

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 allergen

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Summary

Background Current studies on mechanisms underlying allergen-induced pulmonary inflammation and asthma are hampered by the lack of appropriate physiological *in vivo* models that reflect the natural route of allergen exposure and sensitization.

Objective To generate and phenotype a transgenic mouse strain expressing the T cell receptor (TCR) specific for an immunodominant domain of the major inhalant allergen *Dermatophagoides pteronyssinus* species of house dust mite (Der p 1), for the development of an *in vivo* model of allergic asthma.

Methods Der p 1 transgenic mice were generated using TCR- $\alpha\beta$ derived from a CD4⁺ T cell hybridoma reactive with Der p 1 residues p 110–131. The frequency and functional activity of peripheral T cells were determined and parameters of airway inflammation assessed following allergen challenge of the airways with Der p 1.

Results CD4⁺ T cells are functionally active, exhibiting dose-dependent proliferation and IL-4 production on primary stimulation with Der p 1 or Der p 1, p 110–131 *in vitro*, independent of *in vivo* antigen priming. On sensitization of the airways with allergen, in the absence of systemic priming or the application of adjuvants, the TCR transgenic mice develop airway inflammation characterized by a marked lymphocytic and eosinophilic infiltrate with goblet cell hyperplasia and enhanced mucin production.

Conclusion The Der p 1 TCR transgenic mice provide a model for investigating the pathophysiological mechanisms of pulmonary inflammation following sensitization by exposure of the airways to allergen and for investigating the mode of action and efficacy of novel immunotherapeutics.

Keywords airway inflammation, allergy, *in vivo* animal models, transgenic mice

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Introduction

Allergic asthma is a complex inflammatory disease of the lung characterized by airway eosinophilia, increased airway hyper-responsiveness (AHR), mucus hypersecretion and elevated serum IgE levels [1]. Allergen reactive CD4⁺ T-helper type 2 (Th2) cells, which express the cytokines IL-4, IL-5, IL-9 and IL-13, are central in initiating and maintaining allergic responses in pre-disposed individuals [2]. Studies using mice deficient in single or multiple cytokine genes have demonstrated that although IL-5, IL-9 and IL-13 contribute towards the magnitude of the response, IL-4 is essential for initial activation of Th2 effector functions and the switch to IgE

production [3]. The activation of CD4⁺ T cells involves ligation of the T cell receptor (TCR) by processed antigen presented in association with major histocompatibility (MHC) class II molecules expressed on professional antigen-presenting cells.

The TCR- $\alpha\beta$ heterodimer confers antigen specificity through the use of different α and β chain variable (V), diversity (D) and joining (J) gene segments that undergo somatic rearrangement during thymocyte development [4]. Analysis of T cell responses to defined peptide–MHC class II complexes suggests that particular V α and V β region gene segments confer antigen specificity irrespective of the MHC class II haplotype [5], possibly because of the insertion or deletion of nucleotides in the complementary determining region 3 junctional region [6]. Many chronic T cell-mediated inflammatory diseases characterized by the existence of dominant effector T cell epitopes within the antigen [7] are associated with restricted TCR- $\alpha\beta$ chain usage [8, 9].

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Allergic asthma is characterized by episodes of acute CD4⁺Th2-mediated inflammation induced on repeated exposure of the airways to allergen. At the population level of a single allergenic protein, the number of T cell epitopes recognized is diverse, reflecting the range of human leucocyte antigen (HLA) restriction specificities [10]. In spite of this, there is evidence of dominant antigenic regions within the major allergens, as well as cross-reactivity between allergens [11]. This is determined at the level of T cell recognition, by the selection of a limited TCR V α and V β repertoire. Clinical studies have revealed a disproportionate increase in expression of certain V α and V β subsets in individuals allergic to allergens present in birch pollen, cat dander, or house dust mite (HDM) [12–14]. Furthermore, analysis of the TCR sequence of HDM reactive T cell clones and peripheral blood T lymphocytes demonstrated sequence identity, suggestive of clonal expansion of a limited number of dominant CD4⁺ T cell clones *in vivo* because of chronic allergen exposure [15]. Similarly, in murine models of allergic disease, the introduction of specific disease-associated V β T cell subsets was sufficient to confer and maintain an IgE phenotype in non-sensitized mice [16].

The HDM species *Dermatophagoides pteronyssinus* is a source of indoor inhalant allergens and a major cause of allergic asthma. The major group I allergen (Der p 1) has been cloned, sequenced and immunodominant T cell epitopes defined [17]. One such region, located within residues 110–131, is immunodominant for Der p 1 reactive T cells isolated from atopic individuals as well as high responder strains of mice, expressing the H-2^b haplotype [18]. We have recently cloned and sequenced the TCR V α β domain derived from a murine T cell hybridoma reactive with Der p 1, p 110–131 and used the cDNA to generate a Der p 1 transgenic strain.

A limitation of murine *in vivo* models of allergic asthma is the low frequency of allergen reactive effector CD4⁺ T cells in the periphery. The induction of an asthmatic phenotype depends on the prior systemic sensitization with allergen adsorbed to adjuvants such as alum, which non-specifically drive Th2 responses, and thus do not represent physiological sensitization. As a consequence, the opportunity to perform objective mechanistic studies may be limited.

Here, we report that mice expressing a Der p 1, p 110–131 specific V α 3.2 and V β 14 TCR as a transgene rapidly develop an allergic asthmatic phenotype characterized by pulmonary eosinophilia and localized expansion of specific T cells, following exposure of the airways to Der p 1. In the absence of localized allergen challenge, mice exhibit normal lung physiology. The induction of this phenotype does not depend on prior systemic sensitization with allergen, thereby avoiding the use of adjuvants. These transgenic mice may, therefore, provide a suitable model for defining pathophysiological mechanisms of pulmonary inflammation and assessing the mode of action and efficacy of new therapeutic agents.

Materials and methods

Animals

Mice transgenic for the TCR V α 3.2 V β 14 were generated at the Babraham Institute by DNA microinjection into

fertilized mouse eggs (C57BL/6J $\times$ CBA/CA). Mice positive for the transgene were backcrossed for five generations onto inbred C57BL/6 mice (Charles River or Harlan, UK). Animal care and experimental procedures were conducted in accordance with the animal ethics regulations of the Home Office, UK.

Reagents

Der p 1 was affinity purified from spent mite medium using McAb 4C1 (Indoor Biotechnologies Ltd, Cardiff, UK). The synthetic peptide Der p 1; p 110–131 [18] was synthesized with a free carboxyl terminus and HPLC purified (Advanced Biotechnology Centre, Imperial College, London, London).

