Culture (R.I. Freshney ed. 1986); Immobilized Cells and Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide to Molecular Cloning (1984); the Methods in Enzymology series (Academic Press, Inc.), especially volumes 154 & 155; Gene
15 Transfer Vectors for Mammalian Cells (J.H. Miller and M.P. Calos eds. 1987, Cold Spring Harbor Laboratory); Immunochemical Methods in Cell and Molecular Biology (Mayer and Walker, eds. 1987, Academic Press, London); Scopes, (1987) Protein Purification: Principles and Practice, Second Edition (Springer Verlag, N.Y.); and Handbook of Experimental Immunology, Volumes I-IV (D.M. Weir and C. C. Blackwell eds. 1986).

20 As used herein, the term "polypeptide" includes any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e. peptide isosteres. This term refers both to short chains (peptides and oligopeptides) and to longer chains (proteins).

The polypeptide of the present invention may be in the form of a mature protein or may be
25 a pre-, pro- or prepro- protein that can be activated by cleavage of the pre-, pro- or prepro- portion to produce an active mature polypeptide. In such polypeptides, the pre-, pro- or prepro- sequence may be a leader or secretory sequence or may be a sequence that is employed for purification of the mature polypeptide sequence.

The polypeptide of the first aspect of the invention may form part of a fusion protein. For
30 example, it is often advantageous to include one or more additional amino acid sequences

which may contain secretory or leader sequences, pro-sequences, sequences which aid in purification, or sequences that confer higher protein stability, for example during recombinant production. Alternatively or additionally, the mature polypeptide may be fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol).

In a further preferred embodiment, a polypeptide of the invention, that may comprise a sequence having at least 85% of homology with INSP221, is a fusion protein.

These fusion proteins can be obtained by cloning a polynucleotide encoding a polypeptide comprising a sequence having at least 85% of homology with INSP221 in frame to the coding sequences for a heterologous protein sequence.

The term "heterologous", when used herein, is intended to designate any polypeptide other than a human INSP221 polypeptide, or its splice variant INSP186.

Example of heterologous sequences, that can be comprised in the soluble fusion proteins either at N- or at C-terminus, are the following: extracellular domains of membrane-bound protein, immunoglobulin constant regions (Fc region), multimerization domains, domains of extracellular proteins, signal sequences, export sequences, or sequences allowing purification by affinity chromatography.

Many of these heterologous sequences are commercially available in expression plasmids since these sequences are commonly included in the fusion proteins in order to provide additional properties without significantly impairing the specific biological activity of the protein fused to them (Terpe K, Appl Microbiol Biotechnol, 60: 523-33, 2003). Examples of such additional properties are a longer lasting half-life in body fluids, the extracellular localization, or an easier purification procedure as allowed by the a stretch of Histidines forming the so-called "histidine tag"(Gentz et al., Proc Natl Acad Sci USA, 86: 821-4, 1989) or by the "HA" tag, an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell, 37: 767-78, 1994). If needed, the heterologous sequence can be eliminated by a proteolytic cleavage, for example by inserting a proteolytic cleavage site between the protein and the heterologous sequence, and exposing the purified fusion protein to the appropriate protease. These features are of particular importance for the fusion proteins since they facilitate their production and use in the preparation of

pharmaceutical compositions. For example, the protein used in the examples (INSP221) can be purified by means of a hexa-histidine peptide fused at the C-terminus of INSP221. When the fusion protein comprises an immunoglobulin region, the fusion may be direct, or via a short linker peptide which can be as short as 1 to 3 amino acid residues in length or longer, for example, 13 amino acid residues in length. Said linker may be a tripeptide of the sequence E-F-M (Glu-Phe-Met), for example, or a 13-amino acid linker sequence comprising Glu-Phe-Gly-Ala-Gly-Leu-Val-Leu-Gly-Gly-Gln-Phe-Met (SEQ ID NO: 29) introduced between the sequence of the substances of the invention and the immunoglobulin sequence. The resulting fusion protein has improved properties, such as an extended residence time in body fluids (half-life), increased specific activity, increased expression level, or the purification of the fusion protein is facilitated.

In a preferred embodiment, the protein is fused to the constant region of an Ig molecule. Preferably, it is fused to heavy chain regions, like the CH2 and CH3 domains of human IgG1, for example. Other isoforms of Ig molecules are also suitable for the generation of fusion proteins according to the present invention, such as isoforms IgG₂ or IgG₄, or other Ig classes, like IgM or IgA, for example. Fusion proteins may be monomeric or multimeric, hetero- or homomultimeric.

In a further preferred embodiment, the functional derivative comprises at least one moiety attached to one or more functional groups, which occur as one or more side chains on the amino acid residues. Preferably, the moiety is a polyethylene (PEG) moiety. PEGylation may be carried out by known methods, such as the ones described in WO99/55377, for example.

Polypeptides may contain amino acids other than the 20 gene-encoded amino acids, modified either by natural processes, such as by post-translational processing or by chemical modification techniques which are well known in the art. Among the known modifications which may commonly be present in polypeptides of the present invention are glycosylation, lipid attachment, sulphation, gamma-carboxylation, for instance of glutamic acid residues, hydroxylation and ADP-ribosylation. Other potential modifications include acetylation, acylation, amidation, covalent attachment of flavin, covalent attachment of a haeme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid derivative, covalent attachment of

phosphatidylinositol, cross-linking, cyclization, disulphide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, GPI anchor formation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination.

Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl terminus in a polypeptide, or both, by a covalent modification is common in naturally-occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention.

The modifications that occur in a polypeptide often will be a function of how the polypeptide is made. For polypeptides that are made recombinantly, the nature and extent of the modifications in large part will be determined by the post-translational modification capacity of the particular host cell and the modification signals that are present in the amino acid sequence of the polypeptide in question. For instance, glycosylation patterns vary between different types of host cell.

The polypeptides of the present invention can be prepared in any suitable manner. Such polypeptides include isolated naturally-occurring polypeptides (for example purified from cell culture), recombinantly-produced polypeptides (including fusion proteins), synthetically-produced polypeptides or polypeptides that are produced by a combination of these methods.

The functionally-equivalent polypeptides of the first aspect of the invention may be polypeptides that are homologous to the INSP221 polypeptides. Two polypeptides are said to be "homologous", as the term is used herein, if the sequence of one of the polypeptides has a high enough degree of identity or similarity to the sequence of the other polypeptide. "Identity" indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. "Similarity" indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. Degrees of identity and similarity can be readily calculated (Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988;

Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and
5 Devereux, J., eds., M Stockton Press, New York, 1991).

Homologous polypeptides therefore include natural biological variants (for example, allelic variants or geographical variations within the species from which the polypeptides are derived) and mutants (such as mutants containing amino acid substitutions, insertions or deletions) of the INSP221 polypeptides. Such mutants may include polypeptides in
10 which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; among the basic residues Lys and Arg;
15 or among the aromatic residues Phe and Tyr. Particularly preferred are variants in which several, i.e. between 5 and 10, 1 and 5, 1 and 3, 1 and 2 or just 1 amino acids are substituted, deleted or added in any combination. Especially preferred are silent substitutions, additions and deletions, which do not alter the properties and activities of the protein. Also especially preferred in this regard are conservative substitutions. Such
20 mutants also include polypeptides in which one or more of the amino acid residues includes a substituent group.

In accordance with the present invention, any substitution should be preferably a "conservative" or "safe" substitution, which is commonly defined a substitution introducing an amino acids having sufficiently similar chemical properties (e.g. a basic,
25 positively charged amino acid should be replaced by another basic, positively charged amino acid), in order to preserve the structure and the biological function of the molecule.

The literature provide many models on which the selection of conservative amino acids substitutions can be performed on the basis of statistical and physico-chemical studies on the sequence and/or the structure of proteins (Rogov SI and Nekrasov AN, 2001). Protein
30 design experiments have shown that the use of specific subsets of amino acids can produce foldable and active proteins, helping in the classification of amino acid "synonymous"

substitutions which can be more easily accommodated in protein structure, and which can be used to detect functional and structural homologs and paralogs (Murphy LR et al., 2000). The groups of synonymous amino acids and the groups of more preferred synonymous amino acids are shown in Table 1.

- 5 Specific, non-conservative mutations can be also introduced in the polypeptides of the invention with different purposes. Mutations reducing the affinity of the PMP-22/EMP/MP20/Claudin-like molecule may increase its ability to be reused and recycled, potentially increasing its therapeutic potency (Robinson CR, 2002). Immunogenic epitopes eventually present in the polypeptides of the invention can be exploited for developing
10 vaccines (Stevanovic S, 2002), or eliminated by modifying their sequence following known methods for selecting mutations for increasing protein stability, and correcting them (van den Burg B and Eijsink V, 2002; WO 02/05146, WO 00/34317, WO 98/52976).

Preferred alternative, synonymous groups for amino acids derivatives included in peptide mimetics are those defined in Table 2. A non-exhaustive list of amino acid derivatives also
15 include aminoisobutyric acid (Aib), hydroxyproline (Hyp), 1,2,3,4-tetrahydro-isoquinoline-3-COOH, indoline-2carboxylic acid, 4-difluoro-proline, L- thiazolidine-4-carboxylic acid, L-homoproline, 3,4-dehydro-proline, 3,4-dihydroxy-phenylalanine, cyclohexyl-glycine, and phenylglycine.

By "amino acid derivative" is intended an amino acid or amino acid-like chemical entity
20 other than one of the 20 genetically encoded naturally occurring amino acids. In particular, the amino acid derivative may contain substituted or non-substituted, linear, branched, or cyclic alkyl moieties, and may include one or more heteroatoms. The amino acid derivatives can be made de novo or obtained from commercial sources (Calbiochem-Novabiochem AG, Switzerland; Bachem, USA).

- 25 Various methodologies for incorporating unnatural amino acids derivatives into proteins, using both *in vitro* and *in vivo* translation systems, to probe and/or improve protein structure and function are disclosed in the literature (Dougherty DA, 2000). Techniques for the synthesis and the development of peptide mimetics, as well as non-peptide mimetics, are also well known in the art (Golebiowski A et al., 2001; Hruby VJ and Balse PM, 2000;
30 Sawyer TK, in "Structure Based Drug Design", edited by Veerapandian P, Marcel Dekker Inc., pg. 557-663, 1997).

Typically, greater than 30% identity between two polypeptides is considered to be an indication of functional equivalence. Preferably, functionally equivalent polypeptides of the first aspect of the invention have a degree of sequence identity with the INSP221 polypeptides, or with active fragments thereof, of greater than 80%. More preferred
5 polypeptides have degrees of identity of greater than 85%, 90%, 95%, 98%, 98.5%, 99% or 99.5% respectively.

The polypeptides of the first aspect of the invention also include fragments of the INSP221 polypeptides and fragments of the functional equivalents of the INSP221 polypeptides, provided that those fragments retain cytokine receptor activity (preferably IL-9 receptor
10 activity or IL-9 receptor-like protein activity) or have an antigenic determinant in common with the INSP221 polypeptide. Preferably, the fragments of the INSP221 polypeptides and fragments of the functional equivalents of the INSP221 polypeptides retain IL-9 antagonistic activity.

As used herein, the term "fragment" refers to a polypeptide having an amino acid sequence
15 that is the same as part, but not all, of the amino acid sequence of the INSP221 polypeptides or one of its functional equivalents. The fragments should comprise at least n consecutive amino acids from the sequence and, depending on the particular sequence, n preferably is 7 or more (for example, 8, 10, 12, 14, 16, 18, 20 or more). Small fragments may form an antigenic determinant.

20 Nucleic acids according to the invention are preferably 10-400 nucleotides in length, preferably 50-350 nucleotides, preferably 100-300, preferably 150-250, preferably 175-225 nucleotides in length. Polypeptides according to the invention are preferably 5-150 amino acids in length, preferably 10-130, preferably 25-120, preferably 50-100 amino acids in length.

25 Fragments of the full length INSP221 polypeptides may consist of combinations of 1 or 2 neighbouring exon sequences in the INSP221 polypeptide sequences, respectively. These exons may be combined with further mature fragments according to the invention. For example, such combinations include exons 1 and 2, exons 1 and 3 and so on. Such fragments are included in the present invention. Fragments may also consist of

combinations of different domains of the INSP221 protein. For example a fragment may consist the extracellular domain of INSP221 as recited above.

Such fragments may be "free-standing", *i.e.* not part of or fused to other amino acids or polypeptides, or they may be comprised within a larger polypeptide of which they form a part or region. When comprised within a larger polypeptide, the fragment of the invention most preferably forms a single continuous region. For instance, certain preferred embodiments relate to a fragment having a pre - and/or pro- polypeptide region fused to the amino terminus of the fragment and/or an additional region fused to the carboxyl terminus of the fragment. However, several fragments may be comprised within a single larger polypeptide.

The polypeptides of the present invention or their immunogenic fragments (comprising at least one antigenic determinant) can be used to generate ligands, such as polyclonal or monoclonal antibodies, that are immunospecific for the polypeptides. Such antibodies may be employed to isolate or to identify clones expressing the polypeptides of the invention or to purify the polypeptides by affinity chromatography. The antibodies may also be employed as diagnostic or therapeutic aids, amongst other applications, as will be apparent to the skilled reader.

The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art. The term "antibody" or "immunoglobulin" is intended to encompass both polyclonal and monoclonal antibodies. The preferred antibody is a monoclonal antibody reactive with the antigen. The term "antibody" is also intended to encompass mixtures of more than one antibody reactive with the antigen (e.g., a cocktail of different types of monoclonal antibodies reactive with the antigen). The term "antibody" is further intended to encompass whole antibodies, biologically functional fragments thereof, single-chain antibodies, and genetically altered antibodies such as chimeric antibodies comprising portions from more than one species, bifunctional antibodies, antibody conjugates, humanized and human antibodies. Biologically functional antibody fragments, which can also be used, are those peptide fragments derived from an antibody that are sufficient for binding to the antigen. Antibody as used herein is meant to include the entire antibody as well as any antibody fragments (e.g. F(ab')₂, Fab', Fab, Fv) capable of specifically binding

the epitope, antigen or antigenic fragment of interest. It will be appreciated that Fab and F(ab')₂ and other fragments of the antibodies useful in the present invention may be used for the detection and quantitation of their antigens according to the methods disclosed herein for intact antibody molecules. Such fragments are typically produced by proteolytic
5 cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')₂ fragments).

By "binds specifically" it is meant high avidity and/or high affinity binding of an antibody to a specific polypeptide i.e., INSP221. Antibody binding to its epitope on this specific polypeptide is preferably stronger than binding of the same antibody to any other epitope.
10 Antibodies which bind specifically to INSP221 may be capable of binding other polypeptides at a weak, yet detectable, level (e.g., 10% or less of the binding shown to the polypeptide of interest). Such weak binding, or background binding, is readily discernible from the specific antibody binding to the compound or polypeptide of interest, e.g. by use of appropriate controls.

15 As regards the antibodies mentioned herein throughout, the term "monoclonal antibody" is meant to include monoclonal antibodies, chimeric antibodies, fully humanized antibodies, antibodies to anti-idiotypic antibodies (anti-anti-Id antibody) that can be labelled in soluble or bound form, as well as fragments thereof provided by any known technique, such as, but not limited to enzymatic cleavage, peptide synthesis or recombinant techniques. A
20 monoclonal antibody contains a substantially homogeneous population of antibodies specific to antigens, which populations contain substantially similar epitope binding sites.

Monoclonal antibodies of the invention may be modified for use as therapeutics. One embodiment is a "chimeric" antibody in which a portion of the heavy (H) and/or light (L) chain is identical with or homologous to a corresponding sequence in antibodies derived
25 from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to a corresponding sequence in antibodies derived from another species or belonging to another antibody class or subclass. Also included are fragments of such antibodies, so long as they exhibit the desired biological activity.

30 The term "chimeric antibody" thus refers to an antibody in which the constant region comes from an antibody of one species (typically human) and the variable region comes

from an antibody of another species (typically rodent). Hence, chimeric antibodies are molecules of which different portions are derived from different animal species, such as those having the variable region derived from a murine Mab and a human immunoglobulin constant region. Chimeric antibodies are primarily used to reduce immunogenicity in application and to increase yields in production, for example, where murine Mabs have higher yields from hybridomas but higher immunogenicity in humans, such that human/murine chimeric Mabs are used.

In another embodiment, a monoclonal antibody of the invention is a "humanized" antibody. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source that is non-human. Humanization can be performed, for example, using methods described in the art, by substituting at least a portion of a rodent complementarity-determining region for the corresponding regions of a human antibody.

Thus, the term "humanized antibody" or "humanized immunoglobulin" refers to an immunoglobulin comprising a human framework, at least one and preferably all complementarity determining regions (CDRs) from a non-human antibody, and in which any constant region present is substantially identical to a human immunoglobulin constant region, i.e., at least about 85-90%, preferably at least 95% identical. Hence, all parts of a humanized immunoglobulin, except possibly the CDRs, are substantially identical to corresponding parts of one or more native human immunoglobulin sequences. See, e.g. Queen et al., U.S. Pat. Nos. 5,530,101; 5,585,089; 5,693,762; and 6,180,370 (each of which is incorporated by reference in its entirety).

"Fully humanized antibodies" are molecules containing both the variable and constant region of the human immunoglobulin. Fully humanized antibodies can be potentially used for therapeutic use, where repeated treatments are required for chronic and relapsing diseases such as autoimmune diseases. One method for the preparation of fully human antibodies consist of "humanization" of the mouse humoral immune system, i.e. production of mouse strains able to produce human Ig (Xenomice), by the introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated. The Ig loci are exceedingly complex in terms of both their physical structure and the gene rearrangement and expression processes required to ultimately produce a broad immune response. Antibody diversity is primarily generated by combinatorial

rearrangement between different V, D, and J genes present in the Ig loci. These loci also contain the interspersed regulatory elements, which control antibody expression, allelic exclusion, class switching and affinity maturation. Introduction of unrearranged human Ig transgenes into mice has demonstrated that the mouse recombination machinery is compatible with human genes. Furthermore, hybridomas secreting antigen specific hu-
5 mAbs of various isotypes can be obtained by Xenomice immunisation with antigen.

