

Transgenic Models in Allergic Responses

Michael Hausding, Kerstin Sauer, Joachim H. Maxeiner and Susetta Finotto*

Laboratory of Cellular and Molecular Lung Immunology, I Medical Clinic, Mainz, Germany

Abstract: The immunoresponses are mediated by cells presenting the antigen to T cells. The transcription factors involved in the differentiation of T helper cells enclose T-bet (Th1), *c-maf* (Th2), GATA-3 (Th2), Foxp3 (T reg) and ROR γ T (Th17). They are regulated in allergic asthma. The use of murine models either as germline or as tissue specific transgenic mice has given decisive immunological tools to understand the importance of selected transcription factors or cytokines. Tissue specific transgenic lines have been generated into the Clara Cell or CD2 promoter directing tissue- and immune cells specific expression of the gene of interest. We identified T cell transcription factors important for asthma – such as T-bet, *c-maf*, GATA-3. Transgenic and knockout murine models of these transcription factors provided very important information for the human disease. Regarding to the pathogenesis of chronic asthma, we generated transgenic lines overexpressing IL-18 and analyzed a dominant negative mutant of the TGF- β receptor II. These models will offer to us a great input for the understanding of the T cell memory and the processes like airway remodelling. Beside DNA microinjection and stem cell transfer the On/Off systems like Cre-lox models have helped to understand the role of selected genes in different steps of experimental disease. Moreover, the transgenic model provide reliable models for the pre-clinical approval of therapy for allergic asthma to develop more efficient compounds and functional antibodies.

Key Words: Transgenic mice, allergic asthma, T regulatory cells, CD2 promoter, T-bet, TGF- β 1.

INTRODUCTION

Generation of Transgenic Mice and their Importance to Analyse the Pathogenesis of Allergic Asthma

The term transgenic animal refers to an animal in which there has been a deliberate targeted modification in the genome - the material responsible for inherited characteristics - in contrast to spontaneous mutations as documented e. g. in the S(pontaneous)C(ombined) I(mmuno) D(eficiency) mice. Foreign DNA is transferred to the progeny by using recombinant DNA technology. To generate transgenic mice, male and female mice are coupled, and the fertilized eggs are washed out of the female's oviduct. The designated DNA construct is then directly microinjected into the pronuclei of these eggs, then inserted in the uterus of a pseudopregnant mouse, which has been mated with a vasectomized male mouse. In hormonally pregnant female mice, the fertilized eggs are applied in the uterus. Some of the offsprings have the transgene integrated into their genome. The first gene manipulated organism was created in 1973, the *E. coli* bacteria. This system combined and replicated genetic information from different species [1]. The first chimeric mice were generated a year later [2]. The cells of two different embryos from different strains were combined together at an early stage of development, the blastocyte, to form a single embryo that subsequently developed into a chimeric adult, exhibiting characteristics of each strain. The intercross between the F1 generation then led to the expression of the gene in homozygous mice to a 25% of F2. This is the basis of the

transfer of gene manipulated stem cells into host mother blastocytes described later. The combination of developmental biology and genetically modified DNA transfer induced the fast development of the techniques for the creation of transgenic animals. DNA microinjection in the pronucleus of fertilized eggs was the first technique on mammals and was first applied to mice and then to various other species such as e.g. rats or rabbits as described in Fig. (1A) [3]. As seen before in bacteria and some plants, also animals can be genetically modified by viral infection. Since the genetic modification occurs only in those cells that become infected, this cellular clone may be rapidly eliminated by the immune system. Permanent genetic modification of entire animals has been achieved in mice by retrovirus-mediated transgenesis [4]. In fact, retroviral transfection, with modified DNA integrated in the viral DNA, has been used to infect mouse embryos for targeted integration of foreign DNA (Fig. 1B). As similar technique, the embryonic stem (ES) cell-mediated gene transfer was developed three years later. Chimeric mice were generated by the transfer of *in vitro* manipulated stem cells into blastocysts (Fig. 1C). Target DNA expression takes place when a reporter plasmid is integrated by transfection of the ES cell *via* non-homologous recombination to the ES cells genome [5]. These transfected cells are implanted into a blastocyte (an early embryo), which is then implanted into the uterus of a female mouse. Offspring will be chimera containing some cells derived from the unmodified cells of the blastocyte and some derived from the modified stem cell. By selecting mice whose germ cells developed from the modified cell and interbreeding them, mice that contain the genetic modification in all of their cells will be born (Fig. 1C). When the transgene has integrated into the germ cells, the so-called germ line chimeras are then inbred for 10 to 20

*Address correspondence to this author at the Laboratory of Cellular and Molecular Lung Immunology, I. Medical Clinic, University of Mainz, Obere Zahlbacher Strasse 63, Room 1-359-1-369, 55131 Mainz, Germany; Tel: 49-06131-3933376; Fax: 49-06131-557444; E-mail: finotto@mail.uni-mainz.de