Preparation of DNA constructs for microinjection

The TCR- α 3.2/ β 14 transgene was derived from the Der p 1, p 110–131 reactive CD4⁺ T cell hybridoma 1AD2. Complete TCR- $\alpha\beta$ chain nucleotide sequences were obtained by sequencing the cDNA. mRNA from 1AD2 was reverse transcribed (OligoTex direct mRNA extraction kit; Qiagen, Crawley, UK) with MMLV reverse transcriptase and oligo dT primers (Gibco BRL, Life Technologies, Paisley, UK) and cDNA amplified by PCR using Advantage high-fidelity DNA polymerase (BD Biosciences, Clontech, Palo Alto, CA, USA) β and primers specific for TCR- α /TCR- β 5'/3' sequences for the variable and constant regions of TCR V α 3.2, V β 14 (Fig. 1b). The *Eco*RI and *Xba*I restricted TCR- α and TCR- β DNA sequences (derived as PCR products) were run on 1.2% (w/v) agarose gels, and appropriate bands were excised, cleaned (QIAquick spin columns: Qiagen), ligated into the 8 kb pTexIIIa vector and transformed into *E. coli* XL1-Blue (Stratagene, La Jolla, CA, USA). The p-Bluescript KS⁺ (Stratagene)-derived pTexIIIa vector (Fig. 1a) incorporated a human CD2 cassette (transcription initiation site, first intron of the human CD2 gene and 2 polyadenylation sites in the 3' untranslated region of the gene: a kind gift of Dr D. Kioussis, NIMR, Mill Hill, London, UK). Ampicillin-resistant clones were screened by restriction digests and DNA sequencing. Plasmids containing TCR- α and TCR- β inserts were purified and cut with *Not*I to generate full-length TCR constructs each with an upstream human CD2 promoter and 3' poly-A sequences. The linearized 6 kb constructs were subjected to agarose gel electrophoresis, extracted, cleaned (QIAquick columns: Qiagen), concentrated and diluted for microinjection in Ultra-pure grade low salt buffer (5 mM Tris-HCl, pH 7.4, 0.1 mM EDTA). Founders and offspring generated by backcrossing onto C57/BL6J were screened for transgene expression by PCR (MJ Research Peltier thermal cyclers, Cambridge Scientific Products, Cambridge, MA, USA: PTC-200) using genomic DNA (Qiagen DNeasy kit): hot-start 94 °C for 2 min, amplification (94 °C, 30 s; 63 °C, 30 s; 72 °C, 30 s) for a total of 25 cycles, followed by 72 °C for 7 min. PCR products were visualized on 2% (w/v) agarose gels after electrophoresis.

Induction of pulmonary inflammatory responses to allergen

Anaesthetized (Avertin 2,2,2,-tribromoethanol 0.1 mg/g) mice were challenged twice at a 5-day interval by intratracheal instillation of Der p 1 (20 μ g in 50 μ L saline; endotoxin free:

Gibco BRL, Life Technologies) into the airways. Control mice received saline alone. Previous intratracheal instillation of Evans blue dye has shown that using this method, allergen is dispersed throughout the lung. After 24 h, mice were killed and parameters of lung inflammation assessed. Blood samples were centrifuged at 2000 g, and sera were collected and stored at -70°C for antibody levels.

Isolation and culture of lymphocytes for proliferation assays and cytokine production

Single-cell suspensions were prepared from spleen or pooled tracheal lymph nodes draining the airways, and red blood cell lysis was performed. Cells were cultured at 1×10^5 /well in 96-well tissue culture plates (Costar, Corning, NY, USA) in a final volume of 0.2 mL RPMI 1640 medium supplemented with 5% fetal calf serum (FCS; Gibco, Paisley, UK), 50 μM 2-mercaptoethanol, 2 mM L-glutamine and 10 U penicillin/streptomycin (Gibco). Cells were stimulated in triplicate with either Der p 1, or peptide: Der p 1; p 110–131 (0.1–100 μg /mL). As controls, cells were cultured in media alone, with an irrelevant antigen, HA (haemagglutinin), or as a polyclonal stimulus in wells coated with 5 μg /mL anti-CD3 and anti-CD28 (145 2C11 and 37.51; Pharmingen, San Diego, CA, USA). After 48 h supernatants were collected, stored at -70°C and plates pulsed with 1 μCi /well of [^3H TdR]. Labelled cells were harvested after a further 18 h in culture and [^3H TdR] uptake determined using a beta scintillation counter. Supernatants were assayed in duplicate for the presence of IL-4, using ELISA. Antibodies were purchased and used according to the instructions of the manufacturer (DuoSet mouse ELISA; R&D Systems, Oxford, UK). Sensitivity of IL-4 > 15.6 pg/mL.

Flow cytometric analysis

CD4⁺ T cells expressing the transgenes TCR V α 3.2/V β 14 were identified by flow cytometric analysis. Briefly, splenocytes resuspended in PBS/1% bovine serum albumin (BSA) were stained at 4°C with allophycocyanin or FITC-conjugated hamster anti-mouse CD3 ϵ ⁺ (145-2C11), anti-B220 (CD45R)-phycoerythrin (PE), anti-CD4 (LYT4)-FITC (RM4-5), or anti-CD8 (Ly-2)-PE (53-6.7) to determine cell ratio. The percentage of CD4⁺ T cells expressing the transgenes was determined using anti-mouse V β 14 TCR (clone 14-2) followed by PE-conjugated mouse anti-rat IgM (clone G53-238). Stained cells were washed, fixed in 0.4% formalin in PBS and flow cytometric analysis performed on the Becton Dickinson (Oxford, UK) FACSCalibur. Antibodies, including isotype matched controls, were purchased from BD Pharmingen.

Characterization of the cellular infiltrate in the airways

To obtain bronchoalveolar lavage (BAL) fluid, trachea were exposed, cannulated using a 27-gauge silicon tubing and lungs gently lavaged by instillation of 500 μL of cold PBS. The volume of BAL recovered, total cell numbers and cellular composition of samples were determined. Samples were microfuged (700 g, 5 min, 4°C), re-suspended in PBS/10% BSA and 1×10^5 cells spun onto poly (L-lysine)-coated slides by cytocentrifugation (3 min at 300 g; Cytospin 5, Thermo Shandon Inc., Pittsburgh, PA, USA). Slides were

air dried, fixed in methanol and stained with Diff/Quick using standard procedures. Differential cell counts were performed in duplicate on coded slides, with at least 300 cells counted per slide.

Histological and immunohistochemical analysis of lung tissue

Lungs were excised, inflated under constant pressure and fixed in 4% formalin in PBS prior to embedding in paraffin. Sections (3–4 μm) were assessed for general morphology and cellular infiltrate using haematoxylin and eosin. Mucous-producing goblet cells were identified using periodic acid-Schiff/alcan blue to detect neutral and acid mucins.

For immunohistochemical analysis, paraffin sections were de-waxed, re-hydrated and microwave treated with Vector antigen retrieval solution. Endogenous peroxidase was blocked with H_2O_2 , after which sections were washed in PBS and pre-incubated with normal goat serum (Sigma Aldrich, Poole, UK; 1:5 dilution in PBS) to block non-specific binding and primary antibody (hamster anti-mouse CD3 ϵ (145-2C11) or rat anti-mouse B220 CD45R; RA3-6B2; BD Pharmingen) added. Slides were incubated at 4°C overnight, washed in PBS, biotinylated secondary antibodies (goat anti-hamster (1:150 dilution in PBS; Vector Laboratories, Inc., Burlingame, CA, USA)) or polyclonal goat anti-rat added (BD Biosciences, Pharmingen) and incubated at room temperature for 30 min. As a control, parallel sections were stained with secondary antibody alone. Sections were washed and bound antibody visualized using avidin–biotin–peroxidase complex (R.T.U. Vectastain ABC-HRP reagent; Vector Laboratories) and 3,3' diaminobenzidine tetra-hydrochloride chromogenic substrate (brown colouration). Sections were counterstained blue with haematoxylin, washed, dehydrated, mounted in Pertex and examined by light microscopy (Leica DMR TCS SP2, Leica Microsystems A/S, Herlev, Denmark). All slides were coded and viewed by more than one investigator.

Extraction and measurement of eosinophils numbers in lung tissue

Lungs were weighted and homogenized in Hanks balanced salt solution (HBSS; Gibco) containing 10 mM HEPES (pH 7.4). Following red blood cell lysis, cells were re-homogenized in HBSS containing 0.5% hexadecyltrimethylammonium bromide (HTAB; pH 7.4) and freeze thawed in liquid nitrogen. Levels of eosinophil peroxidase in lung homogenates were determined using the O-phenylenediamine substrate (Sigma; OPD 1.5 mM, H_2O_2 6.6 mM; pH 8). The reaction was stopped using H_2SO_4 (4 M) and plates read in a MRX microplate reader at 490. Data were analysed using Revelation software.