By “substantially greater affinity” we mean that there is a measurable increase in the affinity for a polypeptide of the invention as compared with the affinity for other related polypeptides in the prior art.

10 Preferably, the affinity is at least 1.5-fold, 2-fold, 5-fold 10-fold, 100-fold, 10^3 -fold, 10^4 -fold, 10^5 -fold or 10^6 -fold greater for a polypeptide of the invention than for other related polypeptides in the prior art.

Preferably, there is a measurable increase in the affinity for a polypeptide of the invention as compared with known IL-9 receptors.

15 Preferably, there is a measurable increase in the affinity for a polypeptide of the invention as compared with natural transmembrane IL-9 receptors.

If polyclonal antibodies are desired, a selected mammal, such as a mouse, rabbit, goat or horse, may be immunised with a polypeptide of the first aspect of the invention. The polypeptide used to immunise the animal can be derived by recombinant DNA technology
20 or can be synthesized chemically. If desired, the polypeptide can be conjugated to a carrier protein. Commonly used carriers to which the polypeptides may be chemically coupled include bovine serum albumin, thyroglobulin and keyhole limpet haemocyanin. The coupled polypeptide is then used to immunise the animal. Serum from the immunised animal is collected and treated according to known procedures, for example by
25 immunoaffinity chromatography.

Monoclonal antibodies to the polypeptides of the first aspect of the invention can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies using hybridoma technology is well known (see, for example, Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology*

Today 4: 72 (1983); Cole *et al.*, 77-96 in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985).

Panels of monoclonal antibodies produced against the polypeptides of the first aspect of the invention can be screened for various properties, i.e., for isotype, epitope, affinity, etc.

- 5 Monoclonal antibodies are particularly useful in purification of the individual polypeptides against which they are directed. Alternatively, genes encoding the monoclonal antibodies of interest may be isolated from hybridomas, for instance by PCR techniques known in the art, and cloned and expressed in appropriate vectors.

- Chimeric antibodies, in which non-human variable regions are joined or fused to human
10 constant regions (see, for example, Liu *et al.*, Proc. Natl. Acad. Sci. USA, 84, 3439 (1987)), may also be of use.

- The antibody may be modified to make it less immunogenic in an individual, for example by humanisation (see Jones *et al.*, Nature, 321, 522 (1986); Verhoeyen *et al.*, Science, 239, 1534 (1988); Kabat *et al.*, J. Immunol., 147, 1709 (1991); Queen *et al.*, Proc. Natl. Acad.
15 Sci. USA, 86, 10029 (1989); Gorman *et al.*, Proc. Natl. Acad. Sci. USA, 88, 34181 (1991); and Hodgson *et al.*, Bio/Technology, 9, 421 (1991)). The term "humanised antibody", as used herein, refers to antibody molecules in which the CDR amino acids and selected other amino acids in the variable domains of the heavy and/or light chains of a non-human donor antibody have been substituted in place of the equivalent amino acids in a human antibody.
20 The humanised antibody thus closely resembles a human antibody but has the binding ability of the donor antibody.

In a further alternative, the antibody may be a "bispecific" antibody, that is an antibody having two different antigen binding domains, each domain being directed against a different epitope.

- 25 Phage display technology may be utilised to select genes which encode antibodies with binding activities towards the polypeptides of the invention either from repertoires of PCR amplified V-genes of lymphocytes from humans screened for possessing the relevant antibodies, or from naive libraries (McCafferty, J. *et al.*, (1990), Nature 348, 552-554; Marks, J. *et al.*, (1992) Biotechnology 10, 779-783). The affinity of these antibodies can
30 also be improved by chain shuffling (Clackson, T. *et al.*, (1991) Nature 352, 624-628).

Antibodies generated by the above techniques, whether polyclonal or monoclonal, have additional utility in that they may be employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA). In these applications, the antibodies can be labelled with an analytically-
5 detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme.

Preferred nucleic acid molecules of the second and third aspects of the invention are those which encode the polypeptide sequences recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID
10 NO:28 and functionally equivalent polypeptides. These nucleic acid molecules may be used in the methods and applications described herein. The nucleic acid molecules of the invention preferably comprise at least n consecutive nucleotides from the sequences disclosed herein where, depending on the particular sequence, n is 10 or more (for example, 12, 14, 15, 18, 20, 25, 30, 35, 40 or more).

15 The nucleic acid molecules of the invention also include sequences that are complementary to nucleic acid molecules described above (for example, for antisense or probing purposes).

Nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance cDNA, synthetic DNA or genomic
20 DNA. Such nucleic acid molecules may be obtained by cloning, by chemical synthetic techniques or by a combination thereof. The nucleic acid molecules can be prepared, for example, by chemical synthesis using techniques such as solid phase phosphoramidite chemical synthesis, from genomic or cDNA libraries or by separation from an organism. RNA molecules may generally be generated by the *in vitro* or *in vivo* transcription of DNA
25 sequences.

The nucleic acid molecules may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

The term "nucleic acid molecule" also includes analogues of DNA and RNA, such as those
30 containing modified backbones, and peptide nucleic acids (PNA). The term "PNA", as

used herein, refers to an antisense molecule or an anti-gene agent which comprises an oligonucleotide of at least five nucleotides in length linked to a peptide backbone of amino acid residues, which preferably ends in lysine. The terminal lysine confers solubility to the composition. PNAs may be pegylated to extend their lifespan in a cell, where they
5 preferentially bind complementary single stranded DNA and RNA and stop transcript elongation (Nielsen, P.E. *et al.* (1993) *Anticancer Drug Des.* 8:53-63).

A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:2 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:1. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:4 may be identical to the
10 coding sequence of the nucleic acid molecule shown in SEQ ID NO:3. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:6 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:5. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:8 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:7. A nucleic acid molecule which
15 encodes the polypeptide of SEQ ID NO:10 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:9. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:12 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:11. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:14 may be identical to the coding sequence of the nucleic acid
20 molecule shown in SEQ ID NO:13. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:16 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:15. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:18 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:17. A nucleic acid molecule which encodes the
25 polypeptide of SEQ ID NO:20 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:19. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:22 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:21. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:24 may be identical to the coding sequence of the nucleic acid
30 molecule shown in SEQ ID NO:23. A nucleic acid molecule which encodes the polypeptide of SEQ ID NO:26 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:25. A nucleic acid molecule which encodes the

polypeptide of SEQ ID NO:28 may be identical to the coding sequence of the nucleic acid molecule shown in SEQ ID NO:27. These molecules also may have a different sequence which, as a result of the degeneracy of the genetic code, encodes a polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 or SEQ ID NO:28. Such nucleic acid molecules that encode the polypeptide of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 or SEQ ID NO:28 may include, but are not limited to, the coding sequence for the mature polypeptide by itself; the coding sequence for the mature polypeptide and additional coding sequences, such as those encoding a leader or secretory sequence, such as a pro-, pre- or prepro- polypeptide sequence; the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with further additional, non-coding sequences, including non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription (including termination signals), ribosome binding and mRNA stability. The nucleic acid molecules may also include additional sequences which encode additional amino acids, such as those which provide additional functionalities.

The nucleic acid molecules of the second and third aspects of the invention may also encode the fragments or the functional equivalents of the polypeptides and fragments of the first aspect of the invention. Such a nucleic acid molecule may be a naturally occurring variant such as a naturally occurring allelic variant, or the molecule may be a variant that is not known to occur naturally. Such non-naturally occurring variants of the nucleic acid molecule may be made by mutagenesis techniques, including those applied to nucleic acid molecules, cells or organisms.

Among variants in this regard are variants that differ from the aforementioned nucleic acid molecules by nucleotide substitutions, deletions or insertions. The substitutions, deletions or insertions may involve one or more nucleotides. The variants may be altered in coding or non-coding regions or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or insertions.

The nucleic acid molecules of the invention can also be engineered, using methods generally known in the art, for a variety of reasons, including modifying the cloning, processing, and/or expression of the gene product (the polypeptide). DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic
5 oligonucleotides are included as techniques which may be used to engineer the nucleotide sequences. Site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, introduce mutations and so forth.

Nucleic acid molecules which encode a polypeptide of the first aspect of the invention may
10 be ligated to a heterologous sequence so that the combined nucleic acid molecule encodes a fusion protein. Such combined nucleic acid molecules are included within the second or third aspects of the invention. For example, to screen peptide libraries for inhibitors of the activity of the polypeptide, it may be useful to express, using such a combined nucleic acid molecule, a fusion protein that can be recognised by a commercially-available antibody. A
15 fusion protein may also be engineered to contain a cleavage site located between the sequence of the polypeptide of the invention and the sequence of a heterologous protein so that the polypeptide may be cleaved and purified away from the heterologous protein.

The nucleic acid molecules of the invention also include antisense molecules that are partially complementary to nucleic acid molecules encoding polypeptides of the present
20 invention and that therefore hybridize to the encoding nucleic acid molecules (hybridization). Such antisense molecules, such as oligonucleotides, can be designed to recognise, specifically bind to and prevent transcription of a target nucleic acid encoding a polypeptide of the invention, as will be known by those of ordinary skill in the art (see, for example, Cohen, J.S., Trends in Pharm. Sci., 10, 435 (1989), Okano, J. Neurochem. 56,
25 560 (1991); O'Connor, J. Neurochem 56, 560 (1991); Lee *et al.*, Nucleic Acids Res 6, 3073 (1979); Cooney *et al.*, Science 241, 456 (1988); Dervan *et al.*, Science 251, 1360 (1991)).

The term "hybridization" as used here refers to the association of two nucleic acid molecules with one another by hydrogen bonding. Typically, one molecule will be fixed to a solid support and the other will be free in solution. Then, the two molecules may be
30 placed in contact with one another under conditions that favour hydrogen bonding. Factors that affect this bonding include: the type and volume of solvent; reaction temperature; time

of hybridization; agitation; agents to block the non-specific attachment of the liquid phase molecule to the solid support (Denhardt's reagent or BLOTTO); the concentration of the molecules; use of compounds to increase the rate of association of molecules (dextran sulphate or polyethylene glycol); and the stringency of the washing conditions following
5 hybridization (see Sambrook *et al.* [supra]).

The inhibition of hybridization of a completely complementary molecule to a target molecule may be examined using a hybridization assay, as known in the art (see, for example, Sambrook *et al.* [supra]). A substantially homologous molecule will then compete for and inhibit the binding of a completely homologous molecule to the target molecule
10 under various conditions of stringency, as taught in Wahl, G.M. and S.L. Berger (1987; Methods Enzymol. 152:399-407) and Kimmel, A.R. (1987; Methods Enzymol. 152:507-511).

"Stringency" refers to conditions in a hybridization reaction that favour the association of very similar molecules over association of molecules that differ. High stringency
15 hybridisation conditions are defined as overnight incubation at 42°C in a solution comprising 50% formamide, 5XSSC (150mM NaCl, 15mM trisodium citrate), 50mM sodium phosphate (pH7.6), 5x Denhardts solution, 10% dextran sulphate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1X SSC at approximately 65°C. Low stringency conditions involve the hybridisation
20 reaction being carried out at 35°C (see Sambrook *et al.* [supra]). Preferably, the conditions used for hybridization are those of high stringency.

Preferred embodiments of this aspect of the invention are nucleic acid molecules that are at least 70% identical over their entire length to a nucleic acid molecules encoding the INSP221 polypeptides (SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ
25 ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, or SEQ ID NO:26), and nucleic acid molecules that are substantially complementary to these nucleic acid molecules. Preferably, a nucleic acid molecule according to this aspect of the invention comprises a region that is at least 80% identical over its entire length to a nucleic acid molecule having the sequence given in
30 SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21,

SEQ ID NO:243, SEQ ID NO:25 or SEQ ID NO:27 or a nucleic acid molecule that is complementary thereto. In this regard, nucleic acid molecules at least 90%, preferably at least 95%, more preferably at least 98%, 98.5%, 99% or 99% identical over their entire length to the same are particularly preferred. Preferred embodiments in this respect are
5 nucleic acid molecules that encode polypeptides which retain substantially the same biological function or activity as the INSP221 polypeptide.

The invention also provides a process for detecting a nucleic acid molecule of the invention, comprising the steps of: (a) contacting a nucleic probe according to the invention with a biological sample under hybridizing conditions to form duplexes; and (b)
10 detecting any such duplexes that are formed.

As discussed additionally below in connection with assays that may be utilised according to the invention, a nucleic acid molecule as described above may be used as a hybridization probe for RNA, cDNA or genomic DNA, in order to isolate full-length cDNAs and genomic clones encoding the INSP221 polypeptides and to isolate cDNA and
15 genomic clones of homologous or orthologous genes that have a high sequence similarity to the gene encoding these polypeptides.

In this regard, the following techniques, among others known in the art, may be utilised and are discussed below for purposes of illustration. Methods for DNA sequencing and analysis are well known and are generally available in the art and may, indeed, be used to
20 practice many of the embodiments of the invention discussed herein. Such methods may employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase (US Biochemical Corp, Cleveland, OH), Taq polymerase (Perkin Elmer), thermostable T7 polymerase (Amersham, Chicago, IL), or combinations of polymerases and proof-reading exonucleases such as those found in the ELONGASE Amplification System marketed by
25 Gibco/BRL (Gaithersburg, MD). Preferably, the sequencing process may be automated using machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno, NV), the Peltier Thermal Cycler (PTC200; MJ Research, Watertown, MA) and the ABI Catalyst and 373 and 377 DNA Sequencers (Perkin Elmer).

One method for isolating a nucleic acid molecule encoding a polypeptide with an
30 equivalent function to that of the INSP221 polypeptides is to probe a genomic or cDNA library with a natural or artificially-designed probe using standard procedures that are

recognised in the art (see, for example, "Current Protocols in Molecular Biology", Ausubel *et al.* (eds). Greene Publishing Association and John Wiley Interscience, New York, 1989,1992). Probes comprising at least 15, preferably at least 30, and more preferably at least 50, contiguous bases that correspond to, or are complementary to, nucleic acid sequences from the appropriate encoding gene SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25 or SEQ ID NO:27) are particularly useful probes. Such probes may be labelled with an analytically-detectable reagent to facilitate their identification. Useful reagents include, but are not limited to, radioisotopes, fluorescent dyes and enzymes that are capable of catalysing the formation of a detectable product. Using these probes, the ordinarily skilled artisan will be capable of isolating complementary copies of genomic DNA, cDNA or RNA polynucleotides encoding proteins of interest from human, mammalian or other animal sources and screening such sources for related sequences, for example, for additional members of the family, type and/or subtype.

In many cases, isolated cDNA sequences will be incomplete, in that the region encoding the polypeptide will be cut short, normally at the 5' end. Several methods are available to obtain full length cDNAs, or to extend short cDNAs. Such sequences may be extended utilising a partial nucleotide sequence and employing various methods known in the art to detect upstream sequences such as promoters and regulatory elements. For example, one method which may be employed is based on the method of Rapid Amplification of cDNA Ends (RACE; see, for example, Frohman *et al.*, PNAS USA 85, 8998-9002, 1988). Recent modifications of this technique, exemplified by the MarathonTM technology (Clontech Laboratories Inc.), for example, have significantly simplified the search for longer cDNAs. A slightly different technique, termed "restriction-site" PCR, uses universal primers to retrieve unknown nucleic acid sequence adjacent a known locus (Sarkar, G. (1993) PCR Methods Applic. 2:318-322). Inverse PCR may also be used to amplify or to extend sequences using divergent primers based on a known region (Triglia, T. *et al.* (1988) Nucleic Acids Res. 16:8186). Another method which may be used is capture PCR which involves PCR amplification of DNA fragments adjacent a known sequence in human and yeast artificial chromosome DNA (Lagerstrom, M. *et al.* (1991) PCR Methods Applic., 1, 111-119). Another method which may be used to retrieve unknown sequences is that of

Parker, J.D. *et al.* (1991); Nucleic Acids Res. 19:3055-3060). Additionally, one may use PCR, nested primers, and PromoterFinderTM libraries to walk genomic DNA (Clontech, Palo Alto, CA). This process avoids the need to screen libraries and is useful in finding intron/exon junctions.

- 5 When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. Also, random-primed libraries are preferable, in that they will contain more sequences that contain the 5' regions of genes. Use of a randomly primed library may be especially preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of
10 sequence into 5' non-transcribed regulatory regions.

In one embodiment of the invention, the nucleic acid molecules of the present invention may be used for chromosome localisation. In this technique, a nucleic acid molecule is specifically targeted to, and can hybridize with, a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the
15 present invention is an important step in the confirmatory correlation of those sequences with the gene-associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found in, for example, V. McKusick, Mendelian Inheritance in Man (available on-line through Johns Hopkins University Welch
20 Medical Library). The relationships between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localised by genetic linkage to a particular
25 genomic region, any sequences mapping to that area may represent associated or regulatory genes for further investigation. The nucleic acid molecule may also be used to detect differences in the chromosomal location due to translocation, inversion, etc. among normal, carrier, or affected individuals.

The nucleic acid molecules of the present invention are also valuable for tissue
30 localisation. Such techniques allow the determination of expression patterns of the polypeptide in tissues by detection of the mRNAs that encode them. These techniques

include *in situ* hybridization techniques and nucleotide amplification techniques, such as PCR. Results from these studies provide an indication of the normal functions of the polypeptide in the organism. In addition, comparative studies of the normal expression pattern of mRNAs with that of mRNAs encoded by a mutant gene provide valuable
5 insights into the role of mutant polypeptides in disease. Such inappropriate expression may be of a temporal, spatial or quantitative nature.

