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In Vivo Models of Inflammation

2nd Edition, Volume II

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Preface to the first edition

The purpose of this volume in the series *Progress in Inflammation Research* is to provide the biomedical researcher with a description of the state of the art of the development and use of animal models of diseases with components of inflammation. Particularly highlighted are those models which can serve as *in vivo* correlates of diseases most commonly targeted for therapeutic intervention. The format is designed with the laboratory in mind; thus it provides detailed descriptions of the methodologies and uses of the most significant models. Also, new approaches to the development of future models in selected therapeutic areas have been highlighted. While emphasis is on the newest models, new information broadening our understanding of several well-known models of proven clinical utility is included. In addition, we have provided coverage of transgenic and gene transfer technologies which will undoubtedly serve as tools for many future approaches. Provocative comments on the cutting edge and future directions are meant to stimulate new thinking. Of course, it is important to recognize that the experimental use of animals for human benefit carries with it a solemn responsibility for the welfare of these animals. The reader is referred to the section on current regulations governing animal use which addresses this concern.

To fulfill our purpose, the content is organized according to therapeutic areas with the associated models arranged in subcategories of each therapeutic area. Concepts presented are discussed in the context of their current practice, including intended purpose, methodology, data and limitations. In this way, emphasis is placed on the usefulness of the models and how they work. Data on activities of key reference compounds and/or standards using graphs, tables and figures to illustrate the function of the model are included. The discussions include ideas on a given model's clinical correlate. For example, we asked our contributors to answer this question: How does the model mimic what is found in human clinical practice? They have answered this question in many interesting ways.

We hope the reader will find the information presented here useful for his or her own endeavours investigating processes of inflammation and developing therapeutics to treat inflammatory diseases.

October, 1998

Douglas W. Morgan
Lisa A. Marshall

Preface to the second edition

Since our first edition of "*In Vivo* Models of Inflammation" published in 1999, there has been amazing progress, and an abundance of exciting new information in inflammation research: new technologies, new therapeutics, new understanding of inflammatory processes, … and on and on, have emerged in the past 6 years. Supporting all of this are the fundamentals of inflammation research, i.e., the animal models, known mechanisms, and therapeutic standards, that have continued to provide the basis for generating these advances. Given the great progress, we have chosen to provide a second edition to our original text.

The second edition of "*In Vivo* Models of Inflammation" comes to you in two volumes and provides an update of the models included in first edition with expanded coverage and more models. Again, these volumes emphasize the standard models regarded as the most relevant for their disease area. The intent is to provide the scientist with an up-to-date reference manual for selecting the best animal model for their specific question. Updates on previously described models are specifically focused on references to any additional pharmacology that has been conducted using these systems. The sections on arthritis models have been expanded and now include models relating to osteoarthritis. New areas described herein include models of neurogenic, cancer, and vascular inflammation. Additionally, coverage of *in vivo* technologies includes updates on transgenic and gene transfer technologies, and has also been expanded to include chapters on stem cells and nanotechnologies.

The second edition continues to emphasize that conducting *in vivo* research carries with it a great responsibility for animal respect and welfare. The coverage of this concern has been extended to include chapters describing current regulations in the United States, the United Kingdom, and Japan.

The ultimate aim of the second edition is to provide current best practices for obtaining the maximum information from *in vivo* experimentation, while preserving the dignity and comfort of the animal.

We hope the information provided here helps in advancing the reader's endeavors in investigating processes of inflammation and in developing therapeutics to treat inflammatory diseases.

May, 2006

Christopher S. Stevenson
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Asthma

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Introduction

Since the publication of the first edition of "*In Vivo* Models of Inflammation" in 1999, evidence has continued to accumulate suggesting that asthma is predominantly a chronic inflammatory condition of the lower airways, characterized by varying degrees of airway obstruction or hyperresponsiveness and long-term ultrastructural abnormalities that may mitigate the effectiveness of some of the currently available therapies [1, 2]. These structural changes, which include airway edema, airway epithelial sloughing, increased airway wall thickening (smooth muscle hypertrophy and mucus gland hyperplasia), extracellular matrix abnormalities, and increased airway vascularity all contribute to airway remodeling and fibrosis. The dominant inflammatory cells responsible for initiating or propagating the aforementioned airway pathophysiology and subsequent ultrastructural changes include the T cell (Th2), mast cell, eosinophil, basophil, and macrophage [2, 3].

