Abstract:

The invention relates to a genetically modified animal comprising a nucleic acid encoding a T cell receptor (TCR). The nucleic acid has been recombinantly introduced into the animal. The TCR is specific for an immunogenic fragment of the mite allergen BIo t 5.
GENETICALLY MODIFIED ANIMAL AND METHOD OF OBTAINING THE SAME

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority of US provisional application No. 61/162,055, filed March 20, 2009, the content of which being hereby incorporated by reference in its entirety for all purposes.

FIELD OF THE INVENTION

[0002] The present invention relates to a genetically modified animal comprising a nucleic acid encoding a T cell receptor (TCR) specific for an immunogenic fragment of a mite allergen and a method of obtaining the same. The present invention also relates to an animal obtained by reproduction of the genetically modified animal, a method of generating a T cell line specific for an immunogenic fragment of the mite allergen, a method of immunizing an animal with a T cell specific for an immunogenic fragment of the mite allergen; and a method of testing the efficacy of therapeutic agents using the animals of the present invention.

BACKGROUND OF THE INVENTION

[0003] Allergic diseases represent a big health problem worldwide and up to 30% of the populations in developed countries suffer from allergic diseases such as rhinitis, conjunctivitis, atopic dermatitis, atopic asthma or bronchial asthma. One of the major triggers for the development of allergic diseases such as asthma is sensitivity to dust mite allergens. Exposure to dust mite allergens activates a cascade of inflammatory reactions which involves an Immunoglobulin E (IgE) antibody response. These IgE molecules bind to high affinity Fc receptors (FcεRI) on the surfaces of mast cells (in tissue) or white blood cells such as basophils (in the blood circulation). A subsequent exposure to the allergen causes the allergen to bind and crosslink these IgE molecules, resulting in a stimulation of the respective e.g. mast cells. This causes a rapid release of, among others, inflammatory mediators such as histamine and of newly formed mediators such as prostaglandins and leukotrienes. The secretion of such inflammatory mediators result in
the manifestation of allergy symptoms such as increased vascular permeability, contraction of bronchial smooth muscles and increased secretion of mucus.

[0004] Evidence suggests that the IgE plays a major role in allergic diseases such as asthma. The synthesis of IgE is a result of collaboration between subsets of T helper cells (Th cells), CD4+ and B cells. It is well known that T helper cells such as Th2 cells play a pivotal role in atopic allergy and Th-2 cytokines such as IL-4, IL-5 and IL-13 contribute significantly to the pathogenesis of allergic diseases caused by dust mites for example. The most important mite species of indoor allergen source in both Europe and Australia, as well as worldwide, is *Dermatophagoides pteronyssinus* (Der p) in which Der p1 and Der p2 are the major allergens. *Blomia tropicalis* (Blo t) is clinically the most important mite species in subtropical and tropical regions and Blo t 5 is the major allergen. Most of the *Blomia tropicalis* allergens only have 30-40% sequence identity with their *Dermatophagoides* counterparts. It has been well established that the allergen from *Blomia tropicalis* is the triggering factor for allergic asthma especially in the subtropical and tropical regions. Accordingly, the isolation and characterization of the allergen of this mite species is important in the study of the development of the allergic disease, for diagnostic as well as therapeutic purposes.

[0005] Animal models are useful tools to dissect the underlying mechanism in the development of allergic diseases and to test the efficacy of the immunotherapy. The studies using the genetically modified mice expressing the TCR provided the fundamental knowledge in the understanding of Thl/Th2 cell development. The mouse model having the TCR specific for the ovalbumin (OVA) antigen has been commonly used in the studies of allergic diseases. In one study, a genetically modified mouse specific for OVA antigen were immunized and was shown to exhibit airway hyperreactivity after limited exposure to OVA aerosols (Wilder JA, et al., *J Leukoc Biol*, (2001), 69(4), 538-47). Although there was an accumulation of OVA-specific T cells in the lungs of these mice, no significant increases in OVA-specific IgG or total serum IgE were observed. In another study, transgenic Balb/c mice which are specific for the epitope of OVA were exposed to OVA caused lung inflammation having characteristics of both Th1 and Th2 immune response (Knott PG, et al., *Am J Respir Crit Care Med.*, (2000), 161: 1340-8). However, these methods were insufficient to directly induce airway
hyperreactivity. Although OVA-TCR transgenic mice are widely used in the study of allergic diseases, OVA is not a suitable aeroallergen in the induction of allergic respiratory diseases in man. Therefore, OVA is not a clinically relevant aeroallergen for human since it does not reflect the natural route of allergen exposure and sensitization. Accordingly, the generation of a mouse model of TCR genetically modified mice for clinically relevant aeroallergen may provide a useful tool in the study of the development of allergic diseases in humans.

[0006] Current animal models for allergic diseases in particular for mite allergens are unsatisfactory. Thus there is still a need for an animal model for example, to evaluate the underlying mechanism in developing allergic diseases in humans or develop new drugs.

[0007] Accordingly, it is an objective of the present invention to provide an animal model for example, for the study of human allergic diseases caused by dust mites in particularly Blomia tropicalis. This objective is solved by the subject matter of the appending independent claims.

SUMMARY OF THE INVENTION

[0008] In one aspect the present invention provides a genetically modified animal. The genetically modified animal includes a nucleic acid encoding a T cell receptor (TCR). The nucleic acid has been recombinantly introduced into the animal and the TCR is specific for an immunogenic fragment of the mite allergen BlO t 5.

[0009] In another aspect the invention provides an animal obtained by reproduction of the genetically modified animal as described above.

[0010] In a further aspect the invention provides a method of obtaining a genetically modified animal. The method includes recombinantly introducing a nucleic acid encoding a TCR as described above.

[0011] In a further aspect the invention provides a method of generating a T cell line specific for an immunogenic fragment of the mite allergen BlO t 5. The method includes administering to an animal a BlO t 5 peptide or a fragment thereof, isolating the T cell specific for an immunogenic fragment of the mite allergen BlO t 5 from the
spleenocyte of the animal and further administering to the isolated T cell the BIo t 5 peptide or a fragment thereof.

[0012] In yet a further aspect the invention provides a method of immunizing an animal comprising administering a T cell. The TCR is specific for an immunogenic fragment of the mite allergen BIo t 5.

[0013] In yet another aspect the invention provides a method of testing the efficacy of a therapeutic agent for an allergic disease. This method includes administering to a genetically modified animal or an animal as described above, the therapeutic agent.

[0014] In a further aspect the invention provides a method of testing the suitability of a compound for the treatment of an allergic disease. This method includes administering to a genetically modified animal or an animal as described above, the compound.

[0015] In another aspect the invention provides a method of identifying a compound being suitable for the treatment of an allergic disease. This method includes administering to a genetically modified animal or an animal as described above, the compound.

**BRIEF DESCRIPTION OF THE DRAWINGS**

[0016] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0017] **Figure 1** depicts IgE and IgG1 immune responses (Figure IA) and cytokine profiles of IL-4, IL-13, IL-5 and IFN-γ (Figure IB) in mice sensitized with recombinant BIo t 5 peptides described herein.

[0018] **Figure 2** depicts cytokine profiles (IL-4, IFN-γ, IL-5, IL-10 and IL-13) of a Blot 5 specific T cell line obtained according to the present invention.

[0019] **Figure 3** depicts the specificity of a BIo t 5 T cell line obtained from Figure 2 to BIo t 5, when stimulated with the respective recombinant BIo t 5 peptides.

[0020] **Figure 4** depicts the induction of lymphocytes ("Lym"), monocytes ("Mono"), Neutrophils ("Neu") and eosinophils ("Eos") infiltration in the lungs of mice
when immunized with Bio t 5 specific T cells and Bio t 5 antigen.

[0021] Figure 5 shows the characterization of TCRβ gene of a Bio t 5 specific T cell line obtained according to the present invention by flow-cytometry, polymerase chain reaction (PCR) and gel electrophoresis.

[0022] Figure 6 shows the characterization of TCRα gene of Vβ3+ Bio t 5 specific T cells obtained according to the present invention by PCR and gel electrophoresis.

[0023] Figure 7 shows the PCR products of rearranged VαJα and VβDβJβ genes of Bio t 5 specific T cells as depicted in Figure 7A (containing TCRVα11 and Jα34 genes of size ~620bp) and Figure 7B (containing TCR Vβ3 and Jβ2.4 of size ~580bp) respectively.

[0024] Figure 8 depicts schematically pTαcass and pTβcass vectors used in cloning the respective PCR products of TCR VαJα and TCR VβDβJβ from Figure 7, to obtain the constructs of pTα-Bt5α (Figure 8A) and pTβ-Bt5β (Figure 8B) respectively.

[0025] Figure 9 depicts the expression levels of CD4+ TCRVa+11Vβ3+ T cells obtained from the peripheral blood of a genetically modified mouse of the present invention (Figure 9A) and a wild type mouse (Figure 9B).

[0026] Figure 10 depicts the expression levels of CD4+ TCRVa+11Vβ3+ T cells obtained from the peripheral blood of a genetically modified mouse crossed with a wild type mouse.

[0027] Figure 11 shows the genomic DNA sequence encoding the Vα11+Jα34 domain which is identified as (SEQ ID NO: 1), and the genomic DNA sequence encoding the Vβ3+Dβ2+Jβ2.4 domain which is identified as (SEQ ID NO: 2).

[0028] Figure 12 shows the cDNA sequence encoding the Vα8+Jα28 domain which is identified as (SEQ ID NO: 3); cDNA sequence encoding the Vα4+Jα27 domain which is identified as (SEQ ID NO: 4); cDNA sequence encoding the Vβ4+Dβ2+Jβ2.7 domain which is identified as (SEQ ID NO: 5); and cDNA sequence encoding the Vβ6+Dβ1+Jβ2.4 domain which is identified as (SEQ ID NO: 6).

[0029] Figure 13 shows the airway hyper-responsiveness model of a genetically modified mouse of the present invention.
[0030] Figures 14A and B show the representative Fluorescence Activated Cell Sorting (FACS) plots showing the staining of transcription factor GATA-3 (Figure 14A) and transcription factor T-bet (Figure 14B) in CD4+ T cells of the genetically modified mice as described in Example 12.

[0031] Figures 14C-F show the representative FACS plots showing the proportion of eosinophils (indicated as "EOS") and macrophages (indicated as "MAC") in Blο t 5 adoptive hosts that were challenged intranasally (i.n.) with Blο t 5 peptide.

[0032] Figure 15 shows the eosinophil and neutrophil infiltration into BALF and lung tissues of CD4+ T cell adoptive transfer recipient mice after Blο t 5 challenge.

[0033] Figure 16 measures the airway hyperreactivity in CD4+ T cell adoptive transfer recipient mice followed by Blο t 5 challenge (indicated as circle: "Th2-BT5"); compared to CD4+T cell adoptive transfer recipient mice followed by PBS (indicated as triangle: "Th2-PBS") and control mice.

[0034] Figure 17 shows the Hematoxylin and eosin (H&E) staining of lung sections of CD4+ T cell adoptive transfer recipient mice (Figures 17A & B) challenged intranasally with Blο t 5 peptide and control mice (Figures 17C & D).

[0035] Figure 18 shows the Periodic acid-Schiff (PAS) staining of lung sections of CD4+ T cell adoptive transfer recipient mice (Figures 18C & D) challenged intranasally with Blο t 5 peptide and control mice (Figures 18 A & B).

