

Transgenic mice which overproduce Th2 cytokines develop spontaneous atopic dermatitis and asthma

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Abstract

We have investigated the role of Th2 cytokines in the development of atopic diseases using transgenic mice carrying large genomic segments containing IL4, IL13 and IL5 genes and overexpressing these Th2 cytokines. *In vitro* stimulated, but not unstimulated, Th2 cells from the transgenic mice expressed high levels of IL4, IL13 and IL5 compared to those from non-transgenic mice. The transgenic mice developed spontaneous atopic dermatitis and airway inflammation against environmental allergens. The affected regions for atopic dermatitis covered the entire body including skin in the face, ear, eye-lid, neck, hind region and tail. Histological features showed thickened epidermis and dermis and infiltration of large numbers of inflammatory cells in the affected regions. The transgenic mice also showed airway inflammation characteristic of asthma, including infiltration of inflammatory cells and hypertrophy of airway epithelial cells. These mice also expressed high level of serum IgE, which is a hallmark of atopic diseases. In summary, this study provides additional evidence that Th2 cytokines play key roles in atopic diseases.

Introduction

Atopic diseases including asthma and atopic dermatitis are multifactorial diseases influenced by genetic predisposition and environmental factors (1–3). Atopic diseases have a complex and chronic pathogenesis that may have common causes, which are characterized by high IgE production, cellular tissue infiltration to the site of allergic reactivity by diverse effector cells, including T lymphocytes, eosinophils, monocytes/macrophages and mast cells (4). Co-ordination of the mobilization, activation and trafficking of these effector cells to sites of inflammation is controlled by a complex milieu of cytokines and chemokines chiefly derived from activated CD4 T helper cells and resident cells. This process is dependent on the production of the cytokines IL4, IL5 and IL13 by allergen-specific Th2 cells that are generated from naive precursor T cells (5,6).

Several Th2 cytokine genes are clustered in a 120 kb region in chromosome 11 in mouse and in a 160 kb region in chromosome 5 in human. In a previous study, we generated transgenic mice containing 120 kb of the murine Th2 cytokine locus including the IL4, IL5 and IL13 genes (7). The CD4 T cells from these transgenic mice overexpress Th2 cytokines but only upon antigenic stimulation.

Using this transgenic mouse model, we examined the effects of the systemic overproduction of Th2 cytokines on the

development of allergic diseases. We found that overexpression of Th2 cytokines in this transgenic mouse causes spontaneous atopic dermatitis and airway inflammation reminiscent of asthma against environmental allergens, providing additional evidence that supports the critical role of Th2 cytokines in the pathogenesis of atopic diseases.

Methods

Transgenic mice

Generation of BAC transgenic mice was described in the previous study in detail (7). Briefly, BAC DNA containing IL4, IL13 and IL5 was cloned from 129/svj mouse ES BAC library (GenomeSystems). An 800 base pair IL4 promoter-luciferase reporter construct was inserted into the BAC DNA by *in vitro* transposition to monitor IL4 promoter activity. Reporter integrated BAC DNA (B137) was prepared by removing vector sequence (pBeloBAC11) from the parental plasmid constructs by digesting with *Not*I. DNA was separated by pulse-field electrophoresis (Bio-Rad, CHEF-DR II system), then concentrated by running in a perpendicular direction in 4% low melting agarose gel. The DNA band was cut out, dialyzed in 20 ml of TENPA buffer (10 mM Tris pH 7.5, 1 mM EDTA, 100 mM NaCl,

30 μ M spermine, 70 μ M spermidine) for 1.5 h, and digested with agarase (NEB) at 42°C for 2 h, and dialyzed in injection buffer (10 mM Tris pH 7.5, 0.1 mM EDTA, 100 mM NaCl, 30 μ M spermine, 70 μ M spermidine) for 2 h. B137 DNA was injected into fertilized eggs of (B6 $\times$ C3H)F1, and transgenic mice were generated as previously described (8). Transgene positive founders were screened and the integrity of the transgene was confirmed by PCR. Founders were crossed with B6 mice, and founders and F1 progenies were used for experiment.

