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(54) Title: TREATMENT OF IL-25 MEDIATED DISEASES WITH TLR ANTAGONISTS

(57) Abstract: The invention provides methods and compositions for use in treating or preventing an IL-25 mediated disease such as asthma. The methods and compositions involve using TLR1 or TLR2 antagonists, such as antibody molecules which bind TLR1 or TLR2, or soluble TLR1 or TLR2 receptors. The invention also provides methods of screening for antagonists of TLR1 or TLR2.



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TREATMENT OF IL-25 MEDIATED DISEASES WITH TLR ANTAGONISTSField of the Invention

The present invention relates to a method of treatment of IL-25-mediated disease, particularly asthma, which comprises administration of a toll-like receptor (TLR)1 or TLR2 antagonist.

Background to the Invention

Dysregulated type-2 responses in the skin, respiratory and gastrointestinal systems are linked to allergic disorders<sup>5</sup>, but the aetiology of these responses remains poorly understood. They include the induction of immunoglobulin IgE, eosinophilia, expansion of T helper 2 cells, goblet cell hyperplasia, mucus secretion into the airways and airways hyperreactivity<sup>6</sup>. In contrast to well-characterised innate signals that initiate type-1 immunity<sup>7,8</sup> the innate factors that initiate allergic responses have remained largely elusive.

The identification and characterisation of pattern-recognition receptors, including TLRs, c-type lectins, RIG-I-like receptors, and NOD-like receptors and their ability to activate cytokines such as IL-12 allows for a relatively straightforward explanation for the onset of type-1 immunity. By contrast, although many of the downstream type-2 effector cytokines have been defined and characterised (Fallon, P.G., et al., *Immunity* 17:7-17. (2002), and Coffman, R.L., and T. von der Weid. *J Exp Med* 185:373-375. (1997)), the critical pathways involved in the induction of type-2-induced allergic airways disease are not yet fully understood and represent a significant gap in our knowledge of type-2 immunity.

IL-25 (IL-17E) is a member of the recently described family of IL-17 cytokines. However, its functional role is divergent from other family members and it is associated with type-2 immunity<sup>9, 10</sup>. The acute induction of IL-25 has been reported to play important roles in helminth infection<sup>11 12</sup> and airway disease<sup>4</sup>. A number of cellular sources of IL-25 have been identified including macrophages<sup>13</sup>, mast cells<sup>14</sup>, and epithelial cells<sup>1</sup>. The present inventors, and others, have shown that IL-25 functions upstream of the cardinal type-2 cytokines IL-4, IL-5 and IL-13, initiating their expression by a number of cell lineages including an innate non-B, non-T, cKit+ cell type<sup>11</sup> and Th2 cells<sup>3</sup>.

Asthma is a common chronic inflammatory disorder of the airways. The

number of sufferers has increased dramatically over recent decades and the World Health Organisation estimates that in the region of 300 million people worldwide suffer from asthma. Allergic asthma is characterised by uncontrollable airways hyperresponsiveness (AHR) induced by a variety of provocative stimuli and is associated with type-2 inflammatory infiltrates into the lungs.

A critical factor in the development of allergic asthma is the genesis of an inappropriate type-2 immune response dominated by secretion of interleukin (IL)-4, IL-5 and IL-13, following exposure to normally innocuous stimuli. IL-25 has also been shown to play a role in the initiation and regulation of type-2 immune responses, preceding IL-4, 5, 13 induction, in asthma-like allergic lung inflammation<sup>1-4</sup>. However, despite the increasing prevalence of asthma and allergic disorders in the developing world the innate molecular pathways by which allergic asthma is initiated remain unclear.

#### Disclosure of the Invention

Using IL-25 expression to screen for activators of the innate pulmonary type-2 response, the present inventors have identified that the toll-like receptors TLR1 and TLR2 play a critical role in stimulating IL-25 expression and in the onset of natural allergen-evoked type-2 immunity.

IL-25 secretion from primary airway epithelial cells (AEC) stimulated by an asthma-inducing allergen, Ragweed pollen (RWP), is described herein and is shown to be inhibited using soluble TLR1 or TLR2 *in vitro*. Furthermore, a neutralising anti-TLR2 antibody is shown to reduce IL-25 secretion from AEC following stimulation with RWP.

MyD88 is an adaptor protein that is essential for signalling by all TLRs, except TLR3<sup>18</sup>. Allergen-stimulated-IL-25 secretion is shown herein to be defective in AEC from *myd88*<sup>-/-</sup> and *tlr2*<sup>-/-</sup> mice. Using a TLR2 Fc/fusion protein it is shown herein that the RWP allergen binds TLR2.

Using a mouse model of pulmonary allergic inflammation described herein, allergen-induced airways eosinophilia and pulmonary expression of IL-13 is shown *in vivo* to be MyD88- and TLR2-dependent.

Thus, TLR1 and TLR2 may be useful therapeutic targets for treatment or prevention of an IL-25 mediated disease, such as asthma and other

allergic disorders.

Accordingly, a first aspect of the invention provides antagonists of TLR1 and TLR2 for use in a method of treating or preventing an IL-25 mediated disease.

Preferably, antagonists described herein disrupt or block signalling from TLR1 or TLR2. More preferably, antagonists described herein reduce or inhibit at least one of IL-25 production; IL-25-mediated AHR; IL-13 production; IL-25-mediated IL-5 production; IL-25-mediated IL-8 production; and airways eosinophilia. TLR1/2 signalling may be induced by a TLR ligand such as a natural allergen, for example, RWP.

An antagonist may bind an extracellular domain of TLR1 or an extracellular domain of TLR2. For example, an antagonist may bind a polypeptide comprising amino acids 25-580 of human TLR1 substantially as set out in SEQ ID NO:1, or a polypeptide comprising amino acids 19-587 of human TLR2 substantially as set out in SEQ ID NO:2.

In some embodiments, an antagonist may compete with TLR ligand binding to TLR1 and/or TLR2. For example, an antagonist may compete with a natural allergen or a synthetic TLR ligand such as Pam3CSK4 and/or may bind a ligand binding domain of TLR1 or TLR2.

In some embodiments, an antagonist may disrupt TLR1-TLR2 heterodimerisation, for example by binding to TLR1 or TLR2 and blocking TLR1 interaction with TLR2.

In other embodiments, an antagonist may inhibit or reduce expression of TLR1 or TLR2. For example, an antagonist may inhibit or reduce at least one of transcription of a TLR1- or TLR2-encoding gene; translation of TLR1- or TLR2-encoding mRNA; or secretion of TLR1 or TLR2.

An antagonist may comprise an antibody molecule which binds TLR1 or TLR2. In some embodiments, an antibody molecule may comprise an antibody fragment, such as a Fab, F(ab')<sub>2</sub>, or scFv antibody fragment. In other embodiments, an antibody molecule may comprise a whole antibody.

In some embodiments, an antibody molecule may comprise a human framework region.

In preferred embodiments, an antibody molecule for use according to

the invention binds TLR1 or TLR2 with high affinity and/or specificity.

In other embodiments, an antagonist may comprise an isolated soluble extracellular domain of TLR1 or TLR2. For example, an antagonist may comprise a polypeptide comprising amino acids 25-580 of human TLR1 substantially as set out in SEQ ID NO:1, or a polypeptide comprising amino acids 19-587 of human TLR2 substantially as set out in SEQ ID NO:2.

Antagonists for use as described herein may comprise an Fc fusion protein. For example, an antagonist may comprise a soluble TLR1/Fc fusion or a soluble TLR2/Fc fusion.

The invention further provides a method of treating or preventing IL-25 mediated disease, comprising administering to a subject in need of treatment an effective amount of an antagonist of TLR1 or TLR2.

An antagonist as described herein may be in the form of a pharmaceutical composition, additionally comprising a pharmaceutically acceptable excipient.

In some embodiments, a method of treating or preventing IL-25 mediated disease may comprise administering to a subject in need of treatment an effective amount of an antagonist or a pharmaceutical composition as described herein.

The invention further provides the use of an antagonist as described herein, for example in the form of a pharmaceutical composition, in the manufacture of a medicament for treatment of an IL-25 mediated disease.

In preferred embodiments of the invention, the IL-25 mediated disease is asthma.

Another aspect of the invention provides a method of screening for an antagonist of TLR1 or TLR2 comprising determining the IL-25 expression level in a cell in the presence of a test compound, wherein a decrease in the amount of IL-25 expression in the presence relative to the absence of test compound is indicative that the compound is an antagonist of TLR1 or TLR2.

Such a method may further comprise determining binding of the test compound to TLR1 or TLR2.

These and further aspects of the invention are described in further detail below and with reference to the accompanying examples.

#### Description of the Figures

Figure 1 shows induction of IL-25 in primary epithelial cells. (a) IL-25 production from AEC stimulated with increasing doses of RWP. (b) IL-25 production from AEC stimulated with RWP (1 µg/ml) and the indicated TLR ligands, TLR1/2 - Pam3CSK4 (250 ng/ml), TLR4 - LPS (500 ng/ml), TLR2 - HKLM (Heat killed *Listeria monocytogenes*) (10<sup>8</sup> cells/ml), TLR2/6 - FSL-1 (500 ng/ml), and TLR7 - ssRNA40 (5 µg/ml). Results shown are representative of three independent experiments and data are mean and s.e.m of triplicate cultures. (c) AEC express cell surface TLR1 and TLR2. (d) TLR2/Fc fusion protein binds plate-bound RWP. Data shown are mean and S.D.

Figure 2 shows epithelial cells lacking TLR2 and MyD88 fail to produce IL-25 upon ligation with RWP. (a) IL-25 production from stimulated *tlr2*<sup>-/-</sup> or wildtype AEC. (b) IL-25 production from stimulated *myd88*<sup>-/-</sup> AEC. (c) IL-25 production from AEC incubated with signalling inhibitors and stimulated with RWP. Results shown are representative of two independent experiments and data are mean and s.e.m of triplicate cultures. (d) IL-25 production by AEC following RWP stimulation in the presence of TLR2/Fc, TLR6/Fc or rmTLR1 proteins. (e) IL-25 production by AEC incubated with anti-TLR2 neutralising antibody, or control IgG1, in the presence of RWP. \$ indicates P < 0.05 comparing two differing doses of anti-TLR2. ND, not detected.