DETAILED DESCRIPTION OF THE INVENTION

[0036] In one aspect the present invention provides a genetically modified animal. The genetically modified animal includes a nucleic acid encoding a T cell receptor (TCR). The nucleic acid has been recombinantly introduced into the animal and the TCR is specific for an immunogenic fragment of the mite allergen Blο t 5. Therefore, the genetically modified animal of the present invention does not include an animal being only immunised with Blο t 5 or a fragment thereof. However, the invention does include an animal which has received a nucleic acid that encodes such a TCR for example, either by gene transfer or by simply receiving (for example by adoptive transfer) T cells isolated from another animal that itself stably expresses a nucleic acid encoding a TCR specific
for an immunogenic fragment of BIo t 5. Thus, an example of such an animal is illustrated in Examples 13 and 14 which received CD4+ T cells isolated from the lymph nodes of genetically modified mice according to the present invention.

[0037] The present invention is based on the surprising finding that it is possible to establish a significantly high level of expression of TCR α and β domains specific for an immunogenic fragment of the mite allergen BIo t 5 in the peripheral T cells of the genetically modified animal, relative to the wild type animal. Previously, a mouse expressing the TCR specific for Der p 1 was generated to investigate the development of allergic asthma caused by Dermatophagoides pteronyssinus. (Jarman ER, et. al., Clin Exp Allergy, (2005), 35(7), 960-9). However, a relatively low level of TCRα and β domains specific for Der p 1 was expressed in the T cells of the mouse model. In addition, this mouse model was lost due to unknown reasons. Therefore, the low expression levels and the instability of the cell line limit the opportunity to develop further characterization studies on the development of allergic diseases in an animal model.

[0038] In this context, the term "wild type" in reference to an animal or an amino acid sequence as used herein, means a phenotype, genotype or a gene that predominates in a natural population of organisms or strain of organisms in contrast to the forms that were genetically altered in a natural or artificial environment.

[0039] A genetically modified animal according to the present invention can refer to an animal which is engineered to carry a gene of the same species. It is also possible to generate a genetically modified animal that is engineered to carry a gene of a different species as long as the MHC molecules of the two species are compatible. The term "animal" can include all vertebrate, including humans. It can also include an individual animal in all stages of development, including embryonic and foetal stages. To obtain a genetically modified animal according to the present invention, the genetic material of the animal can be modified using genetic engineering techniques such as recombinant DNA technology. Recombinant DNA can for example be produced through the addition of a relevant DNA into an existing organismal genome, such as a plasmid, to code for or alter different traits for a specific purpose. The genetically modified animal used in the present invention may be a mammal, hi some embodiments, the mammal may include a rodent, Canis, Ungulate, Felidae, Leporidae, and Macaque. Examples of a rodent
include, but are not limited to, a mouse, rat, squirrel, chipmunk, gopher, porcupine, beaver, hamster, gerbil, guinea pig, chinchilla, prairie dog, and groundhog. Examples of Canis include, but are not limited to a dog, wolf, coyote and jackal. Examples of Ungulate include, but are not limited to a horse, donkey, zebra, sheep, pig, goat, camel, giraffe and moose. Examples of Felidae include, but are not limited to a cat, caracal, cougar, cheetah and leopard. Examples of Leporidae include, but are not limited to a rabbit, hare and jackrabbit. An example of a Macaque includes a rhesus monkey.

[0040] The animal as used in the present invention may be a mouse. Different sub-species of the mouse can be used in the present invention. Examples of these sub-species can include, but are not limited to Mus (musculus) musculus, Mus (musculus) castaneus, Mus (musculus) domesticus, Mus musculus gentilulus, Mus musculus homourus, Mus musculus molossinus, Mus musculus bactrianus, Mus musculus praetextus and Mus musculus wagneri. Different strains and sub-strains of the mouse can be used in the present invention. Examples of such strains can include, but are not limited to C57BL/6 and Balb/c mouse. In some embodiments, the animal used in the present invention may be a C57BL/6 mouse.

[0041] The genetically modified animal of the present invention includes a nucleic acid which has been recombinantly introduced into the animal and which encodes a T cell receptor (TCR) specific for an immunogenic fragment of the mite allergen BIo t 5. The term "nucleic acid" as used herein refers to any nucleic acid molecule in any possible configuration, such as a linearized single stranded, a double stranded or a combination thereof. Nucleic acids may include, but are not limited to DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), analogues of the DNA or RNA generated using nucleotide analogues or using nucleic acid chemistry, cDNA synthetic DNA, a copolymer of DNA and RNA, oligonucleotides, and PNA (protein nucleic acids). DNA or RNA may be of genomic or synthetic origin and may be single or double stranded. A respective nucleic acid may furthermore contain non-natural nucleotide analogues and/or be linked to an affinity tag or a label. A nucleic acid molecule such as DNA can be regarded to be "capable of expressing a nucleic acid molecule or a coding nucleotide sequence" or capable "to allow expression of a nucleotide sequence" if it contains regulatory nucleotide sequences which contain
transcriptional and translational information and such sequences are "operably linked" to nucleotide sequences which encode the T cell receptor. An operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequences sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene sequence expression may vary from organism to organism, but shall, in general include a promoter region which, in prokaryotes, contains only the promoter or both the promoter which directs the initiation of RNA transcription as well as the DNA sequences which, when transcribed into RNA will signal the initiation of the synthesis. Such regions will normally include non-coding regions which are located 5' and 3' to the nucleotide sequence to be expressed and which are involved with initiation of transcription and translation such as the TATA box, capping sequence, CAAT sequences.

[0042] The nucleic acid used in the present invention can be obtained from any animal described herein so long as the nucleic acid encodes for a T cell receptor (TCR) and wherein the TCR is specific for an immunogenic fragment of the mite allergen BIo t 5. In some embodiments, the nucleic acid encoding a TCR defined above may be obtained from another animal of the same species. In other embodiments, the nucleic acid may be obtained from an animal of another species wherein the TCR is compatible. The nucleic acid according to the present invention may be an isolated nucleic acid encoding a TCR specific for an immunogenic fragment of the mite allergen BIo t 5. The term "isolated" indicates that naturally occurring matter or a naturally occurring sequence has been removed from its normal cellular (e.g. intracellular) environment. Thus, the matter or sequence may be in a cell-free solution or suspension etc., or placed in a different cellular environment. The term does not imply that the matter or sequence is the only the matter or sequence present, but that it is essentially free (usually about 90 - 95% pure at least) of other matter naturally associated with it.

[0043] The nucleic acid as described herein can be stably integrated into the genome of the genetically modified animal. The nucleic acid may, for example, become linearised prior to integration into the genome of the animal. The nucleic acid may be comprised in a vector, for example an expression vector. The nucleic acid may also be present in two separate vectors. Such a vector is capable of integrating the nucleic acid
described above and can comprise a sequence coding for restriction cleavage site which adjoins the nucleic acid encoding for the TCR in 5' or/and 3' direction. The vector can also contain replication sites and control sequence derived from a species compatible with the host that is used for expression. In this context, the term "vector" relates to a single or double-stranded circular nucleic acid molecule that can be introduced, e.g. transfected into cells and replicated within or independently of a cell genome. A circular double-stranded nucleic acid molecule can be cut and thereby linearised upon treatment with restriction enzymes. An assortment of nucleic acid vectors, restriction enzymes, and the knowledge of the nucleotide sequences cut by restriction enzymes are readily available to those skilled in the art.

[0044] A nucleic acid molecule encoding a TCR specific for an immunogenic fragment of the mite allergen Blot5 can be inserted into a vector by cutting the vector with restriction enzymes and ligating the two pieces together. Any vectors suitable for genetic cloning that are known to the person skilled in art can be used in the present invention. Examples of these vectors may include but are not limited to pUC18, pUC19, BR322, pBR325, pBR327, pBR328, pMB9, pCRI, RSF2124, pBR313, pACYC177 pACVC184, pCR2.1-TA, pVA, pCK (described in Sha C. et al, Nature 335, (1988), 271-274), hCD2 (described in Zhumabekov T. et al, Journal of Immunological Methods 185 (1995) 133-140), P3A9cbTCR and pES4 (described in Barnden J. et al, Immunology and Cell Biology, 76, (1998) 34-40), pTexIIIa (described in Jarman ER, et. al., Clin Exp Allergy, (2005), 35(7), 960-9) and pTOαcass and pTBcass vectors (described in Kouskoff V. et al, Journal of Immunological Methods 180 (1995) 273-280). As an illustrative example of obtaining a nucleic acid for integration into the genome of the animal according to the present invention, the variable region of the TCR α and TCR β genes can be cloned into the pCR2.1-TA cloning vector to obtain the two separate constructs pCR2.1-Bt5α and pCR2.1-Bt5β respectively. The TCR α and TCR β genes can be isolated from the respective pCR2.1-Bt5α and pCR2.1-Bt5β constructs by genetic engineering techniques known to persons skilled in the art. The isolated TCR α and TCR β gene fragments can be re-introduced into the respective pTOαcass and pTBcass vectors by genetic engineering technology, to obtain the constructs pTor-Bt5α and pTβ-Bt5β respectively (cf. Figure 8 and the following examples). These constructs for example, contain the nucleic acid disclosed herein for integration into the genome of the animal
according to the present invention.

[0045] The nucleic acid encoding the TCR specific for an immunogenic fragment of the mite allergen Blot 5 and/or in particular a vector disclosed herein may be contained within a host cell capable of expressing the nucleic acid. Thus, the invention is also directed to a (recombinant) host cell which contains at least one vector as defined above. The host cell disclosed herein can also contain a nucleic acid as defined above. The host cell as used herein includes any host cells that can be used as expression systems to produce recombinant proteins in particular the TCR as described herein. Exemplary host cells are prokaryotic host cells, such as, but are not limited to, Escherichia coli (E. coli), Bacillus subtilis, and Salmonella typhimurium; and eukaryotic host cells including mammalian, avian, fungal, and insect cells. Various methods of introducing the vector into the host cell are known to persons skilled in the art, such as transformation, transfection, electroporation and biolistics. As will be further illustrated in the examples below, the constructs pTα-Bt5α and pTβ-Bt5β disclosed herein can be transformed into E. coli cells to express the respective TCRα and TCR β genes.

[0046] The TCRs expressed on the surface of the T cell are responsible for recognizing antigens, polypeptides, polysaccharides, bacteria and viruses. T cells belong to a group of T lymphocytes which play an important role for cell-mediated immunity. The two main subsets of T cells that are involved in cell-mediated immunity are the T helper (Th) cell and the cytotoxic T (Tc) cell. The Th cell expresses a CD4 glycoprotein on the surface to recognize an antigen and thereby activates the Th cell. Similarly, the cytotoxic T cell (Tc) expresses a CD8 glycoprotein on the surface to recognize and thus activates the Tc cell. Generally, the TCR expressed on the surface of the T cell includes an α chain and a β chain which are linked together by a disulphide bond.

[0047] The genetically modified animal described herein includes the nucleic acid that encodes a TCR α and β chain. Generally, the TCR α chain comprises a variable domain (V), a joining domain (J) and a constant domain (C). The TCR β chain comprises a variable domain (V), a diversity domain (D), a joining domain (J) and a constant domain (C) (see for example, Clements CS et al, Curr. Opin. Struct. Biol. 2006, 16, pp 787-95). hi some embodiments, the nucleotide sequence encoding the TCR domain includes the sequences Vα1+ Jα34 (SEQ ID NO: 1), Vβ3+Dβ2+ Jβ2.4 (SEQ ID NO:
2), Vα8+ Jα28 (SEQ ID NO: 3), Vα4+ Jα27 (SEQ ID NO: 4), Vβ4+Dβ2+Jβ2.7 (SED ID NO: 5) and Vβ6+Dβ1+Jβ2.4 (SEQ ID NO: 6). In some embodiments, the nucleotide sequence of the TCR α chain of the genetically modified animal includes the sequences of Vα1 and Jα34. In other embodiments, the nucleotide sequence of the TCR β chain of the genetically modified animal includes the sequences of VB3 and Jβ2.4. In this context, the genetically modified animal of the present invention also includes a nucleic acid encoding a TCR, wherein the TCR Vα11 and Vβ3 genes (TCR Val^α 11 + ) are expressed (Figure 9, Example 10). As will be further illustrated in the examples and in Figures 9 and 10, more than 90% and 50% of CD4+ and CD8+ T cells of the genetically modified mouse respectively expressed the TCR Vβ3 domain. Furthermore, the percentage of CD4+TCR Vα11+Vβ3+ T cells was significantly increased from 0.19% (in wild type) to 65.7% in the genetically modified mouse.