As alluded to in the previously published chapter on *in vivo* asthma models, much of this evidence continues to be assimilated using various *in vivo* animal models (from mouse to primate), and the caveat that these models are not replicas of the human disease and may not be predictive of human outcome still holds. Nonetheless, there has been a relative explosion in areas such as genomics, bioinformatics, and molecular pharmacology, which has helped us to better understand these various animal models and apply this understanding to the human condition.

This chapter highlights and pays particular attention to the following with respect to animal asthma models: (1) introduction of any new models or procedural changes within existing models, (2) new insights into pathogenesis or pathophysiology provided by the respective animal models of asthma, and (3) novel pharmacological approaches or new drugs tested in these models.

It should be noted that in many of the rodent studies summarized below, airway reactivity or bronchial responsiveness is commonly measured utilizing enhanced pause (Penh). The use of Penh as a measure of airway resistance (in the absence of

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direct measurement of this variable) remains a controversial subject [4], and is considered beyond the scope of this chapter.

Mouse models

The growing use of the mouse asthma model is likely due to the expanding availability of specific molecular, immunological and genetic tools to more completely explore and delineate possible inflammatory mechanisms contributing to the underlying pathophysiology. Excellent reviews [5–7] systematically outline the strengths and weaknesses associated with the mouse model, some of which are summarized in Table 1.

There now appears to be a mounting trend to use naturally occurring, airborne sensitizing allergens [e.g., house dust mite (HDM), ragweed, cockroach antigen, etc.] as opposed to ovalbumin (OVA) in the mouse model because these allergens are also known to precipitate asthma in humans [5, 6]. Repeated (10 day) intranasal exposure of BALB/c mice to purified HDM (*Dermatophagoides pteronyssinus*) in the absence of adjuvants produced an non-tolerant inflammatory response characterized by: (1) airway accumulation of eosinophils, Th2 lymphocytes, macrophages, and dendritic cells; (2) serum IgE increases; (3) mucous hypersecretion; and (4) airway hyperreactivity (AHR) that appeared to be partially mediated by GM-CSF [8]. Kim et al. [9] recently examined the effect of dexamethasone in a BALB/c mouse model that utilized a HDM extract composed of high concentrations of cockroach allergens for both intraperitoneal sensitization (day 0) and intratracheal challenge (days 14 and 21). Dexamethasone both prevented and reversed AHR and inflammation in this study. Interestingly, Fattouh et al. [10] have demonstrated that co-sensitization with intranasal HDM and aerosol OVA daily for 5 weeks produces a dramatic non-tolerant allergic response to aerosolized OVA characterized by eosinophilia, AHR, and elevation in splenocyte-derived Th2-cytokines (IL-4, IL-5, and IL-13). They suggest that HDM allergen may act in a number of ways to condition or alter the immunological environment, including airway epithelial disruption, promotion of a Th2-polarized cytokine responses, and direct proteolytic actions. These studies suggest that mouse asthma models developed using (multiple) environmental inhaled allergens may produce a non-tolerant pathophysiological response comparable to (if not better than) OVA that may be pharmacologically modified by reference or therapeutically novel classes of compounds. In an extension of these types of studies, investigators have now demonstrated that exposure to diverse external stimuli such as cigarette smoke [11–13], respiratory infection via *Mycoplasma pneumoniae* [14], and diesel exhaust [15] can exacerbate allergen-induced airway hyperresponsiveness, airway inflammation and remodeling.

In addition to what influence the types of sensitizing allergens has on the mouse model, there appears to be a large pathogenetic distinction between use of “acute”

Table 1 - Some comparison of human asthma and mouse asthma models

Characteristic	Human	Mouse
Airway inflammation	Sustained eosinophilia noted	Degree of eosinophilia variable; lack of degranulation and few eosinophils in epithelium; may be influenced by strain utilized and length of allergen exposure
Airway remodeling	Evidence of epithelial/goblet cell hyperplasia, subepithelial fibrosis, airway wall thickening	Mouse strain-, sensitization-, and challenge-dependent remodeling as per human noted; requires prolonged intermittent or continuous allergen exposure
Bronchoconstriction, airway hyperreactivity (AHR) and late phase response (LPR)	Sustained AHR and LPR (noted during asymptomatic periods)	Transient AHR and weak LPR; AHR requires high doses of agonist; unresponsive to many of the mediators associated with pathogenesis of asthma
Cytokines involvement	IL-4, IL-5, IL-13	Similar to human condition
Disease onset and progression	Spontaneous	Induced
Immunology	IgE/Th2-mediated	Mix of IgE/IgG and Th1/Th2-mediated events depending on mouse strain, sensitization, and challenge utilized; requires systemic immunization to see IgE mediation
Mast cell	Present in airways	Not noted in respiratory mucosa