Figure 3 shows *in vivo* expression of TLR2/MyD88 is essential to IL-25 production and pulmonary inflammation. C57Bl/6, *tlr2*<sup>-/-</sup> and *myd88*<sup>-/-</sup> mice were subject to RWP treatment 24 hrs after the last treatment. (a) Total BAL cell number. (b) Percentage cell counts. (c) Lung eosinophil numbers. (d) IL-25 levels in BAL fluid. (e) IL-13 levels in BAL fluid. (f) IL-5 mRNA levels in lung tissue. (g) IL-4 mRNA levels in lung tissue. Data represent mean and s.e.m., *tlr2*<sup>-/-</sup> experiments were repeated twice with n = 4-5/group, *myd88*<sup>-/-</sup> challenge was conducted with n = 6/group.

Figure 4 shows allergen induced IL-13 and eosinophilia is IL-25 dependent. Wildtype Balb/c or *il25*<sup>-/-</sup> or *il17br*<sup>-/-</sup> mice were challenged with RWP. (a) BAL cell counts (b) BAL cell percentages. (c) BAL eosinophil numbers. (d) IL-13 levels in BAL. (e) IL-25 levels

in BAL. Results shown are representative of two independent experiments, n = 4/group. Data represent mean and s.e.m.

Figure 5 shows that Type-2 inflammation occurs in the absence of TLR4. Wildtype or TLR4 deficient mice were challenged with RWP. (a) BAL cell counts. (b) IL-13 levels in BAL fluid.

#### Detailed Description of the Invention

The data described herein define for the first time a molecular pathway from a natural allergen to the development of allergic airway inflammation via toll-like receptor signalling.

Toll-like receptors, or TLRs, are a family of at least ten highly conserved mammalian pattern recognition receptor proteins (TLR1 - TLR10) which recognise pathogen-associated molecular patterns (PAMPs) and act as key signalling elements in innate immunity. TLR polypeptides have a characteristic structure that includes an extracellular (or extracytoplasmic) domain having leucine-rich repeats, a transmembrane domain, and an intracellular (or cytoplasmic) domain that is involved in TLR signalling.

TLRs include but are not limited to human TLRs.

Human TLR-1 (GeneID: 7096; Nucleic acid: NM\_003263.3; GI:41350336; Protein: NP\_003254.2; GI:41350337, SwissProt: Q15399), also referred to in the art as CD281, may be cloned or synthesised by reference to the sequences of TLR-1 available in the art.

Human TLR-2 (GeneID: 7097; Nucleic acid: NM\_003264.3; GI:68160956; Protein: NP\_003255.2; GI:19718734; SwissProt: O60603), also referred to in the art as CD282, may be cloned or synthesised by reference to the sequences of TLR-2 available in the art. The extracellular domain of human TLR2 is available from commercial sources (R&D Systems, Cat No. 2616-TR-050) as a histidine-tagged fusion protein.

The invention provides antagonists of TLR1 and TLR2 for use in a method of treating or preventing an IL-25 mediated disease.

An "antagonist" includes any agent that partially or fully blocks, prevents, inhibits, or neutralizes a biological activity of a target molecule, such as TLR1 or TLR2, for example, by reducing, interfering with, blocking, down-regulating, or otherwise preventing; i) the interaction or binding of the target molecule with ligand; ii) the

interaction or binding of the target molecule with a binding partner or downstream effector (e.g dimerisation); iii) the expression or secretion of the target molecule or; iv) the activation and/or modification of the target protein.

An "antagonist of TLR1 or TLR2" may also be referred to as a "TLR1 or TLR2 antagonist" and includes an antagonist of TLR1 or an antagonist of TLR2. Also included are antagonists of both TLR1 and TLR2. Such antagonists may be referred to as "TLR1/TLR2 antagonists" or "TLR1/2 antagonists".

Antagonists for use according to the invention include natural or synthetic peptides and polypeptides, antibody molecules, nucleic acids, ligands, blockers, inhibitors, and modulators, as well as pharmaceutically acceptable salts and solvates of these agents. The term "antagonist polypeptide" may be used to refer to antagonist antibody molecules and other antagonist polypeptides such as soluble TLR1 or TLR2 receptors.

The term "polypeptide" is used herein as a generic term to refer to native protein, fragments, homologs or analogs of a relevant polypeptide sequence.

Biological activity of TLR1 or TLR2 includes stimulating at least one of IL-25 production; IL-25-mediated AHR; IL-13 production; IL-25-mediated IL-5 production; IL-25-mediated IL-8 production; and airways eosinophilia.

An antagonist may bind a mammalian TLR1 or TLR2, such as human, mouse, rat, sheep, cow, or dog TLR1 or TLR2. Preferred antagonists bind human TLR1 or human TLR2.

In some embodiments, an antagonist binds an extracellular domain of TLR1 or TLR2.

An extracellular domain of TLR1 may be a polypeptide comprising amino acids 25-580 of human TLR1 substantially as set out in SEQ ID NO:1. In other embodiments, an extracellular domain of TLR1 may be a polypeptide comprising amino acids 23-581, amino acids 24-581, amino acids 25-581, amino acids 23-580, or amino acids 24-580 of human TLR1 substantially as set out in SEQ ID NO:1.

An extracellular domain of TLR2 may be a polypeptide comprising amino acids 19-587 of human TLR2 substantially as set out in SEQ ID NO:2.

By "substantially as set out" it is meant that the relevant amino acid sequence will be either identical or highly similar to the specified regions of which the sequence as set out herein.

Furthermore, an extracellular domain of TLR1 or TLR2 may be a variant, homolog or analog of a human TLR1 or TLR2 extracellular domain. Variant polypeptides are discussed in more detail below.

TLR1 and TLR2 sequences from several mammals as well as humans, and methods for identifying, sequencing and cloning TLR1 and TLR2 homologues are readily available to the skilled person.

An antagonist may be any molecule which competes with a TLR ligand for binding to TLR1 and/or TLR2. A "TLR ligand", which may also be referred to as a "ligand for TLR" or a "TLR agonist", refers to a molecule that binds a TLR extracellular domain and induces TLR-mediated signalling. In one embodiment, a TLR ligand may be a natural ligand, i.e. a TLR ligand that is found in nature. Examples of natural TLR ligands include allergens such as RWP. In another embodiment, a TLR ligand may be a non-natural or synthetic ligand. Examples of synthetic TLR ligands include Pam3CSK4.

TLR2 is known to form a heterodimer with either TLR1<sup>19</sup> or TLR6<sup>20</sup>. Accordingly, an antagonist of TLR1 or TLR2 may block, prevent or disrupt TLR1-TLR2 heterodimerisation, for example, by binding TLR1 or by binding TLR2 and blocking or disrupting interaction of the two receptors. In some embodiments, an antagonist may bind TLR1 or TLR2 and sterically alter a heterodimerisation surface of the bound TLR, such that the TLR1-TLR2 interaction is disrupted or blocked.

The binding of antagonists to TLR1 or TLR2 may be determined by any convenient technique, such as surface plasmon resonance and isothermal calorimetry. The activity of TLR1 or TLR2 in the presence of a putative ligand or antagonist may be determined by any conventional technique. For example, TLR1 or TLR2 activity may be determined using assays for IL-25 production.

An antagonist for use according to the invention may be an antibody molecule which binds TLR1 or TLR2. In preferred embodiments, the antibody molecule binds TLR1 or TLR2 with high affinity and/or specificity.

An antagonist of TLR1, such as an antibody molecule, may bind TLR1

and show no binding or substantially no binding to other members of the TLR family, such as TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9 and/or TLR10. An antagonist of TLR2, such as an antibody molecule, may bind TLR2 and show no binding or substantially no binding to other members of the TLR family, such as TLR1, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9 and/or TLR10. Furthermore, an antagonist, such as an antibody molecule, may bind both TLR1 and TLR2 and show no binding or substantially no binding to other members of the TLR family, such as TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9 and/or TLR10.

Antibody molecules for use according to the invention may be cross-reactive for TLR1 or TLR2 from different species. For example, an antibody molecule may bind both human TLR1 and mouse TLR1, or may bind both human TLR2 and mouse TLR2.

Typically, specificity may be determined by means of a binding assay such as ELISA employing a panel of antigens.

Binding of an antibody molecule described herein with TLR1 or TLR2 may be abolished by competition with recombinant TLR1 or TLR2.

Binding affinity and neutralisation potency of different antibody molecules described herein can be compared under appropriate conditions using routine techniques.

The term "antibody molecule" describes an immunoglobulin whether natural or partly or wholly synthetically produced. It has been shown that fragments of a whole antibody can perform the function of binding antigens. Thus reference to an antibody also covers any polypeptide or protein comprising an antibody binding fragment.

Generally, an antibody molecule comprises a heavy chain variable region (VH domain) which is paired with a light chain variable region (VL domain) to provide an antibody antigen binding domain, although in some embodiments, a VH domain alone may be used to bind antigen.

Antibody molecules include any binding member or substance having an antibody antigen-binding site with the required specificity and/or binding to TLR1 and/or TLR2. Examples of antibody molecules include immunoglobulin isotypes and their isotypic subclasses; antibody fragments; engineered antibody molecules; and any other polypeptide comprising an antibody antigen-binding site, whether natural or wholly or partially synthetic. Chimeric molecules comprising an

antigen binding domain, or equivalent, fused to another polypeptide are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023.

Examples of antibody molecules include (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. *et al.*, *Nature* 341, 544-546 (1989)) which consists of a VH domain; (v) isolated CDR regions; (vi) F(ab')<sub>2</sub> fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird *et al.*, *Science*, 242, 423-426, 1988; Huston *et al.*, *PNAS USA*, 85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (PCT/US92/09965) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804; P. Holliger *et al.*, *Proc. Natl. Acad. Sci. USA* 90 6444-6448, 1993). Fv, scFv or diabody molecules may be stabilised by the incorporation of disulphide bridges linking the VH and VL domains (Y. Reiter *et al.*, *Nature Biotech*, 14, 1239-1245, 1996). Minibodies comprising a scFv joined to a CH3 domain may also be made (S. Hu *et al.*, *Cancer Res.*, 56, 3055-3061, 1996). Antibody molecules and methods for their construction and use are described in Holliger & Hudson, *Nature Biotechnology* 23(9):1126-1136 (2005).

Where bispecific antibody molecules are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. *Current Opinion Biotechnol.* 4, 446-449 (1993)), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in *E. coli*. Diabodies (and many other polypeptides such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against TLR1 or TLR2, then a library can be made where the other arm is varied and an antibody of appropriate specificity selected. Bispecific whole antibodies may be made by

knobs-into-holes engineering (J. B. B. Ridgeway *et al*, Protein Eng., 9, 616-621, 1996).