[0048] In some embodiments, the TCR specific for the immunogenic fragment of the mite allergen BIo t 5 has a "specific binding affinity" to a fragment of the mature BIo t 5 wild type sequence. The term "specific binding affinity" as used herein is determined by measuring the physiological response that a TCR expressing cell shows to the incubation of the cell with BIo t 5 or an immunogenic fragment thereof, and is usually determined in a suitable in vitro or in vivo assay, such as a proliferation assay. A TCR expressing cell that shows a desired physiological response above a particular (for example, predetermined) threshold value when brought in contact with a respective immunogenic fragment, thus comprises a TCR having specific binding affinity. A TCR having "specific binding affinity" to a fragment (peptide) of the mature BIo t 5 wild type sequence can for example refer to the T cell antigenic (peptide) specificity which is determined by a minimum threshold of TCR binding that must be achieved to activate the T cell (see for example, J.D. Stone et al, Immunology, Feb 2009, 162(2), 165-176). In this context, the "specific binding affinity" or the specificity of the TCR may be measured by mapping the TCR with a series of overlapping BIo t 5 peptides in a T cell proliferation assay which is known to persons of average skill in the art. Such T cell proliferation assay is described in Example 4 and also in V. von. Baehr et al, Journal of Immunological Methods, 251 (2001), 63-71. Other assays for detecting the specific binding affinity include, but are not limited to a cytometric analysis of the expression profile of T cell activation markers, T cell cytokine production profiling by ELISA, a

[0049] The Blo t 5 peptide or an immunogenic fragment thereof may be a recombinant Blo t 5 peptide. In this context, the term "recombinant and/or synthetic Blo t 5 peptide" used in the present invention refers to peptides shorter than the full length sequence of the mature Blo t 5 wild type sequence, for example, fewer than about 50, fewer than about 40, fewer than about 30, or fewer than about 20 amino acids and which can be generated using any technique well known to those of ordinary skill in the art such as recombinant DNA techniques as described in Goh LT, et al., Biotechnology Letters (2001);23:661-5. In some embodiments, the Blo t 5 peptide or an immunogenic fragment thereof can include a fragment that is shorter than the full length sequence of the mature Blo t 5 wild type sequence. The Blo t 5 or an immunogenic fragment thereof can include mutation(s) of the mature Blo t 5 wild type sequence. Such mutations can include substitutions, deletions and also insertions that can be introduced into the mature amino acid sequence as long as the resulting polypeptide folds into a three-dimensionally stable structure. In further embodiments, the Blo t 5 peptide or an immunogenic fragment thereof may include any overlapping amino acid residues that cover the full length of the mature Blo t 5 wild type sequence. Examples of the overlapping amino acid residues of Blo t 5 may include, but are not limited to Blo t 5 fragments that comprise or consist of amino acid residue 1 to 45 of the mature Blo t 5 wild type sequence, amino acid residue 40 to 80 of the mature Blo t 5 wild type sequence and amino acid residue 70 to 117 of the mature Blo t 5 wild type sequence.

[0050] Any overlapping amino acid residues that cover the full length of the mature Blo t 5 wild type sequence can be used in the context of the present invention. The Blo t 5 peptide or an immunogenic fragment thereof may comprise or consist of amino acid residue 1 to 16 of the mature Blo t 5 wild type sequence, amino acid residue 4 to 19 of the mature Blo t 5 wild type sequence, amino acid residue 7 to 22 of the mature Blo t 5 wild type sequence, amino acid residue 10 to 25 of the mature Blo t 5 wild type sequence, amino acid residue 13 to 28 of the mature Blo t 5 wild type sequence, amino acid residue 16 to 31 of the mature Blo t 5 wild type sequence, amino acid residue 19 to 34 of the mature Blo t 5 wild type sequence, amino acid residue 22 to 37 of the mature
BiO t 5 wild type sequence, amino acid residue 25 to 40 of the mature BiO t 5 wild type sequence, amino acid residue 28 to 43 of the mature BiO t 5 wild type sequence, amino acid residue 31 to 46 of the mature BiO t 5 wild type sequence, amino acid residue 34 to 49 of the mature BiO t 5 wild type sequence, amino acid residue 37 to 52 of the mature BiO t 5 wild type sequence, amino acid residue 40 to 55 of the mature BiO t 5 wild type sequence, amino acid residue 43 to 58 of the mature BiO t 5 wild type sequence, amino acid residue 46 to 61 of the mature BiO t 5 wild type sequence, amino acid residue 49 to 64 of the mature BiO t 5 wild type sequence, amino acid residue 52 to 67 of the mature BiO t 5 wild type sequence, amino acid residue 55 to 70 of the mature BiO t 5 wild type sequence, amino acid residue 58 to 73 of the mature BiO t 5 wild type sequence, amino acid residue 61 to 76 of the mature BiO t 5 wild type sequence, amino acid residue 64 to 79 of the mature BiO t 5 wild type sequence, amino acid residue 67 to 82 of the mature BiO t 5 wild type sequence, amino acid residue 70 to 85 of the mature BiO t 5 wild type sequence, amino acid residue 73 to 88 of the mature BiO t 5 wild type sequence, amino acid residue 76 to 91 of the mature BiO t 5 wild type sequence, amino acid residue 78 to 93 of the mature BiO t 5 wild type sequence, amino acid residue 82 to 97 of the mature BiO t 5 wild type sequence, amino acid residue 86 to 101 of the mature BiO t 5 wild type sequence, amino acid residue 90 to 105 of the mature BiO t 5 wild type sequence, amino acid residue 94 to 109 of the mature BiO t 5 wild type sequence, amino acid residue 98 to 113 of the mature BiO t 5 wild type sequence and amino acid residue 102 to 117 of the mature BiO t 5 wild type sequence.

[0051] In one embodiment, the TCR specific for the immunogenic fragment of the mite allergen BiO t 5 has a specific binding affinity to the fragment of amino acid residue 40 to 80 of the mature BiO t 5 sequence, amino acid residue 55-70 of the mature BiO t 5 wild type sequence and amino acid residue 58-73 of the mature BiO t 5 wild type sequence.

[0052] The term "immunogenic", as used herein refers to the capability of evoking an immune response, i.e. of being immunologically active. Accordingly, when used in the present invention, a BiO t 5 or a fragment thereof may in some embodiments in itself be able to cause an immune response, for instance when administered to an individual or animal. It should however be noted that an immunogenic
fragment of an allergen of BIo t 5 need not in itself possess the capability of evoking an immune response. Its capability of being immunologically active may in some embodiments rather depend on the fragment being coupled to additional matter. In some embodiments this coupling to additional matter may for instance occur in vivo, for example by binding to a protein. Thus in some embodiments an immunogenic fragment of an allergen of BIo t 5 may includes, or is, a hapten, which needs to be coupled to a carrier molecule or to a cell in order to show its immunogenic properties. In typical embodiments the BIo t 5 or the fragment thereof is capable of binding to at least one IgE antibody. A respective antibody may for instance be an antibody of an individual or an animal being allergic or sensitive to mites, such as dust mites. Generally, a fragment of BIo t 5 includes at least one epitope. In some embodiments, however, two or more fragments of BIo t 5 may need to be combined to obtain a respective epitope, for instance when coupled to the same carrier molecule. An epitope, also called an antigenic determinant, is a part of an antigen molecule - in this case a BIo t 5 molecule - that can be recognized and bound by an antigen-binding site of an antibody or by a T-cell receptor. Different antibodies and T-cell receptors bind to different epitopes of an antigen.

[0053] The immunogenic fragment of the mite allergen BIo t 5 may include, or is, a polypeptide. By "fragment" in reference to BIo t 5 is meant any amino acid sequence present in the mature and fully active allergen BIo t 5 which is deposited in the UniProtKB/Swiss-Prot database (accession number 096870). The mature and fully active allergen BIo t 5 has 134 amino acids. In some embodiments, the term "fragment" refers to the absence of posttranslational modifications, such as a saccharide or saccharide chain, which are present in BIo t 5. In such embodiments, the amino acid sequence of BIo t 5 may be of any length, whether the entire length or a part of the full length wild type sequence, including a variant, or a mutation of BIo t 5 wild type sequence. In other embodiments the term "fragment" refers to any amino acid sequence present in a polypeptide of BIo t 5 that is shorter than the full length wild type sequence of BIo t 5.

[0054] Apart from the genetically modified animal, in addition, the present invention provides an animal obtained by reproduction of the genetically modified animal as described above. The animal may be reproduced by breeding the genetically modified animal with a wild type animal. In some embodiments, the animal obtained by
reproduction can include any and all animals of all future generations derived therefrom. Therefore, the animal obtained according to the present invention can include a heterozygous or a homozygous animal. The term "homozygous" as used in reference to an animal refers to the state of an animal having two identical alleles at a given locus on homologous chromosomes. The term "heterozygous" as used in reference to an animal refers to the state of an animal having two different alleles at a given locus on homologous chromosomes. In some embodiments, the animal or the wild type animal used in the context of the present invention can include any animal strains and sub¬strains. The animal obtained according to the present invention can be a mammal. The mammal can include a rodent, Canis, Ungulate, Felidae, Leporidae, and Macaque, In some embodiments, the rodent can be a mouse. Different strains and sub¬strains of the mouse can be used according to the present invention. Examples of such strains include, but are not limited to C57BL/6 and Balb/c strains. The sub¬strains of Balb/c mice include Balb/cJ and Balb/cByJ sub¬strains. In some embodiments, the animal used in the context of the invention may be a C57BL/6 mouse.

[0055] The term "breeding" as used herein generally refers to sexual propagating or mating to generate offspring. In this context, an animal obtained by the reproduction of the genetically modified animal may or may not contain a nucleic acid encoding a TCR specific for the immunogenic fragment of the mite allergen B1o t 5. In some embodiments, the animal obtained by the reproduction of the genetically animal can comprise a nucleic acid encoding a TCR, wherein the TCR Vα1 l and Vβ3 genes are expressed (see Figure 10, Example 10). As illustrated in Figure 10, a significant increase in the level of TCR Vα1 l and VB3 chains (98.2%) were expressed in the CD4+ and CD8+ T cells of the offspring of the genetically modified animal.

[0056] The present invention also provides a method of obtaining a genetically modified animal comprising recombinantly introducing a nucleic acid encoding a TCR into an animal, wherein the TCR is specific for an immunogenic fragment of the mite allergen B1o t 5. Various methods of introducing the nucleic acid into an animal for generating a genetically modified animal are known to persons skilled in the art. These methods may include, but are not limited to, microinjection of a desired nucleic acid into fertilized oocytes, injection of genetically engineered embryonic stem cells containing the
desired nucleic acid into fertilized embryos, direct injection of a desired nucleic acid into
muscle tissue and Intra Cytoplasmic Sperm Injection (ICSI). As an illustrative example, the nucleic acid disclosed herein can be introduced into a fertilized egg of the animal by co-injection. The nucleic acid disclosed herein may be in the form of a linearised DNA that does not contain any prokaryotic sequences.