In vitro differentiation of CD4 T cells and measurement of Th2 cytokines

The method we use for *in vitro* differentiation of CD4 T cells was described previously (9). Briefly, CD4 T cells were enriched from spleen cells from transgenic mice by negative selection through depletion using anti-MHC class II (M5/115), anti-NK1.1 (HB191) and anti-CD8 (53-6.7) mAb. Antigen-presenting cells (APC) were prepared by γ -irradiation and negative selection. To differentiate CD4 T cells *in vitro*, 3×10^6 CD4 T cells were cultured with an equal number of APC in 5 ml of Bruff medium with 5% fetal calf serum (Life Technologies, Inc.) and penicillin/streptomycin in the presence of 2.5 μ g/ml Con A and 20 U/ml IL2. For Th2 skewing condition, 1000 U/ml IL4 and 10 μ g/ml XMG1.2 (anti-IFN- γ) antibody were added. After 3 days cells were washed and restimulated with 2.5 μ g/ml Con A for 12 h. IL4, IL5 and IL13 were measured in the supernatant by ELISA.

Total serum IgE measurement

Total serum IgE levels were measured by ELISA. The coating was done with anti-mouse IgE (The Binding Site) in PBS at 4°C overnight; the standard was mouse IgE (IgE-3; PharMingen); and the biotin-labeled secondary antibody was rat anti-mouse IgE(LO-ME-3; BioSource).

Tissue sectioning and staining

Mice were sacrificed by carbon dioxide inhalation. Affected skin regions and lung were excised and immersed in 10% formalin. Tissue processing and histological staining were performed by the Yale Medical School Research Histology, Department of Pathology.

Results

Generation of transgenic mice overexpressing Th2 cytokines

Generation of the BAC transgenic mice overexpressing Th2 cytokines were described previously [(7), Methods]. The transgenic mice contain 120 kb of the murine Th2 cytokine locus including IL4, IL5 and IL13 genes and integrated IL4

promoter-luciferase reporter (Fig. 1). Based on IL4 promoter-reporter expression in T helper cell differentiation, we showed that the BAC transgene recapitulates the endogenous cytokine expression pattern (7), i.e. expression was Th2 specific and dependent on TCR stimulation of the T cells.

To measure the ability to produce Th2 cytokines in this transgenic mouse, splenic CD4 T cells were isolated from the transgenic mice and stimulated with ConA and APC under Th2 polarizing conditions. Th2 cells from most of the B137 transgenic mice lines including that used in this study (B137-29) produced high levels of IL4, IL13 and IL5 in the culture supernatant (Fig. 2). This result indicates that CD4 T cells from this transgenic line have the capacity to produce large amount of Th2 cytokines upon antigenic stimulation. No cytokines were produced in the absence of TCR stimulation (Fig. 2).

The transgenic mice overexpressing Th2 cytokines develop spontaneous atopic dermatitis

All of the transgenic lines made with the construct (B137) developed spontaneous atopic dermatitis, despite the fact that all mice used in this study were kept under specific pathogen-free conditions. The time of onset and severity of disease varied depending on the expression level of Th2 cytokines induced upon TCR ligation (Table 1). The higher the expression levels of Th2 cytokines, the earlier the onset time and the more severe the symptoms of disease (Table 1). The atopic dermatitis affected the entire body including skin in the face, ear, eye-lid, neck, hind region and tail. The lesions manifested hemorrhage and excoriation. The regions affected and the extent of the lesions varied among individuals. One of the lines, B137-29, expressed very high levels of Th2 cytokines and developed the most severe atopic dermatitis (Fig. 3B). In this transgenic line the onset of atopic dermatitis occurred at the age of 5-6 months, and the disease progressed rapidly to the extent that the lesions cover ~20% of the body by the age of 7-8 months, at which point the affected mice had be euthanized for humane reasons.