versus "chronic" challenge protocols based upon the route of sensitization and challenge, concentration and duration of allergen challenge as well as differences between mouse strains utilized. The former are generally used to study aspects of Th2-mediated immune responses, hyperreactivity, airway inflammation, and mucus hypersecretion associated with the model, while the later (although there is evidence of immunological tolerance) may be useful in examining long-term airway remodeling, which is a growing clinical concern. A study reported by Shinagawa and Kojima [16] suggests that chronic allergen instillation (OVA; 3 days/week for up to 12 weeks) but not allergen inhalation (OVA; 5 days/week for up to 4 weeks) produced hallmarks of remodeling (marked airway wall thickening, mucous cell hypertrophy, airway eosinophilia, and collagen deposition) in the A/J mouse strain but to a lesser degree or not at all in BALB/c, C57BL/6, and C3H/HeJ mice. Note that in an acute inhaled-allergen challenge model (OVA; 1.0% for 1 h), the A/J mouse strain exhibited no allergen-induced increases in airway responsiveness to methacholine in the presence of mediocre bronchoalveolar lavage (BAL) accumulation of leukocytes (eosinophils and neutrophils) and cytokine generation [17], suggesting that route and total number of exposures may in fact regulate the pathophysiological response of the airways. Even in less intense chronic mouse models (OVA inhalation or installation for 5–7 days following sensitization), evidence of both peribronchial and perivascular remodeling, including increased smooth muscle mass, collagen expression, and proliferation of epithelial and endothelial cells [18] as well as subepithelial matrix deposition of collagen and proteoglycans [19], have been noted. Overall, the "chronic" mouse models may be useful systems to examine the pharmacology of airway remodeling, and to gain insight into the corresponding human condition.

The allergic mouse continues to be routinely used as a model to explore the efficacy of various antibodies (primarily against cytokines and chemokines) as a therapeutic approach to treat the inflammatory asthmatic process. Some of these studies are summarized in Table 2 [20–24].

Genetically altered mice [severe combined immunodeficiency (SCID), transgenics including knockouts and knockins, etc.] are being increasingly used to delineate the relevance of various mechanisms in the pathobiology of asthma. The use of the SCID mouse, lacking both functional T and B lymphocytes, reconstituted with "sensitized" human peripheral blood mononuclear cells (hPBMC) is allowing investigators to explore components of the human immunobiology of asthma and use reagents especially chemokines and cytokines with limited species cross-reactivity. Duez et al. [25] demonstrated that SCID mice reconstituted intraperitoneally with hPBMC from HDM-sensitive patients and aerosol challenged daily for 4 days with HDM extract exhibited evidence of AHR, increases in human IgE in murine serum, increases in BAL fluid (BALF) IL-5 (but not IL-4 or tumor necrosis factor-α), and human pulmonary infiltrates but no lung eosinophilia. In an extension of this study, Tournoy et al. [26] showed that SCID mice reconstituted intratracheally with hPBMC from nonallergic donors could be driven from a Th1 to a Th2 phenotype (increases in lym-

Table 2 - Representative studies examining the effect of various cytokine or chemokine antibodies in the mouse asthma model

Study	Antibody/route of administration	Inhibition					
		AHR	Cell infiltration BALF/airway	BALF cytokines	Mucus	Remodeling	Other
Justice et al. [20]	CCR3/systemic + aerosol	+	+ EOS	–	+	ND	
Chung et al. [21]	CCR8 (TCA-3)/intranasal	ND	–	–	ND	ND	
Kumar et al. [22]	IL-5, IL-13 or IFN-γ intraperitoneal	+ (IL-13, IFN-γ)	+ EOS (IL-5, IL-13, IFN-γ)	ND	+ (IL-13)	+ collagen (IL-5)	
Cheng et al. [23]	IL-9/intravenous	+	+ EOS, + LYMPH + NEUT	+ IL-4, + IL-5, + IL-13	ND	ND	
Yang et al. [24]	IL-13/intravenous	+	+EOS +LYMPH	+IL-4 +IL-5 +TNF +Eotaxin	+	+Fibrosis	+MMP-9

