



Review

Murine models of hapten-induced asthma

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ABSTRACT

Asthma is a chronic inflammatory disorder of the respiratory tract that is characterized by reversible airflow obstruction and airway hyperresponsiveness. The non-atopic variant of asthma that appears later in life has no allergic background and is more severe and resistant to standard treatment. Hapten-induced asthma models can be utilized to investigate mechanisms behind the development of non-atopic and occupational asthma, in which non-allergic processes seems to play significant role. The development of adequate animal models of non-allergic asthma is a necessary prerequisite both for understanding the pathophysiology of non-allergic asthma and for the possibility of testing new therapies. Still, there is no ideal model that represents all the hallmarks of this complex disease. In this review, we examine the most popular hapten-induced murine models of occupational and non-atopic asthma. For this reason, we describe the most popular sensitizing haptens, sensitization and challenge protocols, symptoms produced by asthma, and advantages and disadvantages of the models.

1. Introduction

According to World Health Organization, asthma is a chronic inflammatory disorder of the respiratory tract that is characterized by reversible airflow obstruction and airway hyperresponsiveness (WHO, 2007). Asthma, which affects about 300 million people worldwide, poses a serious global health problem (GINA, 2018). Although prevention and treatment of the disease are in place, many issues should be investigated. In this respect, experimental animal models of asthma play important roles; the mouse is the most frequently used species. There are many reasons to choose mice in pre-clinical asthma studies. First, a large number of inbred strains are well described. Numerous immunological reagents are available, creating the opportunity to examine specific mechanisms of inflammatory reaction in airways (Shin et al., 2009). Moreover, mouse transgenic models have been developed to assess the influence of particular mediators on asthmatic response (Lloyd, 2007). Other advantages are the shortness of the gestational period, ease of breeding, and the relatively low cost of gaining (Shin et al., 2009). In contrast, due to anatomical differences in airways between mice and humans, translating findings from animal studies to clinic practice should be rational. Additionally, mice do not respond to

constrictive activity of histamine. There are difficulties in designing chronic models of diseases that could be conducted over many months (Patel and Chorawala, 2011). Despite the noted above, mouse models are widely used in both the atopic and non-atopic phenotype of the disease. Bronchial asthma is a heterogeneous disorder that can be distinguished depending on the occurrence of allergic background. In the most common experimental model, the sensitizing compound is a protein allergen, ovalbumin (OVA). The use of the latter leads to allergic variant of asthma with characteristic features of eosinophilia and increased level of IgE antibodies in serum (Zhang et al., 2017; Hwang et al., 2017; Venturini et al., 2018). The pathomechanism of the non-allergic type of the disease has been investigated to a much lesser extent, and the number of animal models is limited.

Non-atopic variant of asthma, also called 'intrinsic asthma' due to the inability to identify external factors causing reaction, is characterized by negative results of allergy skin tests, as well as a lack of increased total IgE concentration and absence of specific IgE in serum (Humbert et al., 1999). A typical feature is an elevated number of neutrophils, which rarely occurs in the airways of atopic asthmatics (Amin et al., 2000). In addition, intrinsic patients display initial signs of the disease later in their lives; no familial predispositions to allergy and

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asthma are observed (Dahlberg and Busse, 2009). Although clinical symptoms in both phenotypes are similar, non-atopic asthma tends to be more severe and resistant to standard doses of inhaled glucocorticoids (Barnes, 2009). Epidemiological studies indicate that association between atopy and asthma concerns less than half of the cases and that a higher proportion can result from an imprecise definition of atopy. As a result of this overestimation, other possible mechanisms of pathological reaction might be insufficiently analyzed (Pearce et al., 1999).

One variant of asthma in which non-allergic processes may play significant role is occupational asthma (Douwes et al., 2002). This phenotype is described as a variable airflow limitation in response to agents present in the workplace (Bardana, 2003). It is estimated that approximately 40% of occupational asthma incidents are caused by chemical substances, including low molecular weight compounds (LMW) (Bernstein, 2003).

