

ORIGINAL PAPER

Immunoglobulin E antibodies enhance pulmonary inflammation induced by inhalation of a chemical hapten

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Summary

Background Occupational exposure to chemicals is an important cause of asthma. Recent studies indicate that IgE antibodies enhance sensitization to chemicals in the skin.

Objective We investigated whether IgE might similarly promote the development of airway inflammation following inhalation of a contact sensitizer.

Methods A model of chemical-induced asthma is described in which introduction of the low-molecular-weight compound, trinitrobenzene sulphonic acid (TNBS), via the respiratory tract was used for both sensitization and challenge. The role of IgE antibodies in the immune response to inhaled TNBS in this model was assessed by comparing the responses of wild-type (WT) and IgE-deficient (IgE^{-/-}) mice on the BALB/c background. Reconstitution of circulating IgE levels by intravenous injection of IgE antibodies into IgE^{-/-} mice before sensitization was performed to confirm the role of IgE in any differences observed between the responses of WT and IgE^{-/-} mice.

Results Intranasal challenge of TNBS-sensitized (but not sham-sensitized control mice) induced intense pulmonary inflammation. Macrophages, eosinophils and lymphocytes, including T, B, natural killer and natural killer T cells, were recruited to the airway and the animals displayed bronchial hyperresponsiveness (BHR) to methacholine. Serum levels of murine mast cell protease-1 (mMCP-1) were elevated suggesting mast cell activation. In contrast, the development of airway inflammation, recruitment of lymphocytes, induction of BHR and production of mMCP-1 were all significantly attenuated in IgE-deficient mice. Reconstitution of IgE^{-/-} mice with IgE (of unrelated antigen specificity) before sensitization partially restored these features of asthma.

Conclusion Our data indicate that IgE antibodies non-specifically enhance the development of airway inflammation induced by exposure to chemical antigens.

Keywords asthma, contact sensitivity, IgE, occupational

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Introduction

Asthma is a chronic disease of the airways characterized by reversible airway obstruction, pulmonary inflammation, mucus hypersecretion and bronchial hyperresponsiveness (BHR). In patients with the atopic form of the disease, asthma is typically driven by T-helper type 2 (Th2)-dominant responses to protein aeroallergens. However, there is a subset of asthmatics in which airway inflammation and BHR are triggered by exposure to small chemical compounds as can occur in some occupational

settings. The most common chemical sensitizers of the airways are diisocyanates, acid anhydrides and some dyes (reviewed in [1]). There is evidence that chemical airway inflammation is also driven by a Th2 response but there may be a role for Th1 cells and their cytokines as well [2].

Asthma is strongly correlated with a genetic predisposition towards atopy, the tendency to develop specific IgE responses following allergen exposure. In pedigree analyses, elevated IgE levels and BHR are co-segregating traits [3], and within cohorts of asthmatic patients, IgE levels have been correlated with disease severity [3, 4]. Inhibition of IgE function using a monoclonal anti-IgE antibody has proven effective in the treatment of a subset

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of asthma patients with known IgE-mediated sensitivity to inhaled protein aeroallergens [5, 6]. In contrast to protein aeroallergen sensitivity, where a role for IgE antibodies in pathogenesis is evident, the contribution of IgE antibodies to airway inflammation induced by chemical sensitizers is not as clear [1]. Production of hapten-specific IgE antibodies has been observed in some patients with chemical sensitivity but the pathogenic function of these antibodies is unknown and there are some chemical sensitizers, especially diisocyanates, which elicit airway responses without any evidence of specific IgE production [7].

We recently reported a novel immunologically non-specific role for IgE antibodies in immune responses to chemical haptens. Using a murine contact sensitivity (CS) model, we showed that IgE antibodies at physiologic levels enhance immune sensitization to the epicutaneously applied contact sensitizer oxazolone [8]. Given the effect of IgE antibodies on immune sensitization to contact sensitizers in the skin, we hypothesized that responses to chemical haptens in the respiratory mucosa, such as those that occur in occupational asthma, might similarly be enhanced by IgE antibodies.

Here we report a novel model of occupational asthma to the chemical trinitrobenzene sulphonic acid (TNBS) in which sensitization and challenge are both performed by intranasal instillation of an aqueous solution. TNBS-exposed animals developed an intense mixed inflammatory response in the lungs along with goblet cell metaplasia, BHR and enhanced levels of circulating murine mast cell protease-1 (mMCP-1). In contrast, TNBS-induced inflammation and BHR were attenuated in IgE^{-/-} mice and the induction of mMCP-1 was impaired. Reconstitution of IgE^{-/-} mice by intravenous (i.v.) injection of IgE antibodies of irrelevant specificity before sensitization partially restored the inflammatory phenotype. These data provide evidence that in the airways, just as in the skin, IgE antibodies serve to promote immune sensitization to chemical antigens.