+, positive effect; –, no effect; BALF, bronchoalveolar lavage fluid; EOS, eosinophils; LYMPH, lymphocytes; NEUT, neutrophil; MMP-9, matrix metalloproteinase-9; ND, not determined.

phocyte-derived IL-4 and IL-5 and decreases in IFN-γ upon intraperitoneal HDM plus adjuvant injection and a subsequent 19-day aerosol exposure to HDM. These animals exhibited AHR and pulmonary infiltration by human T lymphocytes in the absence of eosinophilia, but unlike the previous study exhibited no increases in human IgE in the murine serum. Adjuvant appeared to be necessary to drive the lymphocytes from a Th1 to a Th2 phenotype and elicit AHR. Interestingly, AHR in this human-mouse chimera model was blocked using anti-IL-4/IL-13 (DM-IL-4) or anti-IL-5 (TRFK-5) cytokine therapy, although allergen-induced Th2 cytokine production was not altered. Finally, SCID mice reconstituted intraperitoneally with hPBMC from HDM-sensitive donors primed with human HDM-pulsed dendritic cells and then challenged with HDM for 5 days developed intense pulmonary inflammation consisting of human lymphocytes and mouse eosinophils, increases in BALF IL-4 and IL-5, and elevations in human IgE in murine serum [27]. Based on the above examples, the humanized-SCID mouse asthma model will allow researchers to continue to explore the efficacy of novel therapeutics and mechanisms associated with asthma that may translate directly to the human condition.

The most significant growth in the past 6 years has been noted in terms of the use of transgenic mouse models of asthma especially in the area of cytokine research. An in-depth description of this area is beyond the scope of this chapter; however, some recent studies of interest are summarized below. To date, the use of IL-5 receptor null mice ($^{-/-}$) or monoclonal antibodies have produced equivocal results in establishing the precise role of IL-5 in the pathogenesis of asthma-associated eosinophilia and AHR [28]. Recent investigations, using IL-5 transgenic mice (to augment the eosinophilic response), have demonstrated that subchronic allergen-induced marked airway eosinophilia prevented AHR [29], while chronic allergen-induced eosinophilia enhanced subepithelial and peribronchial fibrosis [30], both through a transforming growth factor-β1 (TGF-β1) effect. The differences in these studies may reflect in part the aforementioned dichotomy associated with using different challenge protocols. In a further modification of the transgenic approach, Shen et al. [31] utilized intratracheal adoptive transfer of allergic eosinophils to eosinophil deficient IL-5$^{-/-}$ mice to demonstrate a probable interdependent relationship between CD4^{+} T cells and eosinophils to initiate allergen-induced elevations in BALF cytokines, AHR, and mucus accumulation. Using both constitutive and inducible overexpression, as well as on/off triple transgenic mice, Elias et al. [32] have shown that IL-13-induced lung inflammation, characterized by airway remodeling, mucus hypersecretion, and fibrosis, is highly regulated via adenosine, chemokine receptor-2, matrix metalloproteinases, vascular endothelial growth factor, TGF-β1, and IL-11. Using IL-10$^{-/-}$ [33] and nitric oxide (NO) synthase2$^{-/-}$ mice [34], it has been recently suggested that NO may have a homeostatic role against allergen-associated immunopathobiology and AHR. There is still a great deal of speculation as to which NO synthase (1, 2 or 3) is bronchoprotective or anti-inflammatory. There is some evidence in transgenic mice overexpressing NOS-2 (the

CC10-rtTA-NOS-2 mouse) suggesting that this NOS is not proinflammatory, and may in fact reduce airway reactivity or provide bronchodilatory NO [35]. Finally, through the use of matrix metalloproteinase (MMP) -9 or -12 knockout mice, various investigators [36–38] have established that these MMPs play a critical role in the pathogenesis of allergen-induced airway inflammation possibly by modulating dendritic cell and T cell trafficking. These aforementioned transgenic-based mouse asthma model systems plus others exploring diverse therapeutic targets such as complement [39], tyrosine kinase inducible T cell kinase [40], NF-κB [41] and IL-17F [42] may provide excellent insight into the complex pathways that regulate asthma as an inflammatory disease.