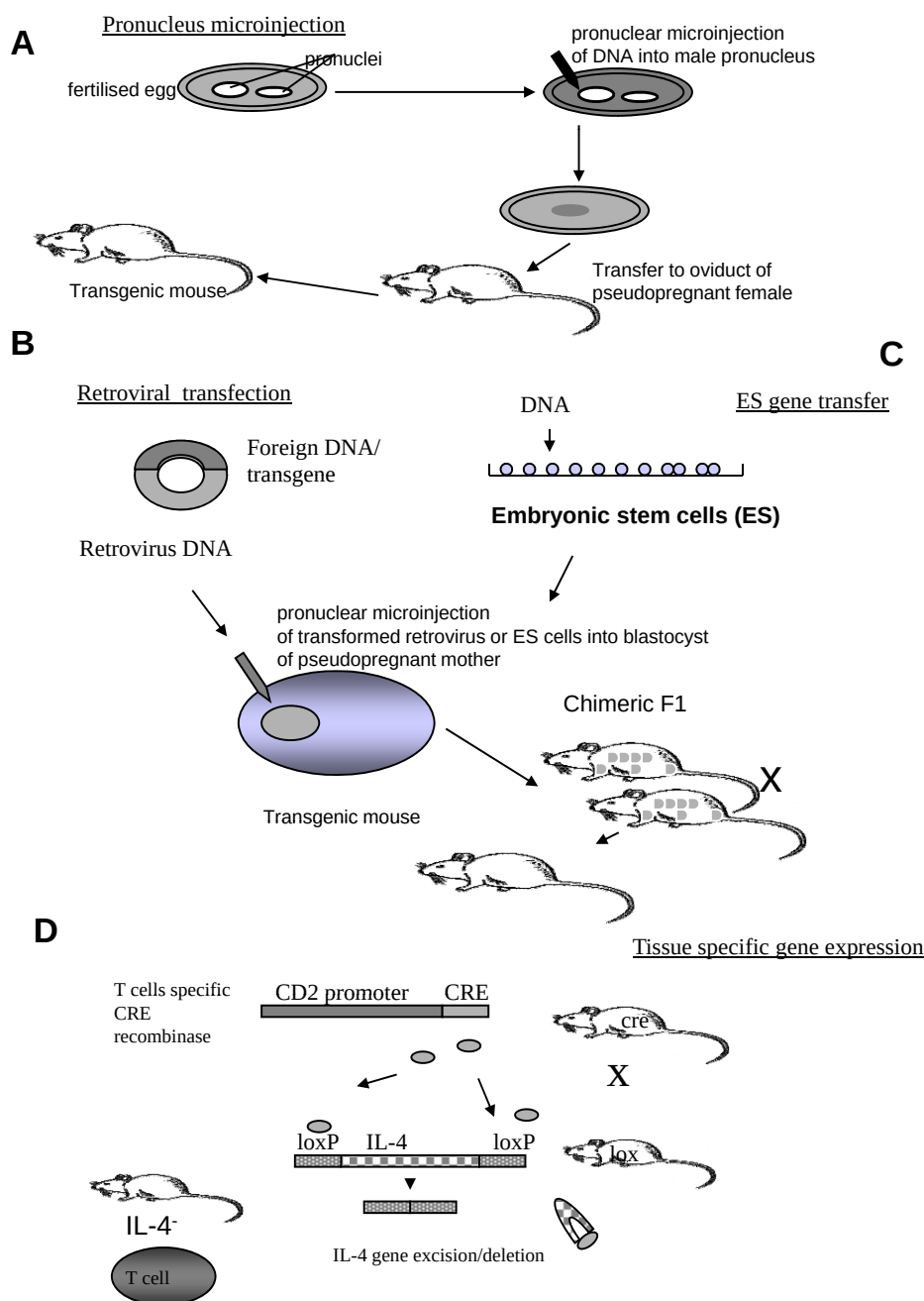


Fig. (1). Important strategies to overexpress structural and functional genes in transgenic mice. **A.** The microinjection of the DNA to be overexpressed by the help of a tissue-specific promoter in fertilized eggs is effective and widely used for horizontal and vertical gene transfer. DNA is injected in the male pronucleus and the embryo is subsequently transferred in the oviduct of a pseudopregnant female mouse. The outbred mice then are germline transgenic so that each cell is overexpressing the target gene and it will be active in the promotor specific tissue or targeted cell type. If the transgenic offspring is characterized by southern blotting or PCR methods the interbred transgenic mice stay genetically stable. In **B.** retroviral transfection is described as one of the techniques to introduce foreign DNA in mice. By the help of retrovirus transducing activity on murine cells the target DNA was cloned into the 3' region of a retroviral plasmid e.g. under the control of a SV19 promotor and transferred to embryonic stem cells and expanded. The infection by intrablastocytic injection is resulting in an expression of the foreign gene in most of germline and structural cells. **C.** A more reliable but similar method is the embryonic stem cell transfer. The expanded ES cells were transfected by non-homologous recombination with a TK promotor and a Neomycine-resistance gene containing plasmid DNA and pre-selected for the target sequence coding between the two selection markers. In positive clone TK promotor was deleted (Gancyclovir resistance) and the Neomycine resistance was conserved. When injected in the early embryos these ES cells lead to an chimeric offspring. In fact, the offspring of the outbred egg in a foster mother is chimeric. If the positively tested mice which express the transgene are interbred the outbred mice become homozygotic for the transgene. **D.** The Cre recombinase Tg mice and the loxP specific gene silencer mice are an ideal model to mimic the loss of a certain unfavorable gene for the disease by using genetic manipulation and recombination [16]. The loxP sites on a vector introduced in one mouse strain flank the gene of interest like an exon of IL-4 upregulated in these mice and by intercross between this strain and Cre mice the IL-4 exon is deleted by Cre recombinase deletion and a conditional knockout mouse represents a different model mouse less susceptible for AHR and display a protected asthma phenotype.

generations until homozygous transgenic animals are obtained and the transgene is present also in every somatic cell. The resulting homozygous embryos then may be conserved in liquid nitrogen and can be used for repeated in-utero-implantation. The success of this method has led to using its potential for investigating a wider range of diseases and conditions. However, models are not strict equivalents, to human disease, so as with any other system care must be taken in drawing conclusions from these data. For medical research, transgenic mice are important for the identification of the function of specific factors in complex homeostatic systems through over- or under-expression of a modified gene (the inserted transgene). Other models use 'knock out' techniques to substitute a functional coding site for a protein by a selection cassette or other DNA fragments to interrupt the regular translation of the intact gene to the functional protein. In molecular biology, the analysis of the regulation of gene expression makes use of the evaluation of a specific genetic overexpression or genotypical gene deletion by 'knock out' at the level of the whole animal. The advantage of the 'knock out' mouse models compared to the overexpression models is that the resulting effect is stronger and more obvious. An innovative solution that resolved this conflict was originated by a team of scientists led by Martin Evans, Oliver Smithies and Mario Capecchi who created what is known as a genetic "knockout". By creating a knockout they proved that it was possible to aim the inserted gene at a precise location in the mouse genome. This gave scientists the ability to replace, or knock out, a specific gene with an inactive or mutated allele. Therefore, knockout animals are considered an investigative technique that allows for a particular gene of interest to be removed in an attempt to define what effect that gene has in the life of the organism. The gene knockout is created by selectively disabling a specific target gene in a particular type of cells called embryonic stem cells. The three scientists received the Lasker Award in 2001 for this achievement and lately they won last year (2007) the nobel prize for medicine for their efforts. The technique has been used to make several thousand different knockout mice. Knockout mice have become one of the most useful scientific tools in helping to understand the human genome and its roles in disease.