A number of studies indicate that mice models of hapten-induced asthma can be utilized to investigate mechanisms behind the development of work-related asthma, as well as non-atopic asthma in the general population. Unfortunately, available models are not uniform in respect to induced reaction, since many of them share both allergic and non-allergic features of asthma. Thus, there is no perfect model that will represent all the hallmarks of this complex disease. The aim of the present review is to present a brief summary of available murine models of occupational and non-atopic asthma, with cross-references including recently published reports.

To this end, a description of most popular sensitizing haptens, sensitization and challenge protocols, produced symptoms of asthma, and advantages and drawbacks of the models are presented.

2. Haptens in murine models of asthma

Haptens are LMW agents (< 5000 Da) that have the ability for unique binding with immunoglobulins and T lymphocyte receptors (antigenicity), but they are not able to elicit an immune response by themselves (immunogenicity). They obtain the latter feature only when attached to large carrier, e.g. a protein molecule. In response to complete adduct, B lymphocytes recognize the hapten and Th lymphocytes

protein carrier (Gołab et al., 2017). There are many compounds classified as chemical sensitizers. Some of them are used in experimental models of asthma, including toluene diisocyanate (TDI), trimellitic anhydride (TMA), dinitrofluorobenzene (DNFB), and picryl chloride (PCL). They can be then divided into those that cause IgE-dependent or IgE-independent asthma (van Houwelingen et al., 2002). Hypothetical mechanism of hapten-induced non-atopic asthma is presented in Fig. 1.

2.1. Toluene diisocyanate model

One of the most common respiratory irritants is toluene diisocyanate, which occurs as a mixture of the 2,4- and 2,6-isomers. It is mainly used in the production of polyurethane foams. Chronic exposure, via inhalation, to 2,4-TDI causes asthma-like symptoms in humans, manifested by bronchial constriction, wheezing, and dyspnea (PubChem, CID = 11,443). Highly reactive diisocyanates include diphenyl-methane diisocyanate and hexamethylene diisocyanate (Świerczyńska-Machura et al., 2012). However, TDI is the most frequently used compound in hapten-induced asthma in mice.

There are numerous experimental protocols with different sensitization and challenge periods (see Table 1). In some of them elevated serum levels of Th2 cytokine, e.g. IL-4 and IgE antibodies, indicate possible allergic mechanism of the reaction (Sun et al., 2007). Although the functional and clinical features of TDI-induced response are similar to those observed in allergic asthma, there are still controversies about substantial differences, including neutrophil-dominated lung inflammation (Sun et al., 2007), low association with atopy, and cause of the pathological process (Park et al., 2012).

Matheson et al. (2005) described two models in which the effect of low-level subchronic exposure to TDI inhalation (20 ppb of TDI for six weeks) and high doses of acute exposure (500 ppb TDI for 2 h) were compared. Mice from the subchronic regimen group demonstrated significant allergic reaction including airway inflammation, eosinophilia, Th1/Th2 cytokine expression in the lung, increased level of serum IgE, and airway hyperresponsiveness (AHR). In contrast, mice that underwent acute exposure showed considerable AHR and specific IgG antibodies, yet there was no increased IgE in the