Although a preponderance of work using the mouse asthma model has centered on transgenic applications and use of antibodies as therapeutic approaches or proof of concept testing, various novel classes of compounds of therapeutic interest have been evaluated since the last edition. The oral efficacy of the selective phosphodiesterase inhibitor, roflumilast (5 mg/kg/day), has been assessed in a chronic mouse model of asthma [43]. Roflumilast, similar to dexamethasone, appears to reduce airway inflammation, subepithelial fibrosis and epithelial hypertrophy. Like the MMPs mentioned above, there seems to be mounting evidence to support the importance of the mast cell-derived protease, tryptase, in the evolution of asthmatic inflammation. Oh et al. [44] have explored the role of tryptase in the mouse asthma model using the orally active reversible tryptase inhibitor, MOL 6131, and shown that it effectively reduced BALF and lung tissue inflammation as well as goblet cell hyperplasia and hypersecretion. Interestingly, when the compound was administered intranasally, the inhibitory effects were more pronounced.

CpG oligonucleotides acting as immunomodulatory agents via the Toll-like receptor (TLR)-9 to ultimately stimulate Th1 cytokine protective effects have also been critically examined. Kline et al. [45] and Jain et al. [46] demonstrated that CpG oligonucleotides may reverse or prevent acute and chronic allergen-induced inflammation and AHR through redirection from a Th2 to a Th1 microenvironment. This may be ultimately mediated by the up-regulation of IL-12 [47]. The importance of the Th1/Th2 balance and IL-12 up-regulation in asthma is further supported by the fact that intraperitoneal treatment with the TLR-2 agonist, PAM3CSK4, reduced lung inflammation, airway hyperresponsiveness, and serum IgE while increasing T-cell or dendritic cell derived IFN-γ, IL-12, and IL-10 in the mouse asthma model.

The mitogen-activated protein kinase (MAPK) family also appears to play an important immunomodulatory role in the mouse lung. Using repeat dosing (7 days) of aerosolized p38α MAPK antisense oligonucleotide, Duan et al. [48] demonstrated that this therapeutic approach effectively reduced allergen-induced eosinophilia, mucus hypersecretion, and AHR. The potential importance of this mechanism is also supported by the findings of Underwood et al. [49], who have demonstrated that the specific p38 MAPK inhibitor, SB 239063, administered orally, essentially abolished allergen-induced BALF eosinophilia in the allergic mouse model. Like-

wise, the combined MAPK/extracellular signal-regulated kinase (ERK) inhibitor, U0126, inhibited allergen-induced lung eosinophilia, increases in BALF IL-4, IL-5, IL-13, and eotaxin, mucus secretion, and airway hyperresponsiveness in the mouse when given via the intraperitoneal route [50].

Guinea pig models

It was noted in the last edition that the allergic guinea pig model has not been substantially altered since its inception some 90 years ago. This is again the case in the intervening years, although there have been modifications as investigators attempt to reproduce the different syndromes that make up human asthma using the guinea pig model. This includes a toluene-2,4-diisocyanate (TDI)-induced model which represents occupational asthma [51]. In this model, guinea pigs were first percutaneously sensitized with TDI followed by five tracheal challenges with a TDI mist. Both early and late responses were seen with only the late phase being inhibited by a corticosteroid. Nishitsuji et al. [52] developed a model of cough variant asthma. Animals were sensitized to OVA and the bronchial responsiveness as well as the cough reflex response to capsaicin were measured 72 h after an OVA inhalation challenge. Studies in humans indicate that exercise-induced asthma (EIA) worsens with increased dietary sodium. Studies in guineas pigs show that hyperpnea-induced airway obstruction, a model for EIA, is worsened in animals fed a high salt diet [53], and that this heightened response was inhibited by leukotriene blockade. This suggests that this guinea pig model has utility to mimic the human condition. Investigators have also examined the effects of different challenges in animals sensitized to OVA. Smith and Johnson [54] demonstrated that 5'-adenosine monophosphate produced a late asthmatic response (LAR) and an AHR in sensitized animals thus mimicking what is seen in atopic individuals. Studies in sensitized guinea pigs challenged with ultrasonically nebulized distilled water indicate a role for neurokinin (NK)-1 receptors in the resultant bronchoconstriction [55].