Tissue Specific Transgenic Mice in the Context of Asthma

Transgenic mice over-express a gene of interest in a highly differentiated cell lineage. This aim can be accomplished by placing in front of the coding sequence of the selected gene, the most specific promoter characterizing the target cell. This method has given a high input in the understanding the pathogenesis of multiple human disease. To generate a transgenic mouse, a DNA construct containing the gene of interest is cloned. In this construct, the gene of interest is linked to a promoter that is designed to drive the expression of this gene in the desired organ and/or tissue or the differentiated cell. After the transfer of the cloned DNA only the tissue specific target cells express the gene of interest e.g. the airway epithelium if a Clara cell promoter construct has been transferred by targeted microinjection in the recipient mouse blastocysts.

a. The Clara Cell Promoter

In murine models of asthma, often a Clara cell 10 promoter with a length of 10 kb is used to target gene expression because it is selectively expressed by the Clara cells that make up to 40% of the epithelium of the murine airway. This described for the IL-9, IL-11 and IL-13 as specifically over-expressed cytokines in the bronchial epithelium and to develop an asthmatic phenotype in this models. Th2 cytokine IL-13 induces mucus metaplasia, eosinophils accumulation in the bronchi, and Th1 cytokine IFN- γ induces inflammation and neutrophilia in the lung in a Clara Cell-promoter driven transgenic model [6]. Contribution of IL-9 to airways inflammation and massive eosinophilia in the lungs was also investigated by a transgenic model using a rat Clara cell 10 promoter for IL-9 overexpression in the rat epithelium ([7] and as indicated in Fig. (3)).

b. The Collagen Promoter

The Collagen (COL1A1) promoter is induced by Bleomycin in fibroblast and fibrotic tissue in the lung by a TGF- β 1 response element in the distal region of the promoter. Bleomycin signals intracellular *via* SMAD3 inhibition and increased collagen production by blockade of intracellular SMAD7 promoter, an inhibitory molecule [8]. Bleomycin's extracellular signaling results from the secretion of more latent TGF- β 1 produced by lung fibroblasts and cleaved to active TGF- β extracellularly. The promoter is activated by Bleomycin and mimics the chronic TGF- β 1 mediated phase of airway remodelling processes in fibroblasts linked to the expression of IL-13 and the IL-13 receptor- α chain.

c. The CD2 Promoter

Beside the possibility to use promoter specific for structural cells, more often an overexpression on cells of the immune system is desired. In this context the human CD2 promoter has been widely used because it allows overexpression of a gene in both CD4, CD8 and NKT cells [9]. The human CD2 was revealed to be a 47-58 kd glycoprotein that is detected exclusively on T lymphocytes. The glycoprotein appears early on the surface of the thymocytes and remains present on all circulating T lymphocytes throughout their life. In contrast to the human CD2, mouse CD2 is also expressed on murine B cells while the expression on lymphocytes resembles the human pattern. The human CD2 enhancer region was studied by Lang et al. and identified to be within a DNase I hypersensitive site in the 3' flanking region [10]. More precisely, two of the three hypersensitive sites that have been classified are located upstream and the third site is positioned 3' to the gene. Important and multifaceted biological processes e.g. differentiation and developmental control of tissue-specific gene expression can best be investigated by using transgenic mice [11]. Therefore, cDNAs encoding human, murine and rat CD2 have been cloned and human CD2 was introduced into the murine genome. All three hypersensitive sites of the CD2 enhancer, which are regulatory elements required for appropriate expression of the human CD2 gene, are enclosed within a 28.5 kb genomic fragment. Examination of the transgenic mice revealed that the CD2 transgene is expressed specifically on T lymphocytes and depended on the copy number while the CD2 expression was independent on the position of integration.

Taken together, the expression of the human CD2 transgene in these mice resembles the human pattern. Few years after the above described studies Zhumabekov and colleagues reported an altered CD2 mini-gene cassette that initiates expression with an effectiveness that is ten times higher than the previous cassette [12]. The impact of the CD2 promoter may be concluded from studies using these transgenic mice that are able to demonstrate general mechanisms of T cell differentiation on gene expression level basing on the fact that the gene is transcribed early on thymocytes and remains stable throughout lineage commitment. This approach offers the important advantage that the foreign gene is likely to be exposed to many differentiation factors, some of which might be expressed only transiently in development. An additional advance of the transgenic mice is the closeness of the major controlling elements of the CD2 gene that allows analysis of the dominant control region in relation to other regulatory regions such as the CD2 enhancer. In the following years, many transgenic mice were created under the control of the human CD2 promoter that allowed analysis of T cell dependent diseases like asthma [13]. In B cells a cloning strategy for the CD11b promoter was published by Dziennis *et al.* [14] and leads in macrophages to a restricted expression of the reporter gene LacZ. In mast cells of a transgenic mouse model the human Fc epsilon RI alpha chain has been expressed while the gene was driven by a human 1.3 kb promoter sequence [15].

d. The Cre-Lox System

Site-specific recombinase have applications in immunology by activating or removing genes at particular stages. The potential core of the Cre/loxP system relies on the use of the P1 phage Cre recombinase and its derivatives to catalyse the excision of the DNA sequence flanked by loxP sites on the chromosome [16]. Use of this system usually involves two mouse lines, one of which carries the transgene with a floxed transcription 'stop sequence' inserted between the promoter and the transgene to silence and another mouse line expressing Cre recombinase (Fig. 1D). Tissue specific expression of the transgene can be achieved by choosing the mouse line expressing Cre under the appropriate tissue-specific promoter to cross with the transgenic line. In addition, to achieve a regulation of transgene expression in a tissue-specific manner, a temporal control of transgene expression can be achieved to circumvent embryonic lethal phenotype. This is usually done by linking the Cre gene with a drug-inducible promoter like TetOn/Off or generating a chimeric Cre protein, which is normally sequestered into the cytoplasm, but whose nuclear translocation is inducible by specific, synthetic steroids.