To examine the histological features of the affected regions, we removed and stained with hematoxylin and eosin tissues in severely affected mice from the B137-29 line and the corresponding unaffected regions from non-transgenic control littermates. Skin from the ear, eye-lid, and hind region from the transgenic mice showed massive cellular infiltration (Fig. 3C-H). All tissues were characterized by thickening of the outer layer and stratum spinosum of the epidermis (Fig. 3D, F and H), and the ear region and hind regions showed inflamed dermis with infiltrating cells (Fig. 3D and H). In the eye-lid, the dermis was shortened, and connective tissues underlying the dermis were completely destroyed by infiltrating cells

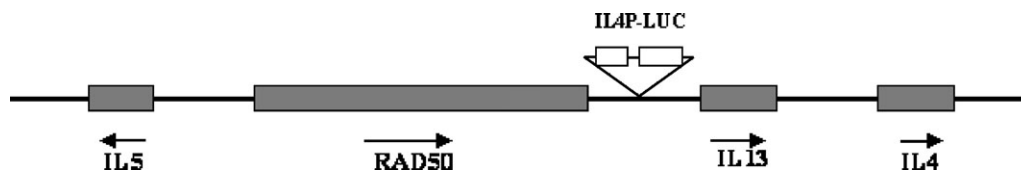


Fig. 1. Diagram of transgene for B137 transgenic mice. BAC DNA (120 kb) containing IL4, IL5 and IL13 was cloned from mouse ES BAC library. BAC DNA was integrated with 800 bp IL4 promoter-luciferase construct to monitor IL4 promoter activity (Methods).

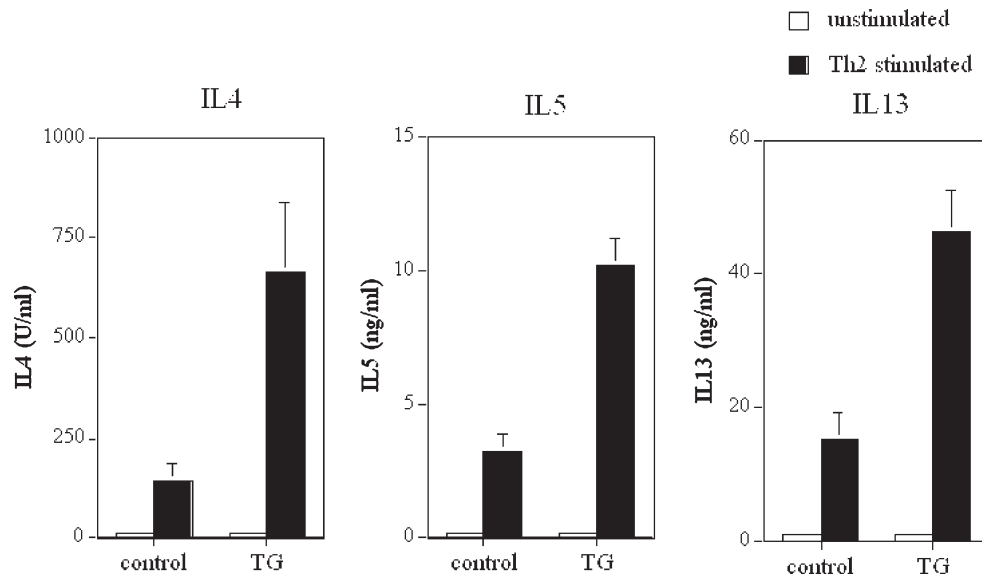


Fig. 2. Production of Th2 cytokines in *in vitro* stimulated CD4 T cells. Splenic CD4 T cells were isolated from three B137–29 transgenic mice and three non-transgenic littermate, and 3×10^6 CD4 T cells were cultured in 5 ml of Bruff's medium with the same number of APC in the presence of 2.5 μ g/ml Con A, 20 U/ml IL2 + APC in Th2 skewing condition for 3 days, washed and restimulated with 2.5 μ g/ml ConA for 12 h. Cytokines were measured from the supernatant by ELISA.

(Fig. 3F). Examination at high magnification showed the infiltrating cells were lymphocytes, macrophages and eosinophils (Fig. 3D, F and H; insets). This histological staining clearly shows characteristic features of atopic dermatitis in transgenic mice overproducing Th2 cytokines.