[0057] The method of obtaining a genetically modified animal may also include, prior to introducing the nucleic acid into the animal, administering to an animal a BIo t 5 peptide or a fragment thereof, isolating T cells specific for an immunogenic fragment of the mite allergen BIo t 5 from the splenocyte or lymphocyte of the animal or a genetically modified animal and isolating a nucleic acid encoding a TCR specific for an immunogenic fragment of the mite allergen BIo t 5 from the T cell. The animal may be stimulated with a BIo t 5 peptide by sensitization methods such as, but are not limited to subcutaneous, intraperitoneal, epicutaneous, intradermal, intranasal and intravenous sensitization methods. As illustrated in Figure 1, BIo t 5 sensitized mice showed elevated levels of IgE and IgG antibody responses (Figure 1A) and elevated production of cytokines (Figure 1B). The T cells specific for an immunogenic fragment of the mite allergen BIo t 5 may be isolated from the animal by extracting the splenocytes of the sensitized animal. The TCR α and β domains of the T cells may be analysed by flow cytometry methods so that the nucleotide sequences of the TCR α and β domains can be isolated. As an illustrative example, the TCR β gene domains characterized by flow-cytometry analysis are shown in Figure 5. The nucleic acid encoding a TCR specific for an immunogenic fragment of the mite allergen BIo t 5 may be isolated from the isolated T cells by various methods such as PCR amplification. The skilled artisan will thus appreciate that other methods of isolating the nucleic acid from the T cells may be employed.

[0058] The present invention further provides a method of generating a T cell line specific for an immunogenic fragment of the mite allergen BIo t 5. The method includes administering to an animal a BIo t 5 peptide or a fragment thereof, isolating the T cell specific for an immunogenic fragment of the mite allergen BIo t 5 from the splenocyte of the animal and further administering to the isolated T cell the BIo t 5 peptide. In some embodiments, the isolated T cell may be stimulated with a recombinant cytokine after
each being stimulated with the recombinant BIo t 5 peptide. As will be illustrated in the examples below, a long term T cell culture specific for an immunogenic fragment of the mite allergen BIo t 5 is established (Figure 2).

[0059] The present invention also provides a method of immunizing an animal. The method includes administering a T cell specific for an immunogenic fragment of the mite allergen BIo t 5. The T cell specific for the immunogenic fragment of the mite allergen BIo t 5 may for example, be obtained from the genetically modified animal according to the present invention. In some embodiments, the T cell specific for an immunogenic fragment of the mite allergen BIo t 5 may be administered into the animal by intravenous injection. The immunization method may also include stimulating the animal with a BIo t 5 peptide or a fragment thereof. As explained above, the BIo t 5 peptide may be administered into the animal by epicutaneous, intradermal, intranasal, (inhalation), intravenous, subcutaneous or intraperitoneal administration. As illustrated in Figure 4, mice immunised with BIo t 5 specific Th 2 cells followed by administration of BIo t 5 peptides resulted in the induction of lung inflammation in mice.

[0060] The genetically modified animal of the present invention may be used for analysis and studies of various mechanisms relating to hypersensitivity and/or inflammatory reactions and is clinically relevant for the study of human allergic diseases. The genetically modified animal can for example, be used to study mechanisms relating to airway responsiveness by administering to the genetically modified animal a BIo t 5 peptide or a fragment thereof. The genetically modified animal is thus a useful tool to study allergic diseases including asthma. Therefore, the genetically modified animal disclosed herein may also be used for testing therapeutic agents including vaccines and drugs targeted against human allergic diseases such as BIo t 5 related allergic diseases. The genetically modified animal is thus a useful model to test the efficacy and predict the outcome of such therapeutic agents. The present invention provides a method of testing the efficacy of a therapeutic agent for an allergic disease. This method includes administering to a genetically modified animal or an animal as described above, the therapeutic agent. The present invention also provides a method for testing the suitability of a compound or identifying a compound being suitable for the treatment of an allergic disease. This method includes administering to a genetically modified animal or an
animal as described above, the compound. The term "therapeutic agent" or "compound"
can be used interchangeably and refers to an agent or remedial agent relating to the
treatment or prevention of allergic diseases or disorders. Examples of therapeutic agents
or compounds may include vaccines, antibodies, small molecules purified from natural
sources, synthetic small molecules, non-steroidal anti-inflammatory drugs and anti-
histamines. The term "allergic diseases" as used herein refer to diseases including
rhinitis, conjunctivitis, atopic dermatitis, atopic asthma and bronchial asthma. The
allergic diseases may include allergies caused by the dust mite allergen such as *Blomia
tropicalis* (BIO t).

**EXEMPLARY EMBODIMENTS OF THE INVENTION**

[0061] Exemplary embodiments of a method of obtaining a genetically modified
animal according to the present invention, a method of generating a T cell line specific
for an immunogenic fragment of the mite allergen, a method of immunising an animal
with the T cell line and a method for developing airway hyper-responsiveness in the
genetically modified animal of the present invention are shown in the appended figures.

[0062] Figure 1A depicts the kinetics of BIO t 5 specific IgE (ng/ml) and IgGl
(µg/ml) antibody responses in C57BL/6 mice sensitized epicutaneously with recombinant
BIO t 5 as described in Example 3 (BIO t 5 is indicated by shaded portion) compared with
mice sensitized with phosphate buffered saline (PBS is indicated by clear portion).

[0063] Figure 1B shows a graphic representation of the cytokines profiles of
splenocytes of BIO t 5 sensitized mice as described in Example 3 (shaded portion)
compared with mice sensitized with PBS as a control (PBS indicated as clear portion).
The concentrations of cytokines IL-4, IL-5, IL-13 and IFN-γ (pg/ml) were determined by
sandwich ELISA. The splenocytes of BIO t 5 sensitized mice show elevated production of
Th2 cytokines such as IL-4, IL-13, IL-5 as well as Th1 cytokine, IFN-γ.

[0064] Figure 2 depicts a graphic representation of the cytokine profiles of a
long-term cultured BIO t 5 specific Th2 cell line. The long-term cultured BIO t 5 specific
Th2 cell line was established after repeatedly stimulating the cells with BIO t 5 in vitro for
six months as described in Example 3. To measure the cytokine profiles of the cultured
BIO t 5 specific Th2 cell line, the BIO t 5 specific T cells were stimulated with 10µg/ml of
BIO t 5 for 72 hours and the culture supernatants were collected for analysis by cytokine
ELISA. This cell line produced high amount of IL-4, IL-5, IL-13 as well as IL-10 but with undetectable IFN-γ (pg/ml and ng/ml).

[0065] **Figure 3A** depicts a graphic representation showing the specificity of a BIo t 5 specific Th2 cell line as described in Example 3 to recombinant BIo t 5 peptides as described in Example 1, using a T cell proliferation assay as described in Example 4. In Figure 3A, the BIo t 5 specific T cells were stimulated with 0.7µM of BIo t 5 (indicated as BIo t 5), GST-fused BIo t 5 overlapping fragments (indicated as "GST-BIo t 5 F1", "GST-BIo t 5 F2" and "GST-BIo t 5 F3") or an irrelevant antigen as control (indicated as GST) (without BIo t 5) for 2 days. The [³H]-thymidine was added and the cells were cultured for another 18 hours. The BIo t 5 specific T cells showed positive response (about 15,000 cpm: counts per minute) to the recombinant GST-BIo t 5 F2 (amino acid residue 40-80 of the mature BIo 15 wild type sequence).

[0066] **Figure 3B** also depicts a graphic representation showing the specificity of the BIo t 5 specific T cell line as described in Example 3 to thirty-three 16 a.a.-length overlapping synthetic peptides as described in Example 1, using a T cell proliferation assay as described in Example 4. In Figure 3B, the BIo t 5 specific T cells were stimulated with 3.5µM of the 16 overlapping synthetic peptides for 2 days. The [³H]-thymidine was added and the cells were cultured for another 18 hours. The BIo t 5 specific T cells reacted strongly to peptide amino acid residues 55-70 (about 12,000 cpm) and showed mild reactivity to peptide amino acid residues 58-73 of the mature BIo t 5 wild type sequence (about 2000 cpm).

[0067] **Figure 4A** depicts a graphic representation of the induction of lymphocytes ("Lym"), monocytes ("Mono"), Neutrophils ("Neu") and eosinophils ("Eos") *:* /?<0.05infiltration in the lungs of mice that received BIo t 5 specific T cells transfer and BIo t 5 challenge. Naïve C57BL/6J mice received intravenous injection with 2.5* 10⁶ of BIo t 5 specific T cells (shaded portion: BIo t 5) or PBS alone (white portion: control) at day 0. All mice received 20 minutes aerosol challenge of 0.1% BIo t 5 at days 1, 2 and 3. The bronchoalveolar lavage fluids (BALF) and lungs were collected at day 5. As shown in Figure 4A, naive mice received cell transfer of BIo t 5 specific Th2 cells showed significantly cellular infiltration as compared to the control mice (cell number (x10^3)). Differential cell counts showed that the most dominant cells were eosinophils.
("Eos"). In addition, the number of lymphocytes was significantly increased in the BAL fluid of mice which received Th2 cell transfer.

[0068] Figure 4B depicts a microscopic image of the lungs of mice which showed eosinophils infiltration and mucus production after receiving BLO t 5 specific T cells transfer and BLO t 5 challenge. Naive C57BL/6J mice received intravenous injection with 2.5x10⁶ of BLO t 5 specific T cells (indicated as "Bt5/Bt5") or PBS alone (indicated as PBS/Bt5) at day 0. All mice received 20 minutes aerosol challenge of 0.1% BLO t 5 at days 1, 2 and 3. The bronchoalveolar lavage fluids (BALF) and lungs were collected at day 5. The lung histopathology showed the mucus production of goblet cells in the mice that received BLO t 5 specific Th2 cells transfer (Bt5/Bt5). (PAS: Periodic acid-Schiff staining, original magnification 200x). Histological examination also demonstrated the existence of perivascular and peribronchial eosinophilic inflammation in the lungs of mice receiving BLO t 5 Th2 cell transfer (Bt5/Bt5).

[0069] Figure 5A shows the characterization of TCR β gene usage of BLO t 5 T cell line as described in Example 3 of the variable region (V) of the TCR β gene by flow-cytometry analysis. Flow cytometry analysis demonstrated that the BLO t 5 cell line expressed Vβ3 (87%) and the rest of the cells expressed Vβ4 (9.8%) and Vβ6 (0.8%).

[0070] Figure 5B shows the characterization of the joining region (J) of the Vβ3+ BLO t 5 specific T cells by PCR amplification by using Vβ3 specific primer paired with 12 Jβ specific primers as indicated in Table 1. Example 6. The PCR analysis demonstrated that the Vβ3+ BLO t 5 specific T cells used only the Jβ2.4 region of the TCR.

[0071] Figure 6 depicts the PCR product of the TCR α gene of ~800bp (indicated by arrow) on a DNA electrophoresis gel. The TCR α gene was isolated from Vβ3+ BLO t 5 specific T cells. To identify the variable region of the TCR α gene of Vβ3+ BLO t 5 specific T cells, 5’RACE of TCR α gene was performed by using the SMART™ RACE cDNA amplification kit (Clontech) as described in Example 7.

[0072] Figure 7A depicts the PCR product of the rearranged VαJα gene amplified from the genomic DNA of BLO t 5 specific T cells on a DNA electrophoresis gel. M indicates the marker for 100bp ladder DNA. The most dominant usage of the TCR α gene, namely Va 11 and Jα34 were selected for the construction of the TCR gene expression vector. The rearranged VαJα were then PCR amplified from the genomic
DNA of the BIo t 5 specific T cells. PCR product of size ~620bp for TCR Va11 and Jα34 was obtained.