The Transfer Potential of Mouse Models of Airway Hyperreactivity for Human Allergic Asthma

Although mouse models do not reproduce all the features of human disease in its entity, after Ovalbumin-sensitization and -challenges, wild type mice develop a clinical syndrome that is similar to allergic asthma [17], which is characterized by eosinophilic lung inflammation [18], increased IgE [19], airway hyperresponsiveness (AHR) [17], mucus hypersecretion [20], and finally, airway remodelling [21]. However, there are differences between human and mouse physiology

that limit the value of mouse models. In fact, patients show increased methacholine-induced AHR even when they are symptom-free, whereas mice exhibit only transient methacholine-induced AHR following allergen exposure. Furthermore, chronic allergen exposure in patients leads to chronic allergic asthma. By contrast, repeated exposures to allergen in sensitized mice causes suppression of disease called immunological tolerance. Also IgE and mast cells, in humans, mediate early- and late-phase allergic responses, but for the generation of allergic asthma in mice these parameters are not essential. Despite, Kung *et al.* [22] present an overview of the clinical aspects of disease in murine models of allergic asthma pointing out the factors influencing the pathophysiological responses during the initiation and perpetuation of disease. Furthermore, Brussee *et al.* [23] and Epstein *et al.* [24] also used the output from murine studies for clinical understanding of the disease and for testing new treatments for allergic asthma in the murine system. Though mouse models of allergic asthma support many present paradigms, although some novel discoveries in mice have to be approved in patients. BALB/c is one of the most widely used and best characterized mouse strains in immunology. For various applications, it is necessary to generate BALB/c transgenic mice. However, using the conventional microinjection technique it is extremely inefficient to produce transgenic BALB/c mice since the one-cell stage BALB/c embryos are highly vulnerable to pronuclear DNA microinjection [25]. Pauwels *et al.* [26] developed in C57/BL6 mice an *in vivo* model of allergic airway inflammation characterized by the presence of IgE antibodies in response to inhaled antigen to an inhaled antigen, peribronchial infiltrates with an increased number of eosinophils, and an enhanced airway responsiveness. In this genetic background the authors investigated the role of different cytokines in the development of the asthma parameters like IgE, AHR and goblet cell metaplasia in the lung and they were performed by using knockout mice or by exogenous administration of cytokines or cytokine antagonists. As described by Brewer *et al.* different strains of mice develop a completely different phenotype referring to changes in airway hyperresponsiveness (Fig. 2), eosinophilia and other asthma parameters [27]. In contrast to C57/BL6 and FVB mice Balb/c mice are able to develop high IgE after allergen exposure and do not diverge in the symptoms development like increased AHR, and eosinophilia like other strains. Most important is the increased response referring to eosinophilia and IgE induced by OVA sensitisation and challenge in the Balb/c mice compared to other mouse strains. As well as Balb/c mice C57/BL6 mice develop an increased AHR and eosinophils in the bronchoalveolar lavage but fail to induce significantly enhanced eosinophilic inflammation in the lung tissue after allergen challenge. Balb/c mice in contrast to C57/BL6 mice develop an enhanced peribronchial eosinophils invasion whereas the C57/BL6 show a rather lateral infiltration of granulocytes in the bronchial tissue [28]. A trait appearing to a different extent in different mouse strain models is difficult to dissect in the complexity of genes responsible for it. This complexity of determination of a single trait or allergic phenotype to the relevant gene modifications responsible for the differences in the asthmatic phenotype between the mouse strains is underlined by the fact, that these differences never have been found to be determined by a differentially ex-

pressed 'gene'. Asthma is likely to be a multiple gene trait or disease, but the different localization of infiltrating eosinophils in the lung of C57/BL6 mice and Balb/c mice and the gene regulating this may better be found if the genome of both strains is better analysed using a knockout-model to find the responsible gene. If one after the other trait may be 'localized' genetically by using the different strains or transgenic mouse models it will help to find the combination of single genes to clarify the pathology of allergic asthma. In the pathophysiology of allergic asthma several inflammatory processes and cell types are involved, including eosinophils, CD4⁺ T cells and neutrophils depending on the pro-inflammatory signalling beginning with the antigen presenting cells driving T cells towards a Th2 or Th1 inflammatory phenotype contributing to the exacerbation or prevention of asthma. Based on *in vivo* and *in vitro* studies the responsiveness of different mouse strains for the basic feature hallmarks of allergic asthma like increased airway hyperreactivity, eosinophilic inflammation, mucus production and substantial skewing towards a Th2 asthma phenotype have variable extent regarding the different strains. The shift towards an allergic Th2 dependent asthma response is regarding all pathophysiological features by the use of ovalbumin sensitisation and challenge is most strikingly obtained in Balb/c mice.

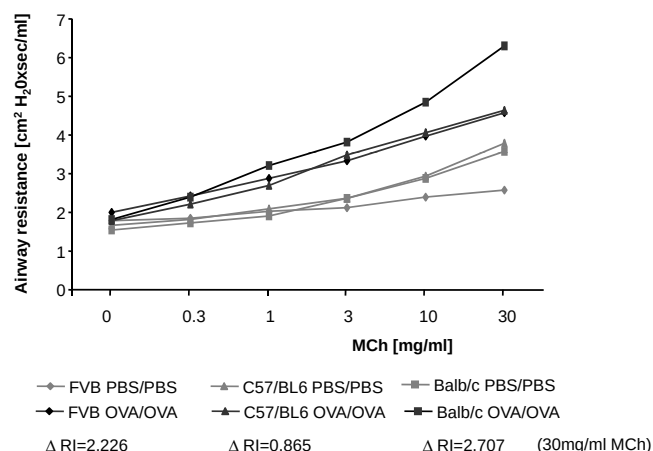


Fig. (2). Lung function and AHR in mice is dependent on the genetical background. In measurements of lung airway resistance the sensitivity of different strains to methacholine was quite different. The resistance is measured in anesthetized mice with a intratracheal tubus allowing to be ventilated and disposed to the bronchoconstrictor. In fact, the lowest change in AHR after allergen (OVA) sensitization and challenge in our asthma model was achieved in C57/BL6 mice corresponding to the low Δ RI value at 30 mg/ml Methacholine. The FVB/N mice displayed a an intermediate responsiveness to methacholine after OVA sensitisation and challenge (OVA/OVA) and the highest values are reached by Balb/c mice. This strain is the most used in the setting of experimental asthma because it develops human disease-like parameters [27].