Antibody molecules may be polyclonal antibodies. Methods of preparing polyclonal antibodies are known to the skilled artisan. Polyclonal antibodies can be raised in a mammal, for example, by one or more injections of an immunizing agent and, if desired, an adjuvant. Typically, the immunizing agent and/or adjuvant will be injected in the mammal by multiple subcutaneous or intraperitoneal injections. The immunizing agent may include the PRO285 and PRO286 polypeptides or a fusion protein thereof. It may be useful to conjugate the immunizing agent to a protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

Alternatively, an antibody molecule may be a monoclonal antibody. A monoclonal antibody is an antibody obtained 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 may be prepared using hybridoma methods, such as those described by Kohler and Milstein, *Nature*, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized *in vitro*.

Monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567.

For production of antibodies or use in immunoassays, whole polypeptides or fragments of native, or recombinant TLR1 or TLR2 may be used, particularly fragments containing an extracellular domain, for example, an extracellular domain substantially as set out in SEQ ID NO:1 or SEQ ID NO:2. Furthermore, antibodies may be raised against extracellular domain fragments.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibody molecules or chimeric molecules which retain the specificity of the original antibody. Such techniques may involve introducing DNA encoding the immunoglobulin variable region, or the complementarity determining regions (CDRs), of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EPA- 184187, GB 2188638A or EP-A-239400.

CDR regions may be grafted into a human framework region. The human framework region may be selected by a number of methods, e.g. by comparing the mouse framework region or mouse V region sequences with known human framework or V region sequences and selecting a human framework region which has the highest, or one of the highest degrees of amino acid similarity or identity. Modifications to framework regions of native human sequences may be made in order to further optimize the resulting CDR-grafted antibodies.

Although antibody molecules comprising a pair of VH and VL domains are preferred, single binding domains based on either VH or VL domain sequences may also be used. It is known that single immunoglobulin domains, especially VH domains, are capable of binding target antigens in a specific manner.

In the case of either of the single chain binding domains, these domains may be used to screen for complementary domains capable of forming a two-domain antibody molecule able to bind TLR1 and/or TLR2, as discussed further herein below.

Antibody molecules may further comprise antibody constant regions or parts thereof. For example, a VL domain may be attached at its C-terminal end to antibody light chain constant domains including human C $\kappa$  or C $\lambda$  chains, preferably C $\lambda$  chains. Similarly, an antibody molecule based on a VH domain may be attached at its C-terminal end to all or part of an immunoglobulin heavy chain derived from any antibody isotype, e.g. IgG, IgA, IgE and IgM and any of the isotype subclasses, particularly IgG1 and IgG4. IgG4 is preferred. Fc regions such as  $\Delta$ nab and  $\Delta$ nac as disclosed in WO99/58572 may be employed.

Framework regions of antibody molecules may also include glycosylation sequences that include one or more glycosylation sites. Depending upon the host cell in which the antibody is expressed, the

pattern of glycosylation may vary. Thus nucleic acid constructs that encode glycosylation sites may be modified to remove the site or alternatively such sites may be introduced into the protein. For example, N-glycosylation sites in eukaryotic proteins are characterized by an amino acid triplet Asn-X-Y, wherein X is any amino acid except Pro and Y is Ser or Thr. Appropriate substitutions, additions or deletions to the nucleotide sequence encoding these triplets will result in prevention of attachment of carbohydrate residues at the Asn side chain. Alteration of a single nucleotide, chosen so that Asn is replaced by a different amino acid, for example, is sufficient to inactivate an N-glycosylation site. Known procedures for inactivating N-glycosylation sites in proteins include those described in U.S. Pat. No. 5,071,972 and EP 276,846.

The term "antigen-binding domain" describes the part of an antibody molecule which comprises the area which specifically binds to and is complementary to part or all of an antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen, which part is termed an epitope. An antigen binding domain may be provided by one or more antibody variable domains (e.g. a so-called Fd antibody fragment consisting of a VH domain). Preferably, an antigen binding domain comprises an antibody light chain variable region (VL) or at least a substantial portion thereof and an antibody heavy chain variable region (VH) or at least a substantial portion thereof.

A substantial portion of an immunoglobulin variable domain will comprise at least the three CDR regions, together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Additional residues at the N-terminal or C-terminal end of the substantial part of the variable domain may be those not normally associated with naturally occurring variable domain regions. For example, construction of antibody molecules made by recombinant DNA techniques may result in the introduction of N- or C-terminal residues encoded by linkers introduced to facilitate cloning or other manipulation steps. Other manipulation steps include the introduction of linkers to join variable domains of the invention to further protein sequences including immunoglobulin heavy chains, other variable domains (for example in the production of diabodies) or protein labels as discussed in more detail below.

In some embodiments, an antagonist may comprise a soluble extracellular domain of TLR1 or TLR2. TLR1 and TLR2 extracellular domains are described in more detail above and in the sequences set out herein. Preferably, a soluble extracellular domain of TLR1 or TLR2 binds a TLR ligand. A soluble extracellular domain may compete with native TLR1 and/or TLR2 for binding to a TLR ligand and may include a fragment of a TLR1 or TLR2 extracellular domain.

An extracellular domain of TLR1 or TLR2 may be a variant polypeptide. Variants may be obtained by means of methods of sequence alteration or mutation and screening. Furthermore, variants include natural allelic variants which are found in one or more individuals within a population and may differ from the reference TLR1 or TLR2 sequence by the addition, deletion, substitution and/or insertion of one or more amino acids, whilst retaining native activity. Particular variants may include one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue) of a reference TLR1 or TLR2 sequence. Variants may have less than about 20 alterations, less than about 15 alterations, less than about 10 alterations or less than about 5 alterations, 4, 3, 2 or 1.

A variant extracellular domain of TLR1 or TLR2 retains the ability of wild-type TLR1 or TLR2 extracellular domain to bind a TLR ligand and may comprise an amino acid sequence which shares greater than about 50% sequence identity with a TLR1 or TLR2 extracellular domain sequence as set out in SEQ ID NO:1 or SEQ ID NO:2, greater than about 60%, greater than about 70%, greater than about 80%, greater than about 90%, greater than about 95%, greater than about 98% or greater than about 99%.

Sequence identity is commonly defined with reference to the algorithm GAP (Genetics Computer Group, Madison, WI). GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) *PNAS USA* 85: 2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) *J. Mol Biol.* 147: 195-197), or the TBLASTN program, of Altschul *et al.* (1990) *supra*, generally employing default parameters. In particular, the psi-Blast algorithm (Nucl. Acids Res. (1997) 25 3389-3402) may be used. Sequence identity and similarity may also be determined using

Genomequest™ software (Gene-IT, Worcester MA USA). Sequence comparisons are preferably made over the full-length of the relevant sequence described herein.

Antagonist polypeptides and nucleic acid encoding antagonist polypeptides described herein will generally be isolated i.e. free or substantially free of material with which they are naturally associated such as other polypeptides or nucleic acids with which they are found in their natural environment, or the environment in which they are prepared (e.g. cell culture) when such preparation is by recombinant DNA technology practised *in vitro* or *in vivo*.

Antagonist polypeptides and nucleic acid encoding antagonist polypeptides may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the molecules will normally be mixed with gelatin or other carriers if used to coat microtitre plates for use in immunoassays, or will be mixed with pharmaceutically acceptable carriers or diluents when used in diagnosis or therapy. Antibody molecules may be glycosylated, either naturally or by systems of heterologous eukaryotic cells (e.g. CHO or NS0 (ECACC 85110503) cells), or they may be (for example if produced by expression in a prokaryotic cell) unglycosylated.

An antagonist polypeptide may also be in a substantially purified form, in which case it will generally comprise the antagonist polypeptide in a preparation in which more than 90%, e.g. 95%, 98% or 99% of the polypeptide in the preparation is an antagonist polypeptide.

In addition to antibody or TLR polypeptide sequences, an antagonist as described herein such as an antibody molecule or a soluble extracellular domain of TLR1 or TLR2 may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen or ligand.

Antagonists may comprise non-naturally occurring peptide and polypeptide fusions comprising antagonist polypeptides as described above wherein the polypeptide is fused to one or more sequences which are not naturally fused to the polypeptide. Sequences which are not naturally fused to the polypeptide may include synthetic amino acid sequences, sequences from a source other than TLR1 or TLR2 or an antibody molecule which binds TLR1 and/or TLR2, or additional copies of the polypeptide sequence itself. In some embodiments, one or more

heterologous amino acids may be joined or fused to the antagonist polypeptide.

By "heterologous" is meant not occurring in the antagonist polypeptide sequence, that is to say usually a chain of amino acids which is not found naturally joined to the antagonist polypeptide sequence.

In some embodiments, a soluble extracellular domain of TLR1 or TLR2 may comprise an Fc fusion protein - i.e. a soluble extracellular domain of TLR1 or TLR2 joined to an Fc region of an immunoglobulin, such as IgG.

In some embodiments, antagonist polypeptides may carry a detectable or functional label, or may be conjugated to a toxin or enzyme (e.g. via a peptidyl bond or linker).

A label can be any molecule that produces or can be induced to produce a signal, including but not limited to fluorescers, radiolabels, enzymes, chemiluminescers or photosensitizers. Thus, binding may be detected and/or measured by detecting fluorescence or luminescence, radioactivity, enzyme activity or light absorbance.

Suitable labels include radiolabels such as  $^{131}\text{I}$  or  $^{99}\text{Tc}$ , which may be attached to antibody molecules using conventional chemistry known in the art of antibody imaging. Labels also include enzyme labels such as horseradish peroxidase, alkaline phosphatase, glucose-6-phosphate dehydrogenase ("G6PDH"), alpha-D-galactosidase, glucose oxydase, glucose amylase, carbonic anhydrase and acetylcholinesterase. Labels include fluorescent labels or fluorescers, such as fluorescein and its derivatives, fluorochrome, rhodamine compounds and derivatives and GFP (GFP for "Green Fluorescent Protein"). Labels further include chemical moieties such as biotin which may be detected via binding to a specific cognate detectable moiety, e.g. labelled avidin.

Where the additional feature is a polypeptide domain or label, the antibody molecule may be produced by recombinant techniques, i.e. by the expression of nucleic acid encoding a fusion of the antibody molecule and the further domain.

All the above described techniques are known as such in the art and in themselves do not form part of the present invention. The skilled person will be able to use such techniques to provide antibody

molecules as described herein using routine methodology in the art.

Another aspect of the invention provides a nucleic acid encoding an antagonist for use as described herein, such as an antibody molecule, a soluble extracellular domain of TLR1 or TLR2, or a nucleic acid antagonist as described below.

Generally, nucleic acid is provided as an isolate, in isolated and/or purified form, except possibly one or more regulatory sequence(s) for expression. Nucleic acid in accordance with the present invention may be provided as part of a recombinant vector.