The transgenic mice also show histological features of asthma

We examined lung section of B137–29 mice to check whether these mice exhibited airway inflammation. Histology of B137–29 mice showed perivascular and peribronchiolar infiltration with inflammatory cells, and hypertrophy of airway epithelial cells (Fig. 4), which are characteristic inflammatory features of asthma. Examination at high magnification showed the presence of lymphocytes and eosinophils infiltration in the lung (Fig. 4B; inset). This result supports the critical role of Th2 cytokines in the development of asthma and also supports the hypothesis that atopic dermatitis and allergic asthma share a common genetic basis (10).

High level of serum IgE in transgenic mice

Circulating IgE is captured on its surface receptor (Fc ϵ R1) present on mast cells and eosinophils. Subsequent encounter with antigens induces Fc ϵ R1 crosslinking, cellular activation and release of various allergic mediators such as histamine, proteases and cytokines (11). A high concentration of total serum IgE is one of the characteristic features of atopic diseases. Therefore we examined total serum IgE level from B137–29 transgenic mice (Fig. 5). The average total serum IgE concentration was ~60-fold higher in the transgenic mice than that in littermate control mice. The increase of serum IgE is probably due to the overproduction of IL4 and IL13 by CD4 T cells, which stimulate B cells to class-switch to IgE (12,13),

Table 1. Disease onset time and progression time depend on expression level of Th2 cytokines

Transgenic mice line	Relative expression level of Th2 cytokines*	Atopic dermatitis onset time (months)	Atopic dermatitis progression time** (months)
B137–29	+++	5–6	2
B137–36	++	7–8	3
B137–75	+	12–14	4–5

*Relative expression level of Th2 cytokines was measured as the same as in Fig. 2.

**Disease progression time is the period from onset to spread to involvement of 20% of the body surface. In the case of B137–75 transgenic mice, the spread was 10% of the body 4–5 months after onset. Animals were required to be euthanized for humane reasons at this point.

manifesting the *in vivo* effect of these cytokines in the transgenic mice.

Discussion

In this study, we investigated the role of systemic overproduction of Th2 cytokines in the pathogenesis of atopic diseases using transgenic mice containing murine Th2 cytokine cluster. We found that physiologic overproduction of Th2 cytokines in this transgenic mouse causes spontaneous atopic dermatitis and asthma, providing additional evidence for the critical role of Th2 cytokines in the pathogenesis of atopic diseases.

It is a generally accepted hypothesis that Th2 cells play key roles in the pathogenesis of atopic dermatitis (14,15), although recent studies propose that Th1 cells also play a role in atopic dermatitis pathology particularly at the later stages of the