Results

T cell receptor V α 3.2 V β 14 transgenic mice derived CD4⁺ T cells exhibit functional activity and specificity for their cognate antigen Der p 1; p 110–131

We have previously demonstrated specificity of the murine T cell hybridoma 1A2D2 from which the TCR V α 3.2 V β 14

transgene was derived, for the immunodominant epitope of Der p 1; p 110–131 (N. Kristensen's thesis, unpublished data). For the purpose of this study, V α 3.2 V β 14 TCR transgenic mice were backcrossed onto the high-responder C57 BL/6J (H-2^b) background. Transgenic mice, generated by backcrossing onto C57/BL6J, were selected on the basis of transgene expression, as determined by RT-PCR using primers specific for V α 3.2 and V β 14 (Fig. 1c). Flow cytometric analysis of TCR V β 14 surface antigen expression demonstrated increased frequencies (between 2% and 12%) in secondary lymphoid tissues obtained from TCR transgenic mice, of CD4⁺ T cells expressing the TCR V α 3.2 and V β 14 gene products (12%; Fig. 1d), in transgenic offspring as compared with FLI or TCR transgene negative littermate controls (2%). Ratios of CD4:CD8 T cells in the periphery of transgenic mice were within the normal range (Fig. 1d).

Using mice expressing the TCR V α 3.2 V β 14 transgene (Figs 1c and d) or as a control wild-type C57 BL/6, we investigated T cell proliferative responses on *in vitro* stimulation with either intact Der p 1 protein or the peptide covering residues, Der p 1; p 110–131. As shown in Fig. 2, spleen (a, b) or lymph node (c, d)-derived cells obtained from unchallenged mice expressing the TCR V α 3.2 V β 14 transgene exhibited dose-dependent responses on stimulation with either Der p 1 (a, c) or peptide Der p 1; p 110–131 (b, d). Responses were greatly enhanced on prior challenge of the airways with Der p 1. Optimal responses to the cognate antigenic epitope Der p 1; p 110–131 were obtained at 10-fold lower concentrations than Der p 1, possibly reflecting the increased frequency of T cells expressing the TCR transgene reactive with the immunodominant epitope of Der p 1. In contrast, wild-type H-2^b mice, when challenged by instillation of Der p 1 into the airways in the absence of prior sensitization with Der p 1 adsorbed to adjuvant, showed negligible responses on *in vitro* stimulation with either Der p 1 or the antigenic peptide.

Differences in the extent of proliferation observed between spleen- and tracheal lymph node-derived cells, obtained from saline as compared with Der p 1 challenged transgenic mice, may reflect the existence of functionally active mature CD4⁺ T cells expressing TCR V α 3.2 V β 14 in the peripheral circulation prior to allergen challenge. This would result in a strong primary and recall systemic T cell response on *in vitro* stimulation of splenocytes in both saline- and Der p 1-challenged mice. The failure of lymphocytes obtained from lymph nodes draining the airways to exhibit marked proliferative responses on stimulation with Der p 1 may reflect a localized tolerogenic milieu in the airway mucosa. Mucosal tolerance has been shown to arise as a consequence of low-level exposure of the airways to environmental allergen [19]. Challenge of the airways with a relatively high dose of Der p 1 allergen could conceivably break tolerance, resulting in localized priming and clonal expansion of T cells expressing the TCR transgene in the lymph nodes draining the airway mucosa. This would be reflected as increased proliferation in response to stimulation with the cognate antigen, as observed here, following challenge of the airways with Der p 1.

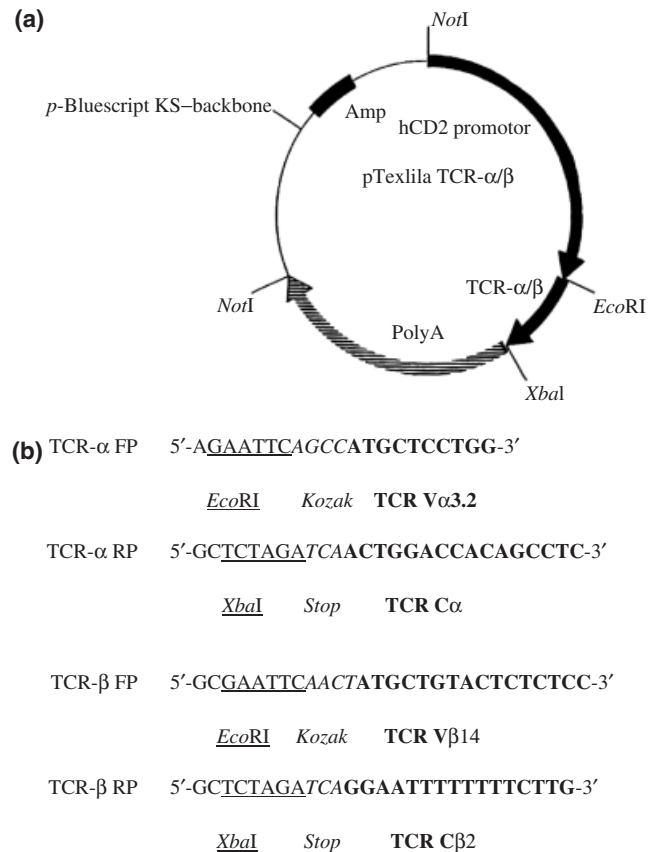


Fig. 1. The plasmid pTexIIIa vector used to generate the DNA constructs (a). Transgene expression as determined by RT-PCR using primers specific for TCR V α 3.2, TCR V β 14 (b). PCR products were visualized on a 2% agarose gel after electrophoresis (cDNA ladder (lane 1), cDNA constructs used, as positive control; 0.9 kb β chain and 0.8 kb α chain (lane 2), C57 BL/6J wild-type-derived cDNA as negative control (lane 3), cDNA derived from transgenic offspring (lanes 4–10)). Genomic expression of TCR V α 3.2 TCR V β 14 cDNA transcripts (lanes 4, 9 and 10: c). Flow cytometric analysis demonstrating cell surface expression of the TCR V β 14 transgene by CD4⁺-gated T lymphocytes, using anti-mouse V β 14 TCR McAb and phycoerythrin (PE)-conjugated secondary antibody. Data presented for (TCR –/–) and transgenic (TCR +/+) mice (d). Ratio of CD4, CD8 and CD3⁺ T lymphocytes in secondary lymphoid organs, as determined using PE-conjugated McAb (d). Representative data presented ($n = 4$). Analysis performed on the Becton Dickinson FACSCalibur. TCR, T cell receptor.