Materials and methods

Animals

BALB/c mice were purchased from Taconic Farms (Germantown, NY, USA). The derivation of IgE^{-/-} mice bred onto a BALB/c background has been previously described [8, 9]. All experiments were carried out in accordance with the IACUC policies and procedures of the Children's Hospital, Boston.

Sensitization of mice

Mice were anaesthetized by isoflurane inhalation. Fifty microlitres of 1% TNBS (Sigma, St. Louis, MO, USA), pH 7.2 or 50 µL of phosphate-buffered saline (PBS) was

applied to the nares on days 0 and 1. Mice were challenged on day 5 with 1% TNBS, and were studied 48 h later for the induction of airway inflammation. To reconstitute IgE^{-/-} mice, 10 µg of IgE antibodies of an irrelevant specificity [anti-dinitrophenol (DNP) IgE; clone SPE-7] were injected i.v. 18 h before the initial sensitization dose as described previously [8]. In some experiments as indicated in the figure legend, some mice received an equivalent amount of anti-DNP IgG (Sigma).

Determination of bronchial hyperresponsiveness

Lung function was assessed by whole-body barometric plethysmography (Buxco Electronics, Wilmington, NC, USA) in live, unrestrained animals as described previously [10]. Briefly, animals were placed in a sealed chamber and exposed to increasing concentrations of methacholine from 33 to 333 mg/mL. Changes in airway resistance to aerosolized methacholine were measured as Enhanced Pause (Penh).

Collection of specimens

For bronchoalveolar lavage (BAL), 1 mL of staining medium (SM) consisting of Hank's balanced salt solution supplemented with 10 mM HEPES buffer at pH 7.2% and 2% newborn calf serum was infused into the trachea and then retrieved using a syringe. BAL leucocytes were counted using a haemocytometer. Erythrocytes were lysed by hypotonic shock and leucocytes were resuspended in SM. Total leucocytes were enumerated and viability was assessed by trypan blue exclusion.

Histological analysis

Lungs were fixed in 10% neutral-buffered formalin. To prevent atelectasis, fixation was performed under mild vacuum. The lungs were embedded in paraffin, and 5 µm sections were cut and stained with haematoxylin and eosin or periodic acid-Schiff stain. The inflammatory infiltrate was examined using standard light microscopy by an investigator unaware of the identity of individual samples.

Flow cytometry

BAL leucocytes were analysed by flow cytometry for the presence of T, B, natural killer (NK) and natural killer T (NKT) cells by staining with antibodies to CD3, CD4, CD8, B220 (CD45R) and DX5 for 30 min on ice. Following the incubation with primary antibody, cells were washed and resuspended in SM containing propidium iodide to differentiate between live and dead cells. All mAbs were conjugated with FITC, PE, APC, PE-Cy5 or APC-Cy7 and were purchased from eBioscience (San Diego, CA, USA) and BD

Biosciences (San Jose, CA, USA). Cells were analysed using the FACS Calibur and FACS Canto flow cytometers and data were processed using either CellQuest (BD Biosciences) or FlowJo (Treestar Inc., Ashland, OR, USA) software.

Enzyme-linked immunosorbent assay

mMCP-1 ELISA was performed on serum and BAL samples using a kit (Moredun Scientific Ltd, Midlothian, Scotland) according to the manufacturer's instructions.

Statistical analysis

Differences in values for various experimental groups were examined for significance using the two-tailed Student's *t* test and ANOVA. All data are shown as the mean response $\pm$ standard error of mean.

Results

Intranasal instillation of trinitrobenzene sulphonic acid sensitizes mice for the development of airway inflammation following inhaled hapten challenge

We previously reported that IgE antibodies enhance immune responses to contact sensitizers in the skin [8] and

hypothesized that IgE might play a similar role in the airways, facilitating reactions to chemical sensitizers such as those responsible for occupational asthma. Various experimental systems have been used to study the development of airway sensitization and inflammatory responses to chemical allergens. In order to determine the effects of IgE on the sensitization of inhaled chemical antigens *in vivo*, we chose to use the water-soluble low-molecular-weight compound TNBS as our model antigen. This chemical has been previously shown to induce contact colitis in both mice and guinea-pigs [11, 12]. Of note, the high-affinity IgE receptor, Fc ϵ RI, enhanced inflammation in the TNBS colitis model, suggesting that IgE might also regulate the development of inflammatory responses to this chemical antigen in the gut [12]. However, a role for TNBS as an inducer of airway allergic inflammation in a murine model of chemical-induced asthma has not previously been described.