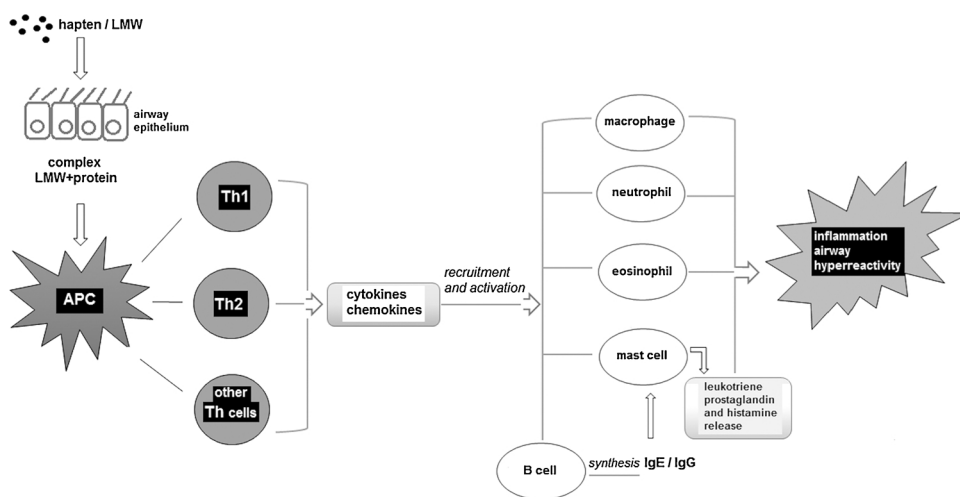


Fig. 1. Hypothetical mechanism of hapten-induced non-atopic asthma. Hapten after conjugation with protein is processed by antigen presenting cells (APC), which leads to differentiation and proliferation of T helper cells. T lymphocytes release cytokines and chemokines triggering recruitment and activation of inflammatory cells including B-cells and mast cells, which also release mediators of bronchoconstriction. DNFB induces mast cell activation and neutrophilic phenotype of asthma, while TDI and TMA eosinophilic, neutrophilic or mixed type depending on protocol applied. TDI and TMA elevate IgE production.

Table 1
Toluene diisocyanate (TDI) asthma model.

Mouse strain	Sensitization phase	Challenge phase	BALF influx	AHR	Cytokines	IgE	Reference
C57BL/6 J female	subchronical: inhalation of 20 ppb of TDI (4 h/5 days/6 weeks) acute: inhalation of 500 ppb of TDI for 2 h	inhalation with 20 ppb of TDI for 1 h, 2 weeks after sensitization	eosinophils, lymphocytes	↑ AHR to methacholine	↑ IL-4, IL-5, TNFα, IFNγ mRNA transcripts	↑ serum IgE	Matheson et al. (2005)
C57BL/6 male	dermal application of 20 µl of 2% TDI on days 1,8	oropharyngeal instillation of 20 µl of 0.01% TDI on day 15	lymphocytes neutrophils, less eosinophils	↑ AHR to methacholine ↑ AHR to methacholine	no increase in cytokine expression ↑ IL4, IL-13, IL-10, IFNγ in auricular lymphocytes	no change ↑ serum IgE	Pollaris et al. (2016)
BALB/c male	dermal application of 20 µl of 0.3% TDI on days 1,7	intranasal instillation of 10 µl 0.1% TDI on days 10,13,16 intranasal instillation of 10 µl of 0.1% TDI on day 10	neutrophils, eosinophils only after third challenge neutrophils	↑ AHR to methacholine ↑ AHR to methacholine	↑ MIP-2, TNFα in BALF ↑ IL-4, IL-13, IFNγ in auricular and cervical lymph node cells ↑ MIP-2 in BALF ↑ IL-4, IFNγ in auricular lymph node cells	↑ serum IgE ↑ serum IgE	Vanoirbeek et al. (2009) Tarkowski et al. (2007)
BALB/c female	subcutaneous injection with 20 µl of TDI on days 0,7,14 intranasal administration of 20 µl of 3% TDI for 5 days; repeated after a 3-week interval	intranasal challenge with 1% TDI from days 21 to 23 inhalation with 1% TDI on day 7 after the second sensitization	- neutrophils, less eosinophils	- ↑ AHR to methacholine	↑ IL-4 in serum	↑ serum IgE -	Sun et al. (2007) Lee et al. (2001)
BALB/cJ/Han/IMP female		inhalation with 3% TDI starting on day 7 after second sensitization (4 h/5 days/5 weeks)	neutrophils, less eosinophils	-	↑ IFNγ, TNFα in BALF	-	Świerczyńska-Machura et al. (2014)

AHR- airway hyperresponsiveness, BALF-bronchoalveolar lavage fluid.

Table 2
Trimellitic anhydride (TMA) asthma model.