Der p 1; p 110–131 reactive CD4⁺ T cells isolated from transgenic mice exhibit a T-helper type 2 phenotype with elevated levels of interleukin-4 production in response to primary antigen stimulation

To define the phenotype of mice transgenic for the Der p 1-specific TCR, we measured levels of IL-4 production by splenocytes isolated from groups of age-matched, wild-type or TCR transgenic mice. Data presented in Fig. 3 revealed that within secondary lymphoid organs, such as the spleen, CD4⁺ T cells are the primary source of IL-4 produced on antigenic stimulation with either allergen or a synthetic peptide covering an immunodominant T cell epitope with Der p 1. The data suggest that CD4⁺ T cells obtained from naïve transgenic mice (TCR +/+ saline) produced increased levels of IL-4 following primary stimulation *in vitro* with Der p 1 or peptide; p 110–131, as compared with allergen-challenged, wild-type (TCR –/– Der p 1) controls. Levels of IL-4

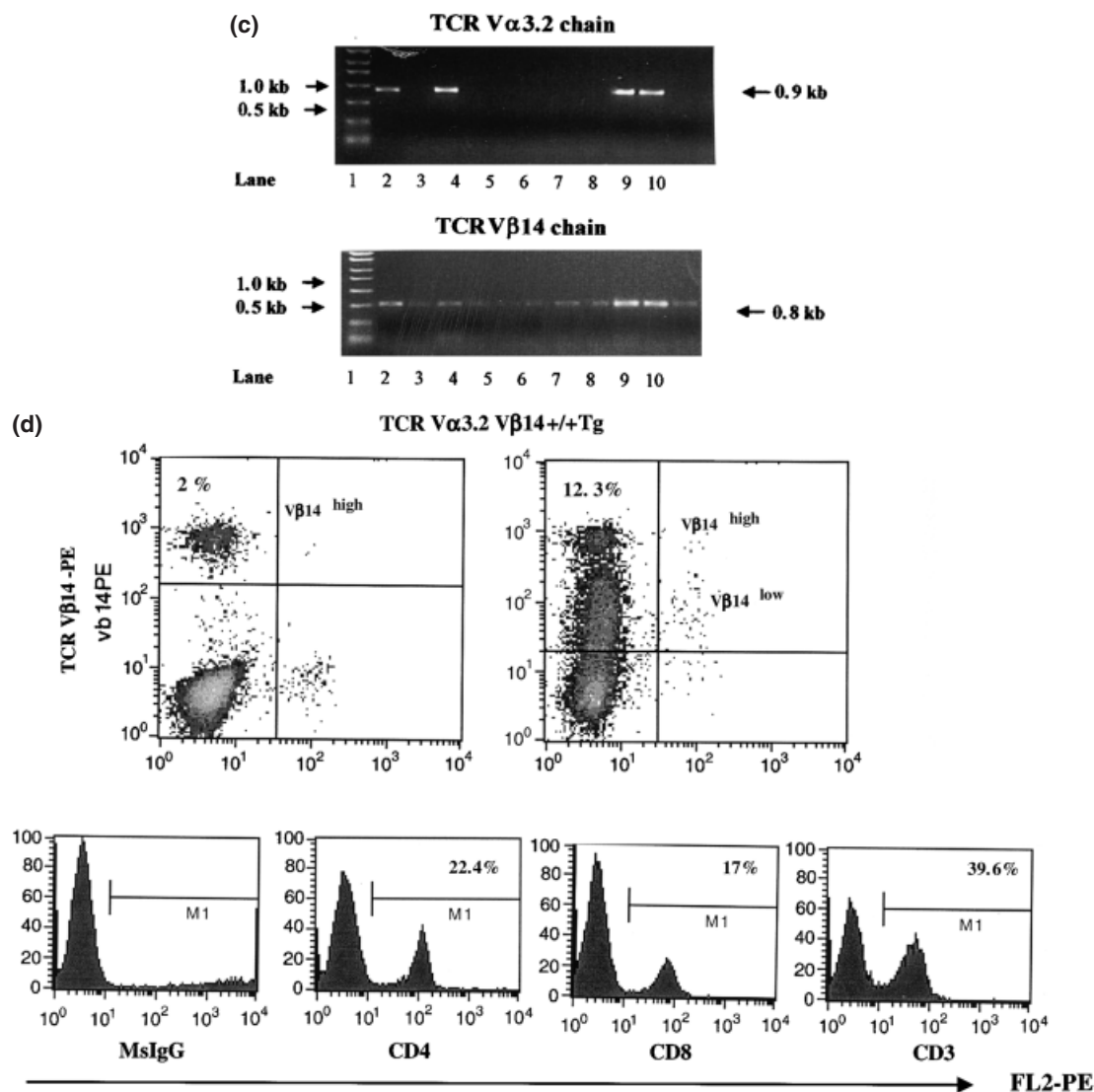


Fig. 1. Continued.

production *in vitro*, on stimulation of splenocytes with Der p 1 or peptide Der p 1; p 110–131, were further increased *on prior* exposure of the airways to allergen (TCR+/+ Der p 1). In contrast, CD4⁺ T cells isolated from Der p 1-challenged, wild-type C57 BL/6J (–/–) mice failed to produce detectable levels of IL-4 (≤ 15 pg/mL) on stimulation. Production of ≥ 2000 pg/mL IL-4 on stimulation by plate bound anti-CD3 and CD28 confirmed cell viability (data not shown).

T cell receptor transgenic mice develop pulmonary inflammatory responses characterized by marked recruitment of lymphocytes into the airways following challenge with Der p 1

The extent of pulmonary inflammation induced in transgenic mice on exposure of the airways to allergen in the absence of prior systemic sensitization is shown in Fig. 3b. Histological and immunohistochemical analysis was performed on lungs obtained from transgenic mice or similarly treated C57 BL/6J controls. In the absence of allergen challenge, TCR- $\alpha\beta$

transgenic mice exhibited normal lung structure (+/+ saline). On secondary exposure of the airways to allergen, transgenic mice developed a marked inflammatory infiltrate consisting of mononuclear cells, principally lymphocytes and multinucleated granulocytic cells. Immunohistological analysis of parallel sections of lung tissue revealed that the cellular infiltrate was predominantly perivascular and peribronchial, with cells located primarily around blood vessels and alveoli but also migrating through the lung parenchyma to the central bronchi (+/+ Der p 1). Similarly treated wild-type C57 BL/6J mice failed to develop airway inflammatory responses in the absence of prior systemic priming with allergen [18]. To quantify the extent of eosinophil infiltration into the lung tissue, eosinophil peroxidase levels were analysed as described previously [18]. Transgenic mice generally exhibited a 50% increase in levels of eosinophil peroxidase, following allergen challenge (TCR- $\alpha\beta$ +/+ tg: 1.283 ± 0.4 OD 490 nm) as compared with wild type [C57 BL/6 –/– 0.80 ± 0.08 OD 490 nm ($P = 0.342$)], which is indicative of an increase in tissue eosinophilia. To further elucidate the cellular infiltrate in allergen-challenged trans-

