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(54) **Title:** ANTIBODY FOR TREATING DIABETES AND AUTOIMMUNE DISEASES

(57) **Abstract:** An isolated antibody comprising an antigen recognition region which comprises six complementarity determining region (CDR) amino acid sequences as set forth in: (i) SEQ ID NOs: 4, 6, 8, 12, 14 and 16; (ii) SEQ ID NOs: 20, 22, 24, 28, 30 and 32; or (iii) SEQ ID NOs: 36, 38, 40, 44, 46 and 48, is disclosed. Methods of producing same as well as pharmaceutical composition comprising same and uses thereof are disclosed.



ANTIBODY FOR TREATING DIABETES AND AUTOIMMUNE DISEASES

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to novel NKp46
5 antibodies and, more particularly, but not exclusively, to the use of same for preventing
and/or treating diabetes and autoimmune diseases.

Type 1 diabetes (T1D) is a multifactorial autoimmune disease in which insulin-
producing beta cells in pancreatic islets are destroyed by autoreactive T cells.
Mononuclear cells infiltrate the pancreatic islets of Langerhans during a variable period
10 of clinically silent inflammation (insulinitis), and eventually T cells destroy insulin-
producing beta cells. Full-blown type 1 diabetes ensues when most beta cells are
destroyed and the pancreas ceases to produce enough insulin. Exogenous insulin must
then be administered for life. To date, the only treatment that frees T1D patients from
their insulin dependency is allogeneic transplantation of islets obtained from deceased
15 donors. However, this treatment suffers from many obstacles among them is the scarcity
of pancreatic islets, and the other is the loss of a large portion of the transplanted islets
within the first weeks of transplantation, even while using immunosuppressive agents.
The reasons accounting for the transplanted islets' rejection are largely unknown.

Type 2 diabetes is characterized by insulin resistance which may be combined
20 with reduced insulin secretion. The defective responsiveness to insulin is believed to
involve the insulin receptor. In the early stage of type 2 diabetes, hyperglycemia can be
reversed by a variety of measures and medications that improve insulin sensitivity or
reduce glucose production by the liver. As the disease progresses, impairment of insulin
secretion occurs, and therapeutic replacement of insulin is often required. Beta cell
25 destruction also occurs in type 2 diabetes, and it has been proposed that one contributing
factor is increased beta cell apoptosis.

Diabetes, especially T1D, is considered to be a T cell mediated disease.
However, recently, a link between the development of diabetes and NK cells has been
suggested [Poirot L. et al., Proc Natl Acad Sci U S A. (2004) 101(21):8102-7]. Poirot et
30 al. disclose that the proportion and number of NK cells, and the timing of their entry to
the pancreas correlate with the severity of T1D in transgenic NOD mice. It has also
been disclosed that depletion of NK cells in transgenic NOD mice models of accelerated

T1D significantly inhibits diabetes development. However, the molecular mechanisms of NK cell involvement in T1D are still largely unknown.

NK cells recognize target cells through a diverse array of activating receptors and a delicate balance between inhibitory and activating signals tightly regulates their activation. The killing mediated by NK cells involves several activating receptors, such as the natural cytotoxicity receptors (NCRs) NKp30, NKp44, NKp46 and NKG2D. NKp30, NKp44 and NKp46 are expressed almost exclusively on NK cells, whereas NKG2D is expressed in additional types of lymphocytes such as CD8+ T cells. NKp46 (NCR-1 in mice) is considered to be the most specific NK marker, however, the NKp46 cellular ligands are still unknown.

The human NKp46 receptor has multiple isoforms including isoform a (GenBank Accession No. CAA04714); isoform b (GenBank Accession No. CAA06872); isoform c (GenBank Accession No. CAA06873), and isoform d (GenBank Accession No. CAA06874). In general the NKp46 receptor comprises two extracellular Ig-like domains of the C2 type, a transmembrane portion and an intracellular segment. The extracellular portion of NKp46 comprises a D1 domain, designated NKp46D1 (corresponding to residues 22-120 of the mature full length protein of isoform a), and a D2 domain, designated NKp46D2, comprising 134 amino acid residues (corresponding to residues 121-254 of the full length protein of isoform a).

Some of the inventors of the present invention have disclosed that both human and mouse β -cells express specific but unknown ligand(s) for the NKp46 receptor [Gur C. et al., Nat Immunol. (2010) 11(2):121-8]. Furthermore, Gur et al. disclosed that the development of diabetes was impaired in mice injected with LDST in the absence of NKp46 and that NKp46-deficient mice had less development of T1D induced by injection of a low dose of streptozotocin. Gur et al. further demonstrated that NKp46 is directly involved in the killing of β -cells by using a chimeric molecule composed of the extracellular portion of NKp46 fused to human IgG1 and showed the existence of NKp46 ligand(s) on pancreas β -cells. Moreover, injection of soluble NKp46 proteins into non-obese diabetic mice during the early phase of insulinitis and the prediabetic stage prevented the development of T1D.

Some of the inventors of the present invention have further demonstrated that human β cells express an unknown ligand for NKp46 and are killed in an NKp46-

dependent manner and that the recognition to the ligand expressed on β cells is confined to domain 2 (NKp46-D2) and not to domain 1 (NKp46-D1) [Gur C et al., J Immunol. (2011) 187(6):3096-103].

U.S. Patent Application Publication No. 2012/0076753 discloses the use of the natural cytotoxicity receptor NKp46 for preventing and treating diabetes, including type 1 and type 2 diabetes. U.S. Patent Application Publication No. 2012/0076753 provides compositions comprising a fragment of the extracellular region of NKp46, an antibody specific for the extracellular region of NKp46, or a combination thereof, for preventing the onset and progression of diabetes.

U.S. Patent Application Publication No. 2008/0274047 discloses methods of treating immunoproliferative and autoimmune disorders (e.g. type 1 diabetes) using antibodies which bind NK cell receptors (e.g. antibodies directed against human NKp46), particularly to deplete cells involved in the immunoproliferative pathology. U.S. 2008/0274047 further discloses that injection of anti-human NKp46 antibodies into transgenic mice expressing human NKp46 resulted in depletion of NK cells in blood, spleen, liver and lung.

U.S. Patent Application Publication No. 2007/0231813 discloses methods and compositions to evaluate the therapeutic response and/or side effects of a subject to a therapeutic composition comprising therapeutic antibodies (preferably antibodies or proteins comprising Fc portions of the G4 subclass) in situations where target neutralization is desired without depletion of a target cell. According to the teachings of U.S. 2007/0231813, the composition may specifically bind an NK receptor inter alia NKp46 and the subject may have juvenile onset diabetes.

U.S. Patent Application Publication No. 2004/0038339 discloses a multifunctional polypeptide comprising (a) a first domain comprising a binding site specifically recognizing an extracellular epitope of the NKG2D receptor complex; and (b) a second domain having receptor or ligand function, wherein said receptor or ligand function may be an antigen binding site of an antibody or fragment thereof directed against e.g. NKp46. According to U.S. Patent Application Publication No. 2004/0038339, the composition may be used for treating autoimmune diseases, e.g. insulin-dependent diabetes mellitus, wherein elimination of the subpopulation of immune cells that causes the disease is desired.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided an isolated antibody comprising an antigen recognition region which comprises six complementarity determining region (CDR) amino acid sequences as set forth in: (i) SEQ ID NOs: 4, 6, 8, 12, 14 and 16; (ii) SEQ ID NOs: 20, 22, 24, 28, 30 and 32; or (iii) SEQ ID NOs: 36, 38, 40, 44, 46 and 48.

According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising as an active ingredient the isolated antibody of some embodiments of the invention and a pharmaceutically acceptable carrier.

According to an aspect of some embodiments of the present invention there is provided a method for preventing or treating diabetes or an autoimmune disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of some embodiments of the invention.

According to an aspect of some embodiments of the present invention there is provided a use of a therapeutically effective amount of the pharmaceutical composition of some embodiments of the invention for the manufacture of a medicament identified for preventing or treating diabetes or an autoimmune disease in a subject in need thereof.

According to an aspect of some embodiments of the present invention there is provided an article of manufacture comprising the isolated antibody of some embodiments of the invention being packaged in a packaging material and identified in print, in or on the packaging material for use in the treatment of diabetes.

According to an aspect of some embodiments of the present invention there is provided an isolated polynucleotide encoding the antibody of some embodiments of the invention.

According to an aspect of some embodiments of the present invention there is provided a method of producing an antibody specific for the extracellular region of NKp46, the method comprising: (a) immunizing a mouse with a NKp46-Ig fusion polypeptide and NCR1-Ig so as to activate antibody producing cells; (b) generating hybridomas from spleen cells of the mouse of step (a) comprising fusing the spleen cells with myeloma cells; (c) isolating RNA from positively selected hybridomas of step (b)

and reverse transcribing the RNA into cDNA; and (d) cloning the cDNA of step (c) into a vector; thereby producing the antibody specific for the extracellular region of NKp46.

According to some embodiments of the invention, the antibody is an antibody fragment.

5 According to some embodiments of the invention, the antibody is selected from the group consisting of a Fab fragment, an Fv fragment and a single chain antibody.

According to some embodiments of the invention, the antibody is a monoclonal antibody.

According to some embodiments of the invention, the antibody is humanized.

10 According to some embodiments of the invention, the antibody is a chimeric antibody.

According to some embodiments of the invention, the diabetes is type 1 diabetes.

According to some embodiments of the invention, the diabetes is type 2 diabetes.

15 According to some embodiments of the invention, the therapeutically effective amount results in an increase in blood insulin levels of the subject following the administering.

According to some embodiments of the invention, the therapeutically effective amount results in reduction in pancreatic beta cell destruction in the subject following the administering.

20 According to some embodiments of the invention, the article of manufacture further comprising insulin.

According to some embodiments of the invention, the nucleic acid sequence of the isolated polynucleotide is as set forth in: (i) SEQ ID NOs: 3, 5, 7, 11, 13 and 15; (ii) SEQ ID NOs: 19, 21, 23, 27, 29 and 31; or (iii) SEQ ID NOs: 35, 37, 39, 43, 45 and 47.

25 According to some embodiments of the invention, step (b) of the method is effected by fusing the spleen cells with the myeloma cells at a ratio of 10:1 in the presence of PEG.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which
30 the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent

specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-D are photographs depicting ELISA screening results of fusion no. 68 hybridomas' antibodies on NKp46-D2-Ig coated plates. Blue wells indicate positive binding to NKp46-D2-Ig.

FIG. 2 is a table summarizing ELISA screening results of fusion no. 68 hybridomas' antibodies on plates coated with human Ig, NKp46-D1-Ig, NKp46-D2-Ig, NKp46-Ig, mouse NCR1-Ig and human LIR1-Ig. Blue highlight indicates specific binding to human NKp46-D2-Ig and full length human NKp46-Ig.

FIG. 3 is a table summarizing flow cytometry analysis results of fusion no. 68 hybridomas' antibodies positive staining on NK92-NKp46 cells (NK92 cells over-expressing NKp46) as compared to natural killer 92 (NK92) cell line. Numbers illustrate mean fluorescence intensity. Of note, hybridoma clone numbers 80.2, 35.4, 67.3, 117.4 and 104.1 bind NK92-NKp46 cells with a significantly higher intensity as compared to NK92 cells.