Mouse strain	Sensitization phase	Challenge phase	BALF influx	AHR	Cytokines	IgE	Reference
BALB/c female	dermal sensitization with 100 µl of 3% TMA on days 1,3; then intratracheal sensitization with TMA-MSA (30 or 400 µg) on day 12	intratracheal instillation of 30 or 400 µg TMA-MSA on days 19,22,23	eosinophils	-	-	↑ IgE in plasma ↑ IgE in BALF supernatant ↑ serum IgE	Regal et al. (2001)
	dermal sensitization with 1% TMA on days 1–3, 8–10, 15–17	inhalation with TMA (0.5 mg /L) for 3 consecutive days, 2 weeks after last sensitization	eosinophils	-	↑eotaxin-1, IL-4, IL-6, KC in BALF ↑IL4 gene expression in lung tissue ↑IL-4, IL-6 production in hilar lymph node	↑ serum IgE	Nishino et al. (2016)
BALB/c male	dermal sensitization on abdomen with 100 µl of 25% TMA for 2 consecutive days in one week (protocol 1) or 2 consecutive days in each of two weeks (protocol 2)	intratracheal instillation with 50 µl of 0.25% TMA 17 days after the last sensitization	neutrophils	-	↑ IFN γ in protocol 1, ↑ IFN γ in protocol 2, in BALF	↑ serum IgE ↑ IgE in BALF only in protocol 2	Sallstad et al. (2003)
	dermal application of 20 µl of 5% TMA on days 1,7	intranasal instillation of 10 µl of 1% TMA on day 10	neutrophils	↑ AHR to methacholine	↑ MIP-2, TNF α in BALF ↑ IL-4, IFN γ in stimulated auricular and cervical lymphocytes	↑ serum IgE	Vanoirbeek et al. (2006)

AHR- airway hyperresponsiveness, BALF-bronchoalveolar lavage fluid.

Table 3
Models of asthma induced with dinitrobenzene derivatives (DNFB, DNCB).

Mouse strain	Sensitization phase	Challenge phase	BALF influx	AHR or tracheal hyperreactivity	Cytokines	IgE	Reference
CD1 female	epicutaneous application of DNFB (50 µl, 0.5 % to the thorax and paws on days 0, 1	intranasal challenge with 50 µl of 0.6% DNS on day 5	macrophages	↑ tracheal hyperreactivity to 5-HT, no reaction to carbachol	-	-	Bozkurt et al. (2016)
			neutrophils	↑ tracheal hyperreactivity to carbachol	-	-	Kraneveld et al. (2005) van der Kleij et al. (2004)
BALB/c male	intratracheal challenge with 50 µl of 0.6% DNS on day 5	intranasal challenge with 50 µl of 0.6% DNS on day 5	mononuclear cells and neutrophils	↑ AHR to methacholine	↑ TNF α in BALF	-	van der Kleij et al. (2004)
			mononuclear cells and neutrophils	↑ tracheal hyperreactivity to carbachol	-	-	Kraneveld et al. (2002)
			mononuclear cells and neutrophils	↑ tracheal hyperreactivity to carbachol	-	-	van der Kleij et al. (2003)
			mononuclear cells and neutrophils	↑ AHR to methacholine	-	-	Kogut et al. (2017)
	dermal application of 20 µl of 0.2% DNCB on each ear on days 1, 7	intranasal instillation of 10 µl of 0.02% DNCB on day 10	neutrophils	no AHR to methacholine	↑ IL-1 α , IL-2, IL-13, TNF α in BALF ↑ IL-1 β , IL-17 A, KC, IL-12p40, RANTES in lung tissue ↑ TNF α in BALF ↑ IL-4, IFN γ in stimulated auricular and cervical lymphocytes	no change	Vanoirbeek et al. (2006)

AHR- airway hyperresponsiveness, BALF-bronchoalveolar lavage fluid, DNFB-dinitrofluorobenzene, DNCB- dinitrochlorobenzene.

serum, no eosinophilia, and no increased cytokines in the lungs. The experiment clearly indicates that pathological reaction in the TDI-induced asthma model depends on the type of exposure protocol. This assumption is confirmed by another study comparing single and multiple intranasal challenges with TDI. In both cases, Th1 and Th2 lymphocytes are involved in inflammatory response. The difference concerns the accumulation of inflammatory cells in bronchoalveolar lavage fluid (BALF). In single challenge protocol, the characteristic feature is neutrophil inflammation, which reaches the levels similar to those demonstrated in a multiple provocation regime. However, only in the multiple challenge protocol was an influx of eosinophils observed (Vanoirbeek et al., 2009).

In other studies, sensitization and challenge with TDI mainly resulted in increased numbers of neutrophils in BALF with a much less pronounced increase in the number of eosinophils (Lee et al., 2001; Tarkowski et al., 2007; Pollaris et al., 2016). These findings again suggest that neutrophils may strongly contribute to the pathogenesis of TDI-induced asthma.