Gene silencing approaches may also be undertaken to down-regulate endogenous expression of a gene encoding a polypeptide of the invention. RNA interference (RNAi) (Elbashir, SM et al., Nature 2001, 411, 494-498) is one method of sequence specific post-
10 transcriptional gene silencing that may be employed. Short dsRNA oligonucleotides are synthesised *in vitro* and introduced into a cell. The sequence specific binding of these dsRNA oligonucleotides triggers the degradation of target mRNA, reducing or ablating target protein expression.

Efficacy of the gene silencing approaches assessed above may be assessed through the
15 measurement of polypeptide expression (for example, by Western blotting), and at the RNA level using TaqMan-based methodologies.

The vectors of the present invention comprise nucleic acid molecules of the invention and may be cloning or expression vectors. The host cells of the invention, which may be transformed, transfected or transduced with the vectors of the invention may be
20 prokaryotic or eukaryotic.

The polypeptides of the invention may be prepared in recombinant form by expression of their encoding nucleic acid molecules in vectors contained within a host cell. Such expression methods are well known to those of skill in the art and many are described in detail by Sambrook *et al* (supra) and Fernandez & Hoeffler (1998, eds. "Gene expression
25 systems. Using nature for the art of expression". Academic Press, San Diego, London, Boston, New York, Sydney, Tokyo, Toronto).

Generally, any system or vector that is suitable to maintain, propagate or express nucleic acid molecules to produce a polypeptide in the required host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-
30 known and routine techniques, such as, for example, those described in Sambrook *et al.*,

(supra). Generally, the encoding gene can be placed under the control of a control element such as a promoter, ribosome binding site (for bacterial expression) and, optionally, an operator, so that the DNA sequence encoding the desired polypeptide is transcribed into RNA in the transformed host cell.

- 5 Examples of suitable expression systems include, for example, chromosomal, episomal and virus-derived systems, including, for example, vectors derived from: bacterial plasmids, bacteriophage, transposons, yeast episomes, insertion elements, yeast chromosomal elements, viruses such as baculoviruses, papova viruses such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, or combinations
10 thereof, such as those derived from plasmid and bacteriophage genetic elements, including cosmids and phagemids. Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained and expressed in a plasmid. The vectors pDONR221_INSP186-94R-106R-6HIS, pDONR221_INSP186-6HIS, pEAK12d_INSP186-6HIS, pDEST12.2_INSP186-6HIS and pCR4-TOPO-INSP221 are
15 preferred examples of suitable vectors for use in accordance with the aspects of this invention relating to INSP221.

Particularly suitable expression systems include microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus
20 expression vectors (for example, baculovirus); plant cell systems transformed with virus expression vectors (for example, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (for example, Ti or pBR322 plasmids); or animal cell systems. Cell-free translation systems can also be employed to produce the polypeptides of the invention.

- 25 Introduction of nucleic acid molecules encoding a polypeptide of the present invention into host cells can be effected by methods described in many standard laboratory manuals, such as Davis *et al.*, Basic Methods in Molecular Biology (1986) and Sambrook *et al.*, [supra]. Particularly suitable methods include calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection,
30 electroporation, transduction, scrape loading, ballistic introduction or infection (see Sambrook *et al.*, 1989 [supra]; Ausubel *et al.*, 1991 [supra]; Spector, Goldman &

Leinwald, 1998). In eukaryotic cells, expression systems may either be transient (for example, episomal) or permanent (chromosomal integration) according to the needs of the system.

The encoding nucleic acid molecule may or may not include a sequence encoding a control sequence, such as a signal peptide or leader sequence, as desired, for example, for secretion of the translated polypeptide into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment. These signals may be endogenous to the polypeptide or they may be heterologous signals. Leader sequences can be removed by the bacterial host in post-translational processing.

- 10 In addition to control sequences, it may be desirable to add regulatory sequences that allow for regulation of the expression of the polypeptide relative to the growth of the host cell. Examples of regulatory sequences are those which cause the expression of a gene to be increased or decreased in response to a chemical or physical stimulus, including the presence of a regulatory compound or to various temperature or metabolic conditions.
- 15 Regulatory sequences are those non-translated regions of the vector, such as enhancers, promoters and 5' and 3' untranslated regions. These interact with host cellular proteins to carry out transcription and translation. Such regulatory sequences may vary in their strength and specificity. Depending on the vector system and host utilised, any number of suitable transcription and translation elements, including constitutive and inducible
- 20 promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the Bluescript phagemid (Stratagene, LaJolla, CA) or pSport1™ plasmid (Gibco BRL) and the like may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (for example, heat shock, RUBISCO and storage
- 25 protein genes) or from plant viruses (for example, viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of the sequence, vectors based on SV40 or EBV may be used with an appropriate selectable marker.
- 30 An expression vector is constructed so that the particular nucleic acid coding sequence is located in the vector with the appropriate regulatory sequences, the positioning and

orientation of the coding sequence with respect to the regulatory sequences being such that the coding sequence is transcribed under the "control" of the regulatory sequences, i.e., RNA polymerase which binds to the DNA molecule at the control sequences transcribes the coding sequence. In some cases it may be necessary to modify
5 the sequence so that it may be attached to the control sequences with the appropriate orientation; i.e., to maintain the reading frame.

The control sequences and other regulatory sequences may be ligated to the nucleic acid coding sequence prior to insertion into a vector. Alternatively, the coding sequence can be cloned directly into an expression vector that already contains the control sequences and an
10 appropriate restriction site.

For long-term, high-yield production of a recombinant polypeptide, stable expression is preferred. For example, cell lines which stably express the polypeptide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate
15 vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells that successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to
20 the cell type.

Mammalian cell lines available as hosts for expression are known in the art and include many immortalised cell lines available from the American Type Culture Collection (ATCC) including, but not limited to, Chinese hamster ovary (CHO), HeLa, baby hamster kidney (BHK), monkey kidney (COS), C127, 3T3, BHK, HEK 293, Bowes melanoma and
25 human hepatocellular carcinoma (for example Hep G2) cells and a number of other cell lines.

In the baculovirus system, the materials for baculovirus/insect cell expression systems are commercially available in kit form from, inter alia, Invitrogen, San Diego CA (the "MaxBac" kit). These techniques are generally known to those skilled in the art and are
30 described fully in Summers and Smith, Texas Agricultural Experiment Station Bulletin No.

1555 (1987). Particularly suitable host cells for use in this system include insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells.

There are many plant cell culture and whole plant genetic expression systems known in the art. Examples of suitable plant cellular genetic expression systems include those described
5 in US 5,693,506; US 5,659,122; and US 5,608,143. Additional examples of genetic expression in plant cell culture has been described by Zenk, *Phytochemistry* 30, 3861-3863 (1991).

In particular, all plants from which protoplasts can be isolated and cultured to give whole regenerated plants can be utilised, so that whole plants are recovered which contain the
10 transferred gene. Practically all plants can be regenerated from cultured cells or tissues, including but not limited to all major species of sugar cane, sugar beet, cotton, fruit and other trees, legumes and vegetables.

Examples of particularly preferred bacterial host cells include *streptococci*, *staphylococci*, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells.

15 Examples of particularly suitable host cells for fungal expression include yeast cells (for example, *S. cerevisiae*) and *Aspergillus* cells.

Any number of selection systems are known in the art that may be used to recover transformed cell lines. Examples include the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) *Cell* 11:223-32) and adenine phosphoribosyltransferase (Lowy, I.
20 *et al.* (1980) *Cell* 22:817-23) genes that can be employed in tk- or apt[±] cells, respectively.

Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dihydrofolate reductase (DHFR) that confers resistance to methotrexate (Wigler, M. *et al.* (1980) *Proc. Natl. Acad. Sci.* 77:3567-70); npt, which confers resistance to the aminoglycosides neomycin and G-418 (Colbere-Garapin, F. *et al.*
25 (1981) *J. Mol. Biol.* 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. Additional selectable genes have been described, examples of which will be clear to those of skill in the art.

Although the presence or absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For

example, if the relevant sequence is inserted within a marker gene sequence, transformed cells containing the appropriate sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a polypeptide of the invention under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells that contain a nucleic acid sequence encoding a polypeptide of the invention and which express said polypeptide may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations and protein bioassays, for example, fluorescence activated cell sorting (FACS) or immunoassay techniques (such as the enzyme-linked immunosorbent assay [ELISA] and radioimmunoassay [RIA]), that include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein (see Hampton, R. *et al.* (1990) *Serological Methods*, a Laboratory Manual, APS Press, St Paul, MN) and Maddox, D.E. *et al.* (1983) *J. Exp. Med.*, 158, 1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labelled hybridization or PCR probes for detecting sequences related to nucleic acid molecules encoding polypeptides of the present invention include oligolabelling, nick translation, end-labelling or PCR amplification using a labelled polynucleotide. Alternatively, the sequences encoding the polypeptide of the invention may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesise RNA probes *in vitro* by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labelled nucleotides. These procedures may be conducted using a variety of commercially available kits (Pharmacia & Upjohn, (Kalamazoo, MI); Promega (Madison WI); and U.S. Biochemical Corp., Cleveland, OH)).

Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes and fluorescent, chemiluminescent or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Nucleic acid molecules according to the present invention may also be used to create transgenic animals, particularly rodent animals. Such transgenic animals form a further aspect of the present invention. This may be done locally by modification of somatic cells, or by germ line therapy to incorporate heritable modifications. Such transgenic animals
5 may be particularly useful in the generation of animal models for drug molecules effective as modulators of the polypeptides of the present invention.

The polypeptide can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulphate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography,
10 hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. High performance liquid chromatography is particularly useful for purification. Well known techniques for refolding proteins may be employed to regenerate an active conformation when the polypeptide is denatured during isolation and or purification.

15 Specialised vector constructions may also be used to facilitate purification of proteins, as desired, by joining sequences encoding the polypeptides of the invention to a nucleotide sequence encoding a polypeptide domain that will facilitate purification of soluble proteins. Examples of such purification-facilitating domains include metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilised
20 metals, protein A domains that allow purification on immobilised immunoglobulin, and the domain utilised in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, WA). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, CA) between the purification domain and the polypeptide of the invention may be used to facilitate purification. One such expression
25 vector provides for expression of a fusion protein containing the polypeptide of the invention fused to several histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification by IMAC (immobilised metal ion affinity chromatography as described in Porath, J. *et al.* (1992), *Prot. Exp. Purif.* 3: 263-281) while the thioredoxin or enterokinase cleavage site provides a means for
30 purifying the polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D.J. *et al.* (1993; *DNA Cell Biol.* 12:441-453).

If the polypeptide is to be expressed for use in screening assays, generally it is preferred that it be produced at the surface of the host cell in which it is expressed. In this event, the host cells may be harvested prior to use in the screening assay, for example using techniques such as fluorescence activated cell sorting (FACS) or immunoaffinity
5 techniques. If the polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the expressed polypeptide. If polypeptide is produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

As indicated above, the present invention also provides novel targets and methods for the screening of drug candidates or leads. These screening methods include binding assays
10 and/or functional assays, and may be performed *in vitro*, in cell systems or in animals.

As indicated above, the present invention also provides novel targets and methods for the screening of drug candidates or leads. These screening methods include binding assays and/or functional assays, and may be performed *in vitro*, in cell systems or in animals.

In this regard, a particular object of this invention resides in the use of an INSP221
15 polypeptide as a target for screening candidate drugs for treating or preventing IL-9 related disorders.

Another object of this invention resides in methods of selecting biologically active compounds, said methods comprising contacting a candidate compound with a INSP221 gene or polypeptide, and selecting compounds that bind said gene or polypeptide.

20 A further other object of this invention resides in methods of selecting biologically active compounds, said method comprising contacting a candidate compound with recombinant host cell expressing a INSP221 polypeptide with a candidate compound, and selecting compounds that bind said INSP221 polypeptide at the surface of said cells and/or that modulate the activity of the INSP221 polypeptide.

25 A "biologically active" compound denotes any compound having biological activity in a subject, preferably therapeutic activity, more preferably a compound having IL-9 activity, and further preferably a compound that can be used for treating INSP221 related disorders, or as a lead to develop drugs for treating IL-9 related disorder. A "biologically active" compound preferably is a compound that modulates the activity of INSP221.

The above methods may be conducted *in vitro*, using various devices and conditions, including with immobilized reagents, and may further comprise an additional step of assaying the activity of the selected compounds in a model of IL-9 related disorder, such as an animal model.

- 5 Preferred selected compounds are agonists of INSP221, *i.e.*, compounds that can bind to INSP221 and mimic the activity of an endogenous ligand thereof.

A further object of this invention resides in a method of selecting biologically active compounds, said method comprising contacting *in vitro* a test compound with a INSP221 polypeptide according to the present invention and determining the ability of said test
10 compound to modulate the activity of said INSP221 polypeptide.

A further object of this invention resides in a method of selecting biologically active compounds, said method comprising contacting *in vitro* a test compound with a INSP221 gene according to the present invention and determining the ability of said test compound to modulate the expression of said INSP221 gene, preferably to stimulate expression
15 thereof.

In another embodiment, this invention relates to a method of screening, selecting or identifying active compounds, particularly compounds active on multiple sclerosis or related disorders, the method comprising contacting a test compound with a recombinant host cell comprising a reporter construct, said reporter construct comprising a reporter gene
20 under the control of a INSP221 gene promoter, and selecting the test compounds that modulate (*e.g.* stimulate or reduce, preferably stimulate) expression of the reporter gene.

The polypeptide of the invention can be used to screen libraries of compounds in any of a variety of drug screening techniques. Such compounds may activate (agonise) or inhibit (antagonise) the level of expression of the gene or the activity of the polypeptide of the
25 invention and form a further aspect of the present invention. Preferred compounds are effective to alter the expression of a natural gene which encodes a polypeptide of the first aspect of the invention or to regulate the activity of a polypeptide of the first aspect of the invention.

Agonist or antagonist compounds may be isolated from, for example, cells, cell-free preparations, chemical libraries or natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors or structural or functional mimetics. For a suitable review of such screening techniques, see Coligan *et al.*,
5 Current Protocols in Immunology 1(2):Chapter 5 (1991).

Binding to a target gene or polypeptide provides an indication as to the ability of the compound to modulate the activity of said target, and thus to affect a pathway leading to IL-9 related disorder in a subject. The determination of binding may be performed by various techniques, such as by labelling of the candidate compound, by competition with a
10 labelled reference ligand, *etc.* For *in vitro* binding assays, the polypeptides may be used in essentially pure form, in suspension, immobilized on a support, or expressed in a membrane (intact cell, membrane preparation, liposome, *etc.*).

Modulation of activity includes, without limitation, stimulation of the surface expression of the INSP221 receptor, modulation of multimerization of said receptor (*e.g.*, the formation
15 of multimeric complexes with other sub-units), *etc.* The cells used in the assays may be any recombinant cell (*i.e.*, any cell comprising a recombinant nucleic acid encoding a INSP221 polypeptide) or any cell that expresses an endogenous INSP221 polypeptide. Examples of such cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, *etc.*).
20 Specific examples include *E. coli*, *Pichia pastoris*, *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces* or *Saccharomyces* yeasts, mammalian cell lines (*e.g.*, Vero cells, CHO cells, 3T3 cells, COS cells, *etc.*) as well as primary or established mammalian cell cultures (*e.g.*, produced from fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, *etc.*).

25 Compounds that are most likely to be good antagonists are molecules that bind to the polypeptide of the invention without inducing the biological effects of the polypeptide upon binding to it. Potential antagonists include small organic molecules, peptides, polypeptides and antibodies that bind to the polypeptide of the invention and thereby inhibit or extinguish its activity. In this fashion, binding of the polypeptide to normal
30 cellular binding molecules may be inhibited, such that the normal biological activity of the polypeptide is prevented.

The polypeptide of the invention that is employed in such a screening technique may be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. In general, such screening procedures may involve using appropriate cells or cell membranes that express the polypeptide that are contacted with a test compound to
5 observe binding, or stimulation or inhibition of a functional response. The functional response of the cells contacted with the test compound is then compared with control cells that were not contacted with the test compound. Such an assay may assess whether the test compound results in a signal generated by activation of the polypeptide, using an appropriate detection system. Inhibitors of activation are generally assayed in the presence
10 of a known agonist and the effect on activation by the agonist in the presence of the test compound is observed.

A preferred method for identifying an agonist or antagonist compound of a polypeptide of the present invention comprises:

(a) contacting a cell expressing (optionally on the surface thereof) the polypeptide
15 according to the first aspect of the invention, the polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and

(b) determining whether the compound binds to and activates or inhibits the polypeptide by
20 measuring the level of a signal generated from the interaction of the compound with the polypeptide.

Methods for generating detectable signals in the types of assays described herein will be known to those of skill in the art. A particular example is cotransfecting a construct expressing a polypeptide according to the invention, or a fragment such as the LBD, in
25 fusion with the GAL4 DNA binding domain, into a cell together with a reporter plasmid, an example of which is pFR-Luc (Stratagene Europe, Amsterdam, The Netherlands). This particular plasmid contains a synthetic promoter with five tandem repeats of GAL4 binding sites that control the expression of the luciferase gene. When a potential ligand is added to the cells, it will bind the GAL4-polypeptide fusion and induce transcription of the
30 luciferase gene. The level of the luciferase expression can be monitored by its activity

using a luminescence reader (see, for example, Lehman *et al.* JBC 270, 12953, 1995; Pawar *et al.* JBC, 277, 39243, 2002).

A further preferred method for identifying an agonist or antagonist of a polypeptide of the invention comprises:

- 5 (a) contacting a labelled or unlabeled compound with the polypeptide immobilized on any solid support (for example beads, plates, matrix support, chip) and detection of the compound by measuring the label or the presence of the compound itself; or
- (b) contacting a cell expressing on the surface thereof the polypeptide, by means of artificially anchoring it to the cell membrane, or by constructing a chimeric receptor being
10 associated with a second component capable of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and
- (c) determining whether the compound binds to and activates or inhibits the polypeptide by comparing the level of a signal generated from the interaction of the compound with the
15 polypeptide with the level of a signal in the absence of the compound.