Nucleic acid sequences encoding a polypeptide or peptide as described herein can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see *Molecular Cloning: a Laboratory Manual*: 3rd edition, Sambrook and Russell, 2001, Cold Spring Harbor Laboratory Press).

In order to obtain expression of the nucleic acid sequences, the sequences can be incorporated in a vector having one or more control sequences operably linked to the nucleic acid to control its expression.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under condition compatible with the control sequences.

Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids, viral e.g. phage phagemid or baculoviral, cosmids, YACs, BACs, or PACs as appropriate. Vectors include gene therapy vectors, for example vectors based on adenovirus, adeno-associated virus, retrovirus (such as HIV or MLV) or alpha virus vectors.

The vectors may be provided with an origin of replication, optionally a promoter for the expression of the said polynucleotide and optionally a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example an ampicillin resistance

gene in the case of a bacterial plasmid or a neomycin resistance gene for a mammalian vector. Vectors may be used *in vitro*, for example for the production of RNA or used to transfect or transform a host cell. The vector may also be adapted to be used *in vivo*, for example in methods of gene therapy. Systems for cloning and expression of a polypeptide in a variety of different host cells are well known. Suitable host cells include bacteria, eukaryotic cells such as mammalian and yeast, and baculovirus systems. Mammalian cell lines available in the art for expression of a heterologous polypeptide include Chinese hamster ovary cells, HeLa cells, baby hamster kidney cells, COS cells and many others.

Promoters and other expression regulation signals may be selected to be compatible with the host cell for which the expression vector is designed. For example, yeast promoters include *S. cerevisiae* GAL4 and ADH promoters, *S. pombe* nmt1 and adh promoter. Mammalian promoters include the metallothionein promoter which is can be included in response to heavy metals such as cadmium. Viral promoters such as the SV40 large T antigen promoter or adenovirus promoters may also be used. All these promoters are readily available in the art.

The vectors may include sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that the polypeptide is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide produced in the host cell is secreted from the cell. Polypeptide can then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the polypeptide is produced and recovering the polypeptide from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of *E. coli*, yeast, and eukaryotic cells such as COS or CHO cells.

Thus, an antagonist polypeptide as described herein may be produced by expressing from nucleic acid encoding the polypeptide (generally nucleic acid according to the invention). This may conveniently be achieved by growing a host cell in culture, containing such a vector, under appropriate conditions which cause or allow expression of the polypeptide. Polypeptides may also be expressed in *in vitro* systems, such as reticulocyte lysate. As noted, methods of making peptides by chemical synthesis are also encompassed by the present invention.

In some embodiments, an antagonist may inhibit or reduce expression of TLR1 or TLR2. A TLR may be expressed by a cell either naturally

or artificially, for example in a recombinant cell. Generally, an expressed TLR is a functional TLR that is capable of inducing a signal in response to interaction with its ligand. A functional TLR may be a full-length TLR or a fragment thereof. An antagonist may inhibit or reduce expression of TLR1 or TLR2 by inhibiting or reducing at least one of transcription of a TLR1- or TLR2-encoding gene; translation of a TLR1- or TLR2-encoding mRNA; or secretion of TLR1 or TLR2 respectively.

In some preferred embodiments, an antagonist specifically inhibits or reduces expression of at least one of TLR1 and TLR2.

Accordingly, in some embodiments, an antagonist may comprise a nucleic acid such as an antisense sequence, DNA sequence, RNA sequence, or PNA sequence that controls a region of a polynucleotide encoding TLR1 or TLR2.

An antagonist may be an antisense compound, particularly an oligonucleotide, for use in modulating the function of nucleic acid molecules encoding TLR1 or TLR2, ultimately modulating the amount of the proteins produced or expressed. This is accomplished by providing oligonucleotides which specifically hybridize with nucleic acids, preferably mRNA, encoding TLR1 or TLR2.

An antisense oligonucleotide which targets a portion of the mRNA of TLR1 or TLR2 may be used. It may be necessary to determine a site or sites within the nucleic acid sequence for the antisense interaction to occur such that modulation of gene expression will result. This may be done by routine experimentation known to persons of skill in the art as such.

The mRNA sequence of TLR1 or TLR2 may be determined by reference to databases such as the Genbank database, the NIH genetic sequence database, an annotated collection of all publicly available DNA sequences. The human TLR1 and TLR2 gene and protein accession numbers are referenced above.

Antisense oligonucleotides may be formulated in accordance with this invention which are targeted wholly or in part to non-coding or coding parts of the mRNA. Thus for example the oligonucleotide may be specifically hybridizable with a transcription initiation site region, a translation initiation codon region, a 5' cap region, an intron/exon junction, coding sequences, a translation termination codon region or sequences in the 5'- or 3'-untranslated region. Once

the target site or sites have been identified, oligonucleotides are chosen which are sufficiently complementary to the target, i.e., hybridize sufficiently well such that stable and specific binding occurs between the DNA or RNA target and the oligonucleotide to give the desired modulation.

The antisense compounds in accordance with this invention preferably comprise from about 5 to about 50 bases in length, e.g. from about 8 to about 30 bases, such as from about 15 to about 30 bases.

Also suitable as an inhibitor is a double-stranded RNA (dsRNA) molecule which is capable of inducing RNA interference. Such molecules have been shown to induce potent and specific antisense-mediated reduction of the function of a gene or its associated gene products.

These double stranded RNA molecules target regions similar to those targeted by antisense oligonucleotides and have similar effects. These double stranded RNA molecules are generally 19-23, e.g. 19-21 base pairs in length, but may range between 8 and 50 bases. The production of siRNA molecules is known as such in the art and it will be appreciated that any desired siRNA targeted to TLR1 or TLR2 may be synthesized by conventional oligonucleotide synthesis techniques. Generally, the dsRNA may comprise two separate annealed strands, e.g. each of about 19-23, such as about 21, bases in length in which one or two of the terminal 3' nucleotides overhang, or to a single stem loop, often referred to as a short hairpin RNA (shRNA) in which the stem comprises about 19-23 (though this may be from 8-50 as above) base pairs.

Antisense and interfering RNAs may include one or more modified e.g. non-naturally occurring internucleoside linkages. Oligonucleotides having modified internucleoside linkages include internucleoside linkages that retain a phosphorus atom and internucleoside linkages that do not have a phosphorus atom. Examples of modified oligonucleotide backbones containing a phosphorus atom therein include, for example, phosphorothioates, chiral phosphorothioates, phosphoro-dithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates, 5'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, selenophosphates and borano-phosphates having normal 3'-5' linkages,

2'-5' linked analogs of these, and those having inverted polarity wherein one or more internucleotide linkages is a 3' to 3', 5' to 5' or 2' to 2' linkage.

Modified oligonucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; riboacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH<sub>2</sub> component parts.

Another aspect of the invention provides a method of treating or preventing an IL-25-mediated disease in a subject (e.g. a human) in need thereof which comprises administering to the subject an effective amount of an antagonist of TLR1 or TLR2 (which may be in the form of a pharmaceutical composition) as described herein. In some embodiments the IL-25 mediated disease may be asthma or an other IL-25 mediated disease or condition such as allergy and colitis, including ulcerative colitis, and Crohn's disease. Asthma includes allergic asthma. Allergic asthma may be induced by a variety of allergens such as pollen, for example RWP, food allergens, animal dander or animal allergens, for example cat Fel d 1, mold (including *Aspergillus*), dust, cockroach antigens and dust mite antigens.

Also provided is the use of an antagonist (including a composition thereof) described herein in the manufacture of a medicament for treatment of an IL-25 mediated disease. The medicament may be for administration to a human or animal subject.

Clinical indications in which a TLR1 or TLR2 antagonist may be used to provide therapeutic benefit include any condition in which TLR1 or TLR2 signalling has pathological consequences, for example conditions involving stimulation of IL-25 expression. Thus in general, an antagonist described herein may be used in the treatment of any IL-25 mediated condition or disease, for example associated with an unwanted Th2 response or type-2 responses. In some embodiments, the antagonist of the invention may be used for the treatment of allergy

and asthma, particularly asthma.

By "treating", it is meant both therapeutic treatment of ongoing disease intended to cure the disease, or to provide relief from the symptoms of the disease, as well as prophylactic treatment to prevent disease in a subject at risk of developing an IL-25 mediated disease.

By "administering" it is meant providing to a subject via any suitable route of administration an antagonist as described herein. Thus such antagonists may be formulated for any suitable route and means of administration. Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient, i.e. the antagonist, with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

By "a subject" it is intended a mammalian subject, including but not limited to a human subject. Non-human mammalian subjects may be treated in accordance with the invention. This includes rodents such as mice, rats or other rodents used as animal models of disease.

A pharmaceutical composition described herein may be administered to a subject or individual. Administration is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

Appropriate doses of antibody are well known in the art; see Ledermann J.A. *et al.* (1991) *Int. J. Cancer* 47: 659-664; Bagshawe K.D. *et al.* (1991) *Antibody, Immunoconjugates and Radiopharmaceuticals* 4: 915-922.

The precise dose will depend upon a number of factors, including whether the antibody is for diagnosis or for treatment, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment or diabody), and the nature of any detectable label or other molecule attached to the antibody. A typical antibody dose will be in the range 0.5 mg - 1.0 g, and this may be administered intravenously as a bolus or as an infusion over several hours as appropriate to achieve the required dose. Other modes of administration include intravenous infusion over several hours, to achieve a similar total cumulative dose. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician.

A further mode of administration employs precoating of, or otherwise incorporation into, indwelling devices, for which the optimal amount of antibody will be determined by means of appropriate experiments.

An antibody molecule in some embodiments may be a monomeric fragment, such as F(ab) or scFv. Such antibody fragments may have the advantage of a relatively short half life and less risk of platelet activation, which may be caused by receptor clustering.

If a whole antibody, is used, it is preferably in a form that is unable to activate and/or destroy platelets. The IgG4 isotype or alternatively "designer" isotypes derived from the IgG1 backbone (novel Fc gene constructs WO99/58572, Clark, Armour, Williamson) are preferred choices. Smaller antibody fragments may be used, such as F(ab')<sub>2</sub>. In addition, whole antibodies or fragments (e.g. F(ab')<sub>2</sub> or diabodies) with dual epitope specificity (e.g. for the epitopes recognised by scFv D9.2) may be used. Although such an embodiment may promote receptor clustering, a high association rate to individual receptors may rule out this problem.

Antibody molecules described herein will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the antibody molecule.