FIGs. 4A-N are graphs illustrating flow cytometry analysis results of fusion no. 68 hybridomas' positive antibodies on isolated primary human NK cells. Of note, clone numbers 80.2 (Figure 4M), 117.4 (Figure 4H) and 104.1 (Figure 4J) bind primary NK cells with a significantly higher intensity than others.

FIGs. 5A-B are photographs depicting analysis of total RNA isolated from positive hybridomas. The isolated total RNA of the sample was run alongside a DNA marker Marker III (TIANGEN, Cat. No: MD103) on a 1.5 % agarose/GelRedTM gel electrophoresis. Figure 5A illustrates the DNA marker Marker III (lane weights in Kb);

Figure 5B Lane M illustrates DNA marker Marker III and Lane R illustrates Total RNA of the hybridoma sample.

FIG. 6 is a photograph depicting antibody fragment amplification. Four microliters PCR products of each sample were run alongside the DNA marker Marker III on a 1.5 % agarose/GelRed™ gel electrophoresis. The PCR products were purified. Lane M illustrates the DNA marker Marker III; Lane 1 illustrates VH; and Lane 2 illustrates VL.

FIGs. 7A-B, 8A-B and 9A-B depict sequencing of antibody fragments from hybridomas with insertions of VH and VL genes. Ten clones from each hybridoma with insertions of VH and VL genes were sent for sequencing of the antibody fragments. The VH and VL genes were found nearly identical. Their consensus sequence was assumed to be the sequence of the antibody produced by the hybridoma antibody. Figures 7A-B illustrate Hybridoma 68.80.2 Sequences; Figures 8A-B illustrate Hybridoma 68.117.4 Sequences; and Figures 9A-B illustrate Hybridoma 68.104.1.31 Sequences.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to novel NKp46 antibodies and, more particularly, but not exclusively, to the use of same for preventing and/or treating diabetes and autoimmune diseases.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

The mechanism of action of natural killer (NK) cells in diabetes and autoimmune diseases is still unknown. Recently, a link between the development of diabetes and NK cells has been suggested, however, the molecular mechanisms involved in this process are still largely unknown. Additionally, a disease-promoting role for NK cells in autoimmune diseases has been recently established. NK cells kill their targets by using a

limited set of NK killer receptors. Amongst these receptors is the NKp46 receptor which plays a critical role in the mechanism of killing targeted cells. However the NKp46 cellular ligands are still unknown.

The present inventors have previously uncovered that both human and mouse β -cells express specific but unknown ligand(s) for the NKp46 receptor [Gur C et al., Nat Immunol. (2010) 11(2):121-8]. Furthermore, the development of diabetes was impaired in mice injected with low doses of streptozotocin (LDST) in the absence of NKp46 and NKp46-deficient mice had less development of type 1 diabetes (T1D) induced by injection of a LDST. The present inventors have further previously demonstrated that NKp46 is directly involved in the killing of β -cells by using a chimeric molecule composed of the extracellular portion of NKp46 fused to human IgG1 and showed the existence of NKp46 ligand(s) on pancreas β -cells. Further findings demonstrate that human β cells are killed in an NKp46-dependent manner and the recognition to the ligand expressed on β cells is confined to domain 2 (NKp46-D2) and not domain 1 (NKp46-D1) [Gur C et al., J Immunol. (2011) 187(6):3096-103 and U.S. Patent Application No. 20120076753].

While reducing the present invention to practice, the present inventors have now generated novel antibodies targeting human NKp46-D2 that can be used as a therapeutic for diabetic patients and patients suffering from autoimmune diseases. The antibodies of the present invention were generated using a unique protocol of immunization. Specifically, mice were immunized with a combination of a NKp46-D2 peptide and a NCR1 peptide (see the Examples sections which follow). This vaccination approach maximizes the generation of antibodies which bind both human NKp46-D2 and the mouse NCR1-D2. Consequently three specific antibodies were generated, each comprising six unique CDR sequences, and all of which specifically bind NKp46. These novel anti-NKp46 antibodies are contemplated for the treatment of disease in cases in which inhibition of NKp46 is warranted (e.g. in diabetes).

Thus, according to one aspect of the present invention there is provided an isolated antibody comprising an antigen recognition region which comprises six complementarity determining region (CDR) amino acid sequences.

According to one embodiment, the amino acid sequences are as set forth in SEQ ID NOs: 4, 6, 8, 12, 14 and 16.

According to a specific embodiment, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8 are arranged in a sequential order (N>C, as CDRs 1-3, respectively) on a heavy chain of the antibody, while SEQ ID NO: 12, SEQ ID NO: 14 and SEQ ID NO: 16 are arranged in a sequential order (N>C, as CDRs 1-3, respectively) on a light chain of the antibody.

According to some embodiments of the invention, the amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, e.g., 100 % sequence homology or identity to the peptide set forth in SEQ ID NOS: 4, 6, 8, 12, 14 or 16, wherein the peptide is capable of binding NKp46 (e.g. the D2 region of human NKp46).

According to another embodiment, the amino acid sequences are as set forth in SEQ ID NOS: 20, 22, 24, 28, 30 and 32.

According to a specific embodiment, SEQ ID NO: 20, SEQ ID NO: 22 and SEQ ID NO: 24 are arranged in a sequential order (N>C, as CDRs 1-3, respectively) on a heavy chain of the antibody, while SEQ ID NO: 28, SEQ ID NO: 30 and SEQ ID NO: 32 are arranged in a sequential order (N>C, as CDRs 1-3, respectively) on a light chain of the antibody.

According to some embodiments of the invention, the amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, e.g., 100 % sequence homology or identity to the peptide set forth in SEQ ID NOS: 20, 22, 24, 28, 30 or 32, wherein the peptide is capable of binding NKp46 (e.g. the D2 region of human NKp46).

According to yet another embodiment, the amino acid sequences are as set forth in SEQ ID NOS: 36, 38, 40, 44, 46 and 48.

According to a specific embodiment, SEQ ID NO: 36, SEQ ID NO: 38 and SEQ ID NO: 40 are arranged in a sequential order (N>C, as CDRs 1-3, respectively) on a heavy chain of the antibody, while SEQ ID NO: 44, SEQ ID NO: 46 and SEQ ID NO: 48 are arranged in a sequential order (N>C, as CDRs 1-3, respectively) on a light chain of the antibody.

According to some embodiments of the invention, the amino acid sequence comprises an amino acid sequence having at least 80 %, at least 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, e.g., 100 % sequence homology or identity to the peptide set forth in SEQ ID NOS: 36, 38, 40, 44, 46 or 48, wherein the peptide is capable of binding NKp46 (e.g. the D2 region of human NKp46).

Homology (e.g., percent homology, identity + similarity) can be determined using any homology comparison software, including for example, the BlastP or TBLASTN software of the National Center of Biotechnology Information (NCBI) such as by using default parameters, when starting from a polypeptide sequence; or the tBLASTX algorithm (available via the NCBI) such as by using default parameters, which compares the six-frame conceptual translation products of a nucleotide query sequence (both strands) against a protein sequence database.

For example, default parameters for tBLASTX include: Max target sequences: 100; Expected threshold: 10; Word size: 3; Max matches in a query range: 0; Scoring parameters: Matrix – BLOSUM62; filters and masking: Filter – low complexity regions.

The term “isolated” refers to at least partially separated from the natural environment e.g., from a cell.

As used herein the term "antibody", refers to an intact antibody molecule and the phrase “antibody fragment” refers to a functional fragment thereof, such as Fab, F(ab')₂, and Fv that are capable of binding to macrophages. These functional antibody fragments are defined as follows: (i) Fab, the fragment which contains a monovalent antigen-binding fragment of an antibody molecule, can be produced by digestion of whole antibody with the enzyme papain to yield an intact light chain and a portion of one heavy chain; (ii) Fab', the fragment of an antibody molecule that can be obtained by treating whole antibody with pepsin, followed by reduction, to yield an intact light chain and a portion of the heavy chain; two Fab' fragments are obtained per antibody molecule; (iii) (Fab')₂, the fragment of the antibody that can be obtained by treating whole antibody with the enzyme pepsin without subsequent reduction; F(ab')₂ is a dimer of two Fab' fragments held together by two disulfide bonds; (iv) Fv, defined as a genetically engineered fragment containing the variable region of the light chain and the variable region of the heavy chain expressed as two chains; and (v) Single chain

antibody ("SCA"), a genetically engineered molecule containing the variable region of the light chain and the variable region of the heavy chain, linked by a suitable polypeptide linker as a genetically fused single chain molecule.

The term "monoclonal antibody" as used herein refers to an antibody obtained
5 from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations that typically include different
10 antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal
15 antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al. (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries, as is known in the art, for example using techniques such as those described in Clackson et
20 al. (1991) *Nature* 352:624-628 and Marks et al. (1991) *J. Mol. Biol.* 222:581-597, and further described hereinbelow.

Methods of producing polyclonal and monoclonal antibodies as well as fragments thereof are well known in the art (See for example, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, New York, 1988,
25 incorporated herein by reference).

Antibody fragments according to some embodiments of the invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (e.g. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Antibody fragments can be obtained by pepsin
30 or papain digestion of whole antibodies by conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')₂. This fragment can be further cleaved using a

thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab' monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, by
5 Goldenberg, U.S. Patent Nos. 4,036,945 and 4,331,647, and references contained therein, which patents are hereby incorporated by reference in their entirety. See also Porter, R. R. [Biochem. J. 73: 119-126 (1959)]. Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may
10 also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

Fv fragments comprise an association of VH and VL chains. This association may be noncovalent, as described in Inbar et al. [Proc. Nat'l Acad. Sci. USA 69:2659-62 (1972)]. Alternatively, the variable chains can be linked by an intermolecular disulfide
15 bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise VH and VL chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the VH and VL domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced
20 into a host cell such as E. coli. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by [Whitlow and Filpula, Methods 2: 97-105 (1991); Bird et al., Science 242:423-426 (1988); Pack et al., Bio/Technology 11:1271-77 (1993); and U.S. Patent No. 4,946,778, which is hereby incorporated by
25 reference in its entirety.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction
30 to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick and Fry [Methods, 2: 106-10 (1991)].

According to an embodiment of the present invention, the CDR amino acid sequences are as set forth in SEQ ID NOs: 4, 6, 8, 12, 14 and 16.

According to an embodiment of the present invention, the CDR amino acid sequences are as set forth in SEQ ID NOs: 20, 22, 24, 28, 30 and 32.

5 According to an embodiment of the present invention, the CDR amino acid sequences are as set forth in SEQ ID NOs: 36, 38, 40, 44, 46 and 48.

It will be appreciated that for human therapy or diagnostics, humanized antibodies are preferably used. Humanized forms of non-human (e.g., murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or
10 fragments thereof (such as Fv, Fab, Fab', F(ab').sub.2 or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody)
15 such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially
20 all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin [Jones
25 et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992)].

Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often
30 referred to as import residues, which are typically taken from an import variable domain. Humanization can be essentially performed following the method of Winter and co-workers [Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature

332:323-327 (1988); Verhoeyen et al., *Science*, 239:1534-1536 (1988)], by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such humanized antibodies are chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be produced using various techniques known in the art, including phage display libraries [Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991)]. The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.*, 147(1):86-95 (1991)]. Similarly, human antibodies can be made by introduction of human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in the following scientific publications: Marks et al., *Bio/Technology* 10,: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368 812-13 (1994); Fishwild et al., *Nature Biotechnology* 14, 845-51 (1996); Neuberger, *Nature Biotechnology* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13, 65-93 (1995).