[0073] Figure 7B indicates the PCR product of the rearranged VβDβJβ gene amplified from the genomic DNA of BIo t 5 specific T cells on a DNA electrophoresis gel. The most dominant usage of the TCR β gene, namely Vβ3 and Jβ2.4 were selected for the construction of the TCR gene expression vector. A PCR product of size ~580bp for TCR Vβ3 and Jβ2.4 gene was obtained (Figure 7B).

[0074] Figure 8A shows the construct of pTα-Bt5α (right vector) obtained by cloning the TCR α gene into Xmal/NolI region of the PTα cassette vector (left vector). The cloning method of the TCRα gene into the PTα cassette vector is described in Example 8.

[0075] Figure 8B shows the construct of pTβ-Bt5β (right vector) obtained by cloning the TCR β gene into the Xhol/Clal region of the PTβ cassette vector (left vector). The cloning method of the TCRβ gene into the PTβ cassette vector is described in Example 8.

[0076] Figure 9 shows the expression levels of the TCRVa +11Vβ3+ in the peripheral T cells of the genetically modified C57BL/6 mice according to an embodiment of the present invention (indicated as "Bt5 Tg" in Figure 9A) compared to wild type C57BL/6 mice (indicated as "wt" in Figure 9B). The expression of the TCRVαllV β3 in the genetically modified mouse was analysed by flow cytometry and such methods can be described in Sha C. et al, Nature 335, (1988), 271-274. In Figure 9, a reduction in the percentage of CD8+ T cells was observed (from 10.1% of the wild type mouse to 4.38% of the genetically modified mouse according to one embodiment of the present invention). This result indicates that the nucleic acid specific for BIo t 5 successfully induced the selection of CD4+ T cells (for example, Vα11 and Vβ3) in the thymus of the genetically modified mouse. Furthermore, more than 90% and 50% of CD4+ and CD8+ T cells respectively expressed the TCR Vβ3 (see Figure 9A). The percentage of CD4+TCR α11+Vβ3+ T cells was significantly increased from 0.19% (in wild type) to 65.7% in the genetically modified mouse.

[0077] Figure 10 shows the expression levels of the CD4+ TCRVa +11Vβ3+ T cells obtained from the peripheral blood of a mouse reproduced by breeding a genetically
modified mouse with a wild type. Both the α and β genes of the TCR were transmitted successfully to the mouse. The percentage of CD4+TCRV α1+Vβ3+ T cells in the mouse was 98.2%.

[0078] Figure 11 shows the genomic DNA sequence encoding the Vα1+Jα34 domain which is identified as (SED ID NO: 1), and the genomic DNA sequence of the encoding the Vβ3+Dβ2+Jβ2.4 which is identified as (SED ID NO: 2).

[0079] Figure 12 shows the cDNA sequence encoding the Vα8+Jα28 domain which is identified as (SEQ ID NO: 3); cDNA sequence encoding the Vα4+Jα27 domain which is identified as (SEQ ID NO: 4); cDNA sequence encoding the Vβ4+Dβ2+Jβ2.7 domain which is identified as (SEQ ID NO: 5); and cDNA sequence encoding the Vβ6+Dβ1+Jβ2.4 domain which is identified as (SEQ ID NO: 6).

[0080] Figure 13 shows the airway hyper-responsiveness model of a genetically modified mouse according to the present invention. The experimental procedure is described in Example 11. Intranasal administration (i.n.) of BIo t 5 induced the genetically modified mouse to develop airway hyper-responsiveness (AHR). The genetically modified mouse (indicated as "Tg") or the genetically modified negative littermates (indicated as "WT") received 50 µg of BIo t 5 (indicated as Bt5) or PBS intranasally for 3 consecutive days. The airway responsiveness was measured on day 4 by the non-invasive whole-body plethysmography as described in Example 11. Data represent ± SEM. *: P < 0.05 as compared with the other 3 groups with the 2-tailed Mann-Whitney U Test. The genetically modified mice (indicated as "shaded square: Tg_Bt5") developed airway-responsiveness on day 4 after 3 times of intranasal BIo t 5 administrations. The airway responsiveness of the genetically modified mice that received the PBS treatment (indicated as "unshaded square: Tg_PBS") and the genetically modified negative littermates (indicated as unshaded triangle: "WT_PBS" and shaded triangle: "WT_Bt5") remained normal under this experimental procedure.

[0081] Figures 14A and 1B show the representative Fluorescence Activated Cell Sorting (FACS) plots indicating the staining of transcription factor GATA-3 (Figure 14A) and transcription factor T-bet (Figure 14B) in CD4+T cells obtained from the genetically modified mice as described in Example 12.

[0082] Figures 14C-F show the representative FACS plots indicating the
proportion of eosinophils (indicated as "EOS") and macrophages (indicated as "MAC") in Bio t 5 adoptive hosts that were challenged intranasally (i.n.) with Bio t 5 peptide. Figure 14C shows the proportion of EOS and MAC in bronchoalveolar lavage fluids (BALF) of mice following adoptive transfer of Th2 cells isolated from the genetically modified mice according to the present invention into wild type recipient mice and challenge with Bio t 5 peptide. Figure 14D shows the proportion of EOS and MAC in BALF of mice following adoptive transfer of Th2 cells isolated from the genetically modified mice according to the present invention into wild type recipient mice and challenge with Bio t 5 peptide. Figure 14E shows the proportion of EOS and MAC in lung tissue of mice following adoptive transfer of Th2 cells isolated from the genetically modified mice according to the present invention into wild type recipient mice and challenge with Bio t 5 peptide. Figure 14F shows the proportion of EOS and MAC in lung tissue of mice following adoptive transfer of Th2 cells isolated from the genetically modified mice according to the present invention into wild type recipient mice and challenge with saline (control).

[0083] Figure 15 shows the eosinophil and neutrophil infiltration into BALF and lung tissues of CD4+ T cell adoptive transfer recipient mice according to the present invention after Bio t 5 challenge. The eosinophil and neutrophil infiltration into BAL fluids and lung tissues of CD4+ T cell adoptive transfer recipient mice after Bio t 5 challenge were studied using FACS. Cell infiltration into BAL fluids and lung tissues expressed as obtained using FACS and cell numbers obtained by multiplying the percentages by the total number of cells isolated from the BAL fluid and lung tissues of the individual mice. Results are expressed as the mean ± stem, from 4 mice * denotes p < 0.05 between the groups indicated, Student's t test.

[0084] Figure 16 measures the airway hyperreactivity in CD4+ T cell adoptive transfer recipient mice according to the present invention followed by Bio t 5 challenge (indicated as circle: "Th2-BT5"); compared to CD4+T cell adoptive transfer recipient mice followed by PBS (indicated as triangle: "Th2-PBS") and control mice. The control mice did not receive CD4+T cells or challenge by Bio t 5. Airway resistance induced in the mice after the administration of different doses of methacholine was measured and expressed as a percentage of baseline airway resistance level. The results are expressed as
the mean ± s.e.m.

[0085] Figure 17 shows the Hematoxylin and eosin (H&E) staining of lung sections. Figures 17A and B shows the H&E staining of the lung sections of adoptive transfer recipient mice according to the present invention. 3 million of the 2-weeks cultured cells were injected intravenously (i.v.) into the mice and challenged intranasally (i.n.) with BIo t-5 protein for 3 consecutive days and culled on day 4. The cellular infiltrates recruited to the periphery of the airways (denoted by arrows). Figures 17 C and D shows H&E staining of the lung sections of control mice which were injected and challenged with sterile PBS. Note that the cellular infiltrates are absent in the control mice. n=2 for all groups.

[0086] Figure 18 shows the Periodic acid-Schiff (PAS) staining of lung sections. Figure 18A and B shows PAS staining of the lung sections of control mice which were injected and challenged with sterile PBS as observed at 2OX and 4OX magnification respectively. The mucus hypersecreting goblet cells are absent in these control mice. Figures 18C and D shows PAS staining of the lung sections of adoptive transfer recipient mice according to the present invention as observed at 2OX and 4OX magnification respectively. 3 million of the 2-weeks cultured cells were injected intravenously (i.v.) into the mice and challenged intranasally (i.n.) with BIo t 5 protein for 3 consecutive days and culled on day 4. Note the mucus hypersecreting goblet cells which are stained more darkly (denoted by arrows). Each section was taken from different mice within the same experimental group. n=2 for all groups.

EXAMPLES

[0087] Mice used in the following illustrative examples were C57BL/6 mice, 6-10 weeks of age, from the Animal Holding Unit, National University of Singapore.

[0088] Statistical comparisons were performed by analysis of means using Student's t-test. All values are shown as mean ± standard deviation (SD). A value of p<0.05 was regarded as significant.

Example 1: Preparation of BIo 15 proteins and peptides

[0089] Stocks of recombinant BIo t 5 used in the following examples were prepared as described previously in Goh LT, et al., Biotechnology Letters (2001);23:661-5. The cDNA of the three overlapping BIo t 5 fragments, which correspond to amino acid
residue 1-45 of the mature BIo t 5 wild type sequence, amino acid residue 40-80 of the mature BIo t 5 wild type sequence and amino acid residue 70-117 of the mature BIo t 5 wild type sequence, were amplified by PCR from the full length BIo t 5 cDNA. The PCR products were cloned into the pGEX-4T expression vector and transformed into E. coli TG-I cells. The GST fused BIo t 5 fragments were purified by glutathione agarose beads from the bacteria lysate after inducing the protein expression by IPTG. A panel of thirty-three 16 aa-length synthetic peptide arrays in PeptSets™ format and the HPLC grade synthetic peptides (80% purity) were custom made by Mimotopes (Australia). The thirty-three 16aa-length synthetic peptides are illustrated in Figure 3B.

**Example 2: Preparation of Antibodies**

[0090] Monoclonal antibodies (mAbs) used in ELISA were rat antibodies to mouse IL-4 (BVD4-1D1), IL-5 (TRFK5), IL-10 (JES052A5; R&D systems (Minneapolis, USA)), IL-13 (38213, R&D systems), IFN-γ (R4-6A2), TNF-α (AF-410-NA, R&D systems) and biotin-conjugated rat antibodies to mouse IL-4 (BVD6-24G2), IL-5 (TRFK4), IL-10 (BAF417; R&D systems), IL-13 (R&D systems), IFN-γ (XMG1.2), TNF-α (BAF410; R&D systems). The recombinant mouse IL-4, IL-5, IL-10 (R&D systems), IL-13 (R&D systems), TNF-α (R&D systems), IFN-γ were used as standards in sandwich ELISA. mAbs used for flow cytometry were PerCP-conjugated anti-CD3ε (145-2C11), PE-conjugated anti-CD4 (RM4.5). The Vb gene usage were analysed by flow cytometry using the mouse Vβ TCR screening panel. All mAbs and reagents were purchased from PharMingen (San Diego, CA) unless otherwise specified. All the antibodies used for detection of cytokines in the cultured T cell supernatant were purchased from PharMingen. The ELISA was performed as described in Huang CH, et al., *J Invest Dermatol.* (2003); 121:289-93.