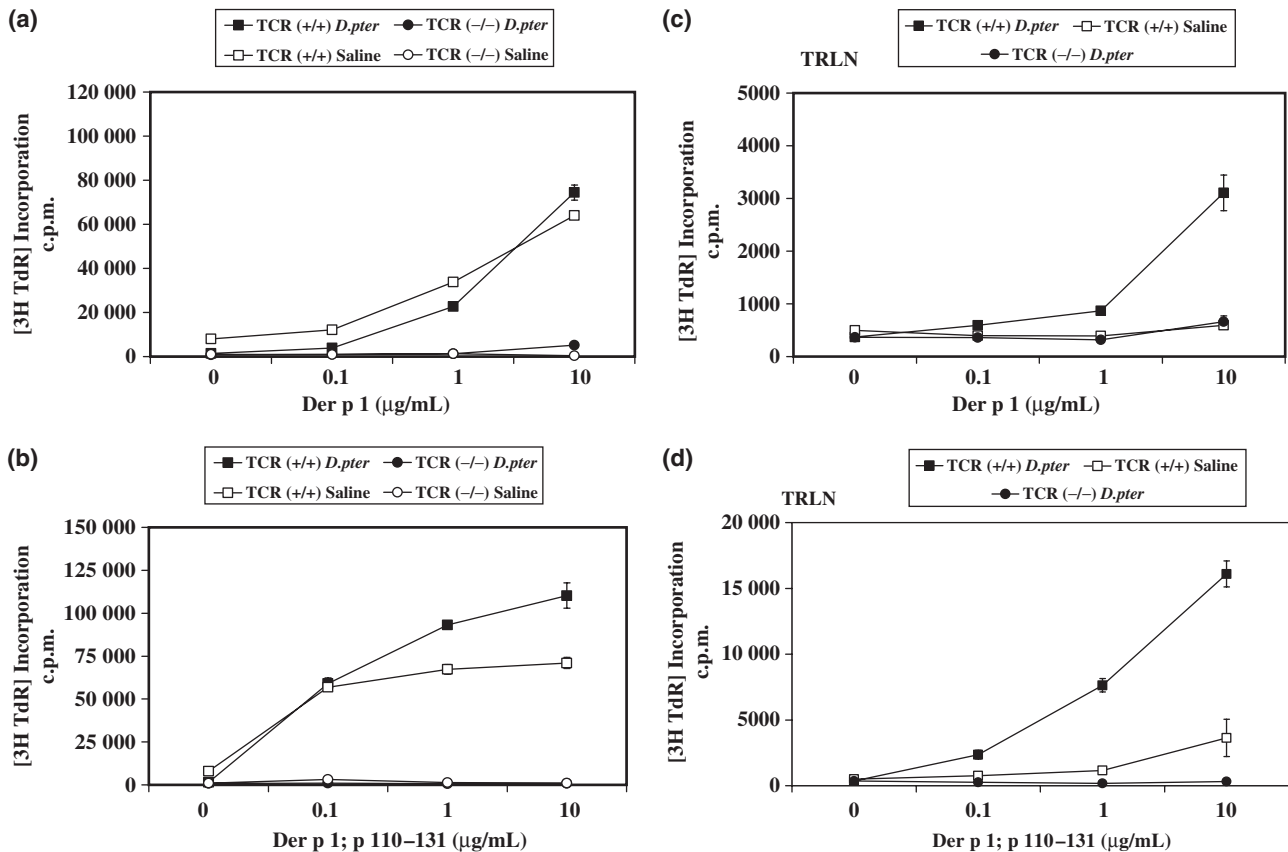


Fig. 2. Dose-dependent responses on primary and recall stimulation of T cells isolated from secondary lymphoid organs of V α 3.2 V β 14 TCR transgenic mice on stimulation with Der p 1 or the immunodominant peptide Der p 1; p 110–131. Proliferative responses of splenocytes (a, b) or draining lymph node (c, d)-derived cells, obtained from TCR transgenic (+/+) or wild-type C57 BL/6J (-/-) mice following challenge of the airways with Der p 1 or saline. Data are representative of three experiments and results presented as mean $\pm$ SEM for triplicates. TCR, T cell receptor.

genics as compared with wild type, we performed differential analysis of cytopins on BAL fluid. As shown in Fig. 3c, although increases in lymphocytes (TCR (+/+) Der p 1: $17.1 \pm 5.9\%$, TCR (-/-) Der p 1: 12 ± 5.8 , $P = 0.273$) as well as polymorphonuclear granulocytes (38%) were observed in TCR- $\alpha\beta$ transgenic mice following secondary challenge of the airways with Der p 1, these values were not significant, reflecting an early response to allergen challenge. The granulocytic infiltrate was composed of eosinophils (TCR (+/+) Der p 1: $23 \pm 9\%$, TCR (-/-) Der p 1: 7.4 ± 4 , $P = 0.144$) as well as neutrophils (TCR (+/+) Der p 1: $15 \pm 8.7\%$, TCR (-/-) Der p 1: 5.9 ± 3.5 , $P = 0.405$). The cellular composition of BAL fluid obtained from similarly challenged wild-type or naïve transgenic mice consisted principally of alveolar macrophages and monocytes (data not shown; 87%).

To further characterize the cellular infiltrate, immunohistochemical analysis was performed on parallel sections of lung tissue (Fig. 4). The mononuclear infiltrate in TCR- $\alpha\beta$ transgenic mice consisted predominantly of CD3⁺ T cells and to a lesser extent B220⁺ B cells. Notably, the percentage of mature CD3⁺ T cells (40%) and B220⁺ B cells as well as the ratio of CD4⁺ (23%) to CD8⁺ (9–16%) T cells in secondary lymphoid organs of Der p 1 TCR- $\alpha\beta$ transgenic mice was within the normal range, as determined by flow cytometry (see data in Fig. 1d). The marked CD3⁺ T cell infiltrate in the lungs of transgenic mice but not wild-type animals may,

therefore, reflect the increased frequency of p 110–131 reactive CD4⁺ T cells arising because of localized priming and expansion of in the draining tracheal lymph nodes and subsequent recruitment into the airways following re-challenge with allergen.

Transgenic mice develop goblet cell hyperplasia and exhibit increased mucin production following allergen challenge of the airways

One of the major problems in asthma is excessive mucus production and the resulting airway obstruction. Mice transgenic for the Der p 1 TCR- $\alpha\beta$ developed increased goblet cell hyperplasia, metaplasia and mucin production following challenge of the airways with Der p 1 as compared with saline alone, even in the absence of prior systemic sensitization. As a principal constituent of mucus, the detection of mucin in goblet cells, which accompany the observed structural changes in the epithelium, are suggestive of increased levels of mucous production in the airways (Fig. 5).

Discussion

There are a number of existing murine models of allergic asthma, all of which have provided a wealth of information on the mechanisms underlying disease pathophysiology (e.g.

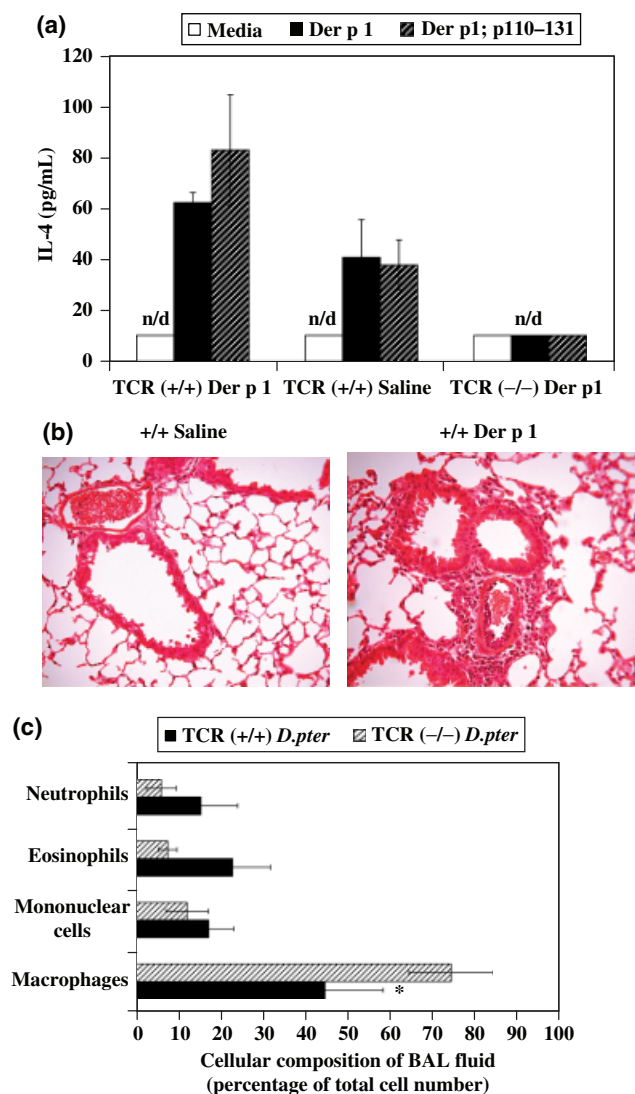


Fig. 3. Mice expressing the V α 3.2 V β 14 TCR transgene develop a T-helper type-2-mediated inflammatory infiltrate on exposure of the airways to Der p 1 allergen. Levels of IL-4 production by lymphocytes obtained from TCR transgenic or wild-type mice on *in vitro* stimulation with Der p 1 or residues Der p 1: p 110–131. Levels of IL-4 < 15 pg/mL were designated as non-detectable (nd) (a). Data as expressed for a representative experiment, mean $\pm$ SEM ($n = 4$). Histological examination of lung sections demonstrating general morphology, as determined by haematoxylin and eosin staining, using tissue derived from mice expressing the V α 3.2 V β 14 TCR transgene (+/+), obtained 24 h after challenge of the airways with saline or Der p 1 (b: magnification $\times 20$ lens). Cellular infiltrate in bronchoalveolar lavage fluid obtained from the airways of transgenic or wild-type mice determined 24 h after challenge with Der p 1, by differential staining (c). TCR, T cell receptor.