According to one embodiment of the present invention, the antibodies are produced essentially as described hereinbelow. Specifically, mice are first immunized with a NKp46-D2-Ig fusion polypeptide and a NCR1-Ig fusion polypeptide and screened for generation of anti-NKp46 specific antibodies. Next hybridomas are generated by fusing spleen cells of mice generating a high titer of specific anti-NKp46 antibodies with myeloma cells (e.g. NSO myeloma cells at a 10:1 ratio, respectively, in the presence of PEG). Hybridomas are then screened for specific binding to human NKp46. Total RNA is isolated from positively selected hybridomas and reverse

transcribed into cDNA. The cDNA is then cloned into an expression vector which is subsequently introduced into a host cell.

According to one embodiment the ratio of spleen cells producing anti-NKp46 antibodies and myeloma cells (e.g. NSO myeloma cells) may be 25:1, 20:1, 15:1, 10:1, 5:1 or 2:1, as can be determined by one of ordinary skill in the art.

Any expression vector may be used in accordance with the present invention. Exemplary mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

Various prokaryotic or eukaryotic cells may be used as host-expression systems to express the polypeptides of some embodiments of the invention. Exemplary cells which may be used include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the coding sequence; yeast transformed with recombinant yeast expression vectors containing the coding sequence, mammalian cells or other protein expression systems. Mammalian expression systems can also be used to express the polypeptides of some embodiments of the invention.

The expression vector (also referred to herein as a "nucleic acid construct") of some embodiments of the invention may further include additional sequences which render this vector suitable for replication and integration in prokaryotes, eukaryotes, or preferably both (e.g., shuttle vectors). In addition, typical cloning vectors may also contain a transcription and translation initiation sequence, transcription and translation terminator and a polyadenylation signal. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof.

Recovery of the recombinant polypeptide is effected following an appropriate time in culture. The phrase "recovering the recombinant polypeptide" refers to collecting the whole fermentation medium containing the polypeptide and need not imply additional steps of separation or purification. Notwithstanding the above,

polypeptides of some embodiments of the invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase
5 chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

According to one embodiment of the present invention, the nucleic acid sequence comprises SEQ ID NOs: 3, 5, 7, 11, 13 and 15.

According to another embodiment of the present invention, the nucleic acid
10 sequence comprises SEQ ID NOs: 19, 21, 23, 27, 29 and 31.

According to yet another embodiment of the present invention, the nucleic acid sequence comprises SEQ ID NOs: 35, 37, 39, 43, 45 and 47.

Of note the nucleic acid constructs may also be used for *in-vivo* use where they are administered to a subject in need thereof (e.g., diabetic), as further described
15 hereinbelow.

As mentioned, antibodies and antibody fragments generated according to the teachings of the present invention serve as inhibitors of NKp46.

The term "NKp46" as used herein refers to any human or non-human homolog, ortholog or isoform of the human natural cytotoxicity receptor known as NKp46,
20 including for example those having GenBank Accession Nos. NP_001138929.1, NP_001138930.1, NP_001229285.1, NP_001229286.1 or NP_004820.1.

According to one embodiment, the antibodies of the present invention bind the D2 region of human NKp46.

According to one embodiment, the antibody is a bi-specific antibody. Bi-specific antibodies can be e.g. monoclonal antibodies that have binding specificities for
25 at least two different antigens. For example, one of the binding specificities can be for NKp46D2 and the other one is for any other antigen, for example a different NK receptor e.g. NKG2D. Methods of generating bi-specific antibodies are disclosed for example, in Suresh et al (Methods in Enzymology 121:210 (1986)).

According to one embodiment, the antibody of the present invention binds
30 NKp46 and inhibits its receptor activity without substantially (e.g., less than 10 %) killing, destroying or eliminating the cell which bears the specific antigen which is

recognized by the particular antibody (e.g. the antibody is a "non-depleting antibody" or a "non-cytotoxic antibody").

Once antibodies are obtained, they may be tested for NKp46 inhibitory activity and binding affinities. Appropriate immunoassays for detecting specific antibody to NKp46 or specific antibody to D2 of NKp46 are known in the art and may be readily used for detecting antibodies according to the present invention. Suitable immunoassays include for example, radioimmunoassays, (RIA), fluorescent immunoassays, (FIA), enzyme-linked immunosorbant assays (ELISA), "sandwich" immunoassays, gel diffusion precipitation reactions, immunodiffusion assays, precipitation reactions, agglutination assays and immunoelectrophoresis assays [see for example, Harlow and Lane, Using Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1999)].

Furthermore, detection of anti-NKp46 antibody can be carried out using surface plasmon resonance, in which NKp46 bound to an appropriate solid substrate is exposed to the specific antibody. Binding of the antibody to NKp46 on the solid substrate results in a change in the intensity of surface plasmon resonance that can be detected qualitatively or quantitatively by an appropriate instrument, e.g., a BiacoreTM apparatus.

One specific use for the antibodies of the present invention is for preventing or treating diabetes in a subject in need thereof.

As used herein "Diabetes" refers to a disease resulting either from an absolute deficiency of insulin (type 1 diabetes) due to a defect in the biosynthesis or production of insulin, or a relative deficiency of insulin in the presence of insulin resistance (type 2 diabetes), i.e., impaired insulin action, in an organism. The diabetic patient thus has absolute or relative insulin deficiency, and may display, among other symptoms and signs, elevated blood glucose concentration, presence of glucose in the urine, excessive discharge of urine (polyuria), increased thirst (polydipsia) and increased hunger (polyphagia). Symptoms may develop quite rapidly (e.g. within weeks or months) in type 1 diabetes, particularly in children. However, in type 2 diabetes symptoms may develop much more slowly and may be subtle or completely absent. Diabetes (both types) may also cause a rapid yet significant weight loss (despite normal or even increased eating) and irreducible mental fatigue. Diabetes as used herein encompasses any stage or type of diabetes, including, but not limited to, type 1 diabetes mellitus, type

2 diabetes mellitus, metabolic syndrome, insulin deficiency syndrome, overt diabetes, pre-diabetes, Latent autoimmune diabetes of adults (LADA), maturity onset diabetes of the young (MODY 1-11) and permanent neonatal diabetes mellitus.

As mentioned above, NK cells may be involved in the etiology of autoimmune diseases. Specifically, NK cells may be involved in direct killing of tissue cells, which could lead to acceleration of autoimmunity. As NK cells kill their targets by using NK killer receptors, including the NKp46 receptor, inhibition of NKp46 using the novel antibodies of the present invention may be beneficial for the prevention or treatment of various autoimmune diseases.

Thus antibodies of the present invention may further be used for preventing or treating autoimmune diseases in a subject in need thereof.

As used herein, the term “autoimmune disease” refers to a disease where the body's immune system attacks its own cells or tissues. Autoimmune diseases include, but are not limited to, cardiovascular diseases, rheumatoid diseases, glandular diseases, gastrointestinal diseases, cutaneous diseases, hepatic diseases, neurological diseases, muscular diseases, nephric diseases, diseases related to reproduction, connective tissue diseases and systemic diseases.

Examples of autoimmune cardiovascular diseases include, but are not limited to atherosclerosis (Matsuura E. *et al.*, *Lupus*. 1998;7 Suppl 2:S135), myocardial infarction (Vaarala O. *Lupus*. 1998;7 Suppl 2:S132), thrombosis (Tincani A. *et al.*, *Lupus* 1998;7 Suppl 2:S107-9), Wegener's granulomatosis, Takayasu's arteritis, Kawasaki syndrome (Praprotnik S. *et al.*, *Wien Klin Wochenschr* 2000 Aug 25;112 (15-16):660), anti-factor VIII autoimmune disease (Lacroix-Desmazes S. *et al.*, *Semin Thromb Hemost*.2000;26 (2):157), necrotizing small vessel vasculitis, microscopic polyangiitis, Churg and Strauss syndrome, pauci-immune focal necrotizing and crescentic glomerulonephritis (Noel LH. *Ann Med Interne (Paris)*. 2000 May;151 (3):178), antiphospholipid syndrome (Flamholz R. *et al.*, *J Clin Apheresis* 1999;14 (4):171), antibody-induced heart failure (Wallukat G. *et al.*, *Am J Cardiol*. 1999 Jun 17;83 (12A):75H), thrombocytopenic purpura (Moccia F. *Ann Ital Med Int*. 1999 Apr-Jun;14 (2):114; Semple JW. *et al.*, *Blood* 1996 May 15;87 (10):4245), autoimmune hemolytic anemia (Efremov DG. *et al.*, *Leuk Lymphoma* 1998 Jan;28 (3-4):285; Sallah S. *et al.*, *Ann Hematol* 1997 Mar;74 (3):139), cardiac autoimmunity in Chagas' disease (Cunha-Neto

E. *et al.*, J Clin Invest 1996 Oct 15;98 (8):1709) and anti-helper T lymphocyte autoimmunity (Caporossi AP. *et al.*, Viral Immunol 1998;11 (1):9).

Examples of autoimmune rheumatoid diseases include, but are not limited to rheumatoid arthritis (Krenn V. *et al.*, Histol Histopathol 2000 Jul;15 (3):791; Tisch R, McDevitt HO. Proc Natl Acad Sci units S A 1994 Jan 18;91 (2):437) and ankylosing spondylitis (Jan Voswinkel *et al.*, Arthritis Res 2001; 3 (3): 189).

Examples of autoimmune glandular diseases include, but are not limited to, pancreatic disease, Type I diabetes, thyroid disease, Graves' disease, thyroiditis, spontaneous autoimmune thyroiditis, Hashimoto's thyroiditis, idiopathic myxedema, ovarian autoimmunity, autoimmune anti-sperm infertility, autoimmune prostatitis and Type I autoimmune polyglandular syndrome. Diseases include, but are not limited to autoimmune diseases of the pancreas, Type 1 diabetes (Castano L. and Eisenbarth GS. Ann. Rev. Immunol. 8:647; Zimmet P. Diabetes Res Clin Pract 1996 Oct;34 Suppl:S125), autoimmune thyroid diseases, Graves' disease (Orgiazzi J. Endocrinol Metab Clin North Am 2000 Jun;29 (2):339; Sakata S. *et al.*, Mol Cell Endocrinol 1993 Mar;92 (1):77), spontaneous autoimmune thyroiditis (Braley-Mullen H. and Yu S, J Immunol 2000 Dec 15;165 (12):7262), Hashimoto's thyroiditis (Toyoda N. *et al.*, Nippon Rinsho 1999 Aug;57 (8):1810), idiopathic myxedema (Mitsuma T. Nippon Rinsho. 1999 Aug;57 (8):1759), ovarian autoimmunity (Garza KM. *et al.*, J Reprod Immunol 1998 Feb;37 (2):87), autoimmune anti-sperm infertility (Diekman AB. *et al.*, Am J Reprod Immunol. 2000 Mar;43 (3):134), autoimmune prostatitis (Alexander RB. *et al.*, Urology 1997 Dec;50 (6):893) and Type I autoimmune polyglandular syndrome (Hara T. *et al.*, Blood. 1991 Mar 1;77 (5):1127).