**Example 3: Generation and Maintenance of BIo 15 specific T cell line**

[0091] Mice were sensitized epicutaneously with recombinant BIo t 5 as described previously as described in Huang CH, et al., *J Invest Dermatol.* (2003); 121:289-93, with the exception that the allergen Der p 8 used by Huang CH, *et al.* was replaced with BIo t 5 in this example.
[0092] Naive C57BL/6 mice were patched with 50 µg of BIo t 5 for 3 times with 2 weeks interval. One week after the last patch, splenocytes were collected and simulated with recombinant Blot 5. The splenocytes were cultured in 24-well plates with RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, antibiotics (IU/ml penicillin and 100 µg/ml streptomycin) (HyClone Laboratories, Logan, UT), 1mM sodium pyruvate and 5.5X10^(-2) mM 2-mercaptoethanol (Life technology, Grand Island, NY). The splenocytes were cultured in the presence of BIo t 5 (10µg/ml). Recombinant IL-2 (Pharmingen) was added to the cultured cell on day 3 and day 5 to a final concentration of 1OU/ml. On day 7, the living cells were harvested by Ficoll-Paque (Amersham Bioscience) density gradient centrifugation and were re-stimulated with 10µg/ml of BIo t 5 in the presence of mitomycin C treated splenocytes of naive mice as antigen presenting cells (APCs). To generate and maintain a long-term cultured BIo t 5 specific T cell line, the cells were stimulated repeatedly with BIo t 5 as described above weekly. The recombinant IL-2 was added to the cultured cell on day 3 and day 5 after each BIo t 5 re-stimulation.

[0093] A significant level of BIo t 5 specific IgE and IgGl but with undetectable IgG2a was seen in BIo t 5 sensitized mice as shown in Figure IA. As shown in Figure IB, splenic cells of BIo t 5 sensitized mice showed elevated production of Th2 cytokines such as IL-4, IL-5, IL-13 as well as Th1 cytokine such as IFN-γ. The methods for detecting BIo t 5 specific antibodies are described in Lieden A, et al., Allergy. (2009);64:304-11, with the exception that Der p 2 used by Lieden A, et al. was replaced by BIo t 5 in this example.

[0094] Therefore, a long-term cultured BIo t 5 specific Th2 cell line was established after repeatedly stimulating the cells with BIo t 5 in vitro for six months. As shown in Figure 2, this cell line produced high amount of IL-4, IL-5, IL-13 as well as IL-10 but with undetectable IFN-γ.

**Example 4: Epitope mapping of BIo 15 specific T cell line**

[0095] The specificity of the BIo t 5 specific T cell line as described in Example 3 was examined by a T cell proliferation assay. In the assay, 5x10^4 of BIo t 5 specific T cells were cultured with 0.7 µM recombinant BIo t 5, GST-BIo t 5(I_{4y}), GST-BIo t 5(I_{4y}^o)
GST-BIo t 5(70-117), GST or with 3.5 µM synthetic peptides in the presence of 2x10^5 antigen presenting cells (APCs) for 48 hours. 0.1µCi/well of [3H]-thymidine was added into each well and the cells were cultured for another 18 hours and the cells were harvested.

[0096] The specificity of the cell line was examined by T cell proliferation assay. By using the three overlapping BIo t 5 fragments, namely amino acid residue 1-45 ("GST-BIo t 5 Fl"), amino acid residue 40-80 ("GST-BIo t 5 F2") and amino acid residue 70-117 ("GST-BIo t 5 F3"), BIo t 5 specific T cells showed positive response to the GST-BIo t 5 F2 BIo t 5 fragment (a.a. 40-80) only (Fig 3A). The fine epitope mapping of the BIo t 5 specific T cell line was performed by using the thirty-three 16 a.a.-length overlapping synthetic peptides. The BIo t 5 specific T cells reacted strongly to synthetic peptide a.a 55-70 and showed mild reactivity to peptide a.a 58-73 (Fig 3B). No positive response was shown in the remaining peptides.

**Example 5: Cell transfer study and airway inflammation induced by BIo 15 specific Th2 cells**

[0097] Long-termed (>10 months) cultured Vβ3+ BIo t 5 specific Th2 cells were harvested 7 days after restimulation with BIo t 5 and washed in sterile PBS. 2.5x10^6 cells in 150µl of PBS. The cells were transferred to recipient mice by intravenous (i.v.) injection into the tail vein. The control mice received i.v. injection of PBS only. The day after cell transfer, all mice receive 20 minutes aerosol inhalation of 0.1% BIo t 5 for 3 consecutive days. The bronchoalveolar lavage (BAL) fluid was collected at one or two days after the last aerosol challenge. To collect the BAL fluid, mice were lavaged three times with 0.8 ml of Hank’s balanced salt solution without calcium and magnesium through tracheostomy. Differential cell counts were performed with cytopsin preparations stained by Liu's stain.

[0098] As shown in Figure 4, naive mice received cell transfer of BIo t 5 specific Th2 cells showed significant cellular infiltration as compared to the control mice which received cell transfer of CD4+ cells from naïve mice (Fig 4A). Differential cell counts showed that the most dominant infiltrated cells were eosinophils. In addition, the number of lymphocytes was significantly increased in the BAL fluid of the mice which received
Th2 cell transfer.

[00099] For histological examination, specimens were obtained from the patched skins at day 50 after sensitization and fixed in 10% buffered neutral formalin immediately. After embedding in the paraffin, sections of 4μm were cut and stained with hematoxylin and eosin. As shown in Figure 4B, histological examination demonstrated the existence of perivesicular and peribronchial eosinophilic inflammation and the increment of mucus production in the lungs of mice receiving Bio t 5 specific Th2 cell transfer.

**Example 6: Analysis of TCR β gene**

[00100] To characterize the TCRβ gene usage of Bio t 5 specific T cells, the cells were stained with a panel of FITC-labeled Vβ specific mAbs (Pharminingen) and analyzed by flow cytometry. To analyze the usage of TCRβ gene of Bio t 5 specific T cells, total RNA was prepared from Bio t 5 specific T cells by using RNeasy Mini kit (Qiagen). Two micrograms of total RNA and 1μg of oligo d(T)18 primer was used to synthesize the first strand cDNA by using MMLV-reverse transcriptase (Promega) at 42°C for 1hr according to the manufacturer's instruction. The polymerase chain reaction (PCR) was performed by using the Vβ3 specific primer (sense) paired with 12 Jβ specific primers (anti-sense). The primers used to synthesize the variable region of the TCR β gene is shown in Table 1 in the following.

**Table 1. Primers used for PCR amplification of TCR β gene**

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5' → 3')</th>
<th>SED ID NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCR (Vβ1.1)</td>
<td>cgactccg[aggccaccaatgctacaagct]</td>
<td>7</td>
</tr>
<tr>
<td>TCR (Vβ1.1)</td>
<td>cgactccgattatactcaacatgtaatgagtcgcttcc</td>
<td>8</td>
</tr>
<tr>
<td>TCR (Jβ1.2)</td>
<td>cgactccgattatactcatatcagctaggtcc</td>
<td>9</td>
</tr>
<tr>
<td>TCR (Jβ1.3)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>10</td>
</tr>
<tr>
<td>TCR (Jβ1.4)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>11</td>
</tr>
<tr>
<td>TCR (Jβ1.5)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>12</td>
</tr>
<tr>
<td>TCR (Jβ1.6)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>13</td>
</tr>
<tr>
<td>TCR (Jβ2.1)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>14</td>
</tr>
<tr>
<td>TCR (Jβ2.2)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>15</td>
</tr>
<tr>
<td>TCR (Jβ2.3)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>16</td>
</tr>
<tr>
<td>TCR (Jβ2.4)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>17</td>
</tr>
<tr>
<td>TCR (Jβ2.5)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>18</td>
</tr>
<tr>
<td>TCR (Jβ2.7)</td>
<td>cgactccgattatactcatataaagcctgcttcc</td>
<td>19</td>
</tr>
</tbody>
</table>
Flow cytometry analysis demonstrated that this T cell line had limited TCR Vβ gene usage. As shown in Figure 5A, most of the cells expressed Vβ3 (87%) and the rest of the cells expressed Vβ4 (9.8%) and Vβ6 (0.8%). The Jβ gene usage of the Vβ3+ cells was analysis by PCR as described above. It is shown in Figure 5B that this Vβ3+ cells used only Jβ2.4 (Fig 5B).

Example 7: Analysis of TCR α gene

To characterize the variable region of TCRα gene usage of B1o t 5 specific T cells (TCR Va gene), 5'RACE of TCR α gene was performed by using the SMART™ RACE cDNA amplification kit (Clontech). The first strand cDNA was synthesized by using the Superscript II (GIBCO) enzyme, one microgram of total RNA and 5'CDS primer according to the manufacturer's instruction. The 5'RACE PCR was then performed by using universal primer mix, gene specific primer for the constant region of TCRα gene (SEQ ID NO: 20 TGAACTGGGGTAGGTGGCGTTGG) and the advantage cDNA polymerase (Clontech) according to the manufacturer's instruction. The PCR product was then cloned into the pCR2.1-TA cloning vector (Invitrogen) and DNA sequencing was performed to identify the TCR α gene.

As shown in Figure 6, a PCR product of ~800bp was obtained. This product was then cloned into the pCR2.1-TA-cloning vector and transformed into the E. coli. The plasmids of twenty-two transformants were purified and DNA sequencing was performed to identify the usage of the TCR α gene of B1o t 5 specific T cells. The sequencing results showed that the majority of the clones (20 out of 22) had the sequences of TCR Vα11 and Jα34. One clone had the sequence of TCR Vα4 and Jα27 and another clone used the TCR Vα8 and Jα28.

Example 8: Cloning of TCR α and B genes into the pTα and pTβ cassette vectors

The genomic DNA of the B1o t 5 specific T cells were purified by genomic DNA isolation kit (Qiagen). The variable region of TCR α gene was PCR amplified by using primers:

(SEQ ID NO: 21) 5'- AACCCGGCCAGCGATTGGACAGGGCCATGCA (sense)
and

(SEQ ID NO: 22) 5'AAAGCGGCCGCTTCAAATTGCTTCACTGTCTCAGAA (anti-sense).

The variable region TCR β gene was amplified by using primers:

(SEQ ID NO: 23) CCGCTCGAGTTTCGTACTTCTAAGCCACCATGGC (sense) and

(SEQ ID NO: 24) CCGATCGATACCCACACAAAAACTATACCCAG (antisense).

The PCR products were subjected to a short extension with Taq DNA polymerase in the presence of dATP and cloned into the pCR2.1-TA cloning vector to obtain the construct pCR2.1-Bt5 α and pCR2.1-Bt5 β. DNA sequencing was performed to verify the TCR genes. The plasmids pCR2.1-Bt5 α and pCR2.1-Bt5 β were digested with XmaI/NotI and XhoI/Clal respectively to obtain the variable gene fragments. The Bt5 α gene fragment was cloned into Xmal/NotI region of the α cassette vector (pT α cass) and the Bt5 β gene fragment was cloned into the XhoI/Clal region of the β cassette vector (pT β cass) to obtain the constructs of pT α-Bt5 α and pT β-Bt5 β respectively. The pT α and pT/3 cassette vectors are described in Kouskoff V. et al., Journal of Immunological Methods 180 (1995) 273-280. The ligation was carried out by T4 DNA ligase (Promega, Madison, WI, USA) at 16°C for overnight. The ligated DNA was purified from the reaction mixtures by DNA clean and concentrator kit (Zymo Research, Orange, CA, USA) and treated with DNA gyrase (Sigma, St Louis, MO, USA) at 37 °C for 30 minutes before being transformed into E. coli strain DH5α. The pT α-Bt5 α and pT β-Bt5/3 constructs were prepared with NucleoBond Xtra Maxi EF kit (Macherey-Nagel, Düren, Germany).