Another complication in the interpretation of data from experimental models of asthma is mouse strain used in the procedure. Not all characteristic effects induced by TDI can be detected in C57Bl/6 and CBA strains, confirming that genetic background has a substantial impact on the observed phenotype of the disease. Moreover, there is no always unequivocal correlation between airway hyperreactivity and the number of inflammatory cells in the lung (De Vooght et al., 2010).

To better understand pathomechanism in chemical-induced asthma, the role of lymphocytes was assessed using severe combined immunodeficiency (SCID) mice and B-lymphocyte knock-out (B-KO) mice. Passive transfer of B-cells obtained from TDI-sensitized animals into naïve wild type, SCID, and B-KO groups followed by oropharyngeal challenge with the same hapten led to airway hyperreactivity and airway inflammation dominated by neutrophils. These results indicate that B-lymphocytes are crucial in the development of asthmatic reaction in mice, without the necessary involvement of T-lymphocytes. Furthermore, asthma-like response was independent of IgE antibodies (De Vooght et al., 2013). Although the complete mechanism of TDI-induced reaction remains unclear, some evidence suggests that it may be different from that of classic IgE-dependent asthma (Świerczyńska-Machura et al., 2014).

2.2. Trimellitic anhydride model

Trimellitic anhydride is a highly reactive compound used in industry in the production of adhesives, polymers, resins, dyes, and as a chemical intermediate to prepare plasticizers. It is one of the main causes of occupational asthma in humans. Inhalation exposure to TMA results in shortness of breath, wheezing, or coughing (PubChem, CID = 11,089). The majority of animal studies concern rat models (especially using the Brown Norway strain); however, there are also a few reports of mouse experiments of TMA-induced asthma.

One study compared cellular infiltration after administration of trimellitic anhydride or ovalbumin followed by intratracheal instillation of TMA conjugated with mouse serum albumin (TMA-MSA) or OVA. In the TMA group, the number of eosinophils in BALF and the level of eosinophil peroxidase in lung tissue homogenates markedly increased (Regal et al., 2001). The observed eosinophilia was comparable to the reaction seen in OVA-sensitized and OVA-challenged mice treated with a similar exposure procedure. Similarly, Nishino et al.

(2016) showed increased numbers of eosinophils next to augmented release of pro-inflammatory Th2 cytokines and chemokines (IL-4, IL-6, KC) in BALF and increased total serum IgE levels of TMA sensitized and inhaled mice. In fact, occupational asthma caused by TMA has many features of protein allergen-induced asthma. Nevertheless, in contrast to response elicited by high molecular weight compound, TMA-induced asthma can persist for months or years after exposure stopping (Valstar et al., 2006).

Acknowledgment that the TMA model induces Th2 skewed response, however, is not that simple, as respiratory sensitization and hypersensitivity is not always accompanied with eosinophilia. In two studies using Balb/c mice, TMA treatment produced neutrophil influx in BALF, increases in IgE, and increased production of mixed Th1 and Th2 cytokines (Sailstad et al., 2003; Vanoirbeek et al., 2006). It seems that much depends on the protocol of TMA sensitization and challenge, and it is possible to evoke both: Th2 and mixed type of Th1/Th2 response. The difference between the latter studies and those where infiltration of eosinophils predominated in the BALF can be associated with recurrence of the challenge phase (Regal et al., 2001; Nishino et al., 2016). Inhalation or intratracheal instillation of TMA leading to eosinophilia took place for three consecutive days, in contrast to the single instillation, which produced neutrophilia (see Table 2). To define the nature of immune response, specific cytokine production by draining lymph node cells was determined following chronic topical exposure to five different acid anhydrides. In each case, acid anhydrides provoked secretion of primarily Th2-type cytokines (Dearman et al., 2000). For generation of Th2-type cell activation by TMA, another study examined mice treated with acid anhydrides along with Th2 cytokine antagonists. Sensitization and challenge with TMA and phthalic anhydride increased the total IgE level in serum and production of IL-4 and IL-10 in the supernatants of spleen cells cultured with concanavalin A. Co-administration with anti-IL-4 and anti-IL-12 modified this Th2 response, resulting in significant elevation of total IgG2a antibodies (characteristic feature of Th1-mediated reaction) in the serum, as well as reduction in IL-4 and IL-10 concentration (Ban and Hettich, 2005). Although it is undisputed that inhalation is the main route of airway sensitization, dermal contact with some chemical respiratory irritants may be also perceived as a potential risk of pulmonary response (Dearman et al., 2000).