Different Transgenic Mouse Models in Asthma and their Impact on the Human Disease

c-maf Tg Mice

c-maf as proto-oncogen is involved in the development of allergic asthma and is linked in expression and in efficacy to

the key transcription factor in atopic asthma, GATA-3. Transgenic mice overexpressing *c-maf* under the control of a T cell-specific promoter showed increased production of the T helper 2 cytokine IL-4 and exhibited elevated serum levels of IgG1 and IgE [29]. *c-maf*, in addition to transactivating the IL-4 promoter, can enhance the production of Th2 cytokines by an IL-4-independent and CD25-mediated mechanism [30]. Recent studies have indicated an important role for *c-maf* in the differentiation of peripheral splenic T cells. In contrast to GATA-3, *c-maf* was able to transactivate the IL-4 promoter but not the IL-5 and IL-13 promoters in peripheral lymphatic organs [31]. However, the latter findings are IL-4 dependent and could be reversed when the *c-maf* Tg animals were backcrossed to an IL-4 deficient background. Furthermore, *c-maf*^{-/-} mice showed greatly impaired IL-4 production, and their T cells spontaneously polarized towards a Th1 phenotype. However, under Th2 differentiating conditions *c-maf*-deficient Th2 cells produced normal amounts of Th2 cytokines indicating that c-Maf is not required for chromatin remodeling at the IL-4/IL-13 locus but rather specifically regulates IL-4 gene expression in peripheral T lymphocytes [32]. Overexpression of *c-maf*, in absence of GATA-3, does not cause airway inflammation and AHR and indicate a different role of c-Maf in regulating cytokine gene expression in lung T cells as compared to peripheral T cells [32]. In particular, c-Maf, in absence of GATA-3 induced selectively IL-5 at the beginning of the Th2 differentiation but suppressed IL-13 production by lung CD4⁺ T cells. The transgenic mouse model is a powerful biological tool because for certain studies the lack of potent antagonists or transcription factors and blocking antibodies for receptors and cytokines makes the transgenic technology to the model of choice.

IL-18 Transgenic Mice

IL-18 was originally identified as an Interferon- γ inducing factor in Th1 cells. It interacts with IL-2 to produce IFN- γ . IL-12 and IL-18 are produced upon antigen exposure and TLR ligation on antigen presenting cells. IL-18 is produced by a variety of different antigen presenting cells and epidermal cells [33]. Many of the IL-18-producing cells can be stimulated *via* activation of their Toll-like receptors (TLR) by environmental factors e.g. CpG and LPS [34].

We generated an IL-18 transgenic mouse under the control of the human CD2 promoter and studied signal transduction in hepatocytes [35]. These mice exhibited increased IFN- γ and TNF- α levels in the serum and showed reduced liver size and weight while the spontaneous Fas dependent apoptosis of hepatocytes was found to be elevated. Signal transduction studies revealed an upregulation of intra-nuclear NF κ B. The adoptive transfer of IL-18 transgenic splenic CD4⁺ T cells in SCID mice resulted in severe liver failure accompanied by periportal fibrosis, liver inflammation and hepatocyte apoptosis. Many studies have been performed concerning the role of IL-18 in murine models of bronchial asthma like Matsubara *et al.* who described a combined treatment with IL-2 and IL-18 that forwards CD4⁺ T cells IFN- γ release in an acute murine model of asthma [36]. In this model, a strong dependency of Th1 development from priming by IFN- γ releasing NK cells was shown. Furthermore, it is of specific interest to understand the role of IL-18 in the development of the Th17 cellular clones which was

shown to express the IL-18 receptor in a setting of allergic asthma [37]. Hereby, the IL-18 transgenic mouse could be the basis of this studies.

Transforming Growth Factor Beta (TGF- β) and Transgenic Mice

TGF- β has positive and negative regulatory effects on differentiation and proliferation of multiple cell types [38] and serves as a signal molecule enhancing fibroblast transformation (Fig. 3). Geiser *et al.* [39] and Shull *et al.* [40] revealed the importance of TGF- β in TGF- β deficient mice, which develop severe autoimmune multiorgan pathology like severe myocarditis, lymphadenopathy and vasculitis and collagen-induced arthritis [41]. The TGF- β family consists of the three isoforms and TGF- β 1 signalling has been recently investigated by Schramm and colleagues to be responsible for the maintenance of liver T cell homeostasis in a transgenic model of autoimmune hepatitis [42]. By creating a mouse targeting the TGF- β receptor blockade on T cell level by introducing the TGF- β -receptor II (TGF- β RII) dominant negative mutant not completely deletes TGF- β signaling in T cells and therefore allows control of T cell responsiveness depending on the local concentration of TGF- β and the extent of T cell stimulation. In a murine model of asthma T cells as a target cell of TGF- β -mediated immune regulation it was demonstrated that impairment of TGF- β signaling in T cells of transgenic mice leads to decreased airway reactivity and no differences in airways Th1 cytokines independently from antigen [43]. In contrast, after injection of OVA adsorbed to alum and challenge with OVA aerosol, transgenic animals showed an increased airway hyperreactivity and inflammation compared with those of wild-type animals. In addition, increased IL-13 levels indicate an important role to TGF- β signaling in T cells in the regulation of airway responses. The influence of TGF- β on immune-suppressive cells like the CD4⁺CD25⁺ T regs has been further on described [44]. These Foxp3 expressing cells are known to suppress CD4⁺ T cells and reduce the proliferation of immunomodulatory T cells in allergic diseases. In contrast to cytokines like IL-6 [45], TGF- β 1 alone has been shown to be required for T reg activity [44] but also for the increased proliferation of T cell clones releasing IL-17 together with IL-6. However, in the presence of IL-6 it can drive the pathophysiological clone Th17 [46] whereby IL-6 inhibits the proliferation of Foxp3 expressing CD4⁺ T cells. In mice overexpression of TGF- β 1 led to an increase of the number of T regulatory cells and therefore, possibly to Th17 clone expansion in these mice would give precious information about the importance of TGF- β induced intracellular transformation and the direct link from SMADs and other transcriptional regulators on the differentiation direction of developing T cells clones.

Chronic Mouse Models and Airway Remodeling

Atopic asthma is characterized by acute airway inflammation and eosinophilia and after immunization the memory T cell may lead to autoimmunity and chronic pathogenesis of the disease. During the step by step repeated allergen challenge the process of airway remodelling takes place. It includes a manifested reorganisation of the lung fibroblast compartment to a rather smooth muscle derived peribron-

chial tissue during myofibroblast transformation. Features of chronic asthma, like collagen deposition around the airways basal membrane, airway fibrosis and smooth muscle hyperplasia, are importantly found in GATA-3 transgenic murine models (Fig. 3). The lack or the overexpression of certain genes of transcription factors like T-bet in a chronic state of the disease including the memory T cell effects has been shown to be essential for the development of tissue fibrosis and remodelling [47]. Controversely, repeated allergen exposure might not lead to induced remodelling of the airways but also to enhanced T cell tolerance mediated by antigen presentation via B cells [48]. Therefore, T-bet deficient mice lack the capability to develop a Th1 memory type and already spontaneously they drop to the airway remodeling process [49]. T-bet Tg mice instead have decreased mucin production in the airways and reduced eosinophilia while GATA-3 Tg mice have increased eosinophilic granulocytes in the BAL and TGF-beta as an inducer of myofibroblast transformation was increased in these transgenic mice [50]. This study clearly demonstrates the importance of generation of transgenic models to define the role of such key transcriptional regulators in allergic asthma *in vivo*.