Thus pharmaceutical compositions for use in accordance with the present invention, may comprise, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or

other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

Therapeutic formulations of antagonists may be prepared for storage by mixing the antagonist having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (see e.g. "Remington: The Science and Practice of Pharmacy", 20th Edition, 2000, pub. Lippincott, Williams & Wilkins.), in the form of lyophilized powder or aqueous solutions. Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronic or polyethylene glycol (PEG).

For the antagonist, such as an antibody molecule, to be used for *in vivo* administration it must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. An antagonist polypeptide, such as an antibody molecule will ordinarily be stored in lyophilized form or in solution.

For solid compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium carbonate, and the like may be used. The antagonist as defined above may be formulated as suppositories using, for example, polyalkylene glycols, acetylated triglycerides and the like, as the carrier. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc, an antagonist as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the

pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see "Remington: The Science and Practice of Pharmacy", 20th Edition, 2000, pub. Lippincott, Williams & Wilkins. The composition or formulation to be administered will, in any event, contain a quantity of the active compound(s) in an amount effective to alleviate the symptoms of the subject being treated.

Dosage forms or compositions containing active ingredient in the range of 0.25 to 95% with the balance made up from non-toxic carrier may be prepared.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, sodium crosscarmellose, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like. Such compositions may contain 1%-95% active ingredient, more preferably 2-50%, most preferably 5-8%.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. For intravenous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as preservatives, stabilisers, antioxidants, wetting or emulsifying agents, pH

buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, triethanolamine sodium acetate, etc.

Parenteral administration may also employ the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained.

The percentage of active compound, i.e. antagonist, contained in such parental compositions is highly dependent on the specific nature thereof, as well as the activity of the compound and the needs of the subject. However, percentages of active ingredient of 0.1% to 10% in solution are employable, and will be higher if the composition is a solid which will be subsequently diluted to the above percentages. Preferably, the composition will comprise 0.2-2% of the active agent in solution.

For topical applications, pharmaceutically acceptable compositions may be formulated in a suitable ointment or gel containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration of antagonists for use as described herein include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. Alternatively, the pharmaceutically acceptable compositions can be formulated in a suitable lotion or cream containing the active components suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include, but are not limited to, mineral oil, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

An antagonist for use as described herein may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated. Other treatments may include the administration of suitable doses of pain relief drugs such as non-steroidal anti-inflammatory drugs (e.g. aspirin, paracetamol, ibuprofen or ketoprofen) or opiates such as morphine; the administration of anti-emetics; or the administration of at least one other compound active against asthma, generally a bronchodilating agent which produces airway relaxation or enhances mucus clearance, e.g. a beta-agonist (e.g. salbutamol, salmeterol), disodium cromoglycate, steroids or an inhibitor of PDE<sub>IV</sub>.

Instead of administering antagonists directly, they may be produced

in target cells by expression from an encoding nucleic acid introduced into the cells, e.g. from a viral vector. A viral vector may be targeted to specific cells to be treated, or it may contain regulatory elements which are switched on more or less selectively by the target cells.

Nucleic acid encoding an antagonist as described herein may thus be used in methods of gene therapy, for instance in treatment of a subject, e.g. with the aim of preventing or curing (wholly or partially) an IL-25 mediated disease or condition.

Vectors such as viral vectors have been used in the prior art to introduce nucleic acid into a wide variety of different target cells. Typically the vectors are exposed to the target cells so that transfection can take place in a sufficient proportion of the cells to provide a useful therapeutic or prophylactic effect from the expression of the desired nucleic acid or peptide. The transfected nucleic acid may be permanently incorporated into the genome of each of the targeted cells, providing long lasting effect, or alternatively the treatment may have to be repeated periodically.

A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see US Patent No. 5,252,479 and WO 93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses.

As an alternative to the use of viral vectors in gene therapy other known methods of introducing nucleic acid into cells include mechanical techniques such as microinjection, transfer mediated by liposomes and receptor-mediated DNA transfer.

Receptor-mediated gene transfer, in which the nucleic acid is linked to a protein ligand via polylysine, with the ligand being specific for a receptor present on the surface of the target cells, is an example of a technique for specifically targeting nucleic acid to particular cells.

Another aspect of the present invention provides a method of screening for an antagonist of TLR1 or TLR2 comprising determining the IL-25 expression level in a cell in the presence of a test compound, wherein a decrease in the amount of IL-25 expression in the

presence relative to the absence of test compound is indicative that the compound is an antagonist of TLR1 or TLR2. Such methods may further comprise determining binding of the test compound to TLR1 or TLR2.

Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure. All documents mentioned in this specification are incorporated herein by reference in their entirety.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

Certain aspects and embodiments of the invention will now be illustrated by way of example and with reference to the figures described above.

### Examples

#### Materials and Methods

##### Mice and experimental allergen exposure

*il25*<sup>-/-</sup> mice on a BALB/c background<sup>11</sup> and *myd88*<sup>-/-</sup><sup>22</sup> mice on a C57BL/6 background were generated as previously described. *tlr2*<sup>-/-</sup> mice on a C57BL/6 background were obtained from The Jackson Laboratory, Maine (<http://www.jax.org/>). C3H/HeJ<sup>LPS-D</sup> mice were obtained from Harlan. *il17br*<sup>-/-</sup> mice on a BALB/c background were created in the inventors' lab using gene-targeting. For allergen exposure, sex and age matched mice were anaesthetised and Ragweed pollen (RWP) (40 µg; Greer Laboratories, <http://www.greerlabs.com/>) or PBS in a volume of 40 µl was applied intra-nasally. This was repeated for 4 days, mice were analysed 24 hrs after the last challenge. All animal experiments were undertaken with the approval of the UK Home Office or the Department of Health and Children, Ireland.

### Primary airway cell culture

Lungs were removed from naïve mice under sterile conditions. AEC were prepared by collagenase D digestion followed by depletion of CD45, CD16/32, and CD11b positive cells as previously described<sup>23</sup>. Cells at a density of  $2 \times 10^5$  were then stimulated for 24 hr as indicated in the description of the figures.

### Signalling inhibitors

An inhibitor of NF- $\kappa$ B transcriptional activation (Calbiochem) was used at 11  $\mu$ M. Inhibitors to IKK $\epsilon$ , MKK1/2, and p38 $\alpha/\beta$  were obtained from Philip Cohen (University of Dundee) and used at the final concentrations of 1  $\mu$ M, 2  $\mu$ M, and 0.1  $\mu$ M respectively.

### Cytokine detection and cell analysis

Following experimental allergen exposure mice were terminally anaesthetised the trachea exposed and lungs lavaged. The resulting lavage fluid was used to detect IL-25 and IL-13. Cells recovered in the lavage were counted, cytopun, Giemsa stained and differential counting performed. IL-13 was detected using a commercially available ELISA (R&D Systems), IL-25 was detected using a direct ELISA format and antibody previously described<sup>4</sup>.

### Statistical methods

Data were plotted in GraphPad Prism, version 4.0, and tested for significance as indicated. Where appropriate data were analysed using the Student's t-Test or a one-way Anova. \* indicates significance of  $P < 0.01$ , \*\* indicates significance of  $P < 0.001$ . \*\*\*  $P < 0.005$ .

### Results

To address the question of what class of ligand and receptor are capable of inducing type-2 cytokines at the mucosal surface and lead to pulmonary inflammation and allergic asthma, a screen was undertaken to identify ligands that would induce IL-25 secretion from primary airways epithelial cells (AEC). The natural allergen Ragweed pollen (RWP) is responsible for the majority of cases of allergic rhinitis in Northern America and is now reported to be widespread throughout Europe with greatly increased rates of sensitisation<sup>15</sup>. In addition RWP has been used as a model allergen in laboratory models of allergic airways disease<sup>16</sup> and experimental allergic conjunctivitis<sup>17</sup>.

Primary AEC were prepared and assessed for their expression of IL-25

in response to various stimuli. AEC exposed to RWP responded by secreting IL-25 in a dose dependent fashion (Fig. 1a), demonstrating that this natural allergen is capable of inducing a potent type-2 response-promoting cytokine. A number of specific TLR ligands were also screened. AEC were exposed to a panel of ligands for TLRs 1 to 7, IL-25 secretion was examined. Significantly, the TLR1/2 ligand Pam3CSK4 stimulated AECs to secrete levels of IL-25 equal to those from RWP stimulated AEC (Fig. 1b), suggesting that a specific TLR signalling pathway is also capable of inducing this type-2-inducing cytokine. FACS analysis of epithelial cells confirmed that both TLR1 and TLR2 were co-expressed on the surface of AEC, using *tlr2*<sup>-/-</sup> AEC as controls (Fig. 1c). Using a TLR2 Fc/fusion protein we confirmed the ability of RWP to bind TLR2 (Fig. 1d). Pam3CSK4, a known ligand of TLR2, also bound the fusion protein, whilst LPS a ligand of TLR4 did not, confirming the specificity of this interaction (Fig. 1d).

MyD88 is an adaptor protein that is essential for signalling by all TLRs, except TLR3<sup>18</sup>. Thus the TLR1/2 ligand Pam3CSK4 does not activate cells from *myd88*<sup>-/-</sup> mice<sup>19</sup>. To confirm the role of a TLR pathway in the induction of IL-25 secretion, AEC were cultured from *myd88*<sup>-/-</sup> mice in the presence of RW, Pam3CSK4 or LPS. IL-25 secretion by *myd88*<sup>-/-</sup> AEC in response to Pam3CSK4 was abolished (Fig. 2b). IL-25 production in response to RWP was also significantly reduced (Fig. 2b), although the data also suggest that a MyD88-independent pathway for IL-25 induction may also exist. Stimulation with LPS resulted in no IL-25 secretion (Fig. 2b), confirming RWP- and Pam3CSK4-induced IL-25 was not endotoxin mediated. AEC cultured from *tlr2*<sup>-/-</sup> mice were used to investigate the specific involvement of TLR2 in IL-25 induction following stimulation with RWP, Pam3CSK4 or LPS (as a negative control). In the absence of TLR2, neither RW nor Pam3CSK4 were capable of stimulating IL-25 expression from AEC (Fig 2a). These data confirm that RWP can induce IL-25 production from AEC through ligation of TLR2, a pathway that is in common with the synthetic TLR2 ligand Pam3CSK4, via a MyD88-dependent signalling pathway. To further elucidate the potential roles of specific components of the MyD88 signalling pathways in the induction of IL-25 by TLR1/2 ligation inhibitors specific for IKK $\epsilon$ , MKK1/2, and p38 $\alpha$ / $\beta$  were employed (Bain et al., Biochem J 408:297-315. (2007)). It was found that inhibitors of IKK $\epsilon$ , MKK1/2 and NF $\kappa$ B profoundly reduced IL-25 production by AEC (Fig. 2c). By contrast, inhibition of p38 $\alpha$ / $\beta$  had no effect on IL-25 secretion (Fig. 2c). These data demonstrate a requirement for canonical TLR2-signalling components in the activation of IL-25 expression in AEC.