Examples of autoimmune gastrointestinal diseases include, but are not limited to, chronic inflammatory intestinal diseases (Garcia Herola A. *et al.*, Gastroenterol Hepatol. 2000 Jan;23 (1):16), celiac disease (Landau YE. and Shoenfeld Y. Harefuah 2000 Jan 16;138 (2):122), inflammatory bowel disease (IBD) including Crohn's disease, ileitis and ulcerative colitis.

Examples of autoimmune cutaneous diseases include, but are not limited to, autoimmune bullous skin diseases, such as, but are not limited to, pemphigus vulgaris, bullous pemphigoid and pemphigus foliaceus.

Examples of autoimmune hepatic diseases include, but are not limited to, hepatitis, autoimmune chronic active hepatitis (Franco A. *et al.*, Clin Immunol Immunopathol 1990 Mar;54 (3):382), primary biliary cirrhosis (Jones DE. Clin Sci (Colch) 1996 Nov;91 (5):551; Strassburg CP. *et al.*, Eur J Gastroenterol Hepatol. 1999 Jun;11 (6):595) and autoimmune hepatitis (Manns MP. J Hepatol 2000 Aug;33 (2):326).

Examples of autoimmune neurological diseases include, but are not limited to, multiple sclerosis (MS, Cross AH. *et al.*, J Neuroimmunol 2001 Jan 1;112 (1-2):1), Alzheimer's disease (Oron L. *et al.*, J Neural Transm Suppl. 1997;49:77), myasthenia gravis (Infante AJ. And Kraig E, Int Rev Immunol 1999;18 (1-2):83; Oshima M. *et al.*, Eur J Immunol 1990 Dec;20 (12):2563), neuropathies, motor neuropathies (Kornberg AJ. J Clin Neurosci. 2000 May;7 (3):191); Guillain-Barre syndrome and autoimmune neuropathies (Kusunoki S. Am J Med Sci. 2000 Apr;319 (4):234), myasthenia, Lambert-Eaton myasthenic syndrome (Takamori M. Am J Med Sci. 2000 Apr;319 (4):204); paraneoplastic neurological diseases, cerebellar atrophy, paraneoplastic cerebellar atrophy and stiff-man syndrome (Hiemstra HS. *et al.*, Proc Natl Acad Sci units S A 2001 Mar 27;98 (7):3988); non-paraneoplastic stiff man syndrome, progressive cerebellar atrophies, encephalitis, Rasmussen's encephalitis, amyotrophic lateral sclerosis, Sydeham chorea, Gilles de la Tourette syndrome and autoimmune polyendocrinopathies (Antoine JC. and Honnorat J. Rev Neurol (Paris) 2000 Jan;156 (1):23); dysimmune neuropathies (Nobile-Orazio E. *et al.*, Electroencephalogr Clin Neurophysiol Suppl 1999;50:419); acquired neuromyotonia, arthrogryposis multiplex congenita (Vincent A. *et al.*, Ann N Y Acad Sci. 1998 May 13;841:482), neuritis, optic neuritis (Soderstrom M. *et al.*, J Neurol Neurosurg Psychiatry 1994 May;57 (5):544) and neurodegenerative diseases.

Examples of autoimmune muscular diseases include, but are not limited to, myositis, autoimmune myositis and primary Sjogren's syndrome (Feist E. *et al.*, Int Arch Allergy Immunol 2000 Sep;123 (1):92) and smooth muscle autoimmune disease (Zauli D. *et al.*, Biomed Pharmacother 1999 Jun;53 (5-6):234).

Examples of autoimmune nephric diseases include, but are not limited to, nephritis and autoimmune interstitial nephritis (Kelly CJ. J Am Soc Nephrol 1990 Aug;1 (2):140).

Examples of autoimmune diseases related to reproduction include, but are not limited to, repeated fetal loss (Tincani A. *et al.*, *Lupus* 1998;7 Suppl 2:S107-9).

Examples of autoimmune connective tissue diseases include, but are not limited to, ear diseases, autoimmune ear diseases (Yoo TJ. *et al.*, *Cell Immunol* 1994 Aug;157 (1):249) and autoimmune diseases of the inner ear (Gloddek B. *et al.*, *Ann N Y Acad Sci* 1997 Dec 29;830:266).

Examples of autoimmune systemic diseases include, but are not limited to, systemic lupus erythematosus (Erikson J. *et al.*, *Immunol Res* 1998;17 (1-2):49) and systemic sclerosis (Renaudineau Y. *et al.*, *Clin Diagn Lab Immunol.* 1999 Mar;6 (2):156); Chan OT. *et al.*, *Immunol Rev* 1999 Jun;169:107).

According to a specific embodiment, the autoimmune disease comprises multiple sclerosis (MS).

According to a specific embodiment, the autoimmune comprises inflammatory bowel disease (IBD).

The term “treating” refers to inhibiting or arresting the development of a disease, disorder or condition and/or causing the reduction, remission, or regression of a disease, disorder or condition or keeping a disease, disorder or medical condition from occurring in a subject who may be at risk for the disease disorder or condition, but has not yet been diagnosed as having the disease disorder or condition. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a disease, disorder or condition, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a disease, disorder or condition.

As used herein, the term “preventing” refers to keeping a disease, disorder or condition from occurring in a subject who may be at risk for the disease, but has not yet been diagnosed as having the disease.

As used herein, the term “subject” refers to an animal, preferably a mammal, most preferably a human being, including both young and old human beings of both genders who suffer from or are predisposed to diabetes.

According to one embodiment, the antibody of the present invention is administered to the subject at a stage of type 1 diabetes comprising pre-insulinitis, early insulinitis, pre-diabetes and/or overt diabetes.

According to another embodiment, the antibody of the present invention is administered to the subject at a stage of type 2 diabetes comprising hyperinsulinemia, pre-diabetes and/or overt diabetes.

According to another embodiment, the antibody of the present invention is administered to a subject with an autoimmune disease at disease onset, during disease remission, during an acute stage of the disease or during a chronic stage of the disease.

Diagnosis of diabetes or of an autoimmune disease is known to one of skill in the art. Thus, for example, tests may be used to diagnose diabetes and pre-diabetes by measuring glucose and insulin levels (e.g. blood or urine levels), including for example, fasting plasma glucose (FPG) test, oral glucose tolerance test (OGTT), random plasma glucose (RPG) test, A1C test and serum insulin level test. Likewise, any number of tests may be used to diagnose autoimmune diseases (depending on the disease type and subject to be tested). Thus, for example, blood tests, ultrasound, CT scan, MRI, etc. may be used as known to one of skill in the art.

The antibody of some embodiments of the invention can be administered to an organism *per se*, or in a pharmaceutical composition where it is mixed with suitable carriers or excipients.

As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

Herein the term "active ingredient" refers to the antibody accountable for the biological effect.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium

phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the pharmaceutical composition can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the pharmaceutical composition to be

formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain
5 tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired,
10 disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and
15 suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as
20 glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for
25 oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an
30 aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage

unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The pharmaceutical composition of some embodiments of the invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredient (antibody) effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g., diabetes) or prolong the survival of the subject being treated.

According to one embodiment, the therapeutically effective amount results in an increase in blood insulin levels and/or in reduction of blood glucose levels (e.g. to normal levels) of the subject following administration of the antibody.

Measurement of blood insulin levels and/or blood glucose levels is known to one of skill in the art, and is described in detail hereinabove.

According to one embodiment, the therapeutically effective amount results in reduction in pancreatic beta cell destruction in the subject following administration of the antibody.

Measurement of pancreatic cell mass may be used according to any method known in the art, as for example, by a biopsy, using magnetic resonance imaging (MRI) or using nuclear imaging techniques. Thus, for example measuring pancreatic cell mass may be carried out by using non-invasive imaging using agents that permit visualization of changes in β -cell mass e.g. using near-infrared fluorescent β -cell imaging agent or using a radioisotope-labeled fluorescent β -cell imaging agent as taught in Reiner et al., Proc Natl Acad Sci USA (2011) 108(31):12815-20; or using a β -cell-specific monoclonal antibody IC2, modified with a radioisotope chelator for nuclear imaging as taught in Moore et al. Diabetes (2001), Vol. 50(10): 2231-2236.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated from animal models (e.g. STZ diabetic mice) to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The

dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

5 Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

10 The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

Compositions including the preparation of the present invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition (e.g. diabetes).

15 Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or
20 dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs
25 or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

30 It will be appreciated that the article of manufacture may further comprise another therapeutic composition for diabetes, e.g. insulin. Thus, for example, the antibodies or fragments thereof can be packaged in one container while insulin may be packaged in a second container both for therapeutic treatment.

According to an embodiment, the insulin composition may comprise any type of insulin known for therapeutics. Thus, for example, insulin of the invention may include rapid-acting insulin (e.g. which typically starts working within a few minutes and lasts for a couple of hours), regular- or short-acting insulin (e.g. which typically takes about 5 30 minutes to work fully and lasts for 3 to 6 hours, intermediate-acting insulin (e.g. which typically takes 2 to 4 hours to work fully and its effect can last for up to 18 hours), or long-acting insulin (e.g. for which there are typically no peak levels in the bloodstream, and can keep working for an entire day).

As used herein the term "about" refers to $\pm 10\%$.

10 The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of" means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the 15 additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures 20 thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should 25 be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies 30 regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges

between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

5 As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

10 It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described
15 embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the
20 following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

25 Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons,
30 Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific

American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Patent Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III
 5 Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for
 10 example, U.S. Patent Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984);
 15 "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course
 20 Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Generation of NKp46 specific antibodies

MATERIALS AND EXPERIMENTAL PROCEDURES

Fusion proteins development and production

Generation of NKp46-Ig2 and NCR1-Ig1 in Simian kidney COS-7 (COS) cells
 30 was carried out as previously described [Gazit R et al., Nat Immunol. (2006) 7(5): 517-

23; Mandelboim O et al., Nature. (2001) 409(6823): 1055-60]. Truncated fusion proteins of NKp46D1-Ig (including the leader peptide 1-21 and residues 1-100) and NKp46D2-Ig (residues 101-235) were generated by polymerase chain reaction (PCR) amplification and cloned into a mammalian expression vector containing the Fc portion of human IgG1 as previously described [Mandelboim O et al., Nature. (2001) 409(6823): 1055-60]. In order to allow expression of NKp46D2-Ig, which lacks its original leader peptide sequence, a methionine start codon was added and the PCR-amplified fragment of NKp46D2 was cloned in frame with the leader peptide of CD5. Sequencing of the constructs revealed that all cDNAs were in frame with the human Fc genomic DNA and were identical to the reported sequences. The production of the fusion proteins in COS cells was previously described [Arnon TI et al., Blood. (2004) 103(2):664-72].

In short, generation of NCR1-Ig1 in COS cells was carried out.

For the production of NKp46D2 in Chinese hamster ovary (CHO) cells, the NKp46D2-Ig fragment was cloned into the pcDNA 3.1 vector. After recloning, the highest protein-producing clone was adapted for special serum-free medium (CHO-SFM II; Gibco, Grand Island, NY), followed by optimization for growth in large-scale cultures. Supernatants were collected and purified on protein-G columns using fast-protein liquid chromatography (FPLC).