Wild-type (WT) BALB/c mice were subjected to intranasal delivery of either PBS or 1% TNBS in PBS on days 0 and 1. Five days after sensitization, all mice were challenged with 1% TNBS and tissues obtained 48 h after challenge were subjected to histopathologic analysis. BALB/c mice sensitized and then challenged with TNBS displayed an intense inflammation of the lung parenchyma, perivascular areas, peribronchial regions and alveolar

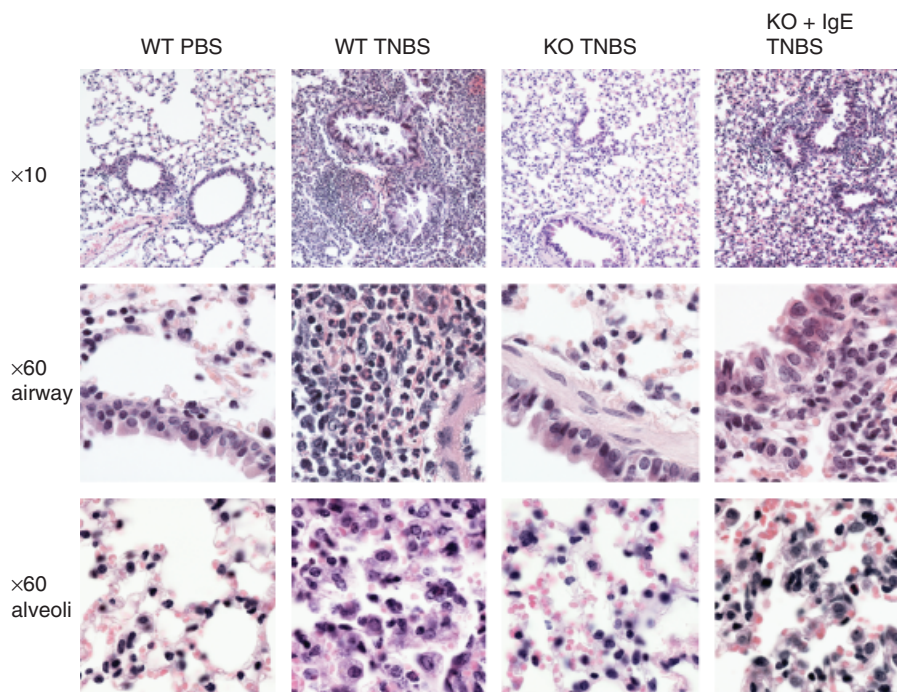


Fig. 1. Lung inflammation induced following inhaled TNBS sensitization and challenge is enhanced in the presence of IgE. WT and IgE^{-/-} mice were given 50 μ L of either PBS or 1% TNBS in PBS on days 0 and 1 intranasally. Additional IgE^{-/-} mice (KO + IgE) received intravenous injections of 10 μ g IgE 18 h before sensitization with TNBS. On day 5, all mice were challenged intranasally with 50 μ L of 1% TNBS and 48 h later lungs were removed and fixed. Haematoxylin and eosin-stained sections were prepared and examined by light microscopy at $\times 10$ (upper panels) and $\times 60$ (middle and lower panels), depicting inflammation around the airways (middle panels) and in alveolar spaces (lower panels). Representative sections are presented. TNBS, trinitrobenzene sulphonic acid; WT, wild-type; PBS, phosphate-buffered saline; KO, knockout.

spaces (Fig. 1). The infiltrating cells were comprised mostly of mononuclear cells but granulocytes, also including some eosinophils (Fig. 1, upper and middle panels). In contrast, the extent of inflammation, both around the airways and in the alveolar spaces, was significantly attenuated in IgE^{-/-} animals (Fig. 1). The development of inflammation was an immunologically specific response, rather than an irritant reaction to TNBS, as confirmed by the absence of inflammation in TNBS-challenged control animals that had been sham sensitized by intranasal administration of PBS 5 days before their TNBS challenge.