TLR2 can form a heterodimer with either TLR1<sup>19</sup> or TLR6<sup>20</sup> thereby increasing the array of ligands recognised, including triacylated and diacylated lipopeptides respectively, and in the case of the TLR2/6 further bacterial products (S. Akira, *et al.*, *Cell* **124** (4), 783 (2006)). To ascertain if TLR1 or TLR6 were involved in the recognition of RWP by TLR2, competition assays were performed *in vitro* using soluble TLR1, TLR2 and TLR6 proteins. It was found that inclusion of either soluble TLR1 or TLR2 was capable of inhibiting RW-induced IL-25 secretion by AEC in a dose dependent fashion (Fig. 2d). In contrast, soluble TLR6 protein had no effect on the secretion of IL-25 (Fig. 2d), confirming that the TLR1/2 heterodimer is responsible for the recognition of RWP leading to IL-25 secretion. In addition, a neutralising anti-TLR2 antibody confirmed that IL-25 secretion from AEC is TLR2 dependent (Fig. 2e).

To formally validate a biological function for TLR1/2-mediated ligation of RWP in an innate type-2 response *in vivo*, an acute allergen-induced mouse model of pulmonary inflammation was used. Following repeated intranasal exposure to RWP intra-nasally over four days, mice developed a localised type-2 inflammation characterised by elevated IL-5, IL-13 and IL-25 production and pulmonary cell infiltration including eosinophils in bronchoalveolar lavage (BAL) (Fig. 3).

To test the hypothesis that RWP binds to TLR1/2 and induces a type-2 immune response *myd88*<sup>-/-</sup> <sup>22</sup> and *tlr2*<sup>-/-</sup> (O. Takeuchi *et al.*, *Immunity* **11** (4), 443 (1999)) mice were exposed to RWP. It was found that cellular infiltration in the lungs was largely prevented in the allergen-treated *myd88*<sup>-/-</sup> and *tlr2*<sup>-/-</sup> mice, compared to allergen-treated wildtype controls (Fig. 3 a,b) with complete ablation of eosinophil infiltrates in both knockout mouse lines (Fig. 3 c). IL-13, IL-5 and IL-4 are key effector molecules in allergic asthma. Neutralisation of IL-13 using antagonists or *il13*<sup>-/-</sup> mice has been shown to reduce cell infiltration and AHR <sup>21</sup> in allergen challenged lungs, inhibition of IL-5 results in amelioration of eosinophilia, and blocking IL-4 decreases Th2 cell differentiation (Kopf, M., *et al.*, *Nature*. 18;362(6417):245-8. (1993)). The absence of either MyD88 or TLR2 resulted in complete abolition of detectable IL-25 and IL-13 in the BAL fluid (Fig. 3 d,e), and IL-5 and IL-4 expression in lung tissue (Fig. 3 f,g) of RWP treated mice when compared to control animals (Fig. 3 d,e,f,g). Importantly, the RWP-challenge of TLR4-deficient mice resulted in normal lung cell infiltration and IL-13 expression, indicating that LPS plays no role in the observed RWP-

induced type-2 phenotype (Fig. 5 a,b). This is an important observation in light of the recently reported role for TLR4-mediated responses to house dust mite allergen in type 2 responses (H. Hammad *et al.*, *Nature medicine* **15** (4), 410 (2009); A. Trompette *et al.*, *Nature* **457** (7229), 585 (2009)), indicating selective specificity of allergen recognition by different TLRs.

The data set out herein clearly demonstrate that the TLR2/MyD88 pathway is essential for the induction of the type-2 pulmonary response to ragweed pollen *in vivo*. To establish whether IL-25 is an essential downstream regulator of this pathway *in vivo* the responses of allergic mice lacking IL-25<sup>11</sup> were analysed. *il25*<sup>-/-</sup> mice were challenged intranasally with RWP and BAL fluid analysed for cytokine production. The absence of IL-25 prevented RWP-induced pulmonary cell infiltration (Fig. 4a,b), including eosinophils in BAL of *il25*<sup>-/-</sup> mice (Fig. 4b,c). This was accompanied by a striking absence of IL-13 in the BAL fluid of the allergen-treated *il25*<sup>-/-</sup> mice when compared to controls (Fig. 4d). As expected IL-25 was not detectable in the BAL of the RWP treated *il25*<sup>-/-</sup> mice but was induced in wild-type animals (Fig. 4e). To substantiate and extend these results, *il17br*<sup>-/-</sup> mice, were generated which are deficient in the IL-25 receptor IL-17BR, and challenged them with RWP.

Allergen challenge of the *il17br*<sup>-/-</sup> mice confirmed that IL-25 production in this setting occurs independently of IL-17BR expression (Fig. 4 e). By contrast, IL-13 protein production was absent in the BAL fluid of RWP exposed mice lacking IL-17BR (Fig. 4 d). Furthermore, BAL eosinophilia was absent in *il17br*<sup>-/-</sup> mice (Fig. 4 c). These data confirm that IL-25 and IL-25-responsive cell populations are essential for the diversification and amplification of the pulmonary response to allergen downstream of TLR1/2 ligation and MyD88 activation.

The results set out herein identify a novel pathway by which the natural allergen RWP binds the pattern-recognition receptor TLR1/2 heterodimer, which is expressed on AEC, resulting in recruitment of MyD88 and triggering MKK1/2, IKK $\epsilon$  and NF $\kappa$ B signalling cascades, and ultimately stimulating IL-25 secretion. Within the lung, secreted IL-25 activates IL-17BR-positive cells to produce the classical type-2 cytokines IL-4, IL-5 and IL-13 resulting in the onset of inflammatory cell infiltration, characterised by eosinophils, into the lungs. and activating IL-17BR-positive cells at the onset of type-2 pulmonary inflammation characterised by IL-13 expression and eosinophil

infiltration.

The data provide compelling evidence for an important link between the positive initiation of type-2 responses to allergens and pattern recognition by TLR1/2.

The results identify TLR signalling as potential therapeutic target and further supports the case for IL-25 as a therapeutic target in a mucosal allergic setting.

Abbreviations

|       |                                      |
|-------|--------------------------------------|
| AEC   | airway epithelial cells              |
| AHR   | Airways hyperreactivity              |
| °C    | Centigrade                           |
| bp    | Base pairs                           |
| CDR   | Complementarity determining region   |
| DNA   | Deoxyribonucleic acid                |
| ELISA | Enzyme linked immuno-adsorbent assay |
| FACS  | Fluorescence activated cell sorting  |
| g     | Grams                                |
| hr    | Hour                                 |
| Ig    | Immunoglobulin                       |
| i.p.  | intraperitoneal                      |
| mAb   | Monoclonal antibody                  |
| min   | Minute                               |
| PBS   | Phosphate buffered saline            |
| PCR   | Polymerase chain reaction            |
| RWP   | Ragweed pollen                       |
| TLR   | toll-like receptor                   |

Sequences

SEQ ID NO:1 *hTLR1 amino acid sequence (786 aa; extracellular domain 25-580 underlined)*

MTSIFHFAII FMLILQIRIQ LSEESEFLVD RSKNGLIHVP KDLSQKTTIL NISQNYISEL  
 WTSIDILSLSK LRILIISHNR IQYLDISVFK FNQELEYLDL SHNKLVKISC HPTVNLKHLD  
 LSFNAFDALP ICKEFGNMSQ LKFLGLSTTH LEKSSVLPIA HLNISKVLLV LGETYGEKED  
 PEGLDQFENTE SLHIVFPTNK EFHFILDVSV KTVANLELSN IKCVLEDNKC SYFLSILAKL  
 QTNPKLSNLT LNNIETTWSN FIRILQLVWH TTVWYFSISN VKLQGQLDFR DFDYSGTSLK  
 ALSIHQVVSD VFGFPQSYIY EIFSNMNIKN FTVSGTRMVH MLCPSKISPF LHLDFSNNLL  
 TDTVFENCGRH LTELETILIQ MNQLKELSKI AEMTTQMKSL QQLDISQNSV SYDEKKGDCS  
 WTKSLLSLNM SSNILTDTIF RCLPPRIKVL DLHSNKIKSI PKQVVKLEAL QELNVAFNSL  
 TDLPGCGSFS SLSVLIIDHN SVSHPSADFF QSCQKMRSIK AGDNPFQCTC ELGEFVKNI  
 QVSSEVLEGW PDSYKCDYPE SYRGTLTKDF HMSELSCNIT LLIVTIVATM LVLAVTVTSL  
 CSYLDLPWYL RMVCQWTQTR RRARNIPLEE LQRNLQFHAF ISYSGHDSFW VKNELLPNLE  
 KEGMQICLHE RNFVPGKSIV ENIITCIEKS YKSIFVLSPN FVQSEWCHYE LYFAHHNLFH  
 EGSNSLILIL LEPIPOYSIP SSYHKLKSLM ARRTYLEWPK EKSKRGLFWA NLRAAINIKL  
 TEQAKK

SEQ ID NO:2 *hTLR2 amino acid sequence (784 aa; extracellular domain 19-587 underlined)*

MPHTLWMVWV LGVIISLSKE ESSNQASLSC DRNGICKGSS GSLNSIPSGL TEAVKSLDLS  
 NNRITYISNS DLQRCVNLQA LVLTSNGINT IEEDSFSSLG SLEHLDLSYN YLSNLSSSWF  
 KPLSSLTFLN LLGNPYKTLG ETSLSFSLTK LQILRVGNMD TFTKIQRKDF AGLTFLEELE  
 IDASDLQSYE PKSLKSIQNV SHLILHMKQH ILLLEIFVDV TSSVECLELR DTDLDTFHFS  
 ELSTGETNSL IKKFTFRNVK ITDESLFQVM KLLNQISGLL ELEFDDCTLN GVGNFRAASN  
 DRVIDPGKVE TLTIRRLHIP RFYLFYDLST LYSALTERVKR ITVENSKVFL VPCLLSQHLK  
 SLEYLDLSEN LMVEEYLKNS ACEDAWPSLQ TLILRQNHIA SLEKTGETLL TLKNLTNIDI  
 SKNSFHSMP ETCQWPEKMKY LNLSSSTRIHS VTGCIPKTLE ILDVSNNNLN LFSNLNPQLK  
 ELYISRNLKM TLPDASLLPM LLVLKISRNA ITTFSKEQLD SFHTLKTLEA GGNNFICSCE  
 FLSFTQEQQ A LAKVLIDWPA NYLCDSPSHV RGQQVQDVRL SVSECHRTAL VSGMCCALFL  
 LILLTGVLCH RFHGLWYMKM MWAQLQAKRK PRKAPSRNIC YDAFVSYSER DAYWVENLMV  
 QELFNFPF KLCLHKRDFI PGKWIIDNII DSIEKSHKTV FVLSNFVKS EWCKYELDFS  
 HFRLFDENND AAILILLEPI EKKAIPQRFK KLRKIMNTKT YLEWPMDEAQ REGFWNLRA  
 AIKS