Probably the most striking change seen since the last edition is the number and class of compounds that have been evaluated in guinea pig models of asthma. There has been a continued interest in the efficacy of new phosphodiesterase (PDE) inhibitors, particularly those specific for PDE4 [56–58]. SCH 351591 and its active metabolite SCH 365351 both inhibited AHR and allergen-induced eosinophilia in the guinea pig [57]. Santing et al. [58] report that allergen-induced bronchoconstriction and AHR can be inhibited by PDE4 inhibitors, whereas inhibition of allergen-induced eosinophilia requires inhibition of both PDE3 and PDE4. There has also been increased interest in immunosuppressives, particularly by inhalation, for the treatment of asthma. Both cyclosporine [59] and FK-506 [60] given by inhalation inhibit AHR and cellular influx following allergen challenge. MX-68, a derivative of methotrexate, given orally inhibits the early and late bronchoconstrictive

response as well as cellular influx [61]. Growing evidence that different prostanoids play either a pro- or anti-inflammatory role in the airways have prompted the synthesis of various new agents. The stable PGE_2 mimetic, misoprostol inhibits both bronchospasm and eosinophilia to an inhaled antigen challenge [62]. The PGD_2 receptor antagonist, S-5751 inhibits eosinophilia [63], whereas the combined TXA_2 synthase inhibitor, 5-LO inhibitor and antihistamine F-1322 inhibits the LAR and eosinophilia in an *Ascaris* model [64]. Investigators have also sought anti-inflammatory effects of recently approved drugs that are thought of as having primarily a bronchodilatory action. Thus, the leukotriene antagonist montelukast has been shown both to inhibit eosinophil influx [65] and to produce apoptosis of eosinophils [66]. The long-acting beta agonist, formoterol, and it R-R isomer have been shown to prevent eosinophilia and to protect against bronchospasm [67]. Interestingly, the long-acting muscarinic antagonist, tiotropium, has been shown to inhibit airway remodeling in a 12-week repeated challenge model [68]. Miscellaneous compounds that have shown efficacy in guinea pig models include ebselen [69], VUF-K-8788 [70], the tyrosine kinase inhibitor, Genistein [71] and the bradykinin B2 receptor antagonist FK-3657 [72].

The guinea pig has continued to be used to investigate the pathophysiology of asthma. The role of neurokinins and their receptors has been extensively studied with evidence for a complex interplay between the three subtypes (NK-1, NK-2, NK-3). NK-1 receptors contribute to AHR and eosinophil, neutrophil and lymphocyte infiltration [73], whereas NK-2 receptors have been reported to mediate late phase hyperreactivity, late phase bronchoconstriction and the influx of neutrophils and lymphocytes but not eosinophils [73, 74]. NK-3 receptors have also been reported to mediate AHR in a severe asthma model [75, 76]. Arginase upregulation has also been reported to contribute to AHR, presumably by lowering NO synthesis from constitutive NO synthases by competing for their common substrate, L-arginine [77]. The complement fragment C3a is also implicated in the bronchoconstrictor response to allergen as guinea pigs with a natural deficiency in the C3a receptor have a reduced response compared to wild-type animals [78]. The ability of an IL-13 binding fusion protein to inhibit eosinophilia and AHR provides further support for a role of IL-13 in asthma pathophysiology [79].

Rat models

The Brown Norway (BN) strain continues to be the primary strain used and the sensitization procedures follow those outlined in the last edition. As in the guinea pig, investigators have also used chemicals, known to produce occupational asthma, as sensitizing and challenge agents in the rat. Thus, both diphenylmethane-4,4'-diisocyanate (MDI) [80] administered dermally or by inhalation [80], and trimellitic anhydride (TMA) administered dermally [81], have been shown to produce IgE

titers and to produce bronchoconstriction and eosinophilia. Dong et al. [82] used HDM antigen with or without the systemic administration of *Bordetella pertussis* in 3-week-old BN rats to establish a model that would more closely reflect the developing immune system of children. They showed that the sensitization process was enhanced when both agents were administered simultaneously. The Flinders sensitive line of rats, which are hyperresponsive to cholinergic stimuli, show enhanced bronchoconstrictor responses and airway inflammation when they are sensitized to OVA and subsequently challenged [83], indicating that neural pathways may play an important role in the asthma phenotype.