For example, a method such as FRET detection of ligand bound to the polypeptide in the presence of peptide co-activators (Norris *et al.*, Science 285, 744, 1999) might be used.

A further preferred method for identifying an agonist or antagonist of a polypeptide of the invention comprises:

- 20 (a) contacting a cell expressing (optionally on the surface thereof) the polypeptide, the polypeptide being associated with a second component capable of providing a detectable signal in response to the binding of a compound to the polypeptide, with a compound to be screened under conditions to permit binding to the polypeptide; and
- (b) determining whether the compound binds to and activates or inhibits the polypeptide by
25 comparing the level of a signal generated from the interaction of the compound with the polypeptide with the level of a signal in the absence of the compound.

In further preferred embodiments, the general methods that are described above may further comprise conducting the identification of agonist or antagonist in the presence of labelled or unlabelled ligand for the polypeptide.

In another embodiment of the method for identifying agonist or antagonist of a polypeptide
5 of the present invention comprises:

determining the inhibition of binding of a ligand to cells which express a polypeptide of the invention (and which optionally have a polypeptide of the invention on the surface thereof), or to cell membranes containing such a polypeptide, in the presence of a candidate compound under conditions to permit binding to the polypeptide, and
10 determining the amount of ligand bound to the polypeptide. A compound capable of causing reduction of binding of a ligand is considered to be an agonist or antagonist. Preferably the ligand is labelled.

More particularly, a method of screening for a polypeptide antagonist or agonist compound comprises the steps of:

- 15 (a) incubating a labelled ligand with a whole cell expressing a polypeptide according to the invention, optionally on the cell surface, or a cell membrane containing a polypeptide of the invention,
- (b) measuring the amount of labelled ligand bound to the whole cell or the cell membrane;
- (c) adding a candidate compound to a mixture of labelled ligand and the whole cell or the
20 cell membrane of step (a) and allowing the mixture to attain equilibrium;
- (d) measuring the amount of labelled ligand bound to the whole cell or the cell membrane after step (c); and
- (e) comparing the difference in the labelled ligand bound in step (b) and (d), such that the compound which causes the reduction in binding in step (d) is considered to be an agonist
25 or antagonist.

Similarly, there is provided a method of screening for a polypeptide antagonist or agonist compound which comprises the steps of:

(a) incubating a labelled ligand with a polypeptide according to the invention on any solid support or the cell surface, or a cell membrane containing a polypeptide of the invention.

(b) measuring the amount of labelled ligand bound to the polypeptide on the solid support,
5 whole cell or the cell membrane;

(c) adding a candidate compound to a mixture of labelled ligand and immobilized polypeptide on the solid support, the whole cell or the cell membrane of step (a) and allowing the mixture to attain equilibrium;

(d) measuring the amount of labelled ligand bound to the immobilized polypeptide or the
10 whole cell or the cell membrane after step (c); and

(e) comparing the difference in the labelled ligand bound in step (b) and (d), such that the compound which causes the reduction in binding in step (d) is considered to be an agonist or antagonist.

The polypeptides may be found to modulate a variety of physiological and pathological
15 processes in a dose-dependent manner in the above-described assays. Thus, the “functional equivalents” of the polypeptides of the invention include polypeptides that exhibit any of the same modulatory activities in the above-described assays in a dose-dependent manner. Although the degree of dose-dependent activity need not be identical to that of the polypeptides of the invention, preferably the “functional equivalents” will exhibit
20 substantially similar dose-dependence in a given activity assay compared to the polypeptides of the invention.

In certain of the embodiments described above, simple binding assays may be used, in which the adherence of a test compound to the polypeptide (e.g. a surface bearing the polypeptide) is detected by means of a label directly or indirectly associated with the test
25 compound or in an assay involving competition with a labelled competitor. In another embodiment, competitive drug screening assays may be used, in which neutralising antibodies that are capable of binding the polypeptide specifically compete with a test compound for binding. In this manner, the antibodies can be used to detect the presence of any test compound that possesses specific binding affinity for the polypeptide.

Assays may also be designed to detect the effect of added test compounds on the production of mRNA encoding the polypeptide in cells. For example, an ELISA may be constructed that measures secreted or cell-associated levels of polypeptide using monoclonal or polyclonal antibodies by standard methods known in the art, and this can be
5 used to search for compounds that may inhibit or enhance the production of the polypeptide from suitably manipulated cells or tissues. The formation of binding complexes between the polypeptide and the compound being tested may then be measured.

Assay methods that are also included within the terms of the present invention are those that involve the use of the genes and polypeptides of the invention in overexpression or
10 ablation assays. Such assays involve the manipulation of levels of these genes/polypeptides in cells and assessment of the impact of this manipulation event on the physiology of the manipulated cells. For example, such experiments reveal details of signalling and metabolic pathways in which the particular genes/polypeptides are implicated, generate information regarding the identities of polypeptides with which the studied polypeptides
15 interact and provide clues as to methods by which related genes and proteins are regulated.

Another technique for drug screening which may be used provides for high throughput screening of compounds having suitable binding affinity to the polypeptide of interest (see International patent application WO84/03564). In this method, large numbers of different small test compounds are synthesised on a solid substrate, which may then be reacted with
20 the polypeptide of the invention and washed. One way of immobilising the polypeptide is to use non-neutralising antibodies. Bound polypeptide may then be detected using methods that are well known in the art. Purified polypeptide can also be coated directly onto plates for use in the aforementioned drug screening techniques.

The polypeptide of the invention may be used to identify membrane-bound or soluble
25 receptors, through standard receptor binding techniques that are known in the art, such as ligand binding and crosslinking assays in which the polypeptide is labelled with a radioactive isotope, is chemically modified, or is fused to a peptide sequence that facilitates its detection or purification, and incubated with a source of the putative receptor (for example, a composition of cells, cell membranes, cell supernatants, tissue extracts, or
30 bodily fluids). The efficacy of binding may be measured using biophysical techniques such as surface plasmon resonance (e.g. supplied by Biacore AB, Uppsala Sweden) and

spectroscopy. Binding assays may be used for the purification and cloning of the receptor, but may also identify agonists and antagonists of the polypeptide, that compete with the binding of the polypeptide to its receptor. Standard methods for conducting screening assays are well understood in the art.

- 5 In another embodiment, this invention relates to the use of a INSP221 polypeptide or fragment thereof, whereby the fragment is preferably a INSP221 gene-specific fragment, for isolating or generating an agonist or stimulator of the INSP221 polypeptide for the treatment of an immune related disorder, wherein said agonist or stimulator is selected from the group consisting of:
- 10 1. a specific antibody or fragment thereof including: a) a chimeric, b) a humanized or c) a fully human antibody, as well as;
2. a bispecific or multispecific antibody,
3. a single chain (e.g. scFv) or
4. single domain antibody, or
- 15 5. a peptide- or non-peptide mimetic derived from said antibodies or
6. an antibody-mimetic such as a) an anticalin or b) a fibronectin-based binding molecule (e.g. trinectin or adnectin).

The generation of peptide- or non-peptide mimetics from antibodies is known in the art (Saragovi et al., 1991 and Saragovi et al., 1992).

- 20 Anticalins are also known in the art (Vogt et al., 2004). Fibronectin-based binding molecules are described in US6818418 and WO2004029224.

- Furthermore, the test compound may be of various origin, nature and composition, such as any small molecule, nucleic acid, lipid, peptide, polypeptide including an antibody such as a chimeric, humanized or fully human antibody or an antibody fragment, peptide- or non-
- 25 peptide mimetic derived therefrom as well as a bispecific or multispecific antibody, a single chain (e.g. scFv) or single domain antibody or an antibody-mimetic such as an

anticalin or fibronectin-based binding molecule (e.g. trinectin or adnectin), etc., in isolated form or in mixture or combinations.

As mentioned above, it is envisaged that the various moieties of the invention (*i.e.* the polypeptides of the first aspect of the invention, a nucleic acid molecule of the second or
5 third aspect of the invention, a vector of the fourth aspect of the invention, a host cell of the fifth aspect of the invention, a ligand of the sixth aspect of the invention, a compound of the seventh aspect of the invention) may be useful in the therapy or diagnosis of diseases. To assess the utility of the moieties of the invention for treating or diagnosing a
10 the following assays refer to the test compound as being a protein/polypeptide, a person skilled in the art will readily be able to adapt the following assays so that the other moieties of the invention may also be used as the “test compound”.

The invention also includes a screening kit useful in the methods for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, that are described above.

15 The invention includes the agonists, antagonists, ligands, receptors, substrates and enzymes, and other compounds which modulate the activity or antigenicity of the polypeptide of the invention discovered by the methods that are described above.

As mentioned above, it is envisaged that the various moieties of the invention (*i.e.* the polypeptides of the first aspect of the invention, a nucleic acid molecule of the second or
20 third aspect of the invention, a vector of the fourth aspect of the invention, a host cell of the fifth aspect of the invention, a ligand of the sixth aspect of the invention, a compound of the seventh aspect of the invention) may be useful in the therapy or diagnosis of autoimmune diseases. To assess the utility of the moieties of the invention for treating or diagnosing an autoimmune disease one or more of the following assays may be carried out.
25 Note that although some of the following assays refer to the test compound as being a protein/polypeptide, a person skilled in the art will readily be able to adapt the following assays so that the other moieties of the invention may also be used as the “test compound”.

	Cells and stimuli	Read-out	Biology rationale	Targeted diseases
T lymphocytes	Fas-Ligand-induced Jurkat T cell death.	Release of LDH.	To regulate T cell death	Autoimmune diseases
	Human PBMC stimulated with the superantigen, TSST.	Proliferation	To modulate T cell proliferation	Autoimmune diseases
		Cytokine secretion.	To modulate T cell cytokine secretion	Autoimmune diseases
T lymphocytes and Antigen presenting cells	Human and Mouse MLR	Proliferation	To modulate T cell proliferation	Autoimmune diseases
		Cytokine secretion.	To modulate T cell cytokine secretion	Autoimmune diseases
	Human PBMC stimulated with either ConA or PHA.	Cytokine secretion.	To modulate T cell cytokine secretion	Autoimmune diseases
Monocytes macrophages and granulocytes	Human PBMC stimulated with LPS	Cytokine secretion.	To modulate macrophage and granulocyte cytokine secretion	Autoimmune diseases
Monocytes	RANTES-induced calcium flux in THP-1.	Calcium flux by FlipR	To induce monocyte activation	Autoimmune diseases
Neutrophils	Human neutrophils stimulated with IL-8	Cytoskeleton reorganization	To modulate neutrophil migration	Autoimmune diseases
B lymphocytes	Human B cell stimulated with goat anti-human IgM antibody and rhIL-4	Survival	To modulate B cell survival	Autoimmune diseases
	Human B cell stimulated plus goat anti-human IgM antibody, rhIL-4 and soluble rhBAFF	Proliferation	To modulate B cell costimulation	Autoimmune diseases
Microglia cells	M-CSF activated microglia cell line	Proliferation	To modulate microglia cell activation	MS

Assays targeting T lymphocyte responses

- **Fas-Ligand-induced T cell death.** This assay will reveal new modulators of receptor mediated cell death.

In this assay, T cell apoptosis is induced by stimulating Jurkat cells (a human T cell line) with recombinant 6 Histidine-tagged Fas Ligand combined with a monoclonal anti 6-his antibody. Death is quantified by release of LDH, a cytoplasmic enzyme released in the culture medium when cells are dying. The read out is a colorimetric assay read at 490nm. T cells have been shown to be pathogenic in many autoimmune diseases, being able to control antigen-specific T cell death is a therapeutic strategy (e.g. anti-TNF α treatment in patient with Crohn's disease).

Human-MLR: proliferation and cytokine secretion. This cell-based assay measures the effects of novel proteins on lymphocyte proliferation and cytokine secretion or inhibition upon stimulation by PBMC from another donor (alloreactivity). These assays address antigen-specific T cell and antigen presenting cell functions, which are crucial cellular responses in any autoimmune diseases. Secreted cytokine (IL-2, 4, 5, 10, TNF- α and IFN- γ) are quantified by CBA.

Note: proliferation and cytokine secretion are independent responses.

15 Mouse-MLR: proliferation. This cell-based assay measures the effects of novel proteins on lymphocyte proliferation or inhibition of mouse spleen cells following stimulation by spleen cells from another donor (mouse strain). This cell-based assay measures the effect of novel proteins on T lymphocyte and antigen presenting cell responses and will be used to confirm activity of positives and hits identified in the h-MLR assays. This assay will be
20 use to select proteins that will be tested in murine models of human diseases.

Human PBMC stimulated with the superantigen, TSST.

Superantigens are strong modulators of the immune system affecting T cells. Superantigens influence immunologically mediated disorders such as IBD, inflammatory skin diseases like atopic dermatitis and psoriasis. In this cellular assay, we are specifically
25 targeting T lymphocyte activation via the TCR but with different requirements than the T cell response to classical antigens, in particular in respect to co-stimulatory molecules.

Human PBMC stimulated with either ConA or PHA. These cell-based assays measure the effects of novel proteins on cytokine secretion induced by two different stimuli acting

on different cells as measured by a cytokine bead array (CBA) assay (IL-2, IFN- γ , TNF- α , IL-5, IL-4 and IL-10).

Most of cytokines can have dual actions, pro or anti-inflammatory, depending on the injury, milieu and cellular target. Any protein with the capability to modulate cytokine secretion may have a therapeutic potential (e.g. decreasing IFN- γ and TNF- α would be beneficial in Th1-mediated autoimmune disease; in contrast decreasing IL-4, IL-5 may be beneficial in Th2-mediated-diseases; inducing IL-10 would be interesting in MS and SLE).

Assays targeting monocyte/macrophages and granulocyte responses

Human PBMC stimulated with LPS. This cell-based assay measures the effects of novel proteins on cytokine secretion (IFN- γ , TNF- α) induced by LPS acting on monocytes/macrophages and granulocytes.

Any protein with the capability to modulate IFN- γ and TNF- α secretion would be beneficial in Th1-mediated autoimmune diseases.

Assays targeting neutrophil responses

Neutrophils are important in inflammation and autoimmune diseases such as Rheumatoid Arthritis. Leukocyte chemo-attractants such as IL-8 initiate a sequence of adhesive interactions between cells and the micro-vascular endothelium, resulting in activation, adhesion and finally migration of neutrophils. The tissue infiltration of neutrophils depends on a reorganisation of cytoskeleton elements associated with specific changes in cell morphology of these cells.

This cell-based assay measures the effect of novel proteins on cytoskeleton reorganization of human neutrophils.

Assays targeting B lymphocyte responses

Autoantibodies as well as infiltrating B cells are thought to be important in the pathogenesis of various autoimmune diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjogren's syndrome and myasthenia gravis. Compelling evidence indicates that a dysregulation in B cell homeostasis could affect immune tolerance

leading to the inappropriate survival of autoreactive B cells producing pathogenic antibodies and sustained inflammation. The identification of new factors that play critical roles in the regulation of B cell proliferation, survival and differentiation following B cell receptor triggering are of high relevance in the development of novel therapies.

- 5 • **B cell proliferation.** This cell-based assay measures the effect of novel proteins on B cell survival.
- **B cell co-stimulation.** This cell-based assay measures the effect of novel proteins on B cell co-stimulation.

Assays targeting monocytes and microglial responses

- 10 • **THP-1 calcium flux.** The Ca^{+} -flux in THP1-cell assay measures the effects of novel proteins on their ability to trigger an intracellular calcium release (a generic second messenger event) from the endoplasmic reticulum.

• **Microglia cell proliferation**

- During proliferation of microglial progenitors, a number of colony-stimulating factors, including some cytokines, are known to play key roles. Among them, M-CSF is crucial for the final step of maturation of macrophages/microglia and is not replaceable by any other factor. The evaluation of this biological response may represent a way to influence the microglial activity and therefore an opportunity to identify molecules with therapeutic potential from MS.
- 15

- 20 A cell-based assay was developed to measure the proliferative response of a microglia cell line to M-CSF. The feasibility and the robustness phases showed optimal results. This assay is in 96 well plates; non-radioactive substrate is required; easily automated.

(i) Cytokine expression modulation assays

- Introduction: The following *in vitro* cell-based tri-replicas assays measure the effects of the protein on cytokine secretion induced by Concanavalin A (Con A) acting on different human peripheral blood mononuclear cells (hPBMC) cells as measured by a cytokine bead array (CBA) assay for IL-2, IFN- γ , TNF- α , IL-5, IL-4 and IL-10.
- 25

The optimal conditions are 100 000 cells/well in 96-well plates and 100µl final in 2 % glycerol. The optimal concentration of mitogen (ConA) is 5 ng/ml. The optimal time for the assay is 48 h. The read-out choice is the CBA.

Purification of Human PBMC from a buffy coat

- 5 The buffy coat 1 to 2 is diluted with DMEM. 25 ml of diluted blood is thereafter slowly added onto a 15 ml layer of Ficoll in a 50 ml Falcon tube, and tubes centrifuged (2000 rpm, 20 min, at RT without brake). The interphase (ring) is then collected and the cells washed with 25 ml of DMEM followed by a centrifuge step (1200 rpm, 5 min). This procedure is repeated three times. A buffy coat gives approximately 600×10^6 total cells.

10 Screening

80 µl of 1.25×10^6 cells/ml are diluted in DMEM+2.5% Human Serum+1% L-Glutamine+1% Penicillin-Streptomycin and thereafter added to a 96 well microtiter plate.