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CLAIMS

1. An antagonist of TLR1 or TLR2 for use in a method of treating or preventing an IL-25 mediated disease.
2. An antagonist for use according to claim 1 which binds an extracellular domain of TLR1 or TLR2.
3. An antagonist for use according to claim 1 or claim 2 which competes with TLR ligand binding to TLR1 and/or TLR2.
4. An antagonist for use according to claim 1 or claim 2 which disrupts TLR1-TLR2 heterodimerisation.
5. An antagonist for use according to claim 1 which inhibits or reduces expression of TLR1 or TLR2.
6. An antagonist for use according to any one of claims 1 to 4 comprising an antibody molecule which binds TLR1 or TLR2.
7. An antagonist for use according to claim 6 wherein the antibody molecule comprises a human framework region.
8. An antagonist for use according to claim 6 or claim 7 which is a Fab, F(ab')<sub>2</sub>, scFv antibody fragment.
9. An antagonist for use according to claim 6 or claim 7 which comprises a whole antibody.
10. An antagonist for use according to claim 1 comprising an isolated soluble extracellular domain of TLR1 or TLR2.
11. An antagonist for use according to claim 10 comprising an Fc fusion protein.
12. A pharmaceutical composition for use in a method of treating an IL-25 mediated disease comprising an antagonist as described in any preceding claim and a pharmaceutically acceptable excipient.
13. A pharmaceutical composition for use according to claim 12 in the form of a lyophilized powder.

14. A method of treating or preventing an IL-25 mediated disease, comprising administering to a subject in need of treatment an effective amount of an antagonist of TLR1 or TLR2.

15. A method of treating or preventing an IL-25 mediated disease, comprising administering to a subject in need of treatment an effective amount of an antagonist as described in any one of claims 1-11 or a pharmaceutical composition as described in claim 12 or claim 13.

16. An antagonist for use, a pharmaceutical composition for use, or a method according to any preceding claim wherein the IL-25 mediated disease is asthma.

17. A method of screening for an antagonist of TLR1 or TLR2 comprising determining the IL-25 expression level in a cell in the presence of a test compound, wherein a decrease in the amount of IL-25 expression in the presence relative to the absence of test compound is indicative that the compound is an antagonist of TLR1 or TLR2.