Mouse immunization

BALB/c mice were injected intraperitoneally (IP) with 40 µg of the antigen NKp46-D2-Ig in Complete Freund's Adjuvant (CFA). Two weeks later mice were injected with the antigens NKp46-D2-Ig and NCR1-Ig respectively every other week in incomplete Freund's adjuvant. After 4 injections mice were injected with boost of the antigen NKp46-D2-Ig in adjuvant. After 2-5 days mice were bled and were screened using ELISA for antibodies in the serum against NKp46-D2-Ig and NCR1-Ig.

Hybridoma development

Spleen cells (approximately 10^8 cells) from high titer mice were fused with NSO myeloma cell line at a ratio of 10:1 respectively in the presence of 50 % PEG. Mature clones were screened by ELISA and approximately 130 hybridomas were detected for producing antibodies binding to NKp46-D2-Ig, none of them were detected for antibodies binding to antigen NCR1-Ig (Figures 1A-D).

Furthermore, the hybridomas' antibodies were screened by ELISA for binding to human Ig, NKp46-D1-Ig, NKp46-D2-Ig, NKp46-Ig, mouse NCR1-Ig & human LIR1-Ig (Figure 2).

ELISA positive clones for NKp46-D2-Ig were used for staining of natural killer 92 (NK92) cell line and NK92 cells over-expressing NKp46 (NK92-NKp46) (Figure 3). Staining of NK92-NKp46 cells was carried out using FACS analysis. In short, cells were harvested, washed and incubated with mAb supernatant followed by washing and incubation with fluorescent anti-mouse commercial Abs. Washed cells were then analyzed in FACSCanto™ II flow cytometer.

Positive hybridomas were screened for staining of human primary natural killer cells isolated from human blood (Figure 4).

Hybridoma sequencing

Total RNA was isolated from the hybridoma cells following the technical manual of TRIzol® Plus RNA Purification System. The total RNA was analyzed by agarose gel electrophoresis (Figures 5A-B). Total RNA was reverse transcribed into cDNA using isotype-specific anti-sense primers or universal primers following the technical manual of SuperScript™ III First-Strand Synthesis System. The antibody fragment was amplified according to the standard operating procedure of RACE of GenScript (Figure 6). Amplified antibody (VH and VL) genes were separately cloned into a standard cloning vector owned by GenScript using standard molecular cloning procedures. Colony PCR screening was performed to identify clones with inserts of correct sizes. No less than ten independent positive colonies were sequenced for each antibody fragment (Figures 7A-B, 8A-B and 9A-B).

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

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

identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. An isolated antibody comprising an antigen recognition region which comprises six complementarity determining region (CDR) amino acid sequences as set forth in:

- (i) SEQ ID NOs: 4, 6, 8, 12, 14 and 16;
- (ii) SEQ ID NOs: 20, 22, 24, 28, 30 and 32; or
- (iii) SEQ ID NOs: 36, 38, 40, 44, 46 and 48.

2. The isolated antibody of claim 1, wherein said antibody is an antibody fragment.

3. The isolated antibody of claim 2, wherein said antibody is selected from the group consisting of a Fab fragment, an Fv fragment and a single chain antibody.

4. The isolated antibody of claim 1, wherein said antibody is a monoclonal antibody.

5. The isolated antibody of any of claims 1-4, wherein said antibody is humanized.

6. The isolated antibody of any of claims 1-4, wherein said antibody is a chimeric antibody.

7. A pharmaceutical composition comprising as an active ingredient the isolated antibody of any of claims 1-6 and a pharmaceutically acceptable carrier.

8. A method for preventing or treating diabetes or an autoimmune disease in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the pharmaceutical composition of claim 7.

9. Use of a therapeutically effective amount of the pharmaceutical composition of claim 7 for the manufacture of a medicament identified for preventing or treating diabetes or an autoimmune disease in a subject in need thereof.

10. The method of claim 8 or use of claim 9, wherein said diabetes is type 1 diabetes.

11. The method of claim 8 or use of claim 9, wherein said diabetes is type 2 diabetes.

12. The method of claim 8, wherein said therapeutically effective amount results in an increase in blood insulin levels of the subject following said administering.

13. The method of claim 8, wherein said therapeutically effective amount results in reduction in pancreatic beta cell destruction in the subject following said administering.

14. An article of manufacture comprising the isolated antibody of any of claims 1-6 being packaged in a packaging material and identified in print, in or on said packaging material for use in the treatment of diabetes.

15. The article of manufacture of claim 14, further comprising insulin.

16. An isolated polynucleotide encoding the antibody of claim 1.

17. The isolated polynucleotide of claim 16, wherein a nucleic acid sequence of said isolated polynucleotide is as set forth in:

- (i) SEQ ID NOs: 3, 5, 7, 11, 13 and 15;
- (ii) SEQ ID NOs: 19, 21, 23, 27, 29 and 31; or
- (iii) SEQ ID NOs: 35, 37, 39, 43, 45 and 47.

18. A method of producing an antibody specific for the extracellular region of NKp46, the method comprising:

- (a) immunizing a mouse with a NKp46-Ig fusion polypeptide and NCR1-Ig so as to activate antibody producing cells;
 - (b) generating hybridomas from spleen cells of said mouse of step (a) comprising fusing said spleen cells with myeloma cells;
 - (c) isolating RNA from positively selected hybridomas of step (b) and reverse transcribing said RNA into cDNA; and
 - (d) cloning said cDNA of step (c) into a vector;
- thereby producing said antibody specific for said extracellular region of NKp46.

19. The method of claim 18, wherein said step (b) is effected by fusing said spleen cells with said myeloma cells at a ratio of 10:1 in the presence of PEG.

FIG. 1A

FIG. 1B

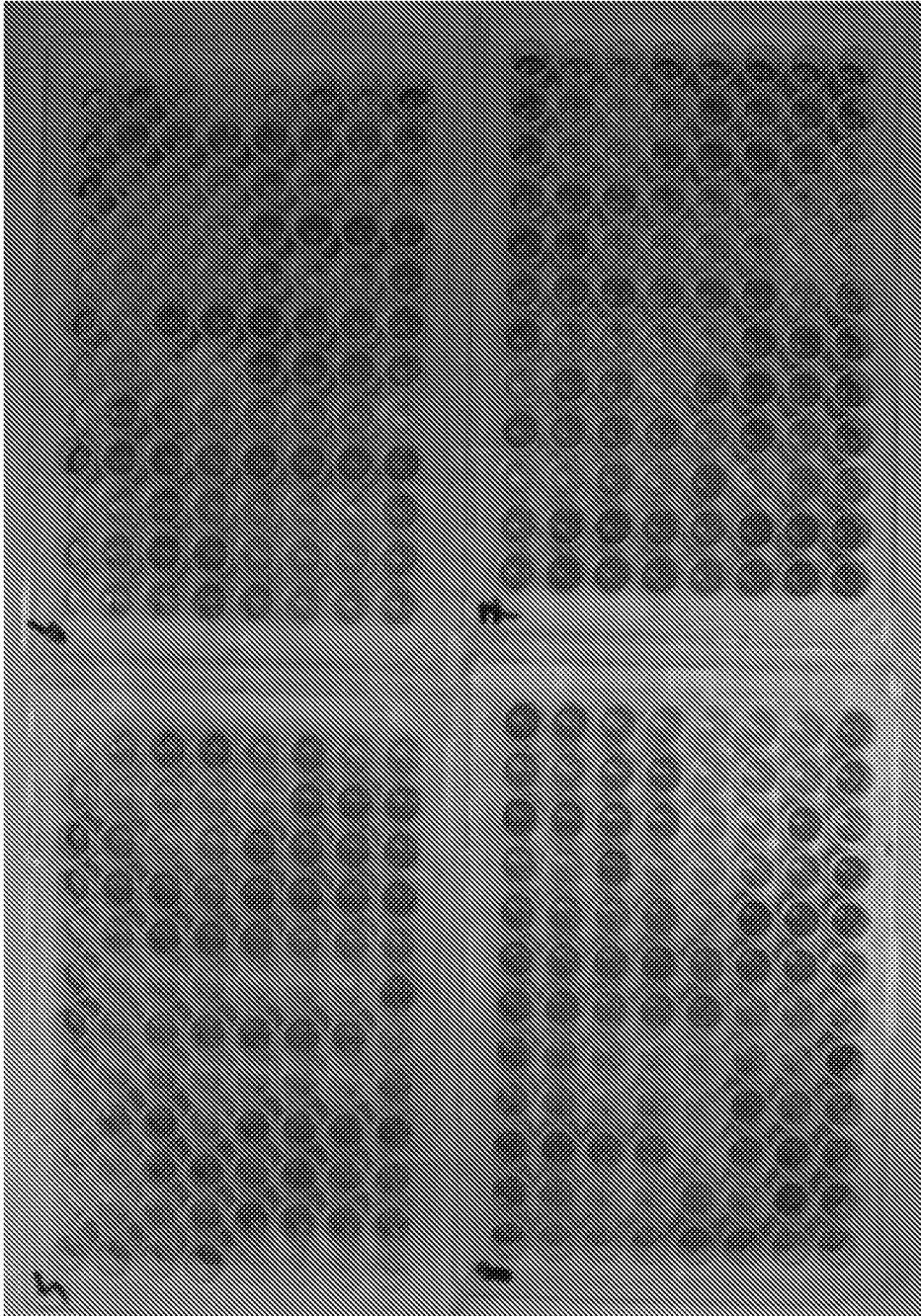


FIG. 1C

FIG. 1D

FIG. 2

Clone number	hu-Ig	NKp46-D1-Ig	NKp46-D2-Ig	NKp46-Ig Full length	mouse NCR1-Ig	human LIR1-Ig
35.4	0.78096264	0.747834962	3.859231911	3.795290632	0.304030685	0.451839834
50.3	0.1404125	0.178159807	2.592789341	2.126616346	0.121619275	0.134352348
33.1	0.1719558	0.165230394	2.514230196	1.98725659	0.114271876	0.191665609
61.1	0.16625075	0.159363099	3.129062456	2.859231911	0.099745896	0.18624598
67.3	0.2193911	0.223317827	3.805756065	3.525055576	0.137887582	0.192852736
83.2	0.15872513	0.124795732	4	3.726133687	0.143482652	0.25948733
80.2	0.22094031	0.212605128	3.326872964	3.014990468	0.152257059	0.335708792
99.1	0.14072679	0.123084434	0.740019267	0.612639955	0.087096428	0.124842106
118.1	0.18572798	0.142560935	3.963695062	3.518172793	0.096498	0.284498353
53.3	0.38666711	0.378858169	3.844508654	3.619199372	0.233312341	0.53296387
117.4	0.89084237	0.832281838	4	3.726133687	0.327381404	0.580159997
12.2	0.9073888	1.071424667	4	3.913389944	0.395661199	0.373889446
22.1	0.32385834	0.248078712	3.297965991	3.128505311	0.118870855	2.881981479
32.2	0.45668712	0.332187211	3.206352318	2.966753486	0.168445763	2.583642583
100.2	0.23738332	0.18146076	3.35035406	3.162664649	0.091037439	2.946881563
104.1	0.29615691	0.177765473	4	4	0.111502489	3.945052952