CONCLUSION

In the last decades research on allergic asthma has focused on the overexpression of relevant genes in several transgenic animal models. Cytokines and other immunomodulatory genes and cells in adaptive immunity particularly affecting the Th2 lineage are transcriptionally regulated. The Th1/Th2 paradigm has been translated in the last decade into different transgenic mice overexpressing Th2 and Th1 transcription factors or lineage specific cytokines. Of particular interest will be the analysis of the interaction between different immune cell lineages e.g. the myeloid dendritic cells or hematopoietic cells like the T helper subsets and the B cells and macrophages (Fig. 3). Our laboratory has also applied different mouse models of asthma by examining key transcription factors functions by using measurements of airway hyperresponsiveness and other pathophysiological parameters. The overexpression of Th2 relevant factors like c-Maf or GATA-3 and the situation in T-bet knockout mice for example have increased the knowledge about the complexity and maintenance of the allergic disease in dependency to genetical and physiological regulatory mechanisms. In the meantime the Th2/Th1 scheme has been extended to comprise other lineage specific differentiation of CD4⁺ T cell differentiation such as the T regulatory cells and the Th17 clone. In the near future it is likely to see the development of tissue specific deficient mice that will prove the role of Th17 and T regulatory cells in different diseases such as asthma. Other important cells which signal for initiation and maintenance of the immune response are the antigen presenting cells and they help the T helper CD4⁺ or CD8⁺ like cells to differentiate and to give instructions on cytokine and molecular basis for the Th1/Th2 balance in different T cell dependent pathologies like allergic asthma. The DC and macrophage like cell types may be analysed in their function by using the CD11b promoter to elucidate the function of those cells in the immunological synapse. One of the major questions evolving is how the T helper cells getting first contact with the allergen, dendritic cells or macrophages influ-

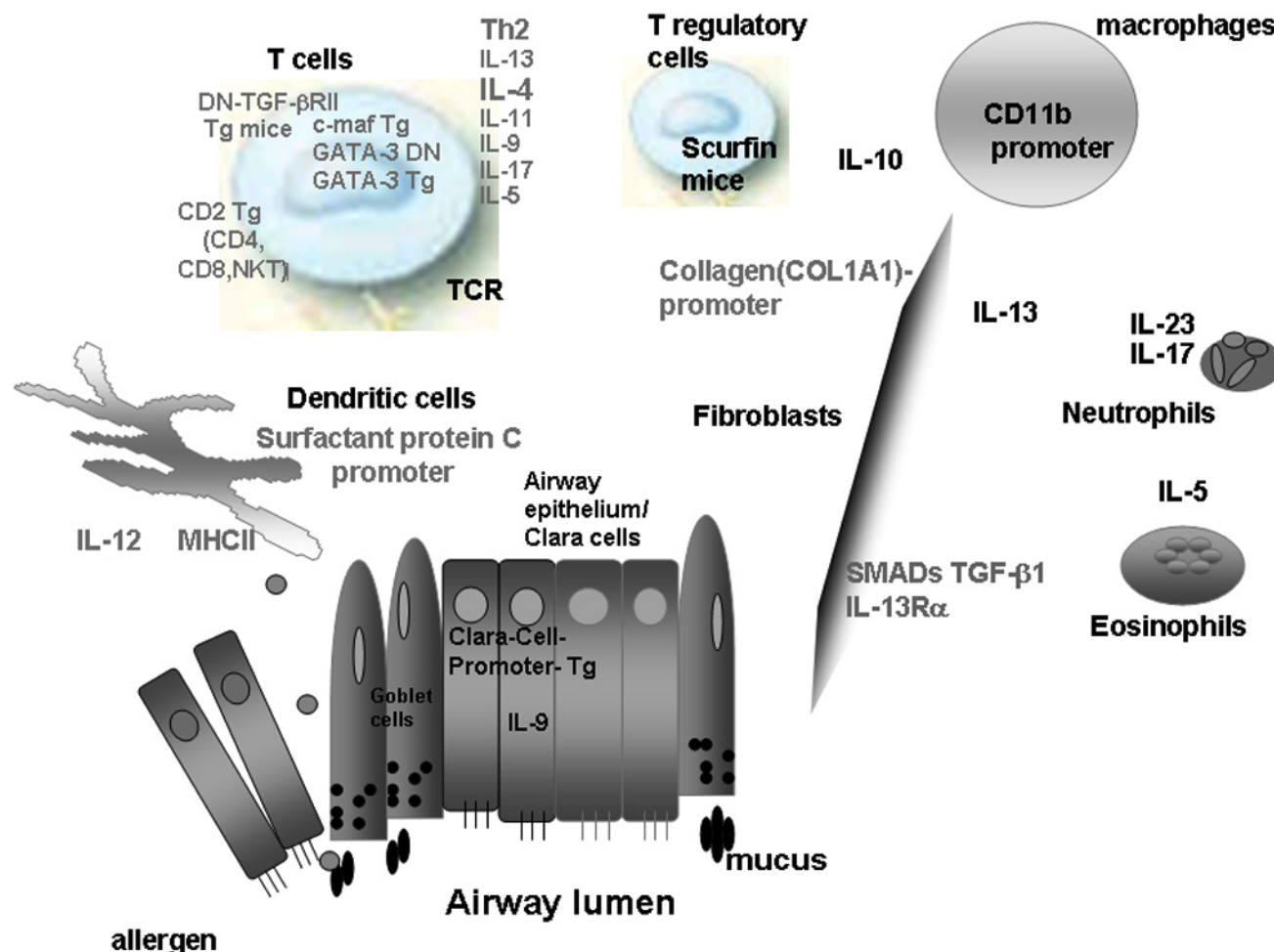


Fig. (3). The cell-specific overexpression or deletion of a gene concerns many types of immune cells to better define factors and cytokine functioning. In the pathophysiology of allergic asthma different cell types play a role and are investigated in tissue specific transgenic mouse models. The central cell type involved in the regulatory mechanisms during the evolvement of a complete immune system are the T helper cells. CD4⁺ Th2 cells release IL-4, IL-13, IL-5 and IL-9 and therefore molecular targets for the overexpression of one of these cytokines to define better its function and often these are directed by the T cell specific CD2 promoter. In the airways epithelium Clara cells have a specialized promoter of 10kb which was an intrinsically expressed vehicle expressing an interleukin (IL-4, IL-13 and IL-9) or other overexpressed gene in the airways leading to an asthmatic phenotype. The macrophages CD11b promoter has also been used for cell-type specific gene expression. In lung fibroblasts the IL-13 responsive receptor α chain has been cloned and transfected and evolved new insights to the development of chronic asthma and myofibroblast transformation and smooth muscle generation in the peripheral lung tissue.