10µl were added per well (one condition per well):

- Proteins are diluted in PBS+20%Glycerol (the final dilution of the proteins is 1/10)

- 15 10µl of the ConA Stimuli are then added per well (one condition per well):

- ConA 50µg/ml (the final concentration of ConA is 5µg/ml)

After 48 h, cell supernatants are collected and human cytokines are measured by Human Th1/Th2 Cytokine CBA Kit Becton-Dickinson.

CBA analysis

- 20 i) Preparation of mixed Human Th1/Th2 Capture Beads

The number of assay tubes that are required for the experiment is determined.

Each capture bead suspension is vigorously vortexed for a few seconds before mixing. For each assay to be analysed, 10µl aliquot of each capture bead is added into a single tube labelled "mixed capture beads". The Bead mixture was thoroughly vortexed.

- 25 ii) Preparation of test samples

Supernatants are diluted (1:4) using the Assay Diluent (20µl of supernatants + 60µl of Assay Diluent). The sample dilution is then mixed before transferring samples into a 96 well microtiter plate conical bottom (Nunc).

iii) Human Th1/Th2 Cytokine CBA Assay Procedure

- 5 50µl of the diluted supernatants are added into a 96 wells microtiter plate conical bottom (Nunc). 50µl of the mixed capture beads are added followed by 50µl addition of the Human Th1/Th2 PE Detection Reagent. The plate is then incubated for 3 hours at RT and protected from direct exposure to light followed by centrifugation at 1500rpm for 5 minutes. The supernatant is then carefully discarded. In a subsequent step, 200µl of wash
10 buffer is twice added to each well, centrifuged at 1500rpm for 5 minutes and supernatant carefully discarded. 130µl of wash buffer is thereafter added to each well to resuspend the bead pellet. The samples are finally analysed on a flow cytometer. The data are analysed using the CBA Application Software, Activity Base and Microsoft Excel software.

Conclusion

- 15 Based on the EC50 value, "best inhibited" cytokine can be determined and the diseases correlated with such cytokine can be found in the literature.

The invention also provides pharmaceutical compositions comprising a polypeptide, nucleic acid, ligand or compound of the invention in combination with a suitable pharmaceutical carrier. These compositions may be suitable as therapeutic or diagnostic
20 reagents, as vaccines, or as other immunogenic compositions, as outlined in detail below.

According to the terminology used herein, a composition containing a polypeptide, nucleic acid, ligand or compound [X] is "substantially free of" impurities [herein, Y] when at least 85% by weight of the total X+Y in the composition is X. Preferably, X comprises at least about 90% by weight of the total of X+Y in the composition, more preferably at least about
25 95%, 98%. 98.5% or even 99% by weight.

The pharmaceutical compositions should preferably comprise a therapeutically effective amount of the polypeptide, nucleic acid molecule, ligand, or compound of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate, or prevent a targeted disease or condition, or

to exhibit a detectable therapeutic or preventative effect. For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, for example, of neoplastic cells, or in animal models, usually mice, rabbits, dogs, or pigs. The animal model may also be used to determine the appropriate concentration range and route
5 of administration. Such information can then be used to determine useful doses and routes for administration in humans.

The precise effective amount for a human subject will depend upon the severity of the disease state, general health of the subject, age, weight, and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities, and
10 tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, an effective dose will be from 0.01 mg/kg to 50 mg/kg, preferably 0.05 mg/kg to 10 mg/kg. Compositions may be administered individually to a patient or may be administered in combination with other agents, drugs or hormones.

15 A pharmaceutical composition may also contain a pharmaceutically acceptable carrier, for administration of a therapeutic agent. Such carriers include antibodies and other polypeptides, genes and other therapeutic agents such as liposomes, provided that the carrier does not itself induce the production of antibodies harmful to the individual receiving the composition, and which may be administered without undue toxicity.
20 Suitable carriers may be large, slowly metabolised macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulphates, and the like; and the salts of
25 organic acids such as acetates, propionates, malonates, benzoates, and the like. A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Pub. Co., N.J. 1991).

Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such
30 as wetting or emulsifying agents, pH buffering substances, and the like, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated

as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals; in particular, human subjects can be
5 treated.

The pharmaceutical compositions utilised in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal or transcutaneous applications (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal,
10 enteral, topical, sublingual, intravaginal or rectal means. Gene guns or hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

15 Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

If the activity of the polypeptide of the invention is in excess in a particular disease state,
20 several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as described above, along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the polypeptide, such as by blocking the binding of ligands, substrates, enzymes, receptors, or by inhibiting a second signal, and thereby alleviating the abnormal condition. Preferably, such antagonists
25 are antibodies. Most preferably, such antibodies are chimeric and/or humanised to minimise their immunogenicity, as described previously.

In another approach, soluble forms of the polypeptide that retain binding affinity for the ligand, substrate, enzyme, receptor, in question, may be administered. Typically, the polypeptide may be administered in the form of fragments that retain the relevant portions.

In an alternative approach, expression of the gene encoding the polypeptide can be inhibited using expression blocking techniques, such as the use of antisense nucleic acid molecules (as described above), either internally generated or separately administered. Modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5' or regulatory regions (signal sequence, promoters, enhancers and introns) of the gene encoding the polypeptide. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature (Gee, J.E. *et al.* (1994) In: Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY). The complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes. Such oligonucleotides may be administered or may be generated *in situ* from expression *in vivo*.

In addition, expression of the polypeptide of the invention may be prevented by using ribozymes specific to its encoding mRNA sequence. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, *et al.*, Curr. Opin. Struct. Biol (1996) 6(4), 527-33). Synthetic ribozymes can be designed to specifically cleave mRNAs at selected positions thereby preventing translation of the mRNAs into functional polypeptide. Ribozymes may be synthesised with a natural ribose phosphate backbone and natural bases, as normally found in RNA molecules. Alternatively the ribozymes may be synthesised with non-natural backbones, for example, 2'-O-methyl RNA, to provide protection from ribonuclease degradation and may contain modified bases.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of non-traditional bases such as inosine, queosine and butosine, as well as acetyl-, methyl-,

thio- and similarly modified forms of adenine, cytidine, guanine, thymine and uridine which are not as easily recognised by endogenous endonucleases.

For treating abnormal conditions related to an under-expression of the polypeptide of the invention and its activity, several approaches are also available. One approach comprises
5 administering to a subject a therapeutically effective amount of a compound that activates the polypeptide, i.e., an agonist as described above, to alleviate the abnormal condition. Alternatively, a therapeutic amount of the polypeptide in combination with a suitable pharmaceutical carrier may be administered to restore the relevant physiological balance of polypeptide.

- 10 Gene therapy may be employed to effect the endogenous production of the polypeptide by the relevant cells in the subject. Gene therapy is used to treat permanently the inappropriate production of the polypeptide by replacing a defective gene with a corrected therapeutic gene.

- Gene therapy of the present invention can occur *in vivo* or *ex vivo*. *Ex vivo* gene therapy
15 requires the isolation and purification of patient cells, the introduction of a therapeutic gene and introduction of the genetically altered cells back into the patient. In contrast, *in vivo* gene therapy does not require isolation and purification of a patient's cells.

- The therapeutic gene is typically "packaged" for administration to a patient. Gene delivery vehicles may be non-viral, such as liposomes, or replication-deficient viruses, such as
20 adenovirus as described by Berkner, K.L., in Curr. Top. Microbiol. Immunol., 158, 39-66 (1992) or adeno-associated virus (AAV) vectors as described by Muzyczka, N., in Curr. Top. Microbiol. Immunol., 158, 97-129 (1992) and U.S. Patent No. 5,252,479. For example, a nucleic acid molecule encoding a polypeptide of the invention may be engineered for expression in a replication-defective retroviral vector. This expression
25 construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding the polypeptide, such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo* (see Chapter 20, Gene Therapy and other Molecular
30 Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics (1996), T Strachan and A P Read, BIOS Scientific Publishers Ltd).

Another approach is the administration of "naked DNA" in which the therapeutic gene is directly injected into the bloodstream or muscle tissue.

In situations in which the polypeptides or nucleic acid molecules of the invention are disease-causing agents, the invention provides that they can be used in vaccines to raise
5 antibodies against the disease causing agent.

Vaccines according to the invention may either be prophylactic (i.e. to prevent infection) or therapeutic (i.e. to treat disease after infection). Such vaccines comprise immunising antigen(s), immunogen(s), polypeptide(s), protein(s) or nucleic acid, usually in combination with pharmaceutically-acceptable carriers as described above, which include
10 any carrier that does not itself induce the production of antibodies harmful to the individual receiving the composition. Additionally, these carriers may function as immunostimulating agents ("adjuvants"). Furthermore, the antigen or immunogen may be conjugated to a bacterial toxoid, such as a toxoid from diphtheria, tetanus, cholera, *H. pylori*, and other pathogens.

15 Since polypeptides may be broken down in the stomach, vaccines comprising polypeptides are preferably administered parenterally (for instance, subcutaneous, intramuscular, intravenous, or intradermal injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the
20 blood of the recipient, and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents.

The vaccine formulations of the invention may be presented in unit-dose or multi-dose containers. For example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use.

25 The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Genetic delivery of antibodies that bind to polypeptides according to the invention may also be effected, for example, as described in International patent application WO98/55607.

The technology referred to as jet injection (see, for example, www.powderject.com) may also be useful in the formulation of vaccine compositions.

A number of suitable methods for vaccination and vaccine delivery systems are described in International patent application WO00/29428.

- 5 This invention also relates to the use of nucleic acid molecules according to the present invention as diagnostic reagents. Detection of a mutated form of the gene characterised by the nucleic acid molecules of the invention which is associated with a dysfunction will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, or susceptibility to a disease, which results from under-expression, over-expression or altered
10 spatial or temporal expression of the gene. Individuals carrying mutations in the gene may be detected at the DNA level by a variety of techniques.

Nucleic acid molecules for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR, ligase chain
15 reaction (LCR), strand displacement amplification (SDA), or other amplification techniques (see Saiki *et al.*, *Nature*, 324, 163-166 (1986); Bej, *et al.*, *Crit. Rev. Biochem. Molec. Biol.*, 26, 301-334 (1991); Birkenmeyer *et al.*, *J. Virol. Meth.*, 35, 117-126 (1991); Van Brunt, J., *Bio/Technology*, 8, 291-294 (1990)) prior to analysis.

In one embodiment, this aspect of the invention provides a method of diagnosing a disease
20 in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to the invention and comparing said level of expression to a control level, wherein a level that is different to said control level is indicative of disease. The method may comprise the steps of:

- a) contacting a sample of tissue from the patient with a nucleic acid probe under stringent
25 conditions that allow the formation of a hybrid complex between a nucleic acid molecule of the invention and the probe;
- b) contacting a control sample with said probe under the same conditions used in step a);
- and c) detecting the presence of hybrid complexes in said samples;

wherein detection of levels of the hybrid complex in the patient sample that differ from levels of the hybrid complex in the control sample is indicative of disease.

A further aspect of the invention comprises a diagnostic method comprising the steps of:

- a) obtaining a tissue sample from a patient being tested for disease;
- 5 b) isolating a nucleic acid molecule according to the invention from said tissue sample; and
- c) diagnosing the patient for disease by detecting the presence of a mutation in the nucleic acid molecule which is associated with disease.

To aid the detection of nucleic acid molecules in the above-described methods, an amplification step, for example using PCR, may be included.

- 10 Deletions and insertions can be detected by a change in the size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labelled RNA of the invention or alternatively, labelled antisense DNA sequences of the invention. Perfectly-matched sequences can be distinguished from mismatched duplexes by RNase digestion or by assessing differences in melting
- 15 temperatures. The presence or absence of the mutation in the patient may be detected by contacting DNA with a nucleic acid probe that hybridises to the DNA under stringent conditions to form a hybrid double-stranded molecule, the hybrid double-stranded molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a mutation associated with disease; and detecting the presence or absence
- 20 of an unhybridised portion of the probe strand as an indication of the presence or absence of a disease-associated mutation in the corresponding portion of the DNA strand.

Such diagnostics are particularly useful for prenatal and even neonatal testing.

- Point mutations and other sequence differences between the reference gene and "mutant" genes can be identified by other well-known techniques, such as direct DNA sequencing or
- 25 single-strand conformational polymorphism, (see Orita *et al.*, Genomics, 5, 874-879 (1989)). For example, a sequencing primer may be used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabelled nucleotides or

by automatic sequencing procedures with fluorescent-tags. Cloned DNA segments may also be used as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. Further, point mutations and other sequence variations, such as polymorphisms, can be detected as described above, for example, through the use of allele-specific oligonucleotides for PCR amplification of sequences that differ by single nucleotides.

DNA sequence differences may also be detected by alterations in the electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing (for example, Myers *et al.*, Science (1985) 230:1242). Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (see Cotton *et al.*, Proc. Natl. Acad. Sci. USA (1985) 85: 4397-4401).

In addition to conventional gel electrophoresis and DNA sequencing, mutations such as microdeletions, aneuploidies, translocations, inversions, can also be detected by *in situ* analysis (see, for example, Keller *et al.*, DNA Probes, 2nd Ed., Stockton Press, New York, N.Y., USA (1993)), that is, DNA or RNA sequences in cells can be analysed for mutations without need for their isolation and/or immobilisation onto a membrane. Fluorescence *in situ* hybridization (FISH) is presently the most commonly applied method and numerous reviews of FISH have appeared (see, for example, Trachuck *et al.*, Science, 250, 559-562 (1990), and Trask *et al.*, Trends, Genet., 7, 149-154 (1991)).

In another embodiment of the invention, an array of oligonucleotide probes comprising a nucleic acid molecule according to the invention can be constructed to conduct efficient screening of genetic variants, mutations and polymorphisms. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see for example: M.Chee *et al.*, Science (1996), Vol 274, pp 610-613).

In one embodiment, the array is prepared and used according to the methods described in PCT application WO95/11995 (Chee *et al.*); Lockhart, D. J. *et al.* (1996) Nat. Biotech. 14: 1675-1680); and Schena, M. *et al.* (1996) Proc. Natl. Acad. Sci. 93: 10614-10619). Oligonucleotide pairs may range from two to over one million. The oligomers are synthesized at designated areas on a substrate using a light-directed chemical process. The

substrate may be paper, nylon or other type of membrane, filter, chip, glass slide or any other suitable solid support. In another aspect, an oligonucleotide may be synthesized on the surface of the substrate by using a chemical coupling procedure and an ink jet application apparatus, as described in PCT application W095/25116 (Baldeschweiler *et al*).

- 5 In another aspect, a "gridded" array analogous to a dot (or slot) blot may be used to arrange and link cDNA fragments or oligonucleotides to the surface of a substrate using a vacuum system, thermal, UV, mechanical or chemical bonding procedures. An array, such as those described above, may be produced by hand or by using available devices (slot blot or dot blot apparatus), materials (any suitable solid support), and machines (including robotic
10 instruments), and may contain 8, 24, 96, 384, 1536 or 6144 oligonucleotides, or any other number between two and over one million which lends itself to the efficient use of commercially-available instrumentation.

In addition to the methods discussed above, diseases may be diagnosed by methods comprising determining, from a sample derived from a subject, an abnormally decreased or
15 increased level of polypeptide or mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, nucleic acid amplification, for instance PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods.

- 20 Assay techniques that can be used to determine levels of a polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art and are discussed in some detail above (including radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays). This aspect of the invention provides a diagnostic method which comprises the steps of: (a) contacting a ligand as described above
25 with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

Protocols such as ELISA, RIA, and FACS for measuring polypeptide levels may additionally provide a basis for diagnosing altered or abnormal levels of polypeptide expression. Normal or standard values for polypeptide expression are established by
30 combining body fluids or cell extracts taken from normal mammalian subjects, preferably humans, with antibody to the polypeptide under conditions suitable for complex formation

The amount of standard complex formation may be quantified by various methods, such as by photometric means.

Antibodies which specifically bind to a polypeptide of the invention may be used for the diagnosis of conditions or diseases characterised by expression of the polypeptide, or in
5 assays to monitor patients being treated with the polypeptides, nucleic acid molecules, ligands and other compounds of the invention. Antibodies useful for diagnostic purposes may be prepared in the same manner as those described above for therapeutics. Diagnostic assays for the polypeptide include methods that utilise the antibody and a label to detect the polypeptide in human body fluids or extracts of cells or tissues. The antibodies may be
10 used with or without modification, and may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. A wide variety of reporter molecules known in the art may be used, several of which are described above.

Quantities of polypeptide expressed in subject, control and disease samples from biopsied tissues are compared with the standard values. Deviation between standard and subject
15 values establishes the parameters for diagnosing disease. Diagnostic assays may be used to distinguish between absence, presence, and excess expression of polypeptide and to monitor regulation of polypeptide levels during therapeutic intervention. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials or in monitoring the treatment of an individual patient.

20 A diagnostic kit of the present invention may comprise:

- (a) a nucleic acid molecule of the present invention;
- (b) a polypeptide of the present invention; or
- (c) a ligand of the present invention.

In one aspect of the invention, a diagnostic kit may comprise a first container containing a
25 nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to the invention; a second container containing primers useful for amplifying the nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease. The kit may further comprise a third container holding an agent for digesting unhybridised RNA.

In an alternative aspect of the invention, a diagnostic kit may comprise an array of nucleic acid molecules, at least one of which may be a nucleic acid molecule according to the invention.

To detect polypeptide according to the invention, a diagnostic kit may comprise one or more antibodies that bind to a polypeptide according to the invention; and a reagent useful for the detection of a binding reaction between the antibody and the polypeptide.