FIG. 3

	NK92 cells	NK92-NKp46 cells
2Ab	3.1	4.2
50.3	5.3	5.25
61.1	5.2	6.3
80.2	6.45	35.3
92.1	4.45	5.8
35.4	5.4	26.4
67.3	4.95	33.95
117.4	6.5	29.7
33.1	4.5	6.2
53.3	4.55	5.3
118.1	4.55	6.35
83.2	4.5	5.2
104.1	4.95	13.95
R&D	26	316
D29A5	6.5	123.5
46-1G1	9.1	81
F-15	4.35	5.4

FIG. 4A

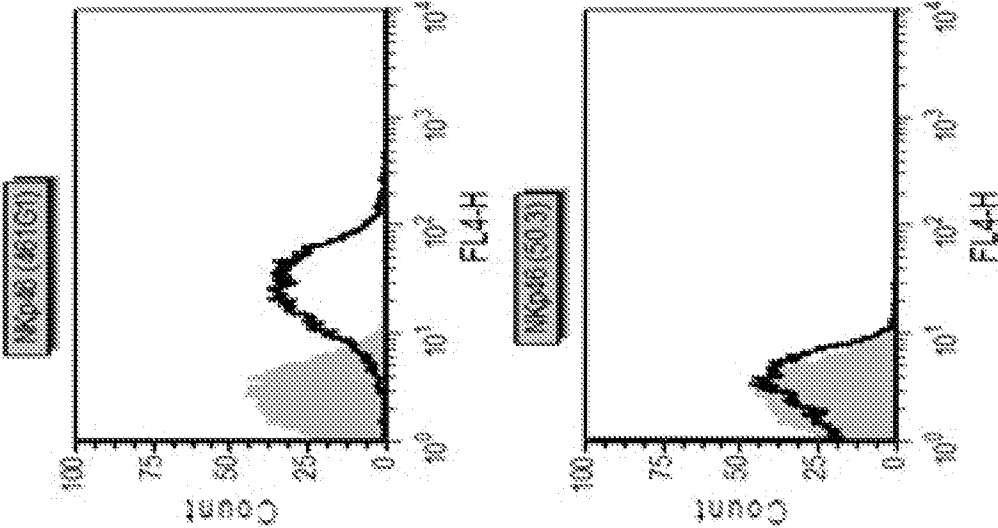


FIG. 4B

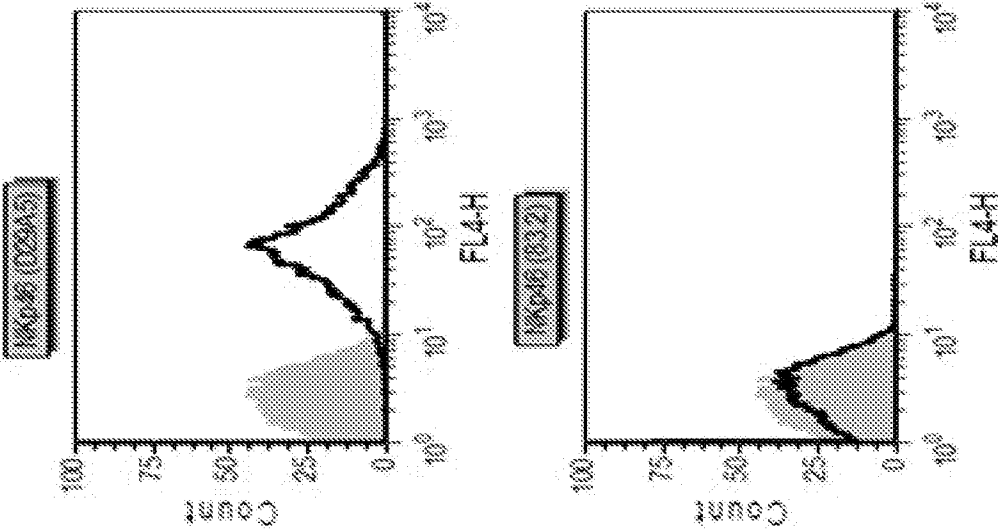


FIG. 4C

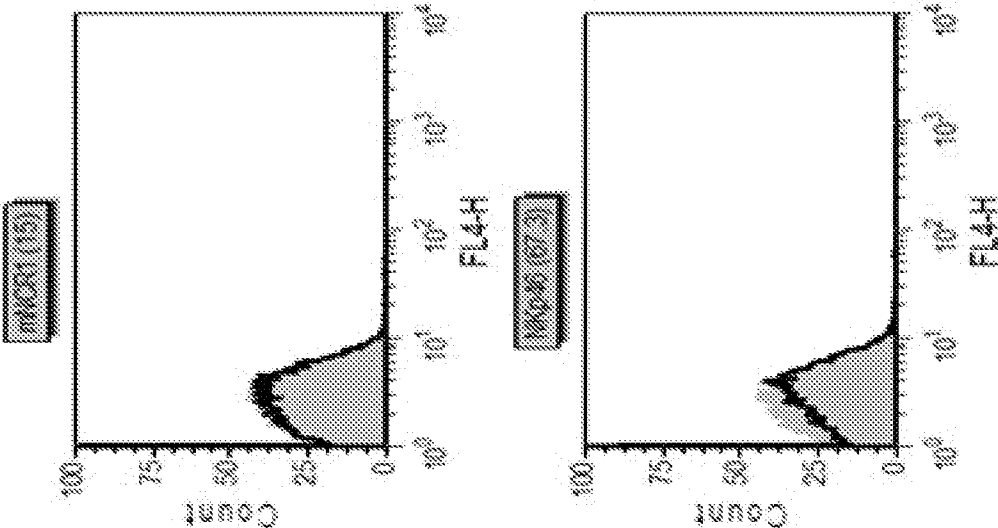


FIG. 4D

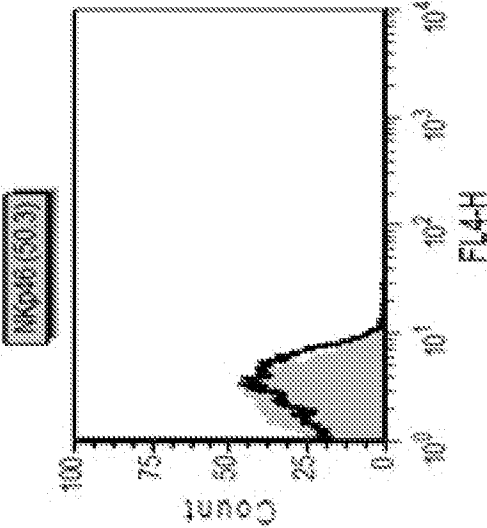


FIG. 4E

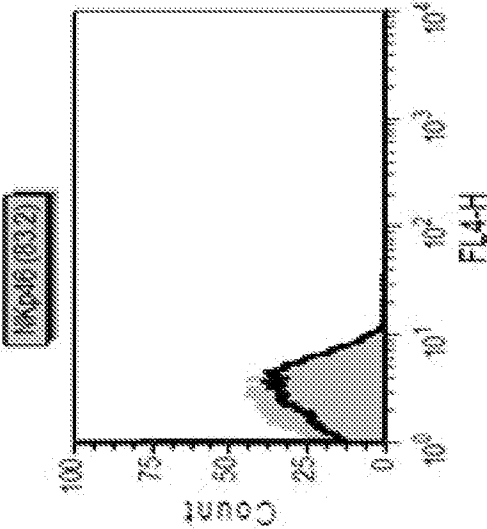
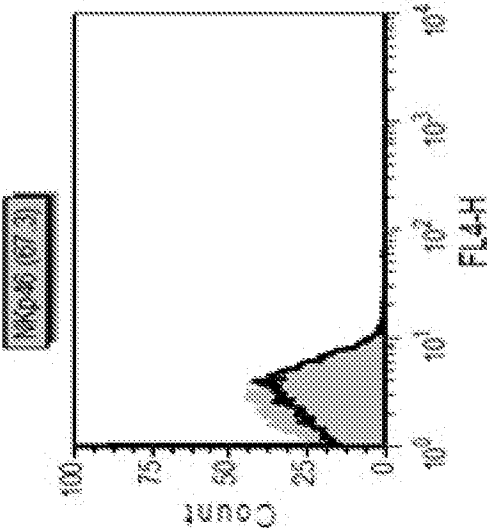