[00105] The most dominant usage of TCR α gene, Vα1 1 plus Jα34, and TCR β gene, Vβ3 plus Jβ 2.4, were selected for the construction of the TCR gene expression vector as described above. The rearranged TCRV αJ α and TCRVβD βI β genes were then PCR amplified from the genomic DNA of the Blot 5 specific T cells. PCR products of size ~620bp (Figure 7A) and ~580bp (Figure 7B) for TCR Vα1land Jα34, and Vβ3 and Jβ2.4, gene respectively were obtained. The TCR α gene was cloned into Xmal/NotI region of the α cassette vector and the TCR β gene was cloned into the XhoI/Clal region of the β cassette vector to obtain the constructs of pT α-Bt5 α (Fig 8A) and pTβ-Bt5 β (Fig 8B) respectively.
Example 9: Generation of CD4 TCR genetically modified mice

[00106] The pTα-Bt5α and pTj5-Bt5|6 constructs were digested with Sal I and Kpn I respectively. The genetically modified mice were generated by co-injection of the linearised DNA fragments, devoid of prokaryotic sequences, into the fertilised eggs of C57BL/6 J mice conducted by Level Biotechnology Inc (Taipei, Taiwan). In this context, the mice generated herein express the TCR specific for an H-2b-restricted epitope of BIo t 5. The mice offspring (F1 mice) were obtained by breeding the genetically modified mice with the wild type mice.

Example 10: Expression of the Vα1 1 and Vβ3 on the T cells of the genetically modified mice

[00107] In the peripheral blood of the genetically modified mice according to the present invention, a reduction in the percentage of CD8+ T cells from 10.1% of the wild type mouse to 4.38% of the genetically modified mouse was observed. This observation indicated that the expression of the TCR Vα1 1 and Vβ3 genes successfully induced the selection of CD4+ T cells in the thymus. Furthermore, more than 90% and more 50% of CD4+ and CD8+ T cells respectively expressed the TCRVβ3 gene. As illustrated in Figure 9, the percentage of CD4+TCRVα1 1+Vβ3+ T cells were significantly increased from 0.19% (in wild type) to 65.7% (in genetically modified mice). Both the TCR α and β genes were transmitted successfully to the F1 mouse according to the present invention. In addition, Figure 10 shows a 98.2% expression of CD4+TCRVα1 1+Vβ3+ in the F1 mice (Fig 10).

Example 11: Intranasal administration of BIo 1 5 protein and the airway hyper-responsiveness model

[00108] The 6-10 weeks old female BIo t 5 specific TCR genetically modified mice obtained from Example 10 (i.e. the genetically modified mice that express TCRVα1 1+Vβ3+) or mice obtained from the reproduction of the genetically modified mice that with the exception that these mice did not express TCRVα1 1+Vβ3+ were used
as control. The mice were received intranasal (i.n.) administration with 50 µg of BIo t 5 in 50 µl PBS or PBS alone for 3 consecutive days. The airway hyper-responsiveness (AHR) was measured one day after the last i.n. instillation. The airway responsiveness was assessed in conscious, unrestrained mice by the whole-body plethysmography (model PLY3211, Buxco Electronics Inc., Troy, New York, USA) as described previously (Hamelmann et al., 1997). This system calculates a dimensionless parameter known as enhanced pause (Penh) reflecting the changes in waveform of the chamber pressure, measured with a transducer (model TRD5100) connecting to preamplifier modules (model MAX2270) and analyzed by BioSystem XA software (model SFT 1410, all from Buxco Electronics). Measurements were made of respiratory rate, tidal volume, pause (PEF/PIF), and Penh ((Te/RT-1) x PEF/PIF), where PEF = peak expiratory flow (ml/s), PIF = peak inspiratory flow (ml/s), Te = expiratory time, and RT = relaxation time). Measurements of methacholine responsiveness were obtained by exposing mice to an aerosol of PBS (baseline readings) and followed by cumulatively increased doses (7.5 to 60 mg/ml) of aerosolized methacholine. The aerosol was generated by a portable ultrasonic nebulizer (model 5500D, DeVilbiss Health Care, Sommerset, PA) and drawn through the chamber for 3 minutes with the Bias Flow Supply (model PLY 1040). The signals were recorded for subsequent 5 minutes. The interval between each dose was 1 minute.

[00109] Referring to Figure 13, the Bt5Tg mice developed airway hyper-responsiveness on day 4 after 3 times of intranasal BIo t 5 administrations. The genetically modified mice (indicated as "shaded square: Tg_Bt5") developed airway-responsiveness on day 4 after 3 times of intranasal BIo t 5 administrations. The airway responsiveness of the genetically modified mice that received the PBS treatment (indicated as "unshaded square: Tg_PBS") and the genetically modified negative littermates (indicated as unshaded triangle: "WT_PBS" and shaded triangle: "WT_Bt5") remained normal.

**Example 12: Degree of polarization of BIo 15 specific T cells of genetically modified mice**

[00110] CD4+ T cells were isolated from the lymph nodes of the BIo t 5-specific T cell receptor genetically modified mice according to the present invention
(indicated as "BT-5") using Magnetic Cell Sorting (MACS) as recommended by the manufacturer (Miltenyi Biotech, Pte Ltd) and resuspended in cell culture medium. Antigen presenting cells (APC) were isolated from the spleen of wild type mice and irradiated with 4000 Rads. The 1 x 10^6 cells/ml purified CD4+ T cells were cultured with irradiated APC at 3.5 x 10^5 cells/ml with 20 µg/ml of Bio t 5, 20 U/ml of IL-2 (Peprotech), 20 ng/ml IL-4 (BD Pharmingen, CA, USA), 10 µg/ml anti-IFN-gamma antibody (eBioscience, CA, USA), 10 µg/ml anti-IL-10 antibody (eBioscience, CA, USA), 10 µg/ml anti-IL-12/IL-23 antibody (eBioscience, CA, USA) and 10 µg/ml monoclonal anti-IFN-gammaR1 antibody (R&D Systems, MN, USA) cell culture medium. Medium was changed at day 3 aspiration 50 % of the medium and replacing with an equal volume of fresh cRPMI together with the same concentrations of antigen, polarizing cytokines and antibodies. The cultures were also re-stimulated with fresh APCs every seven days and this seven-day cycle was repeated over a total of two cycles to generate sufficient numbers of well-polarized CD4+ Th2 cells. The degree of polarization was measured using ELISA (not shown), as well as intracellular transcription factors GATA-3 and T-bet (Figure 14 A, B).

[00111] The BT-5 CD4+ Th2 T cell adoptive transfer recipient mice (wild type mice) according to the present invention that were challenged intranasally (i.n.) with Bio t 5 protein were studied for eosinophil infiltration into their BAL fluids and lung tissues using Flow Cytometry. The results indicate that the proportions of the eosinophils in the BAL fluid (Fig 14 C) and lung (Fig 14 D) of the Bio t 5-challenged mice was higher that in the saline challenged (Fig 14 D BAL, F lung) BT-5 adoptive transfer recipient mice (Student’s t-test, Figure 14C-F).

[00112] Referring to Figure 15, the eosinophil and neutrophil infiltration into BAL fluids and lung tissues of groups of 4 BT-5 Th2 CD4+ T cell recipient mice (wild type mice) after Bio t 5 challenge were studied using Flow cytometry. Results are expressed as the mean ± sem from 4 mice * denotes p < 0.05 ** denotes p<0.01, *** denotes p<0.01 between the groups indicated, Student’s t test.
Example 13: Measurement of airway hyperreactivity in the CD4+ T cell adoptive transfer recipient mice

[00113] The lung function of B10 t 5-challenged BT-5 Th2 cell adoptive transferred recipients (wild type mice) was studied by determining the airway responsiveness to five doses of nebulized methacholine (Figure 16). Results indicate that the B10 t 5-challenged BT-5 Th2 cell adoptive transfer recipients experienced a higher methacholine-induced airway resistance as compared to the control mice. This indicates that the B10 t 5-challenged BT-5 Th2 cell adoptive transfer recipients had increased airway hyperreactivity relative to the control mice and to mice that received BT-5 Th2 cells but were not challenged.

Example 14: Histology analysis of the lungs of B10 15-challenged adoptive hosts

[00114] The lung histology of the BT-5-challenged adoptive hosts was examined in order to study the physiological changes that have taken place in the lungs. Lung sections of the BT-5 adoptive transfer recipients (wild type mice) challenged with B10 t 5 and control mice challenged with saline were stained with the hematoxylin and eosin (H&E) as well as the Periodic acid-Schiff (PAS) stains (Figure 17 and Figure 18). H&E staining of the lung sections revealed the thickening of the airway epithelium as well as the presence of cellular infiltrations surrounding the airways of the BT-5-challenged adoptive hosts (Figure 17). All these features were absent in the control mice. Furthermore, PAS staining of the lung sections also showed that the airways of BT-5-adoptive transfer recipients challenged with B10 t 5 (Fig 17 C, D) compared with mice challenged with saline (Fig 17A, B). BT-5-adoptive transfer recipients challenged with B10 t 5 also had large numbers of visible mucus hypersecreting goblet cells which lined the airways (Figure 18 C, D) compared with mice challenged with saline (Fig 18A, B). Taken together the data clearly show that the B10 t 5-TCR specific genetically modified mice of the present invention represent an excellent tool with which to study allergic diseases such as asthma.

[00115] The listing or discussion of a previously published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge. All documents listed are
hereby incorporated herein by reference.

[00116] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by a preferred embodiment, modification and variation of the invention herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[00117] The invention has been described broadly and generic herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[00118] Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, the skilled artisan will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.
WHAT IS CLAIMED IS:

1. A genetically modified animal comprising a nucleic acid encoding a T cell receptor (TCR), wherein the nucleic acid has been recombinantly introduced into the animal, and wherein the TCR is specific for an immunogenic fragment of the mite allergen BIo t 5.

2. The genetically modified animal according to claim 1, wherein the TCR specific for the immunogenic fragment of the mite allergen BIo t 5 has a specific binding affinity to a fragment selected from the group consisting of amino acid residue 40-80 of the mature BIo t 5 wild type sequence, amino acid residue 55-70 of the mature BIo t 5 wild type sequence and amino acid residue 58-73 of mature BIo t 5 wild type sequence.

3. The genetically modified animal according to claim 1 or 2, wherein the nucleic acid encodes a TCR α chain.

4. The genetically modified animal according to claim 3, wherein the TCR α chain comprises a variable domain (V), a joining domain (J) and a constant domain (C).

5. The genetically modified animal according to claim 3 or 4 wherein the nucleotide sequence of the TCR α chain includes the sequences of the Vcd 1 and Jcθ4 gene.

6. The genetically modified animal according to claim 1 or 2, wherein the nucleic acid encodes a TCR β chain.

7. The genetically modified animal according to claim 6, wherein the TCR β chain comprises a variable domain (V), a diversity domain (D), a joining domain (J) and a constant domain (C).
8. The genetically modified animal according to claim 6 or 7 wherein the nucleotide sequence of the TCR β chain includes the sequences of the Vβ3 and JB2.4 gene.

9. The genetically modified animal according to any of claims 1 to 8, wherein the nucleic acid encoding a TCR specific for an immunogenic fragment of the mite allergen Bio t 5 is comprised in a vector.

10. The genetically modified animal according to any of claims 1 to 9, wherein the nucleic acid is stably integrated into the genome of the animal.

11. The genetically modified animal according to any of claims 1 to 10, wherein the animal is a mammal.

12. The genetically modified animal according to claim 11, wherein the mammal is selected from the group consisting of a rodent, Canis, Ungulate, Felidae, Leporidae, and Macaque.

13. The genetically modified animal according to claim 12, wherein the rodent is selected from the group consisting of mouse, rat, squirrel, chipmunk, gopher, porcupine, beaver, hamster, gerbil, guinea pig, chinchilla, prairie dog, and groundhog.

14. The genetically modified animal according to claim 13, wherein the rodent is a mouse.

15. An animal obtained by reproduction of the genetically modified animal according to any of claims 1 to 14.

16. The animal according to claim 15, wherein the animal is obtained by breeding the genetically modified animal with a wild type animal.