The rat model has also been increasingly used to examine the phenomenon of airway remodeling in the pathogenesis of asthma, as well as potential therapeutic interventions to prevent remodeling. As with many models, the parameters used to elicit remodeling vary considerably between investigators, but one constant is the need to repeatedly challenge the animals with inhaled antigen. Chung's group at the University of London used six OVA challenges every 3rd day [84–86] to elicit remodeling and measured goblet cell hyperplasia, epithelial cell proliferation and airway smooth muscle (ASM) proliferation as indices of the remodeling process. ASM and epithelial proliferation were studied by measuring 5-bromo-2'-deoxyuridine. Using this chronic antigen-challenge paradigm, corticosteroids [84] and a Jun N-terminal kinase (JNK) inhibitor (SP600125) [85] have been shown to inhibit airway remodeling. Xu et al. [87] examined ASM hyperplasia in animals sensitized with OVA with *B. pertussis*, and subsequently challenged with OVA given by inhalation on three occasions, 5 days apart. This study showed a modest increase in ASM cells in the antigen-challenge group compared to the saline-challenge group. Vanacker et al. [88] report on a model where animals were challenged for 28 days with antigen and noticed an increase in goblet cell numbers, epithelial cell proliferation, airway wall area, fibronectin deposition and collagen deposition. These structural changes were inhibited by fluticasone administered daily for the last 2 weeks of the challenge period.

Due to its relevance and utility as a toxicology species, and thus the ability to determine the therapeutic window in the same species, rat models have continued to be used to study the potential of new therapeutic compounds. As noted in the guinea pig, the rat has been used to investigate novel PDE4 inhibitors including NVP-ABE171 [89] and YM976 [90]. The rat has also been used to examine whether parenteral administration of compounds to the lung would provide efficacy with a better safety profile than oral administration. Compounds studied include the "soft" steroids cilcesonide [51] and BNP-166 [91], as well as the macrolide MLD987 [92]. In addition, gene-based therapies have also been studied in the rat, including the use of gene therapy with Galectin 3 [93] and antisense to Syk kinase [94]. As asthma has been increasingly recognized as a chronic disease, mediated via a Th2 imbalance, a considerable number of immunomodulators have been examined for efficacy in the rat asthma model. These can be found in Table 3 [95–105].

Table 3 - The effect of various immunomodulators in the rat asthma model

Compound	Class of compound	Acute/chronic admin.	BHR	Leukocyte infiltration	Other	Ref.
Compound A	IKK β kinase inhibitor	C	Yes	Yes		[95]
SP600125	JNK kinase inhibitor	A	No	Yes		[96]
YM-90709	IL-5 antagonist	?	–	Yes		[97]
SAR943	Immunomodulator	A	No	Yes		[98]
SAR943	Immunomodulator	C	–	No	ASM	[99]
IMM125	Immunomodulator	A	No	Yes	Cytokines	[98]
Suplatast	IgE inhibitor	C	–	Yes	Cytokines	[100]
CGS 21680	Adenosine A2a agonist	A	–	Yes		[101]
SP100030	Transcription factor inhibitor	SubC	–	-	Lymphocytes	[102]
TEI-9874	IgE inhibitor	A	–	Yes	EAR and LAR	[103]
PS-519	Proteasome inhibition	A?	–	Yes		[104]
Leflunomide	Immunomodulator	C	–	-	IgE levels	[105]

– , not examined.

Rabbit models

In comparison with other species, rabbit models have undergone less evolution since the last edition. In an interesting study the hypothesis that systemic allergy and asthma worsens the outcome of cardiovascular complications was examined [106]. The authors found that rabbits sensitized and subsequently challenged by aerosol have increased infarct size and neutrophil infiltration in a model of myocardial ischemia-reperfusion injury compared to non-sensitized rabbits [106]. In a model of gastroesophageal acid reflux, Gallelli et al. [107] demonstrated that the bronchoconstriction elicited by intraesophageal instillation of HCl was mediated via tachykinins acting on NK-1 and NK-2 receptors. Hogman et al. [108] reported that both inhaled histamine and hypertonic saline increase airway reactivity in non-sensitized rabbits and noted that asthmatics have increased airway reactivity after nebulizing hypertonic saline.