ence the regulatory T cell and the newly described IL-17 releasing T helper subsets affecting the pathogenesis of allergic asthma. How does the Th1 or the Th2 lineage influence T regulatory and Th17 lineage cells and vice versa? The next period will see perhaps an expansion of interest in overexpression of gene of the innate immunity such as dendritic cells and NKT cells. Beside this a great interest is also present on the overexpression of gene in structural cells such as epithelial cells because they are sentinel at the entrance of the antigen. The use of transgenic mouse models is a powerful tool to dissect tissue specific function of several genes important for the pathogenesis of allergic asthma and furthermore, germline transgenics offer the opportunity to verify the acute and chronic state of the disease while producing stable transgenesis and same genetic conditions even in different models of investigation.

ACKNOWLEDGEMENTS

The authors thank A. Doganci for her support to generate Fig. (1). The work of S.F. is supported by the Deutsche Forschungs-Gemeinschaft (DFG; SFB548, B8 and Z3) and a MAIFOR grants. M.H. is supported by the Immunointervention Cluster of Excellence (ICE) and a MAIFOR grant from the University of Mainz, Germany. K.S. is supported by the GK1043 'Antigen-specific Immunotherapy' from the Clinic of the University of Mainz.

REFERENCES

- [1] Cohen, S., Chang, A., Boyer, H. & Helling, R. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 3240-3244.
- [2] Brinster R.L. (1974) *J. Exp. Med.*, **140**, 1049-56.
- [3] Gordon, J. W., Scangos, G.A., Plotkin, D.J., Barbosa, J.A., And Ruddle, F.A. *Proc. Natl. Acad. Sci. USA*, **77**, 7380-7384.