[20, 21]). However, one of their major limitations is the failure of animals to develop asthma in response to inhaled allergen, without prior systemic sensitization. This may reflect species-dependent anatomical and physiological differences in the respiratory system. As allergic asthmatics are sensitized to inhalant allergens via the airway mucosa, we were prompted to develop a more physiological model, that would reflect the natural route of allergen exposure [22].

In allergic asthma, CD4⁺ Th2 cells are believed to initiate and perpetuate disease, and their levels are increased in the airways of asthmatics [2]. Numerous studies have demon-

strated that without systemic priming and expansion of the existing antigen specific T cell population, mucosal delivery of soluble antigen in the absence of an immunostimulatory stimulus results in systemic tolerance [18, 23]. To circumvent this phenomenon, we have generated a transgenic strain, in which between 2% and 12% of peripheral CD4⁺ T cells express the V α 3.2 and V β 14 TCR specific for the immunodominant epitope of the major HDM allergen; Der p 1; residues p 110–131. The frequency of allergen reactive CD4⁺ T cells in the peripheral blood of atopic individuals is in the range of 0.001–0.6% with marked increases during the pollen season [24], as reflected in the frequency in expression of selective TCR V β elements [14].

Unimmunized transgenic mice exhibit normal lung physiology and architecture, but rapidly develop an allergic asthmatic phenotype on exposure of the airways to Der p 1. This is characterized by the recruitment of inflammatory cells, goblet cell metaplasia and increased mucin production, resulting eventually in phenotypic changes to the airway epithelium. The mixed granulocytic infiltrate consisting predominantly of eosinophils, and to a lesser extent neutrophils, is indicative of the early inflammatory response. The induction of pulmonary inflammation in Der p 1 transgenic mice does not depend on prior systemic sensitization with non-specific immune-modulating adjuvants, presumably reflecting the high frequency of effector CD4⁺ T cells expressing the specific TCR- $\alpha\beta$. In conventional models of allergic asthma, the requirement for systemic priming prior to allergen challenge may serve to expand the pool of antigen-specific T cells.

CD4⁺ T cells isolated from naive TCR- $\alpha\beta$ transgenic mice, on stimulation with specific antigen produced significant levels of IL-4. Signalling by IL-4, which promotes survival through induction of Bcl-2 expression [25], coupled with low-grade environmental exposure to and presentation of Der p 1-derived antigens in association with MHC class II molecules may be critical for survival of resting naive T cells in the periphery. Furthermore, the production of IL-4 on antigenic challenge of T cells expressing the TCR transgene may account for the rapid recruitment of lymphocytes and inflammatory cells, including eosinophils, and the failure to induce tolerance on mucosal exposure to soluble antigen. IL-4 is thought to play a fundamental role in the pathogenesis of allergic asthma, causing the differentiation of Th2 effector cells from naive CD4⁺ T cell precursors and the induction of IgE isotype switching in B cells [3, 26]. In the airways, signalling through the common IL-4R α chain by IL-4 as well as IL-13 induces upregulation of VCAM-1 on endothelial cells lining the blood vessels, resulting in enhanced recruitment of effector cells [27], and induction of MUC5AC gene expression and increased mucin production by airway epithelial cells [28]. The pronounced goblet cell hyperplasia in transgenic mice following exposure to allergen is most likely associated with elevated levels of Th2 cytokines, including IL-4, produced by antigen specific CD4⁺ Th2 cells which have migrated into the lungs on re-exposure to allergen following antigen-specific priming in the tracheal lymph nodes draining the airways. In addition to IL-4, which is critical for the induction of a Th2 phenotype, elevated levels of IL-5, IL-9 and IL-13 also contribute to the magnitude of the inflammatory response [3].

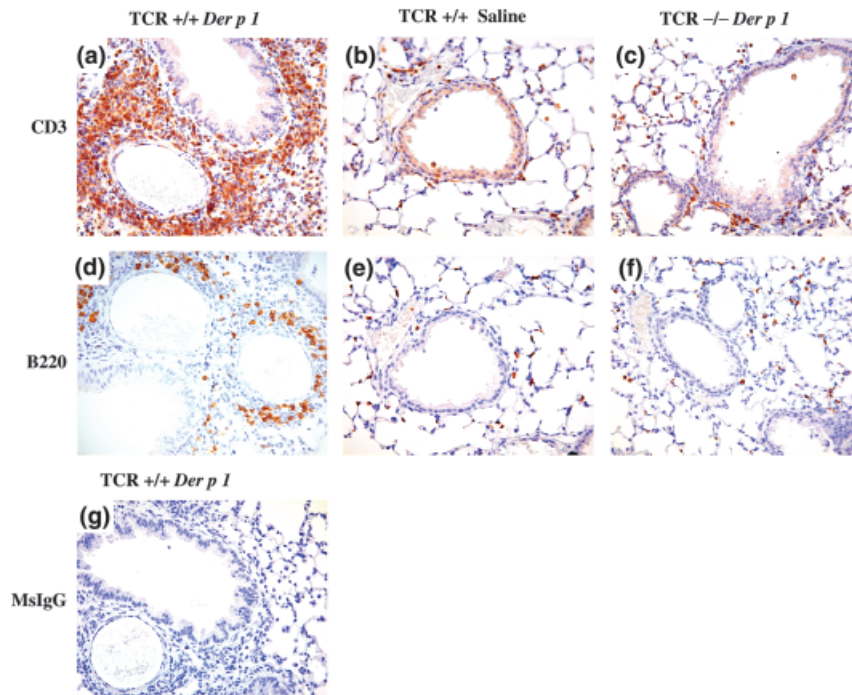


Fig. 4. $V\alpha 3.2 V\beta 14$ TCR (+/+) transgenic mice exhibit increased recruitment of lymphocytes into the lungs following challenge with Der p 1. Immunohistochemical examination of representative lung sections obtained from either groups of mice expressing the TCR $V\alpha\beta$ transgene (+/+), 24 h after challenge of the airways with Der p 1 (a, d, g) or saline (b, e) or wild-type C57 BL/6J (-/-) mice challenged with Der p 1 (c, f). Parallel sections stained with anti-CD3 ϵ (a–c), anti-B220 (d–f) or secondary antibody alone (g). TCR, T cell receptor.