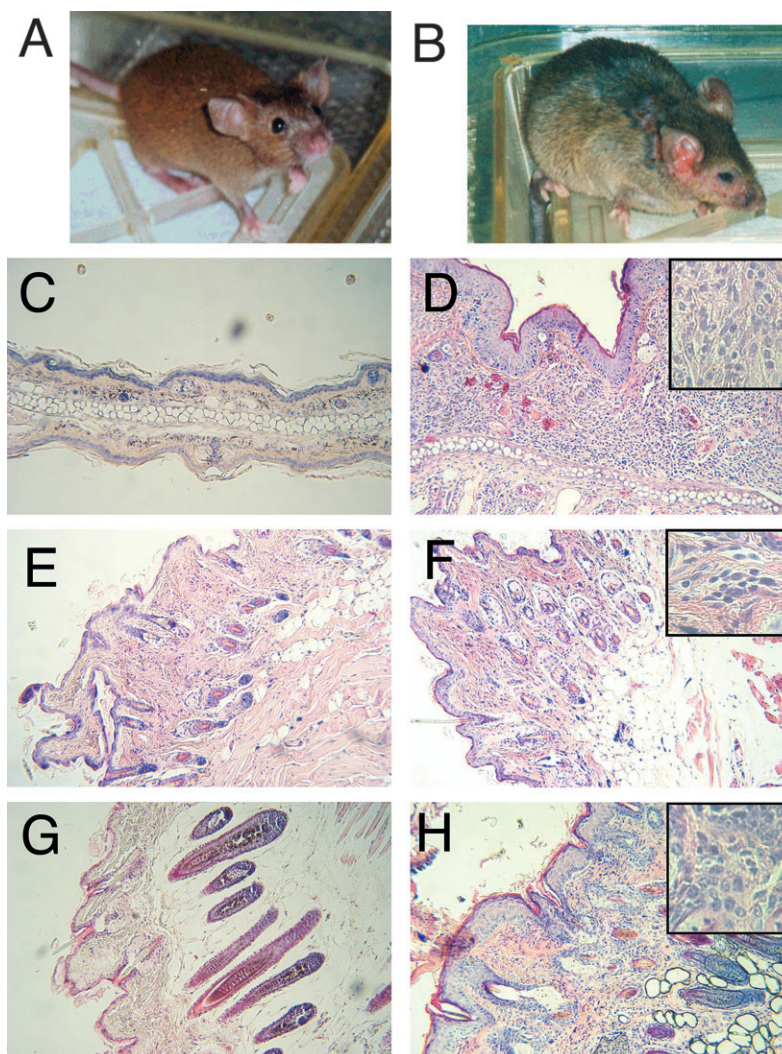


Fig. 3. Appearance and histological features of dermatitic mice. Appearance of a non-transgenic littermate mouse (A) and a dermatitic B137-29 transgenic mouse (B). Dermatitis-affected regions from the transgenic mice (D, F and H) and non-affected counterpart from control mice (C, E and G) were taken and stained with hematoxylin and eosin. (C and D) Ear; (E and F) eye-lid; (G and H) skin from the hind region.

disease process (reviewed in 16). The Th2 hypothesis is mostly based on the following observations; first, the presence of eosinophilia and an elevated level of serum IgE in atopic dermatitis patients (17,18), and second skin-infiltrating T cells in the skin lesions in skin patch test and peripheral blood mononuclear cells (PBMC) from atopic dermatitis patients produced predominantly Th2 cytokines (19,20). In this study by showing that transgenic mice overexpressing Th2 cytokines develop spontaneous severe atopic dermatitis against environmental allergens, we provided additional evidence that supports the hypothesis that Th2 cytokines are critically important contributors of atopic dermatitis.

Histologic analysis showed infiltration of lymphocytes at the dermatitis afflicted skin lesions (Fig. 3). It is therefore possible that these lymphocytes overproduce Th2 cytokine at these sites of inflammation. Previous studies have shown that CLA⁺ (cutaneous-lymphocyte-associated antigen) CD45RO⁺(memory/effector) T cells selectively home to the skin in atopic dermatitis and contact dermatitis (21–23).

It has been shown that CLA⁺ T cells contain and spontaneously release high amounts of preformed IL5 and IL13 (22,23). In addition, CLA⁺ memory/effector T cells induce IgE production in B cells and enhance eosinophil survival by delaying eosinophil apoptosis in atopic dermatitis (22,23). Thus, the cells infiltrating to the skin lesions in our study may include CLA⁺CD45RO⁺ T cells, although the exact nature of the cells was not studied. However, this study does not exclude the possibility that cell types other than T cells may be responsible for the production of Th2 cytokines and disease pathogenesis. Th2 cytokines are produced by many cell types such as mast cells, eosinophils, basophils and stromal cells in the lesions. Spontaneous activation of these cells may also lead to Th2 cytokine overproduction and progression of the diseases. The exact mechanisms for the pathogenesis remain to be studied.