2.3. Models induced by dinitrobenzene derivatives

Dinitrofluorobenzene is a powerful sensitizer that can cause dermatitis and allergic reaction through skin contact or inhalation. Symptoms of exposure to DNFB include irritation of the skin, the eyes, and the mucous membranes (PubChem, CID = 6264). Although some researchers suggest that nitro derivatives of benzene (e.g. dinitrochlorobenzene-DNCB) are typical dermal irritants, which are not able to induce respiratory sensitization (Dearman and Kimber, 1991; Vanoirbeek et al., 2006), it has been proven that epicutaneous sensitization with DNFB and local airway challenge result in non-atopic asthma response (van Houwelingen et al., 2002).

Van der Kleij et al. (2003) developed a model in which mice were dermally sensitized with DNFB and intranasally challenged with water-soluble, cognate compound dinitrobenzene sulphonic acid (DNS). A pulmonary hypersensitivity reaction in sensitized and challenged mice had a two-phase character. In the early phase, which occurred 0–3 h after hapten challenge, mice displayed acute

Table 4
Summary of advantages and disadvantages of hapten-induced models of non-atopic asthma.

Hapten/model	Advantage	Disadvantage
TDI	- dermal sensitization (not invasive) - intranasal challenge or via inhalation (mimics human exposure) - AHR to methacholine - possibility of achieving neutrophil influx to BALF after single-time challenge	- mixed type with features of allergen-induced asthma (eosinophils, IgE, Th2 or Th1/Th2 cytokines) - long-term process of sensitization and many instillations/inhalations required during challenge phase - differences in phenotype between studies depending on mouse strain or protocol used
TMA	- induced asthma can persist for months or years after exposure - dermal sensitization (not invasive) - AHR to methacholine - possibility of achieving neutrophil influx to BALF after single-time challenge	- mixed type with features of allergen-induced asthma (IgE, Th2 or Th1/Th2 cytokines, and after many challenges influx of eosinophils in BALF) - repeated sensitizations and challenges required - differences in phenotype between studies depending on strain or protocol used
DNFB/DNCB	- uniform non-atopic phenotype - (neutrophils in BALF, no IgE in serum, AHR) - single-time challenge - dermal sensitization (not invasive)	- not many studies applying this model

AHR- airway hyperresponsiveness, BALF-bronchoalveolar lavage fluid.

bronchoconstriction and activation of mast cells. However, no hapten-specific IgE antibodies were detected. Features of late phase (24–48 h after DNS administration) included pulmonary edema, infiltration of mononuclear cells and neutrophils into the lung. Also present were mast cell activation, *in vivo* airway responsiveness and *ex vivo* tracheal hyperreactivity. The results provided evidence that mechanisms other than eosinophilic inflammation and atopy may be engaged in the pathogenesis of asthma (van der Kleij et al., 2004). The important role of mast cells was also confirmed by an experiment with mast cell-deficient mice using the same hapten model (Kraneveld et al., 2002). Characteristic features of both early and late phase of reaction were remarkably lower or absent in comparison to the respective normal littermate mice. Furthermore, after mast cell reconstitution, the acute bronchoconstriction, neutrophilia in BALF, tracheal hyperactivity and vascular hyperpermeability were demonstrated anew in DNFB-sensitized and DNS-challenged mice. These findings strongly suggest the contribution of mast cells in the initiation of hapten-induced hypersensitivity reaction in airway (Kraneveld et al., 2002).

In order to explain the pathophysiology underlying this non-atopic response, the role of antigen-specific Ig free light chains (LCs) was examined. Topical sensitization with DNFB and intranasal challenge with DNS induced neutrophil-associated inflammation, airway obstruction, mucosal exudation, and airway hyperreactivity in mice. It appeared that immunization with antigen-specific LCs followed by intra-airway challenge resulted in mast-cell dependent acute bronchoconstriction, demonstrating the possibility of transferring pulmonary hypersensitivity response in a passive way (Kraneveld et al., 2005).