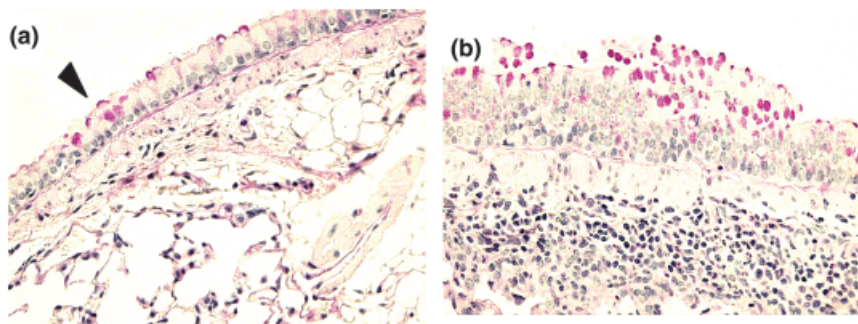


Fig. 5. Allergen-dependent increases in goblet cell hyperplasia and mucin production at sites of cellular recruitment. Histological examination of lung sections obtained from (+/+) transgenic mice challenged with saline (a) or Der p 1 allergen (b), demonstrating increased goblet cell hyperplasia, metaplasia and mucin production, as determined by PAS alcian blue. Magnification $\times 20$ lens, $\times 6.3$ projector Zeiss Axioshop (representative section) (Zeiss Microscopes, Munich, Germany).

We were unable to detect significant increases in specific IgE in sera ([18] for methodology) obtained from mice 5 days after primary challenge of the airways with allergen, presumably reflecting the kinetics of plasma cell differentiation, rather than a lack of CD4 $^{+}$ T cell helper activity [29].

The use of TCR transgenic mice as model systems to study diseases characterized by selective TCR repertoire usage is not novel. Previous studies have demonstrated that mice heterozygous for the D011.10 ovalbumin (OVA) TCR transgene, expressed by 40% of peripheral T cells, developed AHR in response to spasmogenic stimuli on exposure to aerosolized OVA, despite the lack of prior systemic sensitization. Nevertheless, mice failed to develop eosinophilia and goblet cell hyperplasia and levels of IL-4 produced on antigenic challenge were only marginally increased, indicative of a failure to induce Th2-type immunity [30]. Although

associated with asthma, AHR is a prominent feature of a variety of pulmonary disease states, ensuing from changes induced in airway physiology. AHR in asthma is primarily associated with tissue damage caused by the release of inflammatory mediators, but can occur independently of the presence of eosinophils [31]. In patients, sensitization to OVA occurs through the gut mucosa rather than the airways. Others have stipulated that the presence of high levels of endotoxin contaminants may be responsible for the induction of AHR in the absence of eosinophilia [32], as observed by Wilder et al. [30]. We similarly cannot exclude the possibility that low levels of endotoxins in allergen extracts may be responsible for the induction of Th2-mediated airway inflammation [32]. Allergens themselves exhibit enzymatic activity, which may contribute to the pathophysiology of airway inflammation [33]. The transgenic

strain generated here expressed TCR transgenes reactive with Der p 1, a cysteine protease which can disrupt the epithelial architecture of the airways thereby facilitating entry into the mucosa and potentiating development of asthma. Enzymatic cleavage of the IL-2R α subunit may further pre-dispose towards the expansion of CD4⁺ T cells expressing a Th2 phenotype [34].

Here, we demonstrate that the Der p 1 transgenic strain, on exposure of the airways to allergen, develops characteristic features of allergic asthma including goblet cell hyperplasia and increased eosinophil recruitment, although levels of the latter were not significant. The induction of these responses was not dependent on prior systemic sensitization. Eosinophils are known to play a central role in disease pathology, including the induction of AHR (not assessed in this strain). Studies have consistently observed a correlation between increased levels of eosinophils in bronchial biopsies obtained from allergic patients, disease severity and AHR [35]. In addition to the CD4⁺ Th2 cell-derived cytokines, notably IL-4 and IL-13, inflammatory mediators released by eosinophils are directly responsible for tissue damage and changes in lung pathology and are therefore likely to induce increased AHR in this strain [36, 37]. Genetic differences between inbred strains of mice could influence susceptibility to inflammation and AHR on allergen exposure and, consequently, differences between the models used (e.g. [38]). A detailed study demonstrated that among different strains, C57BL/6 mice were less susceptible to induction of TH2-mediated airway inflammation and eosinophilia on allergen challenge in the absence of prior systemic sensitization with OVA adsorbed to alum [39]. Here, the ability to induce airway eosinophilia in the absence of prior systemic sensitization in Der p 1 TCR transgenic on the C57BL/6 background suggests that an increase in the frequency of allergen reactive CD4⁺ T cells expressing a TH2 phenotype may be sufficient to override the tolerogenic microenvironment in the airway mucosa. IL-10 is known to play a role in the induction and maintenance of a tolerogenic phenotype [40]. Analysis of IL-10 and IL-4 ratios in the airways and draining lymph nodes of transgenic and wild-type mice following allergen exposure may give some insight into the underlying mechanisms of mucosal tolerance induction, and suggests that the Der p 1 TCR transgenic mouse described here may provide a useful model system for investigating mucosal tolerance to inhalant allergens.

Allergic asthma is a polygenic trait with multiple genetic loci controlling the asthmatic phenotype. The genetic heterogeneity of the atopic population responsive to HDM allergens suggests that HLA class II restriction specificity is not a major determining factor. Several distinct epitopes have been identified as the target of both human and murine CD4⁺ Th cell immunity to Der p 1. Here, we report that the response generated against a single immunodominant epitope of Der p 1 in mice expressing the specific TCR- $\alpha\beta$ is sufficient to induce an asthmatic phenotype on challenge of the airways. Analysis of TCR gene usage by murine and human effector CD4⁺ T cells suggested limited diversity, despite the heterogeneity in both antigen and HLA restriction specificities. Data obtained from sequencing TCR domain derived from a panel of human Der p 1 reactive T cell clones demonstrated the existence of a limited number of long-lived T cell clones *in vivo*. This finding suggests that a limited

number of CD4⁺ T cells reactive with dominant epitopes may be responsible for maintaining the allergic response on chronic exposure to allergen [15]. If this is the case, it may account for the success of allergen-based immunotherapy and would support the use of limited peptide epitopes rather than the whole allergen as a means of down-regulating effector responses, thereby reducing the risk of triggering anaphylactic reactions in response to IgE receptor crosslinking by conformational epitopes on the allergen.

Our studies indicate that the Der p 1 TCR- $\alpha\beta$ transgenic mice will provide a useful model for defining both the mechanisms underlying the pathophysiology of allergic disease and the immunological basis of mucosal tolerance and immunoregulation. In addition, the TCR- $\alpha\beta$ transgenic could provide a physiological model for the design and testing of novel immunotherapeutic strategies for modulating responses to a clinically relevant inhalant allergen.

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References

- Busquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma: from bronchoconstriction to airway inflammation and remodeling. *Am J Respir Crit Care Med* 2000; 161:1720–45.
- Robinson DS, Hamid Q, Ying S et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992; 326:298–304.
- Fallon PG, Jolin HE, Smith P et al. IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9 and IL-13. *Immunity* 2002; 17:7–17.
- Davis MM, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature* 1988; 334:395–402.
- Boitel B, Ermonval M, Panina-Bordignon P, Mariuzza RA, Lanzavecchia A, Acuto O. Preferential V beta gene usage and lack of junctional sequence conservation among human T cell receptors specific for a tetanus toxin-derived peptide: evidence for a dominant role of a germline-encoded V region in antigen/major histocompatibility complex recognition. *J Exp Med* 1992; 175: 765–77.
- Fink PJ, Matis LA, McElligott DL, Bookman M, Hedrick SM. Correlations between T-cell specificity and the structure of the antigen receptor. *Nature* 1986; 321:219–26.
- Wucherpfennig KW, Ota K, Endo N et al. Shared human T cell receptor V β usage to immunodominant regions of myelin basic protein. *Science* 1990; 248:1016–9.
- Oksenberg JR, Panzara MA, Begovich AB et al. Selection for T-cell receptor V β -D β -J β gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. *Nature* 1993; 362:68–70.
- Acha-Orbea H, Mitchell DJ, Timmermann L, et al. Limited heterogeneity of T-cell receptors from lymphocytes mediating autoimmune encephalomyelitis allows specific immune intervention. *Cell* 1988; 54:263.