The critical role of IL13 in the pathogenesis of asthma is well documented in the recent literature (24–26). Blockade of IL13 by the administration of IL-13-RA2, which binds only IL13,

reverses airway hyper-responsiveness and pulmonary mucous cell hyperplasia (24,26). Recombinant IL13 delivered to mice or overexpression of IL13 in the lung of mice causes an asthma-like phenotype including airway hyper-responsiveness, eosinophilic inflammation, mucous cell hyperplasia and subepithelial fibrosis (24–26). The asthma phenotype shown in the BAC transgenic mice in this study may thus be mediated

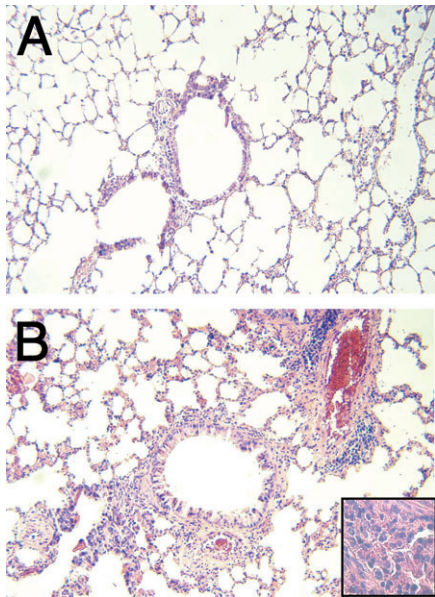


Fig. 4. Histological staining of lung. Lungs from a non-transgenic littermate (A) and a B137-29 transgenic mouse were taken and stained with hematoxylin and eosin.

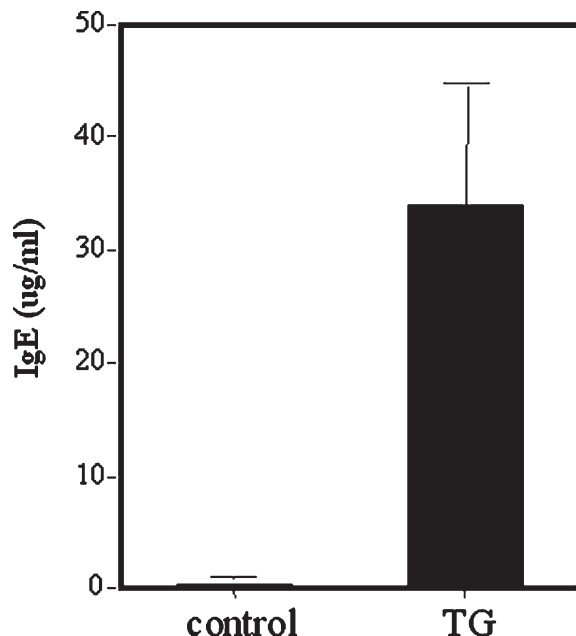


Fig. 5. Total serum IgE concentration. Total IgE levels were measured in the sera from three B137-29 transgenic mice and three non-transgenic littermates by ELISA.

by overproduction of IL13, although IL4 and IL5 also play roles in the disease process.

This study also validated previous genetic linkage studies that the Th2 cytokine cluster is one of the susceptibility regions for atopic diseases. Chromosome 5q31, which includes the IL4, IL5 and IL13 genes, has been shown to be linked with total serum IgE concentration (27,28), eosinophil levels (29), and schistosomiasis resistance (30). Several genetic linkage studies have also shown an association of asthma and atopic dermatitis with chromosome 5q31 (31–33). Many other chromosomal regions have also been shown to be associated with disease susceptibility, suggesting the complex nature of atopic diseases (34–37). Polymorphism or mutation in the IL4 and IL13 genes or in the regulatory regions for the expression of IL4 and IL13 genes have also been linked to atopic diseases (38–42). Therefore, our study of transgenic mice carrying this region provides direct evidence supporting the previous genetic linkage studies suggesting that this region is involved in atopic diseases. Further, our data suggest that the level of cytokine expression must be carefully controlled during evolution to keep a balance between pathology and disease resistance.

In summary, we have provided further support for the critical role of Th2 cytokines in the development of atopic diseases, and additional data that the atopic diseases may share a common cause, i.e. Th2 cytokines. More detailed analysis of mechanisms of pathogenesis awaits further study.

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Abbreviations

BAC	bacterial artificial chromosome
CLA	cutaneous lymphocyte-associated antigen
FcεRI	receptor for Fcε I

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