Such kits will be of use in diagnosing a disease or susceptibility to disease, particularly certain diseases including, but not limited to, immune disorders, such as autoimmune disease, rheumatoid arthritis, osteoarthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis, inflammatory disorders, such as allergy, rhinitis, bronchial hyperresponsiveness, conjunctivitis, glomerulonephritis, uveitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, pancreatitis, digestive system inflammation, sepsis, endotoxic shock, septic shock, cachexia, myalgia, ankylosing spondylitis, myasthenia gravis, post-viral fatigue syndrome, pulmonary disease, respiratory distress syndrome, asthma, chronic-obstructive pulmonary disease, airway inflammation, wound healing, endometriosis, dermatological disease, Behcet's disease, neoplastic disorders, such as melanoma, sarcoma, renal tumour, colon tumour, haematological disease, myeloproliferative disorder, Hodgkin's disease, osteoporosis, obesity, diabetes, gout, cardiovascular disorders, reperfusion injury, atherosclerosis, ischaemic heart disease, cardiac failure, stroke, liver disease, AIDS, AIDS related complex, neurological disorders, male infertility, ageing and infections, including plasmodium infection, bacterial infection and viral infection, particularly human herpesvirus 5 (cytomegalovirus) infection. In a preferred embodiment, such kits will be of use in diagnosing a disease or susceptibility to disease wherein the disease is an autoimmune disease.

Various aspects and embodiments of the present invention will now be described in more detail by way of example, with particular reference to INSP221 polypeptides.

It will be appreciated that modification of detail may be made without departing from the scope of the invention.

Brief description of the Figures

Figure 1: Predicted INSP186 sequence with translation of cds.

Figure 2: Nucleotide alignment of INSP186 cds with the IMAGE clone 5929645 insert sequence.

5 Figure 3: Alignment of the predicted INSP186 amino acid sequence with the corresponding region of IMAGE clone 5929645.

Figure 4: Predicted nucleotide sequence and amino acid translation of the INSP186 variant, referred to herein as INSP221. The position of PCR primers used for the cDNA cloning is indicated by arrows.

10 Figure 5: Amino acid sequence alignment of INSP186, INSP221, INSP076 and INSP076 variant with an original bioinformatics prediction for INSP186.

Figure 6: Nucleotide sequence with translation of the INSP221 PCR product cloned using primers INSP221-CP1 and INSP186-CP2.

TABLE 1

Amino Acid	Synonymous Groups	More Preferred Synonymous Groups
Ser	Gly, Ala, Ser, Thr, Pro	Thr, Ser
Arg	Asn, Lys, Gln, Arg, His	Arg, Lys, His
Leu	Phe, Ile, Val, Leu, Met	Ile, Val, Leu, Met
Pro	Gly, Ala, Ser, Thr, Pro	Pro
Thr	Gly, Ala, Ser, Thr, Pro	Thr, Ser
Ala	Gly, Thr, Pro, Ala, Ser	Gly, Ala
Val	Met, Phe, Ile, Leu, Val	Met, Ile, Val, Leu
Gly	Ala, Thr, Pro, Ser, Gly	Gly, Ala
Ile	Phe, Ile, Val, Leu, Met	Ile, Val, Leu, Met
Phe	Trp, Phe, Tyr	Tyr, Phe
Tyr	Trp, Phe, Tyr	Phe, Tyr
Cys	Ser, Thr, Cys	Cys
His	Asn, Lys, Gln, Arg, His	Arg, Lys, His
Gln	Glu, Asn, Asp, Gln	Asn, Gln
Asn	Glu, Asn, Asp, Gln	Asn, Gln
Lys	Asn, Lys, Gln, Arg, His	Arg, Lys, His
Asp	Glu, Asn, Asp, Gln	Asp, Glu
Glu	Glu, Asn, Asp, Gln	Asp, Glu
Met	Phe, Ile, Val, Leu, Met	Ile, Val, Leu, Met
Trp	Trp, Phe, Tyr	Trp

TABLE 2

Amino Acid	Synonymous Groups
Ser	D-Ser, Thr, D-Thr, allo-Thr, Met, D-Met, Met(O), D-Met(O), L-Cys, D-Cys
Arg	D-Arg, Lys, D-Lys, homo-Arg, D-homo-Arg, Met, Ile, D-Met, D-Ile, Orn, D-Orn
Leu	D-Leu, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Pro	D-Pro, L-I-thioazolidine-4-carboxylic acid, D-or L-1-oxazolidine-4-carboxylic acid
Thr	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Met(O), D-Met(O), Val, D-Val
Ala	D-Ala, Gly, Aib, B-Ala, Acp, L-Cys, D-Cys
Val	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met, AdaA, AdaG
Gly	Ala, D-Ala, Pro, D-Pro, Aib, .beta.-Ala, Acp
Ile	D-Ile, Val, D-Val, AdaA, AdaG, Leu, D-Leu, Met, D-Met
Phe	D-Phe, Tyr, D-Thr, L-Dopa, His, D-His, Trp, D-Trp, Trans-3,4, or 5-phenylproline, AdaA, AdaG, cis-3,4, or 5-phenylproline, Bpa, D-Bpa
Tyr	D-Tyr, Phe, D-Phe, L-Dopa, His, D-His
Cys	D-Cys, S--Me--Cys, Met, D-Met, Thr, D-Thr
Gln	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Asn	D-Asn, Asp, D-Asp, Glu, D-Glu, Gln, D-Gln
Lys	D-Lys, Arg, D-Arg, homo-Arg, D-homo-Arg, Met, D-Met, Ile, D-Ile, Orn, D-Orn
Asp	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Glu	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Met	D-Met, S--Me--Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val

Examples

Example 1: INSP186

Initially, a partial polypeptide sequence was predicted *in silico*, and this sequence is referred to herein as the INSP186 polypeptide. This is a prediction for an interleukin-9
5 receptor-like protein containing 130 amino acids (390 bp) spanning 3 exons. As the prediction lacked a signal peptide it was not expected to be full length. However the predicted sequence did contain a stop codon (see Figure 1).

Interrogation of public EST sequence databases with INSP186 identified an EST which overlapped with the INSP186 predicted sequence, GenBank Accession BC065480
10 corresponding to IMAGE cDNA clone 5929645. The IMAGE clone had been derived from a lymphoma cDNA library.

This IMAGE clone, in cloning vector pOTB7, was purchased from ATCC (plasmid database entry 16671). On sequencing the insert was found to match the predicted INSP186 coding sequence except for 2 nucleotide substitutions leading to amino acid
15 substitutions (W94R and H106R) (figures 4 and 5). An in-frame stop codon was identified 3 amino acids upstream of the first methionine of the INSP186 sequence. The sequence upstream of the first methionine in the image clone corresponded to published genomic sequence of the region, hence the presence of the stop codon was not likely to be due to sequencing errors. We therefore concluded that the first methionine of the INSP186
20 sequence probably represented the start codon. However a signal peptide was not predicted in the sequence, and the nucleotides immediately upstream of this methionine did not contain a consensus Kozak sequence.

INSP186 is a splice variant of another polypeptide, internally referred to as the INSP076 polypeptide, with the alternative splicing occurring near the 3' end of the sequence. A
25 variant of the INSP076 sequence, internally referred to as INSP076var had previously been cloned (internal reference plasmid ID 14419). This sequence contained an alternative exon 1 compared to the original INSP076 prediction.

We therefore attempted to clone the INSP186 prediction using PCR primers which would detect the alternative exon 1. The predicted sequence of the INSP221, containing 125 amino acids (375 bp) and spanning 3 exons is shown in Figure 2.

A pair of PCR primers, INSP221-CP1/INSP186-CP2, was designed to amplify a 375 bp product containing the full length of the predicted INSP221 cds. The INSP221-CP1
5 primer corresponded to 5' end of the alternative exon 1 found in INSP076var, and the INSP186-CP2 primer corresponded to the 3' end of the original INSP186 prediction.

As the INSP186, INSP076 and INSP076var sequences had been derived from lymphoma, glioblastoma (brain) and retina cDNA sources respectively, the INSP221-CP1/INSP186-
10 CP2 primer pair was tested on a range of cDNA libraries and individual cDNA templates derived from these tissues. PCR products were cloned and sequence analysis identified a clone, amplified from a lymph node cDNA template, which contained the INSP221 sequence. The cloned product, pCR4-TOPO-INSP221, is plasmid ID 16795. The cloned sequence also contains the substitutions W9R and H103R. These substitutions may
15 therefore represent polymorphisms. The amino acid alignment of INSP186, INSP221 with INSP076 and INSP076 variant is shown in Figure 5.

Example 2: Cloning of INSP186

Using the cds to search public EST databases we identified a sequence, GenBank
20 Accession BC065480 corresponding to IMAGE cDNA clone 5929645, which matched the INSP186 sequence. The clone was purchased from ATCC and the insert was sequenced using sequencing primers T7 and SP6 (Table 3). The insert sequence was found to contain the INSP186 cds as predicted except for 2 point mutations which lead to the amino acid substitutions W94R and H106R (figures 4 and 5 respectively). IMAGE clone 5929645 is
25 plasmid database ID 16671.

2.1 Construction of mammalian cell expression vectors for INSP186

Plasmid ID 16671 was used as PCR template to generate the ORF containing a C-terminal 6HIS tag and a stop codon. The first stage of this Gateway cloning process involved a two step PCR reaction which generates the ORF of INSP186 flanked at the 5' end by an attB1
5 recombination site and Kozak sequence, and flanked at the 3' end by a sequence encoding an in-frame 6 Histidine (6HIS) tag, a stop codon and the attB2 recombination site (Gateway compatible cDNA). The 2 point mutations leading to W94R and H106R were corrected by site directed mutagenesis after generation of the Gateway entry clone.

10 2.2 Generation of Gateway compatible INSP186 ORF fused to an in frame 6HIS tag sequence.

The first PCR reaction PCR1, (in a final volume of 50 µl) contains respectively: 1 µl (25 ng) of plasmid 16671, 4.0 µl dNTPs (10 mM), 5 µl of 10X Pfx polymerase buffer, 1 µl MgSO₄ (50 mM), 1.0 µl each of gene specific primer (to give a final concentration of 100
15 pmoles) (INSP186-EX1 and INSP186-EX2), and 0.5 µl Platinum Pfx DNA polymerase (Invitrogen). The PCR reaction was performed using an initial denaturing step of 95 °C for 2 min, followed by 30 cycles of 94 °C for 30 s; 64°C for 30 s and 68 °C for 1 min; and a final extension cycle of 68 °C for 5 minutes and a holding cycle of 4 °C. The amplification product was directly purified using the Perfectprep Gel cleanup kit (Eppendorf) and
20 recovered in 50 µl sterile water according to the manufacturer's instructions. A 2 µl aliquot was visualized on 1.6 % agarose gel in 1 X TAE buffer in order to verify that the product was of the expected molecular weight (384bp + 30bp = 414bp).

The second PCR reaction (in a final volume of 50 µl) contained 1 µl of diluted purified PCR1 product (to a final concentration of 10 ng), 4.0 µl dNTPs (10 mM), 5 µl of 10X Pfx
25 polymerase buffer, 1 µl MgSO₄ (50 mM), 1.0 µl of each Gateway conversion primer (GCP Forward and GCP reverse) to give a final concentration of 100 picomoles, and 0.5 µl of Platinum Pfx DNA polymerase. The conditions for the 2nd PCR reaction were: 95 °C for 2 min, followed by 30 cycles of 94 °C for 30 s; 60°C for 30 s and 68 °C for 1 min; and a final extension cycle of 68 °C for 5 minutes and a holding cycle of 4 °C. The PCR gel

purified using the Perfectprep Gel cleanup kit (Eppendorf) and recovered in 50 µl sterile water according to the manufacturer's instructions. A 2 µl aliquot was visualized on 1.6 % agarose gel in 1 X TAE buffer in order to verify that the product was of the expected molecular weight (414bp + 58bp = 472bp).

5 2.3 Subcloning of Gateway compatible INSP186-6HIS ORF into Gateway entry vector pDONR221

- The second stage of the Gateway cloning process involved sub cloning of the Gateway modified PCR product into the Gateway entry vector pDONR221 (plasmid ID 13578, Invitrogen) as follows: 5 µl of gel extracted product from PCR2 was incubated with 1.5 µl
- 10 pDONR221 vector (0.1 µg/µl), 2 µl BP buffer and 1.5 µl of BP clonase enzyme mix (Invitrogen) in a final volume of 10 µl at RT for 1h. The reaction was stopped by addition of 1 µl proteinase K (2 µg/µl) and incubated at 37 °C for a further 10 min. An aliquot of this reaction (2 µl) was used to transform DH5α strain (Invitrogen) as follows: a 50 µl aliquot of DH5α cells was thawed on ice and 2 µl of reaction mixture added. The mixture
- 15 was incubated for 30 min on ice and then heat shocked by incubation at 42 °C for exactly 30 s. Samples were returned to ice and 250 µl of warm SOC media (room temperature) was added. Samples were incubated with shaking (250 rpm) for 1 h at 37 °C. The transformation mixture was then plated on L-broth (LB) plates containing kanamycin (40 µg/ml) and incubated overnight at 37 °C.
- 20 Five numbers of transformants were picked and patched on LB agar plates containing kanamycin (40 µg/ml) and incubated overnight at 37 °C. A scoop of the grown culture from the patched plate was resuspended in 50 µl of water and boiled for 5 minutes to lyse the cells. The cell lysate was centrifuged to remove the cell debris and the supernatant obtained was used as a template for colony PCR screening.
- 25 The PCR mixture (in a final volume of 25 µl) contained 10 µl of the centrifuged cell lysate, 2.0 µl dNTPs (10 mM), 2.5 µl of Taq polymerase buffer, 0.5 µl of screening primers (to give a final concentration of 100 picomoles) (21M13 and INSP186 ATTB1 RP) and 0.5 µl of Taq DNA polymerase.

The conditions for the screening PCR reaction were: 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s; 60°C for 30 s and 72 °C for 1 min; and a final extension cycle of 72 °C for 5 minutes and a holding cycle of 4 °C. The PCR products were loaded onto a 1.6 % agarose gel to verify the fragment size.

- 5 One positive clone was selected and plasmid mini-prep DNA was prepared from 5 ml cultures using QIAprep Spin Miniprep kit (Qiagen). Plasmid DNA (150-200 ng) was subjected to DNA sequencing with 21M13 and M13Rev primers using the CEQ Dye Terminator Cycle sequencing Quick Start Kit (Beckman Coulter P/N 608120) according to the manufacturer's instructions. The primer sequences are shown in Table 3. Sequencing
10 reactions were analyzed on CEQ 2000 XL DNA analysis system (Beckman Coulter P/N 608450). After sequence confirmation, pDONR221_INSP186-94R-106R-6HIS was used for site directed mutagenesis to correct the two mutations.

2.4 Site directed mutagenesis of INSP186-6HIS

- The INSP186 sequence in image clone 5929645 differed from the predicted INSP186 by
15 substitution at three different positions (T280C), (A317G) and (C318T), which leads to two amino acid mutations W94R and H106R. These mutations were not detected in genomic DNA. As it cannot be ruled out that they represent polymorphisms in the INSP186 sequence, the mutations were restored to wild type by site directed mutagenesis using pDONR221_INSP186-94R-106R-6HIS as template.

20 2.5 Gene specific cloning primers for site-directed mutagenesis of INSP186

- Two pairs of PCR primers, INSP186 (R94W) FP, INSP186 (R94W) RP and INSP186 (R106H) FP and INSP186 (R106H) RP (Table 3), were designed such that the primers annealed to opposite strands of the pDONR221_INSP186-6HIS sequence and each primer annealed to 15 – 25 bases on either side of the amino acid to be mutated. The PCR primers
25 were designed following the instructions given in the Instruction manual for QuickChange® II XL Site-Directed Mutagenesis Kit (Stratagene).

2.6 Site-direct mutagenesis of INSP186

Site-directed mutagenesis-1, was carried out using the QuikChange® II Site-Directed Mutagenesis Kit (Stratagene) according to the manufacturer's instructions. The reaction

was performed in a final volume of 50 µl containing 1X reaction buffer, 10 ng plasmid template DNA (pDONR221_INSP186-94R-106R-6HIS), 125 ng INSP186 (R94W) FP, 125 ng INSP186 (R94W) RP, 1 µl dNTP mix, and 2.5 units *PfuUltra* HF DNA polymerase. Thermal cycling was performed using a MJ Research DNA Engine, 5 programmed as follows: 95 °C, 1 min; 18 cycles of 95 °C, 30 sec, 60°C, 1 min, and 68 °C, 3 min 30 sec; followed by a final extension of 68°C for 7 min and a holding cycle at 4 °C.

Dpn I digestion was used to digest the methylated or hemimethylated parental DNA template (plasmid pDONR221_INSP186-94R-106R-6HIS in the sample reaction). 1 µl of *Dpn* I restriction enzyme (10 U/µl, Stratagene) was added to the product of the 10 amplification reaction. The reaction mixture was mixed gently and incubated at 37 °C for 1 hour and then transformed into XL1-Blue supercompetent cells (Stratagene) as follows. A 50 µl aliquot of XL1-Blue cells was thawed on ice and 1 µl of *Dpn* I-treated DNA was added. The mixture was incubated for 30 min on ice and then heat shocked by incubation at 42 °C for exactly 45 s. Sample was returned to ice for 2 min and 250 µl of pre-warmed 15 (42 °C) NZY media was added. Sample was incubated with shaking (220 rpm) for 1 h at 37 °C. The transformation mixture (250 µl on each of 2 plates) was plated on L-broth (LB) plates containing kanamycin (40 µg/ml). Plates were incubated overnight at 37 °C.

2.7 Plasmid DNA preparation and sequencing

One transformant was selected and plasmid mini-prep DNA was prepared from 5 ml 20 cultures using QIAprep Spin Miniprep kit (Qiagen). Plasmid DNA (150-200 ng) was subjected to DNA sequencing with 21M13 and M13Rev primers using the CEQ Dye Terminator Cycle sequencing Quick Start Kit (Beckman Coulter P/N 608120) according to the manufacturer's instructions. The primer sequences are shown in Table 3. Sequencing reactions were analyzed on CEQ 2000 XL DNA analysis system (Beckman Coulter P/N 25 608450). After sequence confirmation of the insert with the corrected mutation, pDONR221_INSP186-6HIS (R94W) was then used for making the second correction (R106H). Site directed mutagenesis-2, was carried out using the protocol and conditions mentioned above. The primers used for site directed mutagenesis-2 were INSP186 (R106H) FP and INSP186 (R106H) RP. Sequence analysis identified a clone which 30 contained the expected INSP186 insert sequence (pDONR221_INSP186-6HIS, plasmid ID 17598).