18. A method according to claim 17 further comprising determining binding of the test compound to TLR1 or TLR2.

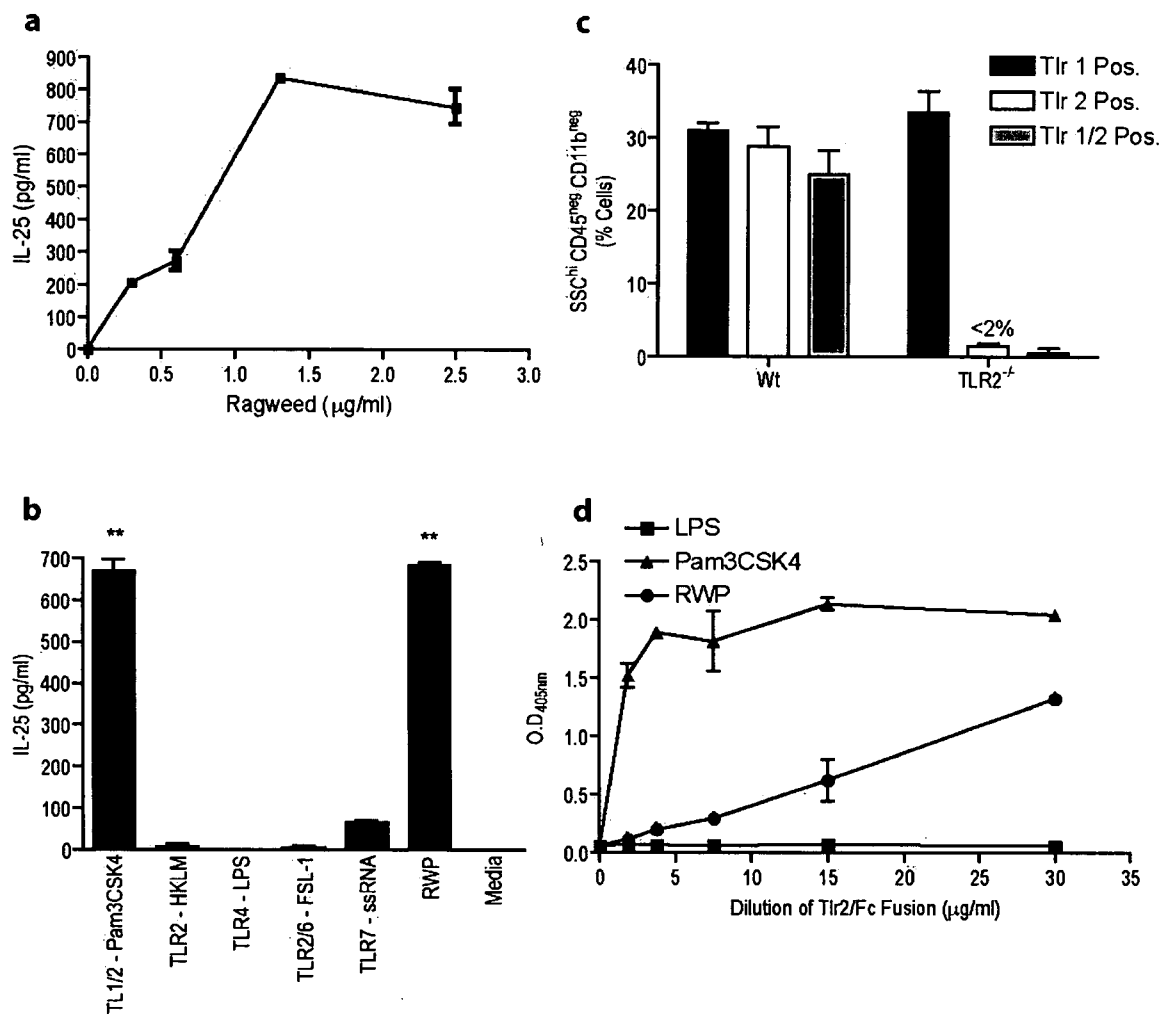


Figure 1

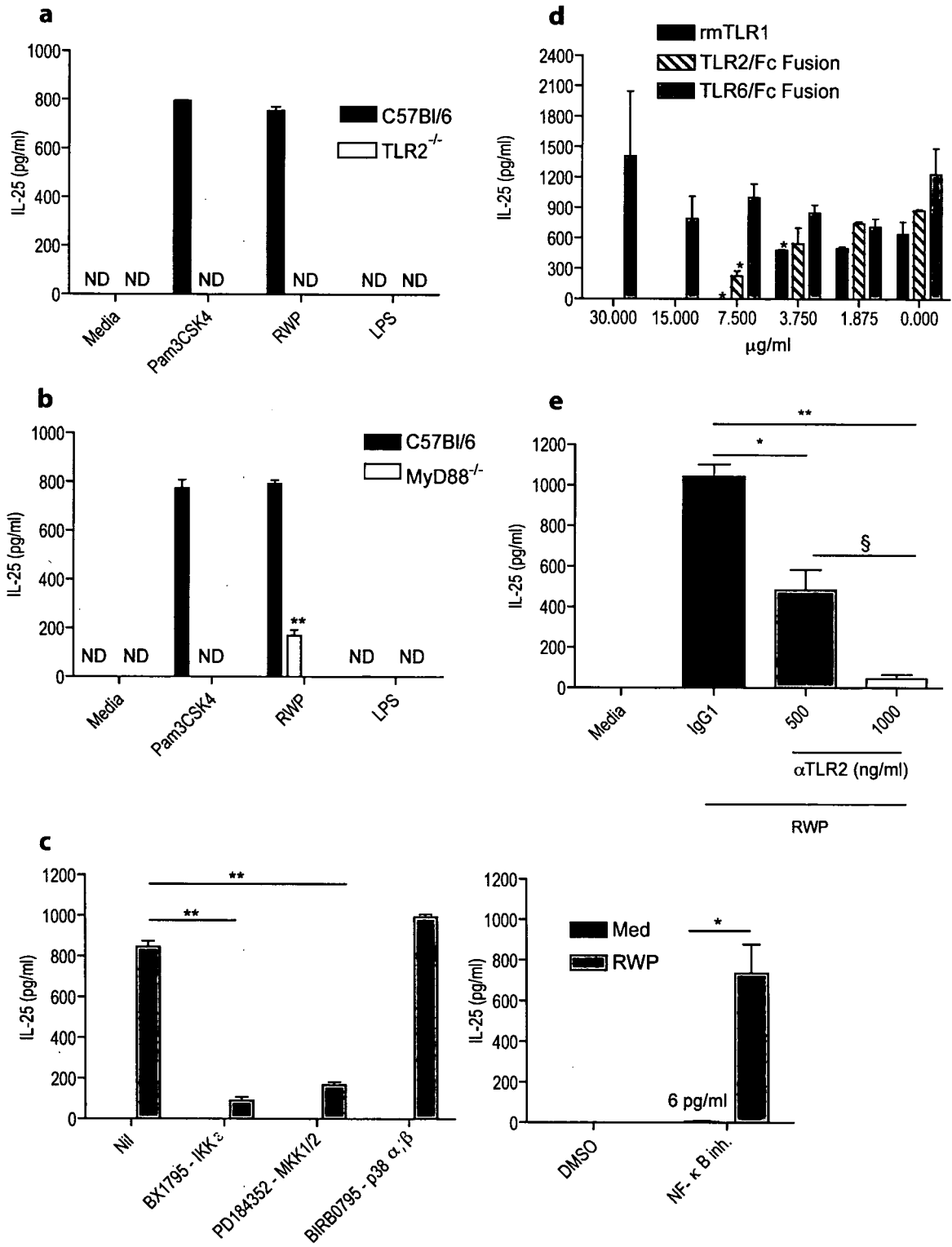


Figure 2

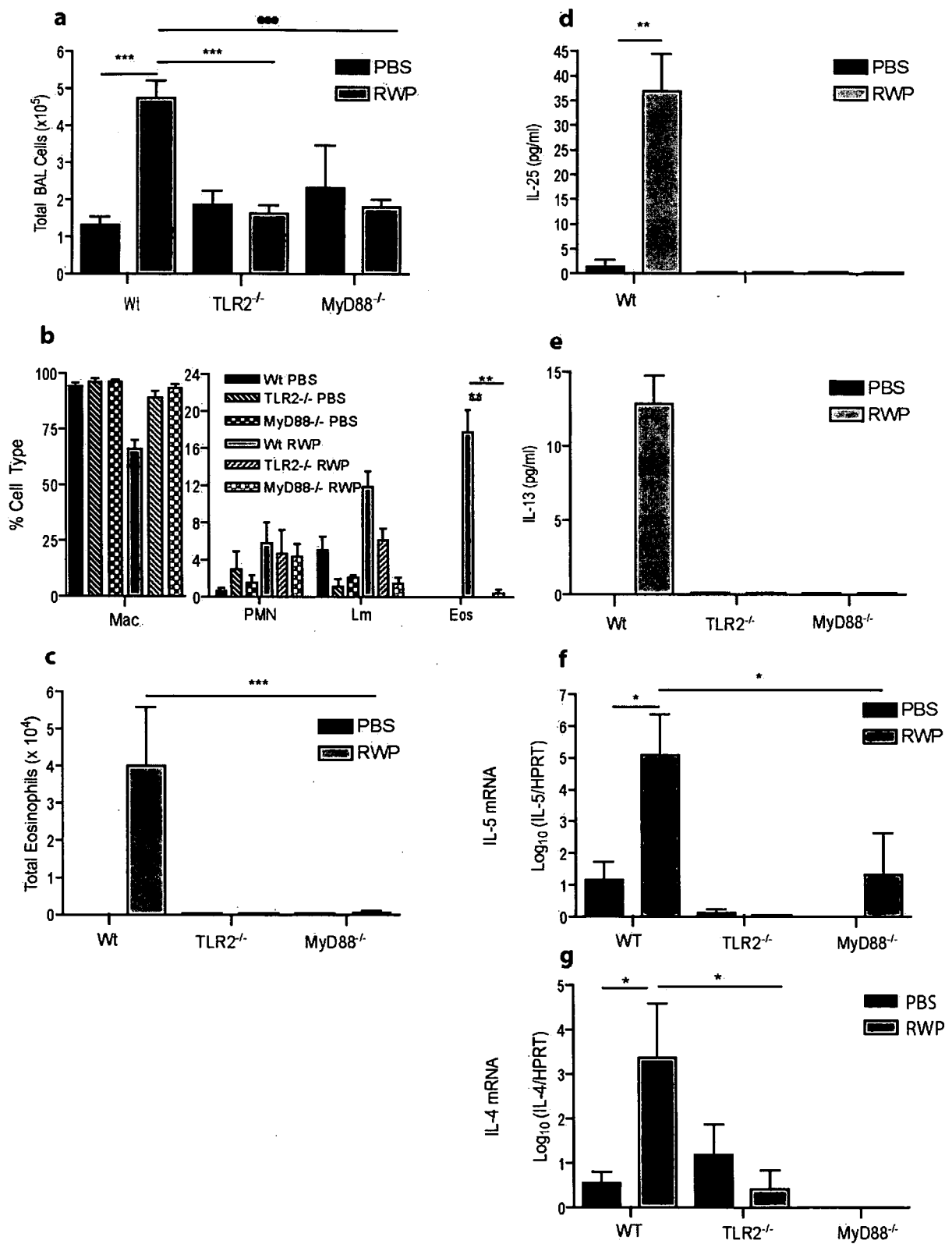


Figure 3

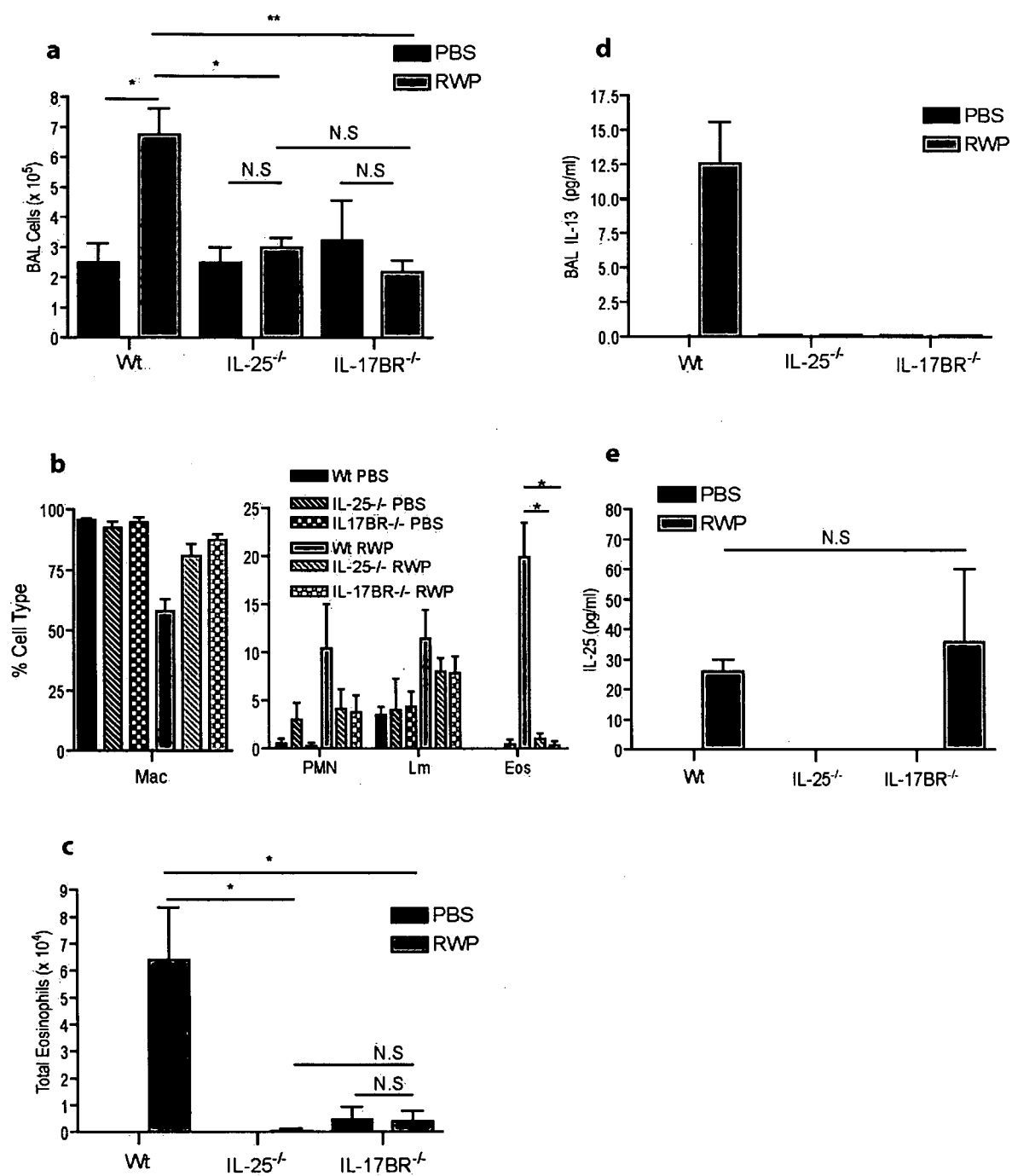


Figure 4

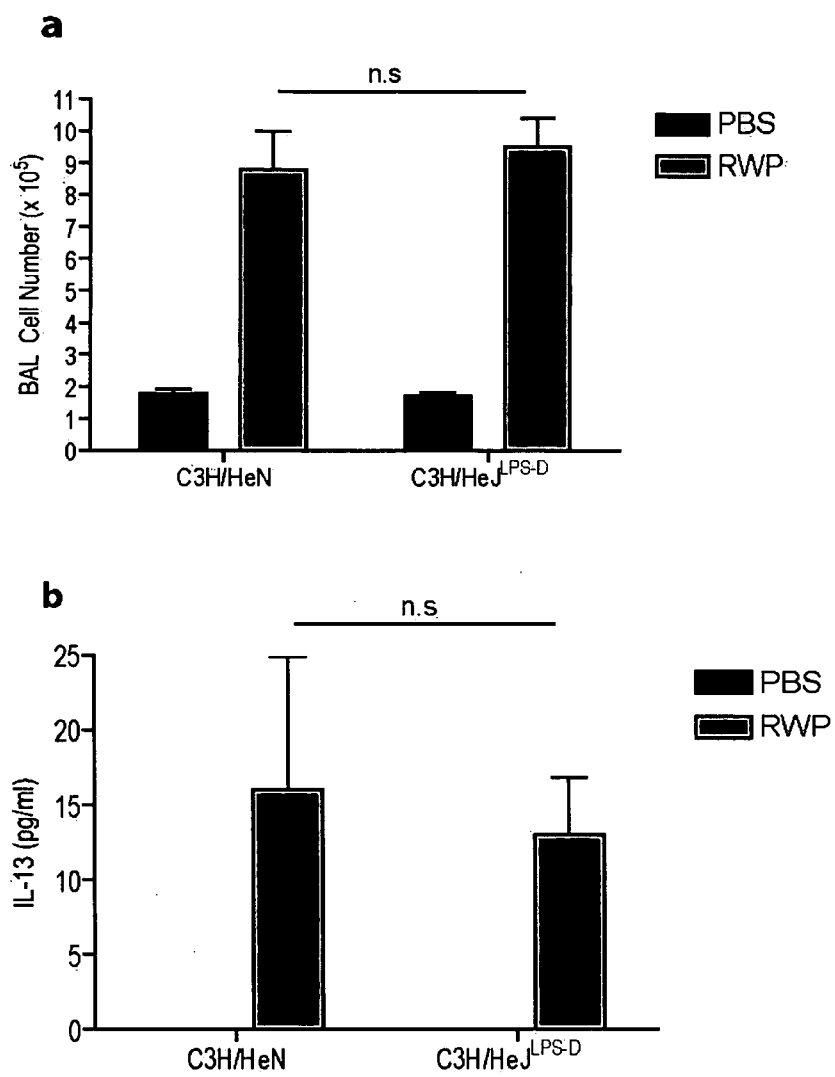


Figure 5