- [4] Jaenisch, R. (1980) *Cell*, **19**, 181-188.
- [5] Gossler, A., Doetschman, T., Korn, R., Serfling, E., and Kemler, R. (1986) *Proc. Natl. Acad. Sci. USA*, **83**, 9065-9069.
- [6] Cutroneo, K.R. (2006) *J. Cell Biochem.*, **97**, 933-939.
- [7] Nohara, K., Pan, X., Tsukumo, S., Hida, A., Ito, T., Nagai, H., Inouye, K., Motohashi, H., Yamamoto, M., Fujii-Kuriyama, Y., Tohyama, C. (2005) *J. Immunol.*, **174**, 2770-2777.
- [8] Zhu, Z., Homer, R.J., Wang, Z., Chen, Q., Geba, G.P., Wang, J., Zhang, Y., and Elias, J.A. (1999) *J. Clin. Invest.*, **103**, 779-788.
- [9] Temann, U.A., Geba, G.P., Rankin, J.A., and Flavell, R.A. (1998) *J. Exp. Med.*, **188**, 1307-1320.
- [10] Lang, G., Wotton, D., Owen, M.J., Sewell, W.A., Brown, M.H., Mason, D.Y., Crumpton, M.J., and Kioussis, D. (1988) *EMBO J.*, **7**, 1675-1682.
- [11] Lang, G., Mamalaki, C., Greenberg, D., Yannoutsos, N., and Kioussis, D. (1991) *Nucleic Acids Res.*, **19**, 5851-5856.
- [12] Zhumabekov, T., Corbella, P., Tolaini, M., and Kioussis, D. (1995) *J. Immunol. Methods*, **185**, 133-140.
- [13] Spik, I., Brenuchon, C., Angeli, V., Staumont, D., Fleury, S., Capron, M., Trottein, F., and Dombrowicz, D. (2005) *J. Immunol.*, **174**, 3703-3708.
- [14] Dziennis, S., Van Etten, R.A., Pahl, H.L., Morris, D.L., Rothstein, T.L., Bloch, C.M., Perlmutter, R.M., and Tenen, D.G. (1995) *Blood*, **85**, 319-329.
- [15] Fung-Leung, W.P., De Sousa-Hitzler, J., Ishaque, A., Zhou, L., Pang, J., Ngo, K., Panakos, J.A., Chourmouzis, E., Liu, F.T., and Lau, C.Y. (1996) *J. Exp. Med.*, **183**, 49-56.
- [16] Kimmel, A.R., and Faix, J. (2006) *Methods Mol. Biol.*, **346**, 187-199.
- [17] Hessel, E.M., Van Oosterhout, A.J., Hofstra, C.L., De Bie, J.J., Garssen, J., Van Loveren, H., Verheyen, A.K., Savelkoul, H.F., and Nijkamp, F.P. (1995) *Eur. J. Pharmacol.*, **293**, 401-412.
- [18] Hogan, S.P., Koskinen, A., and Foster, P.S. (1997) *Immunol. Cell Biol.*, **75**, 284-288.
- [19] Coyle, A.J., Wagner, K., Bertrand, C., Tsuyuki, S., Bews, J., and Heusser, C. (1996) *J. Exp. Med.*, **183**, 1303-1310.
- [20] Dabbagh, K., Takeyama, K., Lee, H.M., Ueki, I.F., Lausier, J.A., and Nadel, J.A. (1999) *J. Immunol.*, **162**, 6233-6237.
- [21] Lloyd, C.M., and Robinson, D.S. (2007) *Eur. Respir. J.*, **29**, 1020-1032.
- [22] Kung, T.T., Jones, H., Adams, G.K. 3rd, Umland, S.P., Kreutner, W., Egan, R.W., Chapman, R.W., and Watnick, A.S. (1994) *Int. Arch. Allergy Immunol.*, **105**, 83-90.
- [23] Brusselle, G.G., Kips, J.C., Tavernier, J.H., van der Heyden, J.G., Cuvelier, C.A., Pauwels, R.A., and Bluethmann, H. (1994) *Clin. Exp. Allergy*, **24**, 73-80.
- [24] Epstein, M.M. (2004) *Int. Arch. Allergy Immunol.*, **133**, 84-100.
- [25] Dinkel, A., Aicher, W.K., Warnatz, K., Burki, K., Eibel, H., and Ledermann, B. (1999) *J. Immunol. Methods*, **223**, 255-260.
- [26] Pauwels, R.A., Brusselle, G.J., and Kips, J.C. (1997) *Am. J. Respir. Crit. Care Med.*, **156**, S78-81.
- [27] Brewer, J.P., Kisselgof, A.B., and Martin, T.R. (1997) *Am. J. Respir. Crit. Care Med.*, **160**, 1150-1156.
- [28] Takeda, K., Haczku, A., Lee, J.J., Irvin, C.G., and Gelfand, E.W. (2001) *Am. J. Physiol. Lung Cell Mol. Physiol.*, **281**, L394-402.
- [29] Ho, I.C., Lo, D., and Glimcher, L.H. (1998) *J. Exp. Med.*, **188**, 1859-1866.
- [30] Hwang, E.S., White, I.A., and Ho, I.C. (2002) *Proc. Natl. Acad. Sci. USA*, **99**, 13026-13030.
- [31] Kim, J.I., Ho, I.C., Grusby, M.J., and Glimcher, L.H. (1999) *Immunity*, **10**, 745-751.
- [32] Hausding, M., Ho, I.C., Lehr, H.A., Weigmann, B., Lux, C., Schipp, M., Galle, P.R., and Finotto, S. (2004) *Eur. J. Immunol.*, **34**, 3401-3412.
- [33] Nakanishi, K., Yoshimoto, T., Tsutsui, H., and Okamura, H. (2001) *Cytokine Growth Factor Rev.*, **12**, 53-72.
- [34] Blease, K., Kunkel, S.L., and Hogaboam, C.M. (2001) *Inflamm. Res.*, **50**, 552-560.
- [35] Finotto, S., Siebler, J., Hausding, M., Schipp, M., Wirtz, S., Klein, S., Protschka, M., Doganci, A., Lehr, H.A., Trautwein, C., Khosravi-Far, R., Strand, D., Lohse, A., Galle, P.R., Blessing, M., and Neurath, M.F. (2004) *Gut*, **53**, 392-400.
- [36] Matsubara, S., Takeda, K., Kodama, T., Joetham, A., Miyahara, N., Koya, T., Swasey, C.H., Okamoto, M., Dakhama, A., and Gelfand, E.W. (2007) *Am. J. Respir. Cell Mol. Biol.*, **36**, 324-332.
- [37] Nakae, S., Iwakura, Y., Suto, H., and Galli, S.J. (2007) *J. Leukoc. Biol.*, **81**, 1258-1268.
- [38] Marie, J.C., Liggitt, D., and Rudensky, A.Y. (2006) *Immunity*, **25**, 441-454.
- [39] Geiser, A.G., Letterio, J.J., Kulkarni, A.B., Karlsson, S., Roberts, A.B., and Sporn, M.B. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 9944-9948.
- [40] Shull, M.M., Ormsby, I., Kier, A.B., Pawlowski, S., Diebold, R.J., Yin, M., Allen, R., Sidman, C., Proetzel, G., Calvin D., Annunziata, N., and Doetschman, T. (1992) *Nature*, **359**, 693-699.
- [41] Schramm, C., Kriegsmann, J., Protschka, M., Huber, S., Hansen, T., Schmitt, E., Galle, P.R., and Blessing, M. (2004) *Arthritis Res. Ther.*, **6**, R114-119.
- [42] Schramm, C., Protschka, M., Kohler, H.H., Podlech, J., Reddehase, M.J., Schirmacher, P., Galle, P.R., Lohse, A.W., and Blessing, M. (2003) *Am. J. Physiol. Gastrointest. Liver Physiol.*, **284**, G525-535.
- [43] Schramm, C., Herz, U., Podlech, J., Protschka, M., Finotto, S., Reddehase, M.J., Kohler, H., Neurath, M.F., Galle, P.R., and Blessing, M. (2003) *J. Immunol.*, **170**, 1313-1319.
- [44] Schramm, C., Huber, S., Protschka, M., Czochra, P., Burg, J., Schmitt, E., Lohse, A.W., Galle, P.R., and Blessing, M. (2004) *Int. Immunol.*, **16**, 1241-1249.
- [45] Doganci, A., Eigenbrod, T., Krug, N., De Sanctis, G.T., Hausding, M., Erpenbeck, V.J., Haddad, el-B., Lehr, H.A., Schmitt, E., Bopp, T., Kallen, K.J., Herz, U., Schmitt, S., Luft, C., Hecht, O., Hohlfeld, J.M., Ito, H., Nishimoto, N., Yoshizaki, K., Kishimoto, T., Rose-John, S., Renz, H., Neurath, M.F., Galle, P.R., and Finotto, S. (2005) *J. Clin. Invest.*, **115**, 313-325.
- [46] Kimura, A., Naka, T., and Kishimoto, T. (2007) *Proc. Natl. Acad. Sci. USA*, **104**, 12099-12104.
- [47] Finotto, S., De Sanctis, G.T., Lehr, H.A., Herz, U., Buerke, M., Schipp, M., Bartsch, B., Atreya, R., Schmitt, E., Galle, P.R., Renz, H., and Neurath, M.F. (2001) *J. Exp. Med.*, **193**, 1247-1260.
- [48] Raimondi, G., Zanoni, I., Citterio, S., Ricciardi-Castagnoli, P., and Granucci, F. (2006) *J. Immunol.*, **176**, 4012-4020.
- [49] Finotto, S., Hausding, M., Doganci, A., Maxeiner, J.H., Lehr, H.A., Luft, C., Galle, P.R., and Glimcher, L.H. (2005) *Int. Immunol.*, **17**, 993-1007.
- [50] Kiwamoto, T., Ishii, Y., Morishima, Y., Yoh, K., Maeda, A., Ishizaki, K., Iizuka, T., Hegab, A.E., Matsuno, Y., Homma, S., Nomura, A., Sakamoto, T., Takahashi, S., and Sekizawa, K. (2006) *Am. J. Respir. Crit. Care Med.*, **174**, 142-151.

Copyright of Current Drug Targets is the property of Bentham Science Publishers Ltd. and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.