2.8 Subcloning of Gateway compatible INSP186 ORF into expression vectors pEAK12d and pDEST12.2

Plasmid DNA (2 µl or approx. 150 ng) of pDONR221_INSP186-6HIS (plasmid ID 17598) was then used in a recombination reaction containing 1.5 µl of either pEAK12d
5 vector or pDEST12.2 vector (0.1 µg / µl), 2 µl LR buffer and 1.5 µl of LR clonase (Invitrogen) in a final volume of 10 µl.

The reaction was stopped by addition of 1 µl proteinase K (2 µg/µl) and incubated at 37 °C for a further 10 min. An aliquot of this reaction (2 µl) was used to transform DH5α strain (Invitrogen) as follows: a 50 µl aliquot of DH5α cells was thawed on ice and 2 µl of
10 reaction mixture added. The mixture was incubated for 30 min on ice and then heat shocked by incubation at 42 °C for exactly 30 s. Samples were returned to ice and 250 µl of warm SOC media (room temperature) was added. Samples were incubated with shaking (250 rpm) for 1 h at 37 °C. The transformation mixture was then plated on L-broth (LB) plates containing ampicillin (100 µg/ml) and incubated overnight at 37 °C.

15 Five numbers of transformants were picked and patched on LB agar plates containing ampicillin (100 µg/ml) and incubated overnight at 37 °C. A scoop of the grown culture from the patched plate was resuspended in 50 µl of water and boiled for 5 minutes to lyse the cells. The cell lysate was centrifuged to remove the cell debris and the supernatant obtained was used as a template for colony PCR screening.

20 The PCR mixture (in a final volume of 25 µl) contained 10 µl of the centrifuged cell lysate, 2.0 µl dNTPs (10 mM), 2.5 µl of Taq polymerase buffer, 0.5 µl of screening primers (to give a final concentration of 100 pmoles and 0.5 µl of Taq DNA polymerase. pEAK12d clones were screened using the primers pEAK12 FP and INSP186 ATTB1 RP and pDEST12.2 clones were screened using the primers 21M13 and INSP186 ATTB1 RP.

25 The conditions for the screening PCR reaction were: 95 °C for 2 min, followed by 30 cycles of 94 °C for 30 s; 60°C for 30 s and 72 °C for 1 min; and a final extension cycle of 72 °C for 5 minutes and a holding cycle of 4 °C. The PCR products were loaded onto a 1.6 % agarose gel to verify the fragment size. One positive clone was selected and plasmid

mini-prep DNA was prepared from 5 ml cultures using QIAprep Spin Miniprep kit (Qiagen).

Plasmid DNA (150 - 200 ng) in the pEAK12d vector was subjected to DNA sequencing with the sequencing primers pEAK12 FP and pEAK12 RP as described above. Plasmid
5 DNA (150 - 200 ng) in the pDEST12.2 vector was subjected to DNA sequencing with the sequencing primers 21M13 and M13Rev as described above.

Sequence confirmed clones were designated as pEAK12d_INSP186-6HIS (plasmid ID 17601) and pDEST12.2_INSP186-6HIS (plasmid ID 17604).

Example 3: Cloning of INSP221

10 3.1 Preparation of human cDNA templates

First strand cDNA was prepared from a variety of human tissue total RNA samples (Clontech, Stratagene, Ambion, Biochain Institute and in-house preparations) using Superscript II or SuperScript III RNase H⁻ Reverse Transcriptase (Invitrogen) according to the manufacturer's protocol.

15 For SuperScript II: Oligo (dT)₁₅ primer (1 µl at 500 µg/ml) (Promega), 2 µg human total RNA, 1 µl 10 mM dNTP mix (10 mM each of dATP, dGTP, dCTP and dTTP at neutral pH) and sterile distilled water to a final volume of 12 µl were combined in a 1.5 ml Eppendorf tube, heated to 65 °C for 5 min and chilled on ice. The contents were collected by brief centrifugation and 4 µl of 5X First-Strand Buffer, 2 µl 0.1 M DTT, and 1 µl
20 RnaseOUT™ Recombinant Ribonuclease Inhibitor (40 units/µl, Invitrogen) were added. The contents of the tube were mixed gently and incubated at 42 °C for 2 min, then 1 µl (200 units) of SuperScript II™ enzyme was added and mixed gently by pipetting. The mixture was incubated at 42 °C for 50 min and then inactivated by heating at 70 °C for 15 min. To remove RNA complementary to the cDNA, 1 µl (2 units) of *E. coli* RNase H
25 (Invitrogen) was added and the reaction mixture incubated at 37 °C for 20 min.

For SuperScript III: 1 µl Oligo(dT)₂₀ primer (50 µM, Invitrogen), 2 µg human total RNA, 1 µl 10 mM dNTP mix (10 mM each of dATP, dGTP, dCTP and dTTP at neutral pH) and sterile distilled water to a final volume of 10 µl were combined in a 1.5 ml Eppendorf tube,

- heated to 65 °C for 5 min and then chilled on ice. For each RT reaction a cDNA synthesis mix was prepared as follows: 2 µl 10X RT buffer, 4 µl 25mM MgCl₂, 2 µl 0.1M DTT, 1 µl RNaseOUT™ (40 U/µl) and 1 µl SuperScript III™ RT enzyme were combined in a separate tube and then 10 µl of this mix added to the tube containing the RNA/primer mixture. The contents of the tube were mixed gently, collected by brief centrifugation, and incubated at 50 °C for 50 min. The reaction was terminated by incubating at 80 °C for 5 min and the reaction mixture then chilled on ice and collected by brief centrifugation. To remove RNA complementary to the cDNA, 1µl (2 units) of *E. coli* RNase H (Invitrogen) was added and the reaction mixture incubated at 37 °C for 20 min.
- 10 The final 21 µl reaction mix was diluted by adding 179 µl sterile water to give a total volume of 200 µl. This represented approximately 20 ng/µl of each individual cDNA template. The templates used in the INSP221 amplification were derived from brain, eye, lymph node, and a Stratagene human universal reference cDNA sample.

3.2 cDNA libraries

- 15 Human cDNA libraries (in bacteriophage lambda (λ) vectors) were purchased from Stratagene, Clontech or Invitrogen, or prepared at the Serono Pharmaceutical Research Institute in λ ZAP, λ GT10, λ GT11, or TriplEx2 vectors according to the manufacturer's protocol (Stratagene, Clontech and Invitrogen). Bacteriophage λ DNA was prepared from small scale cultures of infected *E. coli* host strain using the Wizard Lambda Preps DNA
- 20 purification system according to the manufacturer's instructions (Promega, Corporation, Madison WI). The library template used for the INSP221 amplification were derived from retina.

3.3 Gene specific cloning primers for PCR

- A pair of PCR primers having a length of between 18 and 30 bases were designed to
- 25 amplify the full length of the INSP221 predicted cds using Primer Designer Software (Scientific & Educational Software, PO Box 72045, Durham, NC 27722-2045, USA). PCR primers were optimized to have a T_m close to 55 + 10 °C and a GC content of 40-60%. Primers were selected which had high selectivity for the target sequence (INSP221) with little or no none specific priming.

3.4 PCR amplification of INSP221 from human cDNA templates

Gene-specific cloning primers (INSP221-CP1 and INSP186-CP2, Figure 6, and Table 3) were designed to amplify a cDNA fragment of 375 bp covering the full length of the INSP221 cds. Sequences related to the INSP221 prediction (INSP186, INSP076 and INSP076var) had been cloned from lymphoma, glioblastoma and retina. The primer pair was therefore used with a cDNA library and individual cDNA samples from these sources as PCR templates. PCR was performed in a final volume of 50 µl containing 1X AmpliTaq™ buffer, 200 µM dNTPs, 50 pmoles of each cloning primer, 2.5 units of AmpliTaq™ (Applied Biosystems) and approximately 20 ng of cDNA library or individual cDNA template using an MJ Research DNA Engine, programmed as follows: 94 °C, 2 min; 40 cycles of 94 °C, 1 min, 57 °C, 1 min, and 72 °C, 1 min; followed by 1 cycle at 72°C for 7 min and a holding cycle at 4 °C.

30 µl of each amplification product was visualized on a 0.8 % agarose gel in 1 X TAE buffer (Invitrogen). Products of the expected molecular weight were purified from the gel using the Qiagen MinElute DNA Purification System (Qiagen), eluted in 10 µl of EB buffer (10mM Tris.Cl, pH 8.5) and subcloned directly.

3.5 Subcloning of PCR Products

The PCR products were subcloned into the topoisomerase I modified cloning vector (pCR4-TOPO) using the TA cloning kit purchased from the Invitrogen Corporation using the conditions specified by the manufacturer. Briefly, 4 µl of gel purified PCR product was incubated for 15 min at room temperature with 1 µl of TOPO vector and 1 µl salt solution. The reaction mixture was then transformed into *E. coli* strain TOP10 (Invitrogen) as follows: a 50 µl aliquot of One Shot TOP10 cells was thawed on ice and 2 µl of TOPO reaction was added. The mixture was incubated for 15 min on ice and then heat shocked by incubation at 42 °C for exactly 30 s. Samples were returned to ice and 250 µl of warm (room temperature) SOC media was added. Samples were incubated with shaking (220 rpm) for 1 h at 37 °C. The transformation mixture was then plated on L-broth (LB) plates containing ampicillin (100 µg/ml) and incubated overnight at 37 °C.

3.6 Colony PCR

Colonies were inoculated into 50 µl sterile water using a sterile toothpick. A 10 µl aliquot of the inoculum was then subjected to PCR in a total reaction volume of 20 µl containing 1X AmpliTaqTM buffer, 200 µM dNTPs, 20 pmoles of T7 primer, 20 pmoles of T3 primer, and 1 unit of AmpliTaqTM (Applied Biosystems) using an MJ Research DNA Engine. The cycling conditions were as follows: 94 °C, 2 min; 30 cycles of 94 °C, 30 sec, 48 °C, 30 sec and 72 °C for 1 min. Samples were maintained at 4 °C (holding cycle) before further analysis.

PCR reaction products were analyzed on 1 % agarose gels in 1 X TAE buffer. Colonies which gave PCR products of approximately the expected molecular weight (375 bp + 105 bp due to the multiple cloning site (MCS)) were grown up overnight at 37 °C in 5 ml L-Broth (LB) containing ampicillin (100 µg /ml), with shaking at 220 rpm.

3.7 Plasmid DNA preparation and sequencing

Miniprep plasmid DNA was prepared from the 5 ml culture using a Biorobot 8000 robotic system (Qiagen) or Wizard Plus SV Minipreps kit (Promega cat. no. 1460) according to the manufacturer's instructions. Plasmid DNA was eluted in 80 µl of sterile water. The DNA concentration was measured using a Spectramax 190 photometer (Molecular Devices). Plasmid DNA (200-500 ng) was subjected to DNA sequencing with the T7 primer (Table 3) using the BigDye Terminator system (Applied Biosystems cat. no. 4390246) according to the manufacturer's instructions. Sequencing reactions were purified using Dye-Ex columns (Qiagen) or Montage SEQ 96 cleanup plates (Millipore cat. no. LSKS09624) then analyzed on an Applied Biosystems 3700 sequencer.

Sequence analysis identified a clone, amplified from a lymph node cDNA template, which contained the expected INSP221 sequence. The sequence of the cloned cDNA fragment is shown in Figure 8. The plasmid containing the cloned PCR product is pCR4-TOPO-INSP221 (plasmid ID 16795).

Table 3

Primer	Sequence (5'-3')
INSP221-CP1	ATG CTT CTT GCC ACG CTG TC (SEQ ID NO: 30)
INSP186-CP2	ATG GCC TCC AGC CAC GTC TC (SEQ ID NO: 31)
INSP186-EX1	GCA GGC TTC <u>GCC ACC</u> ATG GGG AAG GGC CGA AGC (SEQ ID NO: 32)
INSP186-EX2	GTG ATG GTG ATG GTG ATG GCC TCC AGC CAC GTC (SEQ ID NO: 33)
GCP Forward	G GGG ACA AGT TTG TAC AAA AAA GCA GGC TTC <u>GCC ACC</u> (SEQ ID NO: 34)
GCP Reverse	GGG GAC CAC TTT GTA CAA GAA AGC TGG GTT TCA <i>ATG GTG</i> <i>ATG GTG ATG GTG</i> (SEQ ID NO: 35)
INSP186 R94W FP	CAA GGA CCC ACC CGA GGA TGG GTC ACC ATG GCT TTG GAG (SEQ ID NO: 36)
INSP186 R94W RP	CTC CAA AGC CAT GGT GAC CCA TCC TCG GGT GGG TCC TTG (SEQ ID NO: 37)
INSP186 R106H FP	GAG CAC CTG GTA GTG TGG CAC GTC CAC AGT GAA GAC CAG (SEQ ID NO: 38)
INSP186 R106H RP	CTG GTC TTC ACT GTG GAC GTG CCA CAC TAC CAG GTG CTC (SEQ ID NO: 39)
pEAK12FP	GCC AGC TTG GCA CTT GAT GT (SEQ ID NO: 40)
pEAK12RP	GAT GGA GGT GGA CGT GTC AG (SEQ ID NO: 41)
21M13	TGT AAA ACG ACG GCC AGT (SEQ ID NO: 42)
M13REV	CAG GAA ACA GCT ATG ACC (SEQ ID NO: 43)
T7	TAA TAC GAC TCA CTA TAG G (SEQ ID NO: 44)
T3	ATT AAC CCT CAC TAA AGG (SEQ ID NO: 45)

Underlined sequence = Kozak sequence

Bold = Stop codon

Italic sequence = His tag

Example 4: Microarray studies

Custom microarrays have been manufactured using Agilent Technologies' (Agilent Technologies Inc, Palo Alto, CA) non-contact in situ synthesis process of printing 60-mer length oligonucleotide probes, base-by-base, from digital sequence files. This is achieved with an inkjet process which delivers extremely small, accurate volumes (picoliters) of the chemicals to be spotted. Standard phosphoramidite chemistry used in the reactions allows for very high coupling efficiencies to be maintained at each step in the synthesis of the full-length oligonucleotide. Precise quantities are reproducibly deposited "on the fly."

10 This engineering feat is achieved without stopping to make contact with the slide surface and without introducing surface-contact feature anomalies, resulting in consistent spot uniformity and traceability. (Hughes et al. (2001) *Nat. Biotech.* Apr; 19(4): 342-7. Expression profiling using microarrays fabricated by an ink-jet oligonucleotide synthesizer).

15 *Probe Synthesis*

Methodologies were carried out according to Agilent instructions. Essentially, cDNA synthesis and subsequent T7 polymerase amplification of Cyanine 3(5)-CTP labeled cRNA probe was carried out using Agilent's low RNA input fluorescent linear amplification kit from a template of 5µg of total RNA according to the kit protocol (version 2 August 2003, Agilent, Palo Alto, CA). cRNA is then fragmented using Agilent's *In Situ* hybridization kit-plus and hybridized both according to Agilent's protocol (Agilent 60-mer oligo microarray processing protocol version 4.1 April 2004, Agilent, Palo Alto, CA).

Microarray Chip Design

- 10,536 probes are on the array
- 25 • 5557 of the probes designed specifically to detect secreted sequences of primary interest
- 1000 probes designed as negative controls
- 500 probes designed as positive controls

- Remainder of the probes were designed to public domain sequences which are known to be either secreted soluble extracellular proteins or membrane bound proteins with an extracellular domain in contact with the extracellular milieu.

Studies specific for INSP221

- 5 INSP221 has 3 exons. We intend to profile the chips using probe synthesized from 10 normal tissues, bone marrow, brain, lung, ovary, PBMCs, placenta, prostate, spleen and testis. Expression reports are obtainable on an exon by exon basis.

Averaging is performed for the data, using the One-step Tukey Bi-Weight Algorithm (Data Analysis and Regression: A Second Course in Statistics", Mosteller and Tukey, Addison-

- 10 Wesley, 1977, pp. 203-209; see also Affymetrix MAS5.0 algorithm). The purpose of this is to define a robust estimate of the average value of a dataset. In this case our datasets will comprise multiple probe expression values for a single exon.

- This custom array is useful for a number of reasons. First, it allows the existence and sequence of the transcript to be confirmed. Second, the tissue distribution of the INSP221
- 15 polypeptide sequence can be evaluated and thus the role of this polypeptide in disease can be clarified. The array can also be used as a diagnostic tool, to diagnose disease incidence in patients with disease conditions with which this polypeptide is correlated. The use of exon-specific probes allows any variance in expression of splice variants of this polypeptide sequence to be evaluated, in general, in specific tissues and in specific disease
- 20 states.

CLAIMS

1. A polypeptide, which polypeptide:
 - i. comprises or consists of the amino acid sequence as recited in SEQ ID NO: 8;
 - ii. is a fragment thereof having cytokine receptor activity (preferably IL-9 receptor activity or IL-9 receptor-like protein activity) or having an antigenic determinant in common with the polypeptide of (i); or
 - iii. is a functional equivalent of (i) or (ii).
2. A polypeptide which is a functional equivalent according to claim 1(iii), characterised in that it is homologous to the amino acid sequence as recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and/or SEQ ID NO:28 and has cytokine receptor activity, preferably IL-9 receptor activity or IL-9 receptor-like protein activity.
3. A polypeptide which is a fragment or a functional equivalent as recited in claim 1 or 2, which has greater than 80% sequence identity with the amino acid sequence recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and/or SEQ ID NO:28, or with an active fragment thereof, preferably greater than 85%, 90%, 95%, 98%, 98.5%, 99% or 99.5% sequence identity.
4. A polypeptide which is a functional equivalent as recited in any one of claims 1 to 3, which exhibits significant structural homology with a polypeptide having the amino acid sequence recited in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and/or SEQ ID NO:28.
5. A polypeptide which is a fragment as recited in claims 1 or 3 having an antigenic determinant in common with the polypeptide of part (i) of claim 1 which consists of 7 or more amino acid residues from the amino acid sequence SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and/or SEQ ID NO:28.

NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26 and/or SEQ ID NO:28.

6. A fusion protein comprising a polypeptide according to any previous claim.

7. A purified nucleic acid molecule which encodes a polypeptide according to any one of
5 the preceding claims.

8. A purified nucleic acid molecule according to claim 7, which comprises or consists of the nucleic acid sequence as recited SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:23, SEQ ID NO:25 and/or SEQ ID NO:27, or is a redundant equivalent or
10 fragment thereof.

9. A purified nucleic acid molecule which hybridizes under high stringency conditions with a nucleic acid molecule according to any one of claim 7 or 8.

10. A vector comprising a nucleic acid molecule as recited in any one of claims 7 to 9.

11. A host cell transformed with a vector according to claim 10.

15 12. A ligand which binds specifically to, and preferably modulates the activity of, a polypeptide according to any one of claims 1 to 6.

13. A ligand according to claim 12, which is an antibody.

14. A compound that either increases or decreases the level of expression or activity of a polypeptide according to any one of claims 1 to 6.

20 15. A compound according to claim 14 that binds to a polypeptide according to any one of claims 1 to 6 without inducing any of the biological effects of the polypeptide.

16. A compound according to claim 15, which is a natural or modified substrate, ligand, enzyme, receptor or structural or functional mimetic.

17. A polypeptide according to any one of claims 1 to 6, a nucleic acid molecule according
25 to any one of claims 7 to 9, a vector according to claim 10, a host cell according to claim

11, a ligand according to claim 12 or claim 13, or a compound according to any one of claims 14 to 16, for use in therapy or diagnosis of disease.

18. A method of diagnosing a disease in a patient, comprising assessing the level of expression of a natural gene encoding a polypeptide according to any one of claims 1 to 6,
5 or assessing the activity of a polypeptide according to any one of claims 1 to 6, in tissue from said patient and comparing said level of expression or activity to a control level, wherein a level that is different to said control level is indicative of disease.

19. A method according to claim 18 that is carried out *in vitro*.

20. A method according to claim 18 or claim 19, which comprises the steps of: (a)
10 contacting a ligand according to claim 12 or claim 13 with a biological sample under conditions suitable for the formation of a ligand-polypeptide complex; and (b) detecting said complex.

21. A method according to claim 18 or claim 19, comprising the steps of:

- 15 a) contacting a sample of tissue from the patient with a nucleic acid probe under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule according to any one of claims 7 to 9 and the probe;
- b) contacting a control sample with said probe under the same conditions used in step a); and
- 20 c) detecting the presence of hybrid complexes in said samples; wherein detection of levels of the hybrid complex in the patient sample that differ from levels of the hybrid complex in the control sample is indicative of disease.

22. A method according to claim 18 or claim 19, comprising:

- 25 a) contacting a sample of nucleic acid from tissue of the patient with a nucleic acid primer under stringent conditions that allow the formation of a hybrid complex between a nucleic acid molecule according to any one of claims 7 to 9 and the primer;

- b) contacting a control sample with said primer under the same conditions used in step a); and
- c) amplifying the sampled nucleic acid; and
- d) detecting the level of amplified nucleic acid from both patient and control samples;
5 wherein detection of levels of the amplified nucleic acid in the patient sample that differ significantly from levels of the amplified nucleic acid in the control sample is indicative of disease.

23. A method according to claim 18 or claim 19 comprising:

- a) obtaining a tissue sample from a patient being tested for disease;
- 10 b) isolating a nucleic acid molecule according to any one of claims 7 to 9 from said tissue sample; and
- c) diagnosing the patient for disease by detecting the presence of a mutation which is associated with disease in the nucleic acid molecule as an indication of the disease.

24. The method of claim 23, further comprising amplifying the nucleic acid molecule to
15 form an amplified product and detecting the presence or absence of a mutation in the amplified product.

25. The method of either claim 23 or 24, wherein the presence or absence of the mutation in the patient is detected by contacting said nucleic acid molecule with a nucleic acid probe that hybridises to said nucleic acid molecule under stringent conditions to form a hybrid
20 double-stranded molecule, the hybrid double-stranded molecule having an unhybridised portion of the nucleic acid probe strand at any portion corresponding to a mutation associated with disease; and detecting the presence or absence of an unhybridised portion of the probe strand as an indication of the presence or absence of a disease-associated mutation.

25 26. A method according to any one of claims 18-25, wherein said disease includes, but is not limited to, immune disorders, such as autoimmune disease, rheumatoid arthritis, osteoarthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis, inflammatory disorders, such as allergy, rhinitis, bronchial hyperresponsiveness,

conjunctivitis, glomerulonephritis, uveitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, pancreatitis, digestive system inflammation, sepsis, endotoxic shock, septic shock, cachexia, myalgia, ankylosing spondylitis, myasthenia gravis, post-viral fatigue syndrome, pulmonary disease, respiratory distress syndrome, asthma, chronic-
5 obstructive pulmonary disease, airway inflammation, wound healing, endometriosis, dermatological disease, Behcet's disease, neoplastic disorders, such as melanoma, sarcoma, renal tumour, colon tumour, haematological disease, myeloproliferative disorder, Hodgkin's disease, osteoporosis, obesity, diabetes, gout, cardiovascular disorders, reperfusion injury, atherosclerosis, ischaemic heart disease, cardiac failure, stroke, liver
10 disease, AIDS, AIDS related complex, neurological disorders, male infertility, ageing and infections, including plasmodium infection, bacterial infection and viral infection, particularly human herpesvirus 5 (cytomegalovirus) infection.

27. Use of a polypeptide according to any one of claims 1 to 6 as a cytokine receptor, preferably as an IL-9 receptor or an IL-9 receptor-like protein and optionally further as an
15 IL-9 antagonist.

28. A pharmaceutical composition comprising a polypeptide according to any one of claims 1 to 6, a nucleic acid molecule according to any one of claims 7 to 9, a vector according to claim 10, a host cell according to claim 11, a ligand according to claim 12 or claim 13, or a compound according to any one of claims 14 to 16.

20 29. A vaccine composition comprising a polypeptide according to any one of claims 1 to 6 or a nucleic acid molecule according to any one of claims 7 to 9.

30. A polypeptide according to any one of claims 1 to 6, a nucleic acid molecule according to any one of claims 7 to 9, a vector according to claim 10, a host cell according to claim 11, a ligand according to claim 12 or claim 13, a compound according to any one of claims
25 14 to 16, or a pharmaceutical composition according to claim 28, for use in the manufacture of a medicament for the treatment of a certain disease including, but not limited to, immune disorders, such as autoimmune disease, rheumatoid arthritis, osteoarthritis, psoriasis, systemic lupus erythematosus, and multiple sclerosis, inflammatory disorders, such as allergy, rhinitis, bronchial hyperresponsiveness,
30 conjunctivitis, glomerulonephritis, uveitis, Crohn's disease, ulcerative colitis, inflammatory bowel disease, pancreatitis, digestive system inflammation, sepsis, endotoxic

shock, septic shock, cachexia, myalgia, ankylosing spondylitis, myasthenia gravis, post-viral fatigue syndrome, pulmonary disease, respiratory distress syndrome, asthma, chronic-obstructive pulmonary disease, airway inflammation, wound healing, endometriosis, dermatological disease, Behcet's disease, neoplastic disorders, such as
5 melanoma, sarcoma, renal tumour, colon tumour, haematological disease, myeloproliferative disorder, Hodgkin's disease, osteoporosis, obesity, diabetes, gout, cardiovascular disorders, reperfusion injury, atherosclerosis, ischaemic heart disease, cardiac failure, stroke, liver disease, AIDS, AIDS related complex, neurological disorders, male infertility, ageing and infections, including plasmodium infection, bacterial infection
10 and viral infection, particularly human herpesvirus 5 (cytomegalovirus) infection.

31. A method of treating a disease in a patient, comprising administering to the patient a polypeptide according to any one of claims 1 to 6, a nucleic acid molecule according to any one of claims 7 to 9, a vector according to claim 10, a host cell according to claim 11, a ligand according to claim 12 or claim 13, a compound according to any one of claims 14
15 to 16, or a pharmaceutical composition according to claim 28.

32. A method according to claim 31, wherein, for diseases in which the expression of the natural gene or the activity of the polypeptide is lower in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, vector, host cell, ligand, compound or composition administered to the
20 patient is an agonist.

33. A method according to claim 31, wherein, for diseases in which the expression of the natural gene or activity of the polypeptide is higher in a diseased patient when compared to the level of expression or activity in a healthy patient, the polypeptide, nucleic acid molecule, vector, host cell, ligand, compound or composition administered to the patient is
25 an antagonist.

34. A method of monitoring the therapeutic treatment of disease in a patient, comprising monitoring over a period of time the level of expression or activity of a polypeptide according to any one of claims 1 to 6, or the level of expression of a nucleic acid molecule according to any one of claims 7 to 9 in tissue from said patient, wherein altering said level
30 of expression or activity over the period of time towards a control level is indicative of regression of said disease.

35. A method for the identification of a compound that is effective in the treatment and/or diagnosis of disease, comprising contacting a polypeptide according to any one of claims 1 to 6, or a nucleic acid molecule according to any one of claims 7 to 9 with one or more compounds suspected of possessing binding affinity for said polypeptide or nucleic acid molecule, and selecting a compound that binds specifically to said nucleic acid molecule or polypeptide.

36. A kit useful for diagnosing disease comprising a first container containing a nucleic acid probe that hybridises under stringent conditions with a nucleic acid molecule according to any one of claims 7 to 9; a second container containing primers useful for amplifying said nucleic acid molecule; and instructions for using the probe and primers for facilitating the diagnosis of disease.

37. The kit of claim 36, further comprising a third container holding an agent for digesting unhybridised RNA.

38. A kit comprising an array of nucleic acid molecules, at least one of which is a nucleic acid molecule according to any one of claims 7 to 9.

39. A kit comprising one or more antibodies that bind to a polypeptide as recited in any one of claims 1 to 6; and a reagent useful for the detection of a binding reaction between said antibody and said polypeptide.

40. A transgenic or knockout non-human animal that has been transformed to express higher, lower or absent levels of a polypeptide according to any one of claims 1 to 6.

41. A method for screening for a compound effective to treat disease, by contacting a non-human transgenic animal according to claim 40 with a candidate compound and determining the effect of the compound on the disease of the animal.

42. The use of an INSP221 polypeptide as a target for screening candidate drugs for treating or preventing an IL-9 related disorder.

43. Method of selecting biologically active compounds comprising:

- (i) contacting a candidate compound with recombinant host cells expressing an INSP221 polypeptide;

- (ii) selecting compounds that bind said INSP221 polypeptide at the surface of said cells and/or that modulate the activity of the INSP221 polypeptide.

Figure 1

```

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Figure 2

INSP186
IMAGE_5929645
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INSP186
IMAGE_5929645
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INSP186
IMAGE_5929645

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INSP186
IMAGE_5929645

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INSP186
IMAGE_5929645

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(Figure 2 ctd)

INSP186
IMAGE_5929645

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IMAGE_5929645

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INSP186
IMAGE_5929645

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INSP186
IMAGE_5929645

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INSP186
IMAGE_5929645

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INSP186
IMAGE_5929645

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Figure 3

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Figure 4

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INSP186-CP2

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→ Position and sense of PCR primer.

Figure 5

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INSP221         --MLLATLSSEMTLCP---SRPVRAQHRDHIVGVTWILLEAFELDCGFIPEARLHVQMAM
INSP076V        --MLLATLSSEMTLCP---SRPVRAQHRDHIVGVTWILLEAFELDCGFIPEARLHVQMAM
                *   .   .:. : .   . * *****
                *   .   .:. : .   . * *****

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                ***** . * .                ** . *

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INSP076V        -----

```

Figure 6

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Position and sense of PCR primers

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Leu His Val Gln Met Ala Met Pro Glu Val Asp Val Ala Glu Lys Glu

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Cys Asp Thr Gly Gln Trp Ser Glu Trp Ser Gln Pro Val Cys Phe Gln
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Ala Pro Gln Arg Gln Gly Pro Thr Arg Gly Trp Val Thr Met Ala Leu
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35 40 45

Leu His Val Gln Met Ala Met Pro Glu Val Asp Val Ala Glu Lys Glu
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Cys Asp Thr Gly Gln Trp Ser Glu Trp Ser Gln Pro Val Cys Phe Gln
65 70 75 80

Ala Pro Gln Arg Gln Gly Pro Thr Arg Gly Trp Val Thr Met Ala Leu
85 90 95

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100 105 110

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Leu Leu Glu Ala Phe Glu Leu Asp Cys Gly Phe Ile Pro Glu Ala Arg
 35 40 45

Leu His Val Gln Met Ala Met Pro Glu Val Asp Val Ala Glu Lys Glu

Cys Asp Thr Gly Gln Trp Ser Glu Trp Ser Gln Pro Val Cys Phe Gln
65 70 75 80

Ala Pro Gln Arg Gln Gly Pro Thr Arg Gly Trp Val Thr Met Ala Leu
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Val Leu Lys Thr Asp Leu Gly Asp Val Ala Gly Gly His
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20 25 30

Leu Leu Glu Ala Phe Glu Leu Asp Cys Gly Phe Ile Pro Glu Ala Arg
35 40 45

Leu His Val Gln Met Ala Met Pro Glu Val Asp Val Ala Glu Lys Glu
50 55 60

Cys Asp Thr Gly Gln Trp Ser Glu Trp Ser Gln Pro Val Cys Phe Gln
65 70 75 80

Ala Pro Gln Arg Gln Gly Pro Thr Arg Gly Trp Val Thr Met Ala Leu
85 90 95

Glu His Leu Val Val Trp His Val His Ser Glu Asp Gln Ser Phe Val

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18

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2006/004845

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K14/54 A61K38/00 G01N33/48 G01N33/50 C12Q1/68
A01K67/027
ADD. C12N1/21 C12N1/15 C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K G01N C12Q A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, EMBL, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/007552 A1 (ARES TRADING SA [CH]; RODRIGUES TANIA MARIA [GB]; FAGAN RICHARD JOSEPH) 22 January 2004 (2004-01-22) cited in the application especially Figure 3 the whole document	1-11, 17-43
X	WO 98/24904 A (MAGAININ PHARMA [US]) 11 June 1998 (1998-06-11) especially Figure 4 the whole document	1-11, 17-43
A	WO 98/08545 A (LUDWIG INST CANCER RES [US]; RENAULD JEAN CHRISTOPHE [BE]; MANY MARY C) 5 March 1998 (1998-03-05) the whole document	1-43
	----- -/--	

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 April 2007

Date of mailing of the international search report

11/05/2007

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Kools, Patrick

INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2006/004845

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHIELDS D C ET AL: "The evolution of haematopoietic cytokine-receptor complexes" CYTOKINE, ACADEMIC PRESS LTD, PHILADELPHIA, PA, US, vol. 7, no. 7, October 1995 (1995-10), pages 679-688, XP002043454 ISSN: 1043-4666 the whole document -----	1-43
A	OZAKI KATSUTOSHI ET AL: "Cytokine and cytokine receptor pleiotropy and redundancy" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 33, 16 August 2002 (2002-08-16), pages 29355-29358, XP002430936 ISSN: 0021-9258 the whole document -----	1-43

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2006/004845

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18, 20-26, 31-34 (partial)
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 12, 14-16, 17, 20, 28, 30, 31-33 (partial)
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Continuation of Box II.1

Although claims 18, 20-26, 34 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claims 31-33 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: 12, 14-16, 17, 20, 28, 30, 31-33 (partial)

Present claim 12 relate to a ligand which has a given desired property or effect, namely specific binding to the polypeptide of the application. However, the description does not provide support and disclosure in the sense of Article 6 and 5 PCT for such ligands having the said property or effect and there is no common general knowledge of this kind available to the person skilled in the art. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of the claim (PCT Guidelines 9.19 and 9.20).

The search of claim 12 was consequently restricted to antibodies having the desired property or effect.

Claims 14-16 are directed to all compounds which either increase or decrease the level of expression or activity of the polypeptide. The description does not provide support and disclosure in the sense of Article 6 and 5 PCT for any such compounds. This non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search of the claim (PCT Guidelines 9.19 and 9.20). The search of claim 14 was consequently restricted to the broad concept of polynucleotides of the present application, the encoded polypeptides and antibodies having the desired property or effect.

Claims 17, 20, 28 30, 31-33 are specific uses of said undefined, and non-supported compounds, or these claims relate to compositions comprising said compounds. Also the search for said claims have been restricted as outlined above.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any

Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2006/004845

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004007552 A1	22-01-2004	AU 2003280966 A1	02-02-2004
WO 9824904 A	11-06-1998	AU 5689198 A	29-06-1998
WO 9808545 A	05-03-1998	AT 252911 T	15-11-2003
		AU 721908 B2	20-07-2000
		AU 4171297 A	19-03-1998
		CA 2263935 A1	05-03-1998
		DE 69725897 D1	04-12-2003
		DE 69725897 T2	26-08-2004
		DK 1007093 T3	26-01-2004
		EP 1007093 A1	14-06-2000
		ES 2210569 T3	01-07-2004
		JP 2000517322 T	26-12-2000
		PT 1007093 T	31-03-2004
		US 5830454 A	03-11-1998
		US 5935929 A	10-08-1999