FIG. 4F



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FIG. 4I

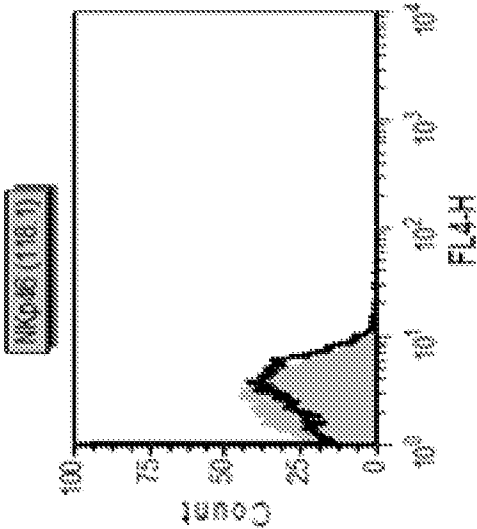


FIG. 4H

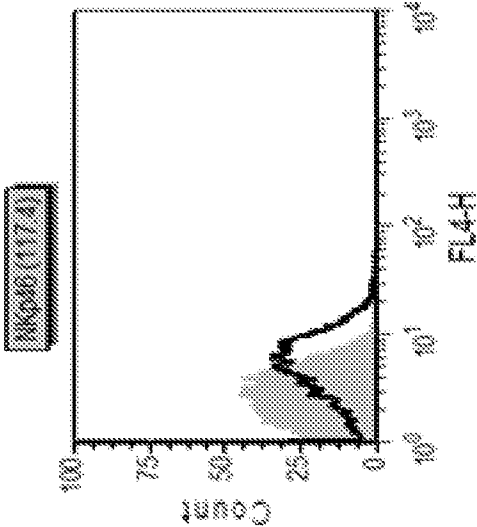


FIG. 4G

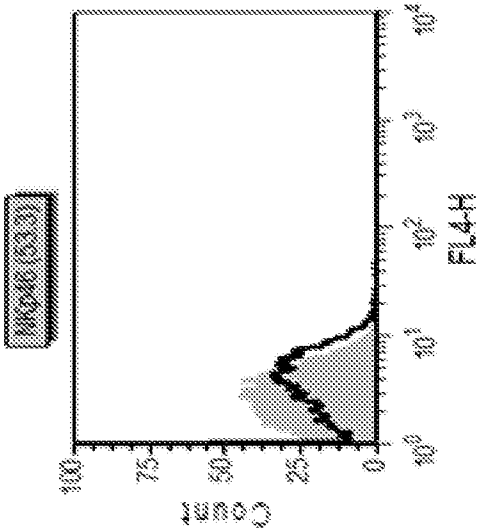


FIG. 4J

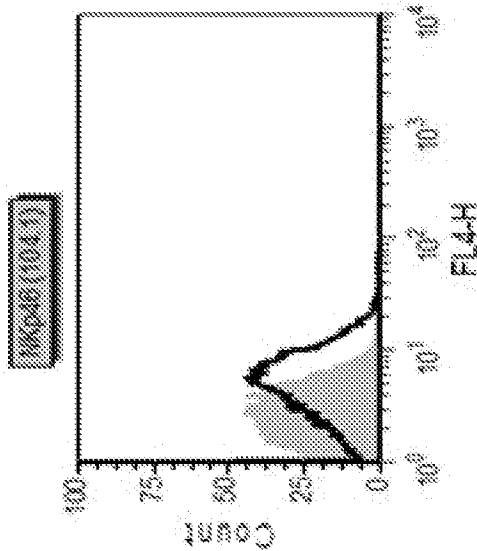


FIG. 4K

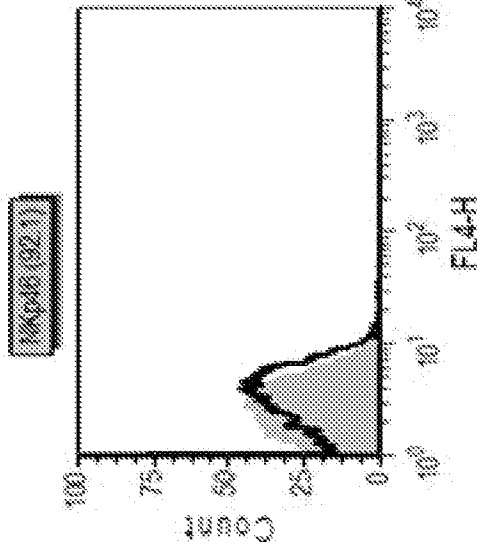


FIG. 4L

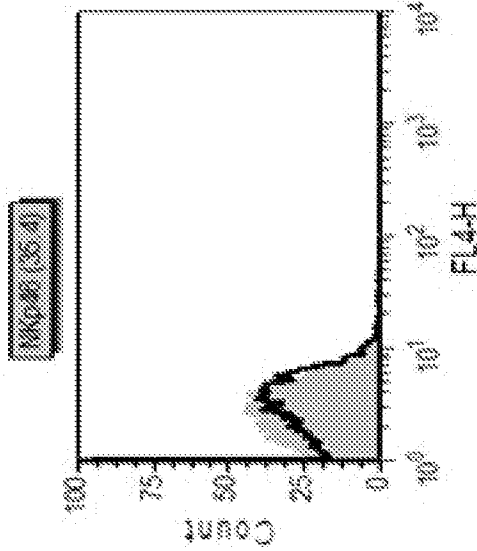


FIG. 4M

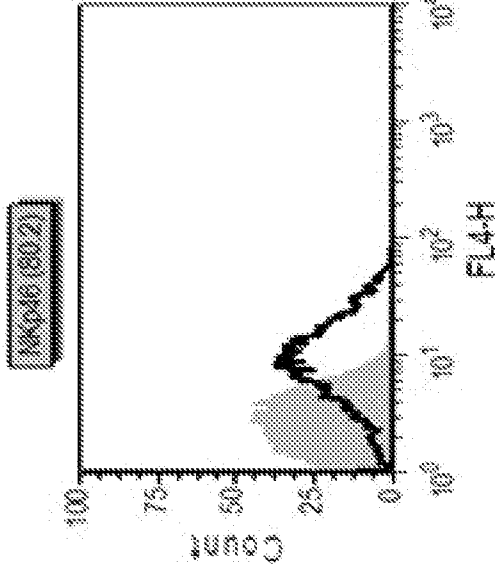


FIG. 4N

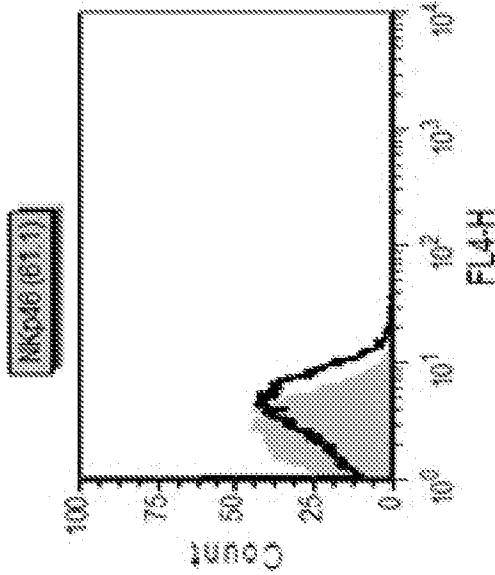


FIG. 5B

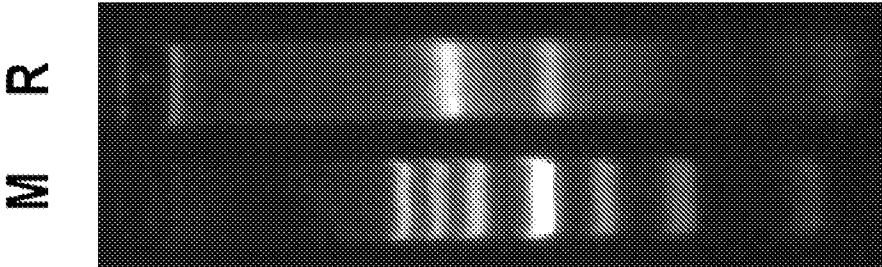


FIG. 5A

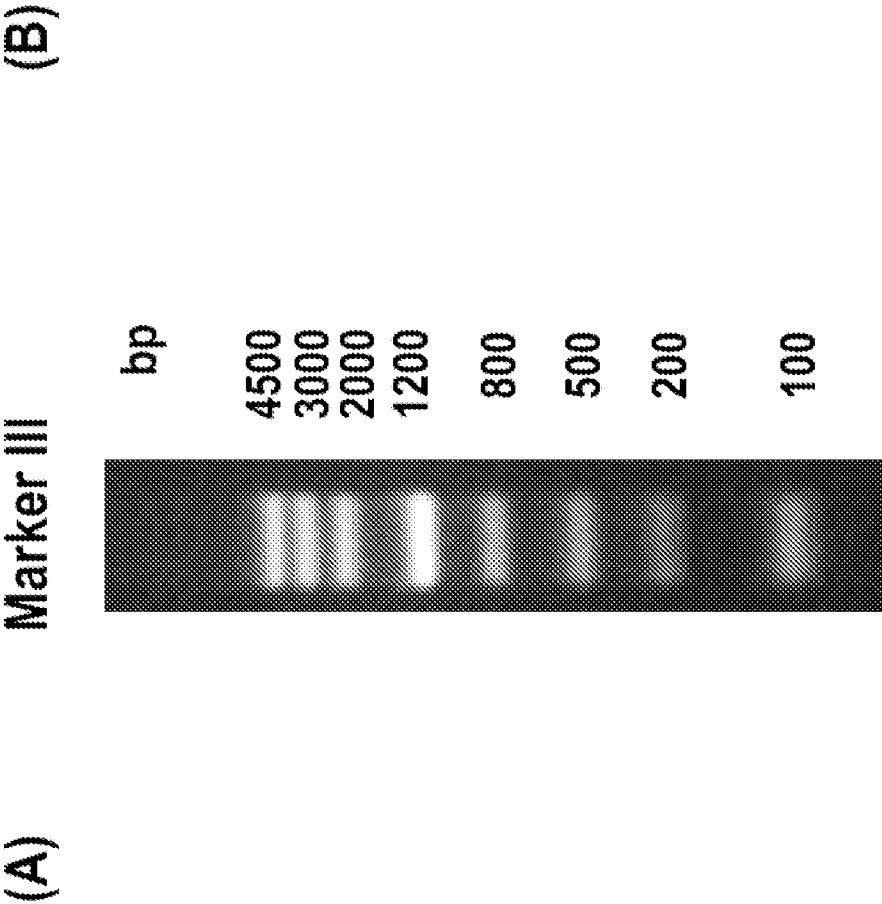
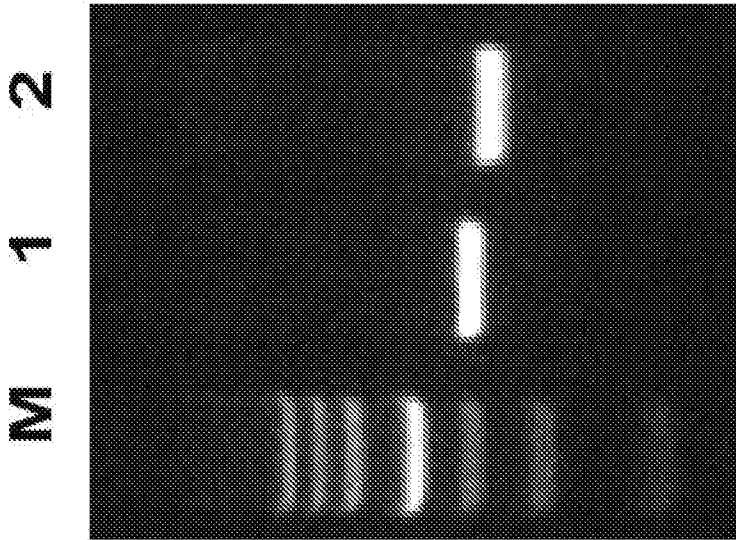


FIG. 6



68.80.2 hybridoma sequences:

FIG. 7A

Heavy chain: DNA sequence (408 bp) **SEQ ID NO: 1**

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

CDR1 - SEQ ID NO: 3

CDR2 - SEQ ID NO: 5

CDR3 - SEQ ID NO: 7

ATGGCTGTCCTGGCGCTACTCCTCTGCGCTGGTGACTTCCCAAGCTGTATCCTTTCCCAG
GTGCAGCTGAAGGAGTCAGGACCTGGCCTGGTGGGCCCTCAGAGCCTGTCCATCA
CATGCACTGTCTCTGGGTCTCATTAACCACTATGGGTAGACTGGGTTCGCCAGTCT
CCAGGAAAGGCTCTGGAGTGGCTGGGAATAATAIGGGTGTGGAAATCACAAATTAT
AATTCAGCTCTCAAAATCCAGACTGAGCATCAGCAAGGACAACTCCAAGAGCCAAAGTTT
CTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCAATGTACTACTGTGCCACATAIG
ATTACGACGGGGGTTTGCTTACTGGGGCCAAAGGACTCTGTCACTGTC
TCTGCA

CDR1 - SEQ ID NO: 4

CDR2 - SEQ ID NO: 6

CDR3 - SEQ ID NO: 8

Heavy chain: Amino acids sequence (136 AA) **SEQ ID NO: 2**

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

MAVLALLCLVTFPSCILSQVOLKESGPGGLVAPSQLSITCTVSGFSLTSYGVDWVRQSPGKG
LEWLGIHWGVGITNYSALKSRLSISKDNSKQVFLKMNSLQIDDTAMYYCATYDYDGGFA
YWGQGLVTVSA

FIG. 7B

CDR1 - SEQ ID NO: 11
 CDR2 - SEQ ID NO: 13
 CDR3 - SEQ ID NO: 15

SEQ ID NO: 9

Light chain: DNA sequence (390 bp)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