The rabbit has continued to be used to investigate the pathophysiology of asthma particularly by investigators using isolated muscle preparations. Grunstein's group has investigated the effects of HDM allergen [109], rhinovirus [110] and IL-1β [111] on contractile and relaxant responses in ASM isolated from the rabbit. They showed that the HDM allergen Der p 1 enhances contractile responses and inhibits relaxant responses via activation of ERK1/2 pathways, and that this is regulated by p38 MAP kinase signaling [109]. Rhinovirus affects ASM responsiveness by an ICAM-1-dependent activation of IL-5 pathways, which in turn releases IL-1β from the ASM [110]. Using *in vivo* models, investigators have examined the role of integrins [112], adenosine [113], tachykinins [114], bradykinin [114] and reactive oxygen species [115] on antigen-induced pulmonary responses. Gascoigne et al. [112] report that VLA4 is involved in acute bronchoconstriction in the rabbit, whereas eosinophil recruitment and infiltration involves VLA4 and LFA-1, and lymphocyte recruitment involves LFA-1 and Mac-1 [112]. Further evidence for a role of adenosine in the allergic response in the rabbit comes from studies using a new selective adenosine A1 receptor antagonist L-97-1 [113]. Other studies have shown a mixed role for bradykinin, neurokinins and reactive oxygen species. Thus, superoxide dismutase inhibits AHR, but has no effect on airway inflammation in a chronic model [115], whereas neurokinins acting via NK-2 receptors inhibit acute bronchoconstriction to antigen, but have no effect on the resultant eosinophilia or AHR [114]. Bradykinin B2 receptors appear to play no role in the airway response to allergen challenge [114].

Canine models

In terms of canine models of asthma, the focus since the first edition of "*In Vivo* Models of Inflammation" published in 1999 has centered on: (1) continued devel-

opment and use of neonatally ragweed-sensitized beagles from allergic parents and (2) use of a dry air challenge model to examine exercise-induced asthma. Although not reviewed in detail here, a ragweed-sensitized dog model of allergic rhinitis has been developed and appears to be useful to study the antiallergic properties of new therapeutic agents [116, 117].

Beagle puppies of allergic, high serum IgE parents sensitized with intraperitoneal ragweed at 24 h post-partum to 22 weeks of age and subsequently exposed to multiple aerosol ragweed challenges exhibited evidence of elevated serum IgE, AHR to histamine, methacholine, and neurokinin A as well as BALF eosinophilia [118–120]. T lymphocytes removed from these animals 4 h following segmental ragweed challenge exhibited evidence of localized activation. In general, while this canine asthma model appears to exhibit many of the characteristics of the human asthmatic condition, it has not been routinely utilized perhaps because of the difficulties associated with housing and maintenance of this particular model.

Hyperventilation dry air challenge of anesthetized dogs can elicit bronchoconstriction and BALF eicosanoid (leukotriene C_4 and E_4; prostaglandin D_2, $F_2\alpha$, and thromboxane B_2) generation [121, 122]. These pathophysiological changes could be attenuated by aerosolized heparin. Using a repeat cold dry air challenge model of hyperpnea, Davis et al. [123] demonstrated evidence of transient airway remodeling characterized by epithelial cell hypertrophy, thickened lamina propria, and tissue accumulation of eosinophils, neutrophils, and mast cells.

Sheep models

The sheep lung exhibits numerous physiological and pathophysiological similarities to humans with respect to lung size, anatomy and development, bronchial circulation and airway innervation, characteristics of mast cells and mucus production, and high serum IgE and allergic inflammation in the lungs after allergen challenge. In addition, they are responsive to bronchospastic agents and modulators that are effective in humans. Two sheep models are currently available, the first and most widely utilized to date using animals having a natural skin sensitivity to *Ascaris suum* [124, 125]. On exposure to aerosolized antigen, an early bronchoconstriction and pulmonary hyperinflation are observed in these animals. A proportion of these early responders called dual responders go on to develop bronchoconstriction 7–8 h later and a nonspecific AHR at 24 h, which can last up to 2 weeks. In addition, lung inflammation characterized 24 h later by BAL and/or tissue accumulation of macrophages, neutrophils, eosinophils, and lymphocytes is evident and correspondingly more pronounced in dual responders. As mentioned in the previous edition, the introduction of a technique (lavage via a double-balloon nasotracheal tube) to isolate and study upper airway epithelial function (i.e., mucus secretion) and inflammation in this model has greatly expanded our understanding of this model [126].