In subsequent experiments, the involvement of different receptor pathways in hapten-stimulated asthma response was also investigated. Bozkurt et al. (2016) demonstrated that DNFB application leads to increased contractile response of isolated tracheas to

5-hydroxytryptamine (5-HT) and elevated numbers of macrophages in BALF. Moreover, there were no changes in response between DNFB and control mice in contraction elicited with carbachol. This suggests that tracheal hyperreactivity to 5-HT does not affect muscarinic receptors in the present non-atopic asthma model. In contrast, in an earlier study, DNS challenge in the same model resulted in carbachol-induced tracheal hyperreactivity. It has been further shown that the tachykinin NK1 receptor plays a crucial role in the development of trachea contractile reaction and the infiltration of neutrophils into the lungs (van der Kleij et al., 2003).

Although the number of experiments with DNFB murine model is limited and the complete pathological mechanism remains unclear, the aforementioned findings and the most recent findings (Kogut et al., 2017) indicate that the DNFB model is repeatable, strongly associated with neutrophils accumulation, and may reflect the characteristic features of non-atopic asthma in humans (see Table 3).

2.4. Picryl chloride model

Picryl chloride is chemically classified as a trinitrochlorobenzene. Exposure to the compound may cause irritation of the skin, the digestive tract, and the upper respiratory tract (PubChem, CID = 6953). Few experiments employ the animal model of PCL-induced asthma. In one study, mice were epicutaneously sensitized with PCL and then challenged by intranasal administration of picryl sulphonic acid (PSA; a water soluble form of PCL). The reaction was characterized by airway obstruction, submucosal infiltration of T cells (but not eosinophils), and prolonged airway hyperreactivity to metacholine. It was demonstrated in adoptive transfer experiments that a specific population of lymphoid cells is essential to elicit this *in vivo* AHR. In addition, AHR did not occur after transfer of the Ag-specific IgE antibody. All of these findings suggest non-atopic mechanism of PCL-induced response (Geba et al., 1997). In another study, sensitization and challenge with

PCL was described as a delayed-type hypersensitivity (DTH) reaction. It served as a model of cellular immunity to examine T-cell-mediated induction of airway hyperreactivity. In fact, hyperreactivity of isolated trachea to carbachol was T-cell-dependent response, as in group with athymic mice hyperreactivity to carbachol was not manifested (Garssen et al., 1991). To enhance understanding of the mechanisms underlying DTH-like reaction, mice dermally sensitized to PCL underwent intranasal challenge with DNS (in place of homologous hapten PSA). In this case, DNS challenge did not induce hypersensitivity-like response judged by unchanged accumulation of mononuclear cells around bronchioles and pulmonary blood vessels. In contrast, challenge with PSA remarkably increased the number of inflammatory cells in the lungs of mice. These results clearly indicated antigen-specific character of DTH-reaction (Garssen et al., 1989).

3. Conclusions

Low molecular weight haptens are fairly diversified group of compounds. All of them require conjugation with endogenous proteins to induce immunological reaction. However, they can act through different mechanisms. This fact is reflected by differences in demonstrated responses: secretion of Th1/Th2 profiles of cytokines, pulmonary infiltration dominated by neutrophils or eosinophils, and correlation with IgE antibodies level (Table 4). Apart from the individual nature of the particular hapten, several reports have shown that the sensitization and challenge paradigm may also have a significant effect on induced inflammation. Long-term sensitization with hapten leads to the development of atopic reaction, whereas short-term sensitization with single-time challenge can result in non-atopic response. Thus, depending on the protocol applied, some haptens such as TDI can be utilized to induce both types of murine model of asthma (Matheson et al., 2005).

However, in view of the complexity of the disease, it is unlikely that the single-mouse model will represent all the hallmarks of the human asthma. It is important to constantly improve experiments related to the relationship between airway dysfunction, pulmonary inflammation, and pathological alteration at the cellular and molecular level (Lloyd, 2007). Although the complete mechanisms of observed processes have not been fully elucidated, experimental murine models of hapten-induced asthma provide crucial insight into the pathophysiology of the disease and may contribute to the development of novel therapeutic approaches.

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