ATGGATTTTCGTGTCAGATTTTCAGCTTCTTGCTAATCAGTGCCTCAGTTGCAATGTC
 AGAGGAGAAATGTGCTCACCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAAA
 AGGTCACCATGACCTGCAGGGCCAGTTCAAGTGTAAAGTTCCAGTTACTTGCAGTGGTAC
 CAGCAGAAATCAGGTGCCTCCCCAACTCTGGATTATAGCACATCCAACTTGGCTTC
 TGGAGTCCCTGCTTCAGTGGCAGTGGGTCTGGGACCCTTACTCTCACAATCA
 GCAGTGTGGAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTACAGTGGTTACCC
 ACTCACGTTCGGCTCGGGACAAAGTTGGAAATAAAA

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CDR1 - SEQ ID NO: 12
 CDR2 - SEQ ID NO: 14
 CDR3 - SEQ ID NO: 16

SEQ ID NO: 10

Light chain: Amino acids sequence (130 AA)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

MDFLVQIFSLISAVMSRGENVLTQSPAIMSASPGEKVTMTCRASSVSSSYLQWYQQ
 KSGASPKLWIYSTSNLASGVPARFSGSGTSYSLTISSVEAEDAATYCCQQYSGVPLTFGSG
 TKLEIK

FIG. 8A

68.117.4 hybridoma sequences:

CDR1 - SEQ ID NO: 19
 CDR2 - SEQ ID NO: 21
 CDR3 - SEQ ID NO: 23

SEQ ID NO: 17

Heavy chain: DNA sequence (426 bp)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

ATGGACTTCAGGCTCAGCTTACTTATTTTGTCTTATTTTAAAGGTGTCAGTGTGAG
 GTGAAGCTGGTGAGTCTGGGGAGGCTTAGTGACGCTGGAGGTCCTGAACCTC
 TCCTGTGCAGCCTCTGGATTACATTTCAGTGACTACGGAAATGGCGTGGTTCGACAGGC
 TCCAAGGAAGGCGCTGAGTGGTAGCATTTATTAGTTATTGACATATAGTATCTACT
 ATGCAGACACTGTGACGGGCCGATTACCAATCTCTAGAGAAAATGCCAAGAACACCCCT
 GTTCCTAGAAAATGAGCAGTCTGAGGTCTGAGGACACGGCCATATATTACTGTGCAAGA
 CAGGGGGCTATGATGGTTACCCCTTACTGTGTTCTGATCTGCTGGGCACAGGGACCA
 CGGTCACCGTCTCCTCA

CDR1 - SEQ ID NO: 20
 CDR2 - SEQ ID NO: 22
 CDR3 - SEQ ID NO: 24

SEQ ID NO: 18

Heavy chain: Amino acids sequence (142 AA)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

MDFRLLIFVLKGVQCEVKLVESGGGLVQPGGSLKLSCAASGFTFSDYGMWVRQAPR
 KGPEWVAFISYLTYSIYADTVTGRFTISRENAKNTLFLEMSSLRSEDTAIYCARQGGYDGY
 PYWYFDVWGTGTTVTVSS

FIG. 8B

CDR1 - SEQ ID NO: 27
 CDR2 - SEQ ID NO: 29
 CDR3 - SEQ ID NO: 31

SEQ ID NO: 25

Light chain: DNA sequence (384 bp)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

ATGGATTTTCAAGTGCAGATTTTCAGCTTCCTGCTAATGAGTGCCTCAGTCATAATGTC
 AGGGGACAAATTGTTCTCACCAGTCTCCAGCACTCATGTCTGCCATTTCCAGGGGAGAA
 GGTCAACCATGACCTGCGAGTGCCAGCTCAAGTGAAGTTACAATGCACCTGGTACCAGCAG
 AAGCCAAGATCCTCCCCCAAACCCCTGGATTATCTCACATCCAAACCTGGCTTCTGGAGTC
 CCTGTTGCTTCAGTGGCAGTGGGCTCTGGACCTCTTACTCTCACAAATCAGCAGCATG
 GAGGCTGAAGATGCTGCCACTTATTACTGCCAGCAGTGAGTAGTTACCCACCCACGTT
 CGGTGCTGGGACCAAGCTGGAGCTGAAA

CDR1 - SEQ ID NO: 28
 CDR2 - SEQ ID NO: 30
 CDR3 - SEQ ID NO: 32

SEQ ID NO: 26

Light chain: Amino acids sequence (128 AA)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

MDFQVQIFSFLLMSASVIMSRGQIVLTQSPALMSAFPGEKVTMTCSASSSVSYMHWYQQ
 KPRSSPKPWYILTSNLASGVPVRFSGSGSWTSYSLTISSMEAEAAATYCCQQWSSYPPTFGA
 GTKLELK

FIG. 9A

68.104.1.31 hybridoma sequences:

CDR1 - SEQ ID NO: 35
 CDR2 - SEQ ID NO: 37
 CDR3 - SEQ ID NO: 39

SEQ ID NO: 33

Heavy chain: DNA sequence (411 bp)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

ATGGAATGGAGCAGAGTCITTAICTTTCTCCTATCAGTAACTGCAGGTGTTCACTCCCA
 GGTCAGCTGCAGCAGTCTGGAGCTGGTAGGCTGGGACTTCAGTGAAGGT
 GTCCTGCAAGGCTTCTGGATACCCCTTCACTAGTTACTTGATAGACTGGGTAAGCAGA
 GGCTGGACAGGGCCTTGAGTGGATTGGAGTGATTATCCTGGAA GTGGTACTAA
 GTACAAATGAGAA GTTCCAGGGCAAGGCAACACTGACTGCAGACAAATCCTCCAGCACT
 GCCTATATGCAGCTCAGCAGCCTGACATCTGAGGACTCTGCGGTCTATTCTGTGCAAG
 GGGGGCTGGGACGGGAAGTTTACTTACTGGGGCCAAGGGACTCTGTCAC TGTCTCT
 GCA

SEQ ID NO: 34 **CDR1 - SEQ ID NO: 36**
SEQ ID NO: 35 **CDR2 - SEQ ID NO: 38**
SEQ ID NO: 36 **CDR3 - SEQ ID NO: 40**

Heavy chain: Amino acids sequence (137 AA)

Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

MEWSRVFIFLLSVTAGVHSQVQLQQSGAELVRPGTSVKVSCKASGYPTSYLIDWVKQRP
 GQGLEWIGVINPGSGGTYNEKFQGKATLTADKSSSTAYMQLSSLTSEDSAVYFCARGGW
 DGKFTYWGGQGLVTVSA

FIG. 9B

	SEQ ID NO: 41		SEQ ID NO: 42	SEQ ID NO: 43
Light chain: DNA sequence (381 bp)				CDR1 - SEQ ID NO: 43
Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4				CDR2 - SEQ ID NO: 45
				CDR3 - SEQ ID NO: 47
	ATGAGGGCTCTGCACAGATTTTGGCTTCTTGTTGCTCTTGTTCCAGGTACCAGATGT			
	GACATCCAGATGACCCAGTCTCCATCCTCCTTATCTGCGCTCTCTGGGAGAAAGAGTCAG			
	TCACACTTGTCGGGCAAGTCAGGACATTGGTAGTAGCTTAAACTGGCTTCAGCAGGAA			
	CCAGATGGAACTATTAAACGCCCTGATCTACGCCACATCCAGTTAGATTCTGGTGTC			
	CAAAAGGTTCAGTGGCAGTAGGTCGGGTGAGATTATCTCTCACCATCAGCAGCCTTG			
	AGTCTGAAGATTTGTAGACTATTACTGTCTACAATAIGCTAGTTCTCCTTACACGTTCC			
	GAGGGGGACCAAGCTGGAAATAAAA			
		CDR1 - SEQ ID NO: 44		
Light chain: Amino acids sequence (127 AA)			CDR2 - SEQ ID NO: 46	
Leader sequence-FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4			CDR3 - SEQ ID NO: 48	
	MRAPAQIFGFLLLFPGTRCDIQMTQSPSSLSASLGERVSLTCRASQDIGSSLNWLQQEPDG			
	TIKRLIYATSSLD SGVPKRFGSRSGDYSLTISSEDFVDYCLQYASSPYTFGGGTKLEIK			

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2014/050684

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P37/00
ADD.

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

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 practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2010/106542 A2 (YISSUM RES DEV CO [IL]; HADASIT MED RES SERVICE [IL]; UNIV BEN GURION) 23 September 2010 (2010-09-23)	1-17
Y	page 1, line 4 - line 7 page 6, line 23 - page 7, line 4 page 7, line 19 - page 9, line 8 page 9, line 17 - line 24 page 11, lines 2-4, 8-10 page 16, line 1 - line 7 page 24, line 11 - line 15 page 27, line 1 - page 28, line 1 claims 1,7,27-29,45; example 5 ----- -/-	18,19



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 application or patent 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

29 October 2014

Date of mailing of the international search report

06/11/2014

Name and mailing address of the ISA/

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Bayer, Annette

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2014/050684

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2012/175613 A1 (INNATE PHARMA [FR]; INST NAT SANTE RECH MED [FR]; NARNI-MANCINELLI EMI) 27 December 2012 (2012-12-27)	18,19
A	page 1, line 4 - line 9 page 3, line 6 - line 14 page 3, line 29 - page 4, line 2 page 5, line 25 - page 8, line 3 page 15, lines 1-12,26 - page 16, line 17; figures 12,15-17 page 21, line 32 - page 31, line 17 claims 1-42; examples 1-3 -----	1-17
A	WARREN H S ET AL: "A novel binding assay to assess specificity of monoclonal antibodies", JOURNAL OF IMMUNOLOGICAL METHODS, ELSEVIER SCIENCE PUBLISHERS B.V.,AMSTERDAM, NL, vol. 305, no. 1, 20 October 2005 (2005-10-20), pages 33-38, XP027659277, ISSN: 0022-1759 [retrieved on 2005-10-20] page 34, left-hand column, paragraph 3 page 37, left-hand column, paragraph 1 - right-hand column, paragraph 1; table 1 -----	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL2014/050684

Box No. 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.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
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:
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 No. 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:

see additional sheet

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 fees, this Authority did not invite payment of additional fees.
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 and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-19

An isolated antibody directed to the extracellular domain of NKp46 and related subject-matter.

1.1. claims: 1-17(partially)

An isolated antibody comprising an antigen recognition region which comprises six complementary region (CDR) amino acid sequences as set forth in SEQ ID NOs: 4, 6, 8, 12, 14 and 16 and related subject-matter.

1.2. claims: 1-17(partially)

An isolated antibody comprising an antigen recognition region which comprises six complementary region (CDR) amino acid sequences as set forth in SEQ ID NOs: 20, 22, 24, 28, 30 and 32 and related subject-matter.

1.3. claims: 1-17(partially)

An isolated antibody comprising an antigen recognition region which comprises six complementary region (CDR) amino acid sequences as set forth in SEQ ID NOs: 36, 38, 40, 44, 46 and 48 and related subject-matter.

1.4. claims: 18, 19

A method of producing an antibody specific for the extracellular region of NKp46 as recited in present claim 18 and related subject-matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IL2014/050684

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2010106542 A2	23-09-2010	DK 2408468 T3	21-07-2014
		EP 2408468 A2	25-01-2012
		ES 2486673 T3	19-08-2014
		US 2012076753 A1	29-03-2012
		US 2013280209 A1	24-10-2013
		WO 2010106542 A2	23-09-2010

WO 2012175613 A1	27-12-2012	EP 2723362 A1	30-04-2014
		US 2014234342 A1	21-08-2014
		WO 2012175613 A1	27-12-2012