17. The animal according to claim 16, wherein the wild type animal is a rodent.
18. The animal according to claim 17, wherein the rodent is a mouse.

19. The animal according to claim 18, wherein the mouse is a C57BL/6 or Balb/c mouse.

20. An isolated nucleic acid encoding a TCR as recited in any of claims 1 to 19.

21. A vector comprising the nucleic acid according to claim 20.

22. A host cell comprising the vector according to claim 21.

23. A method of obtaining a genetically modified animal comprising recombinantly introducing a nucleic acid encoding a TCR into an animal, wherein the TCR is specific for an immunogenic fragment of the mite allergen BIo t 5.

24. The method according to claim 23, wherein the immunogenic fragment of the mite allergen BIo t 5 has a specific binding affinity to a fragment selected from the group consisting of amino acid residue 40-80 of the mature BIo t 5 wild type sequence, amino acid residue 55-70 of the mature BIo t 5 wild type sequence and amino acid residue 58-73 of mature BIo t 5 wild type sequence.

25. The method according to claim 23 or 24, wherein the nucleic acid is introduced into a fertilized egg of the animal.

26. The method according to any of claims 23 to 25 further comprising, prior to introducing the nucleic acid into the animal, administering to the animal a BIo t 5 peptide or a fragment thereof, isolating T cells specific for an immunogenic fragment of the mite allergen BIo t 5 from the splenocyte of the animal and isolating a nucleic acid encoding a TCR specific for an immunogenic fragment of the mite allergen BIo t 5 from the T cell.
27. A method of generating a T cell line specific for an immunogenic fragment of the mite allergen Bio t 5, the method comprising administering to an animal a Bio t 5 peptide or a fragment thereof, isolating the T cell specific for an immunogenic fragment of the mite allergen Bio t 5 from the splenocyte of the animal and further administering to the isolated T cell the Bio t 5 peptide or a fragment thereof.

28. The method according to claim 27, wherein the nucleotide sequence encoding the TCR of the isolated T cell is selected from the sequences consisting of Vβ3, Vβ4, Vβ6, Jβ2.4, Vα4, Vα8, Vα1 l, Jα27, Jα28 and Jα34.


30. The method according to claim 29, wherein the T cell specific for the immunogenic fragment of the mite allergen Bio t 5 is administered into the animal by intravenous injection.

31. The method according to claims 29 or 30, further comprising stimulating the animal with a Bio t 5 peptide or a fragment thereof.

32. The method according to claim 30, wherein the Bio t 5 peptide is administered into the animal by inhalation.

33. The method according to any one of claims 23 to 32, wherein the animal is a mammal.

34. The method according to claim 33, wherein the mammal is a rodent.

35. The method according to claim 34, wherein the rodent is selected from the group consisting of mouse, rat, squirrel, chipmunk, gopher, porcupine, beaver, hamster, gerbil, guinea pig, chinchilla, prairie dog, and groundhog.
36. The method according to claim 35, wherein the rodent is a mouse.

37. The method according to claim 36, wherein the mouse is a C57BL/6 mouse.

38. A method of testing the efficacy of a therapeutic agent for an allergic disease, the method comprising administering to an animal according to any of claims 1 to 19, the therapeutic agent.

39. A method of testing the suitability of a compound for the treatment of an allergic disease, the method comprising administering to an animal according to any of claims 1 to 19, the compound.

40. A method of identifying a compound being suitable for the treatment of an allergic disease, the method comprising administering to an animal according to any of claims 1 to 19, the compound.

41. The method according to any of claims 38 to 40, wherein the allergic diseases is selected from the group consisting of rhinitis, conjunctivitis, atopic dermatitis, atopic asthma and bronchial asthma.
Figure 1
Figure 8
Figure 9

Figure 10
**Vα11+Jα34 (SEQ ID NO: 1)**
CCAGCGATTGGCAGGGCCAATGCAGGGAACCTGGGAAGCTGTGCTGGGTGTGGGTGGTCAGATTTCGC
TTGGAGGTGTGGCTCAGCTAAAGTTCTATTCATTCAGGATCATTCCAGAAATGGTCAGTGGTGAGGAGAAAGAGCT
CACAACAGCTACTCAGCAAGCTCATCATTCATCATCTCAACAGGGTTGGCTTTACCTGGTAGATGAAACACTCTCCTGA
TTTGTTGTGTTCCTCTGTTTTCCAAGCGGAGGTTGAGGAAGTCAGGGTGACGAGAGTTCTCTAGCAGGCCCTGA
GCCTCCAGCGAGGAAACCGGTTCGGCTCTGAGATACAAAATTTACTACTCACCACATGAGGCTGGTGACGCTGGTCC
CGAACAAAGTTCCAGGCGCATCCCAATTGCTGCTGGTTACGGGACAGGACCGAATTTTGAA

**Vβ3+Db2+Jβ2.4 (SEQ ID NO: 2)**
TTTCTGTAATTCCCTAGGCCACCATGGCTACAGGCTTCTCTGTAAACACAGTACTTTGTCTCCTGGGTGGTGCAAGT
AGTCTTAAGAGCAAAAGACAGTCTTGGACCTGGGAAGTGGCAACCACATATCTAAGTATTCCAATTTCTCTGTC
AAGGCCCCCTCTTGCTGTATTACATCTTTTGTCTCTCTCTCTTCTAGGAATTGGAAATTGTCAAAAGATCAT
TCAGACTCCAAATGATCTGTTGAAAGGCGAACAAATGCAAAGATGAGGCTGATCCCTCTAAAGGGAC
ATCCAGTGTATTCTCTGTAACACAAAATAGAACAAATAGTTATCTATATTCTGGAATGAAATTTTGAGGT
GAAGTCTTCCAGCAATAGCACTGGAAAAACGATTTCTCTGCTGAGTGGCTCCTTAAACACTCCTTGGAG
CCTAGAATTCTCAAGCTCCCTTGGACGAGACTCACCTGCTAGGCTGCACGACGTTCCGGGACGTCTC
AAAACACCTTTGTTTGGTGCGGGGACACCGACTATCGGGTCTAGGTAAAAGCTGGGGTGGTGGT

**Figure 11**
Va8+Ja28 (SEQ ID NO: 3)
ATGGCTCTGGACAGGTCTCAATCTGCTCTTCTAGTCTGTGCAGGAGAATGGAGAGCTC
ATGACCAAGAGGAGAGGCTGGTCAACTCTGGCCAGGAGGTTTCTGTGTGTGTGA
ACTGCACACTCAATTCTCTTCTCTTCTGCTATGTGCACTACATCTCATGATTAT
GATGCACAGCTCTGCTCAGGCGCACTGAGCTAGCTGCCTCGACGATGGCCATGGAGT
AAGAGGCTCAGCTTTCTGGAAAAAGGCGACCAATTCTCAGCATCCGA

Va4+Ja27 (SEQ ID NO: 4)
ATGAAGCAATTTCCCAAGTTTAGTCGACTGATGTCAGTTATATCTTGGAGGACCTGAGACTCAGTGAT
CCAGATGCAAGGCCATGAGCTCCCATCAGAAAACGACTCTTCTATTTATATAACTGCACTTATTCAACCACAG
GGTACCAGCACTTTCTCTGTTATCTCCAAGATATTCCTGAGAAAGGGCTCAGCTCTCAGCTCTCTGGGAGTCAACT
GCCACAAACAAGGAAGGAGGAGAGGTTTCTGAAGCTGACTATATGTGATAAAAAGGACCACCTTCCCTGCACCTTGA
GAAAAACCTGCAGGCGAAGATAGATGATCGCTGCTGTAATCTACTGTGATCTAGAGAGAGGGCTGGGCAAAATTAA
CCTTGGGAGTGAGCGGCGAGCTCAAGTGAGCCAA

Vβ4+Dβ2+Jβ2.7 (SEQ ID NO: 5)
ATGGGCTCCATTTTCTCGTGCCTGGCCGTGTCTGTCTGCTGTGTGCAAGGCTCCACGTGCAACCCGAATAATAT
CCAGAAACCAAGATATCTGGGAGCGCTCAGGACGAGGCGGAAAATACTGTAGATGCGAGACAGATATCTAGGCC
ACAATGCTATGTATGTTATAGCAAATGCTAAGAGCCCTCAGTAGGGTTCTCTAGATCATTAATTCTACCA
AAACTTATGAACCATCAGCTAGCTGTTCTCCACACTCAAGAGCTTCAAGAAAAAACCAATTTGACCT
TCAGATCGACGGCTTAAAGGCGCTGACTCGGCAGGATCTTGCTGGCAGACGCCTGCCGCAGACTCCT
ATGAAGAAGCTCTTCTGGGGCGGCGTGAGCTCGTTAG

Vβ6+Dβ1+Jβ2.4 (SEQ ID NO: 6)
ATGAACAGTGTTCTCTGCGAGCAACCTTTCTGCTCTTACTGTGAGGACACATGATGGATGGGC
CATCATATTCAAGCAACCAAATCTCGATGTTGAGAAGGGAAAAACTGACCTTGAATATGCAAGA
ATTGCAATGCTACAACTGACGTCAGCAAGGATCTAGGAGGTAGGACTGACTATTTCTCA
ATACGGAACAGTACCTAAAAGGCGATCGACGTCAGGAGAAGGTTAGATAGTGTTGCAGAGAGAAGTCATC
TCTTCTCTCATTGCAACTCTGGCCAGAAGAGAAGAGATGGCCGTCTTCTGTGCAGACCACTATGGGCC
GGGCTCAAAACACCTTTGACTTTTGGGCGGCACCGACTATCGGTATCG

Figure 12
Figure 13
Figure 14A-B
Figure 14C-F
Figure 17
Figure 18
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl.

AOIK 67/027 (2006.01) C12N 15/12 (2006.01) C12N 5/00 (2006.01) C12N 5/078 (2010.01)
C07K 14/725 (2006.01) C12N 5/07 (2010.01) C12N 5/0783 (2010.01)

Action Date: 30 April 2010

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)

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

EPODOC, WPI & Keywords: TCR, transgenic model, mite and bio 15 and similar terms

Medline, Biobis, hcaplus & keywords: TCR, transgenic model, mite and bio t 5 and similar terms

GenomeQuest: Sequence search on nucleic acid sequences SEQ ID NO: 1-6

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>JARMAN, E.R. et al., Transgenic mice expressing the T cell antigen receptor specific for an immunodominant epitope of a major allergen of house dust mite develop an asthmatic phenotype on exposure of the airways to allergen1, Clinical and Experimental Allergy, 2005, Vol. 35, pages 960-969 Whole document</td>
<td>1-41</td>
</tr>
</tbody>
</table>

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
30 April 2010

Date of mailing of the international search report
04 May 2010

Name and mailing address of the ISA/AU

AUSTRALIAN PATENT OFFICE

PO BOX 200, WODEN ACT 2606, AUSTRALIA

E-mail address: pct@ipaustralia.gov.au

Facsimile No. +61 2 6283 7999

Authorized officer

Esther Ng

AUSTRALIAN PATENT OFFICE

(ISO 9001 Quality Certified Service)

Telephone No: +61 2 6283 3129

Form PCT/ISA/210 (second sheet) (July 2009)
<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>
This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>WO 9507933</td>
<td>AU 37964/93</td>
</tr>
<tr>
<td></td>
<td>AU 77295/94</td>
</tr>
<tr>
<td></td>
<td>AU 84070/91</td>
</tr>
<tr>
<td>CA 2131898</td>
<td>EP 0671945</td>
</tr>
<tr>
<td>US 5512283</td>
<td>US 5643572</td>
</tr>
<tr>
<td>WO 9317703</td>
<td>WO 9201044</td>
</tr>
</tbody>
</table>

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001,