- 10 Young RP, Dekker JW, Wordsworth BP et al. HLA-DR and HLA-DP genotypes and immunoglobulin E responses to common major allergens. *Clin Exp Allergy* 1994; 24:431–9.
- 11 Mohapatra SS, Mohapatra S, Yang M, Parronchi P, Maggi E, Romagnani S. Molecular basis of cross-reactivity among allergen-specific human T cells: T-cell receptor V α gene usage and epitope structure. *Immunology* 1994; 81:15–20.
- 12 Jarman ER, Hawrylowicz CM, Panagiotopoulou E, O'Hehir RE, Lamb JR. Inhibition of human T-cell responses to house dust mite allergens by a T-cell receptor peptide. *J Allergy Clin Immunol* 1994; 94:844–52.
- 13 Kircher MF, Haeusler T, Nickel R, Lamb JR, Renz H, Beyer K. Vb18.1+ and Va2.3+ T-cell subsets are associated with house dust mite allergy in human subjects. *J Allergy Clin Immunol* 2002; 109:517–23.
- 14 Beyer K, Hausler T, Kircher M, Nickel R, Wahn U, Renz H. Specific V β T cell subsets are associated with cat and birch pollen allergy in humans. *J Immunol* 1999; 162:1186–91.
- 15 Wedderburn LR, O' Hehir RE, Hewitt CR, Lamb JR, Owen MJ. In vivo clonal dominance and limited T-cell receptor usage in human T-cell recognition of house dust mite allergens. *Proc Natl Acad Sci USA* 1993; 90:8214–8.
- 16 Renz H, Bradley K, Saloga J, Larsen GL, Gelfand EW. RT cells expressing specific V beta elements regulate immunoglobulin E production and airways responsiveness in vivo. *J Exp Med* 1993; 177:1175–80.
- 17 Yssel H, Johnson KE, Schneider PV et al. T cell activation-inducing epitopes of the house dust mite allergen Der p1. Proliferation and lymphokine patterns by Der p1-specific CD4⁺ T cell clones. *J Immunol* 1992; 148:738–45.
- 18 Hall G, Lund L, Lamb JR, Jarman ER. Kinetics and mode of peptide delivery via the respiratory mucosa determine the outcome of activation vs. TH2 immunity in allergic inflammation of the airways. *J Allergy Clin Immunol* 2002; 110:883–90.
- 19 Holt PG. Immune tolerance and protection against allergic sensitization. *Allergy* 1995; 50 (Suppl.):34–6.
- 20 Hessel EM, Van Oosterhout AJM, Garsen J, Van Loveren H, Savelkoul HFJ, Nijkamp FP. Immediate asthmatic reactions and changes in airway responsiveness after single vs. chronic ovalbumin inhalation in sensitized mice. *Eur J Allergy Clin Immunol* 1993; 48:101.
- 21 Temelkovski J, Hogan SP, Shepard DP, Foster PS, Kumar RK. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolized allergen. *Thorax* 1998; 53:849–56.
- 22 Holt PG, Macaubas C, Stumbles PA, Sly PS. The role of allergy in the development of asthma. *Nature* 1999; 402 (Suppl.):B12–7.
- 23 Wiedermann U, Jahn-Schmid B, Bohle B et al. Suppression of antigen-specific T and B cell responses by intranasal or oral administration of recombinant Bet v1, the major birch pollen allergen, in a murine model of type I allergy. *J Allergy Clin Immunol* 1999; 103:102–10.
- 24 Rimaniol AC, Garcia G, Tills SJ et al. Evaluation of CD4⁺ T cells proliferating to grass pollen in seasonal allergic subjects by flow cytometry. *Clin Exp Immunol* 2003; 132:76–80.
- 25 Vella AT, Dow S, Potter TA, Kappler J, Marrack P. Cytokine/induced survival of activated T cells *in vitro* and *in vivo*. *Proc Natl Acad Sci USA* 1998; 95:3810–15.
- 26 Kopf M, Le Gros G, Bachmann M, Lamers MC, Bluethmann H, Kohler G. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. *Nature* 1993; 362:245–8.
- 27 Rankin JA, Picarella DE, Geba GP et al. Phenotypic and physiological characterization of transgenic mice overexpressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without hyperreactivity. *Proc Natl Acad Sci USA* 1996; 23:7821–5.
- 28 Temann U-A, Prasad B, Gallup MW et al. A novel role for murine IL-4 in vivo: induction of MUC5AC gene expression and mucin hypersecretion. *Am J Respir Cell Mol Biol* 1997; 16: 471–8.
- 29 Calame KL. Plasma cells: finding new light at the end of B cell development. *Nat Immunol* 2001; 2:1103–8.
- 30 Wilder JA, Collie DDS, Bice DE, Tesfaigzi Y, Lyons CR, Lipscomb MF. Ovalbumin aerosols induce airway hyperactivity in naïve DO11.10 T cell receptor transgenic mice without pulmonary eosinophilia or OVA specific antibody. *J Leucocyte Biol* 2001; 69:538–47.
- 31 Corry DB, Folkesson HG, Warnock ML et al. Interleukin 4 but not Interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996; 183:109–117.
- 32 Eisenbarth SC, Piggot DA, Huleatt JW, Visintin I, Herrick CA, Bottomly K. Lipopolysaccharide-enhanced, Toll-like Receptor 4-dependent T helper cell type 2 response to inhaled antigen. *J Immunol* 2004; 172:4527–34.
- 33 Thomas WR. Mite allergens groups I-VII. A catalogue of enzymes. *Clin Exp Allergy* 1993; 23:350–3.
- 34 Shultz O, Sewell HF, Shakib F. Proteolytic cleavage of CD25, the subunit of the human T cell interleukin-2 receptor, by Der p1, a major mite allergen with cysteine protease activity. *J Exp Med* 1998; 87:271–5.
- 35 Wardlaw AJ, Dunnette S, Gleich GJ. Eosinophils and mast cells in bronchoalveolar fluid in subjects with asthma: relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1998; 137:62–9.
- 36 Tomkinson A, Kanehiro A, Rabinovitch N, Joetham A, Cieslewicz G, Gelfand EW. The failure of STAT6-deficient mice to develop airway eosinophilia and airway hyperresponsiveness is overcome by interleukin-5. *Am J Respir Crit Care Med* 1999; 160:1283–91.
- 37 Tomkinson A., Duez C, Lahn M, Gelfand EW. Adoptive transfer of T cells induces airway hyperresponsiveness independently of airway eosinophilia but in a signal transducer and activator of transcription 6-dependent manner. *J Allergy Clin Immunol* 2002; 109:810–6.
- 38 Keen JC, Sholl L, Wills-Karp M, Georas SN. Preferential activation of nuclear factor of activated T cells c correlates with mouse strain susceptibility to allergic responses and interleukin-4 gene expression. *Am J Respir Cell Mol Biol* 2001; 24:58–65.
- 39 Shinakgawa K, Kojima M. Mouse model of airway remodeling. Strain differences. *Am J Respir Crit Care Med* 2003; 168: 959–67.
- 40 Hall G, Houghton CG, Rahbek JU, Lamb JR, Jarman ER. Suppression of allergen reactive Th2 mediated responses and pulmonary eosinophilia by intranasal administration of an immunodominant peptide is linked to IL-10 production. *Vaccine* 2003; 21:549–61.