Remarkably, reversal of the IgE deficiency of the IgE^{-/-} animals by i.v. administration of 10 µg of the monoclonal IgE antibody, SPE-7, 18 h before initial sensitization resulted in a partial rescue of the ability of these mice to display a hapten-specific response in the lungs. Notably, the SPE-7 antibody (anti-DNP specificity) is not directed against the TNBS hapten used for sensitization and challenge. IgE-reconstituted mice displayed perivascular, peribronchial and parenchymal lung inflammation, which while not as intense as that in WT animals was clearly significant compared with sham-sensitized animals. Thus, as had been observed in the cutaneous CS model, the ability of IgE antibodies to restore immune responsiveness to chemical sensitizers is not mediated by their antigenic specificity.

In order to further characterize the pulmonary inflammatory response induced by TNBS sensitization and challenge, we performed cytologic analysis of BAL fluid specimens. Intranasal TNBS induced an intense cellular influx into the airways consisting predominantly of macrophages but also including increased levels of lymphocytes and eosinophils compared with unsensitized TNBS-challenged controls (Fig. 2). The recruitment of cells into the airways was markedly less robust in the IgE^{-/-} mice. Reconstitution of the IgE^{-/-} mice with i.v. IgE (but not i.v. IgG) before TNBS sensitization led to a partial restoration of the response that was most apparent for lymphocytes and eosinophils but also suggested for macrophages.

Analysis of BAL lymphocyte populations by flow cytometry revealed that the numbers of both CD4⁺ and CD8⁺ T cells as well as NK and NKT cells were significantly increased in the BAL of TNBS-sensitized and challenged mice but not in TNBS-challenged non-immune controls (Fig. 3). Again, administration of i.v. IgE before TNBS sensitization led to a partial restoration of the capacity of IgE^{-/-} mice to mount a TNBS-specific inflammatory response.

We have previously determined that the dose used for reconstitution in these studies is sufficient to restore serum IgE levels within the physiological range in injected recipients [8]. At baseline, WT BALB/c mice have serum IgE levels of 200–300 ng/mL (Fig. 4). I.v. administration of anti-DNP IgE initially raises the levels of IgE to more than 600 ng/mL (1 h after transfer). The amount of total IgE

then steadily decreases, presumably related in part to tissue redistribution and binding to mast cell FcεRI, reaching 90 ng/mL at 24 h.

Patients with asthma exhibit increased airway mucus secretion that results from stimulation of subepithelial mucus glands as well as mucus metaplasia of airway epithelial cells. Mucus metaplasia is a feature of many murine models of asthma driven by protein allergen sensitization and challenge. We observed that TNBS, similarly, induced a strong mucus secretion response in the airway epithelium and that this was much less pronounced in IgE-deficient mice (Fig. 5). As with the inflammatory responses, the mucus secretion phenotype was partially rescued by i.v. IgE reconstitution of the IgE^{-/-} mice.

The cardinal physiologic feature of asthma is BHR. BHR manifests as increased sensitivity to stimuli of bronchoconstriction, such as the cholinergic agonist, methacholine. We assessed BHR in TNBS-treated mice by Penh determination following exposure to graded doses of methacholine using whole-body plethysmography of unrestrained animals. A substantial increase in BHR was observed in mice that had been both sensitized and challenged with TNBS (Fig. 6). The response was immunologically specific and not due to a direct bronchoconstricting effect of TNBS as TNBS-challenged sham-sensitized mice did not display BHR. In parallel with our observations on inflammation induced by TNBS inhalation, IgE^{-/-} mice showed an attenuated response, a significant portion of which was corrected by i.v. IgE administration before TNBS sensitization.

Airway inflammation in allergic asthma can be accompanied by evidence of mast cell activation. In order to examine the participation of mast cells in the pulmonary TNBS response, we measured serum levels of mMCP-1, a marker of mast cell load and activation state. TNBS treatment elicited elevated levels of this marker in WT but not IgE^{-/-} mice (Fig. 7).

In summary, we describe a novel model of chemical-induced asthma in which mice sensitized by intranasal instillation of TNBS and then challenged via the same route develop an intense mixed cellular pulmonary infiltrate, goblet cell metaplasia, airway hyperresponsiveness and mast cell activation upon inhaled TNBS challenge. Similar to our previous observations regarding cutaneous CS responses elicited by sensitization and challenge with the chemical, oxazolone in the skin, we found that the reaction to inhaled TNBS is also attenuated in mice lacking IgE and that the response is restored to a significant extent following IgE reconstitution. As in the skin model, the specificity of this IgE is irrelevant, suggesting a non-antigen-specific immunomodulatory effect of IgE antibodies in the process of immune sensitization to chemical haptens.

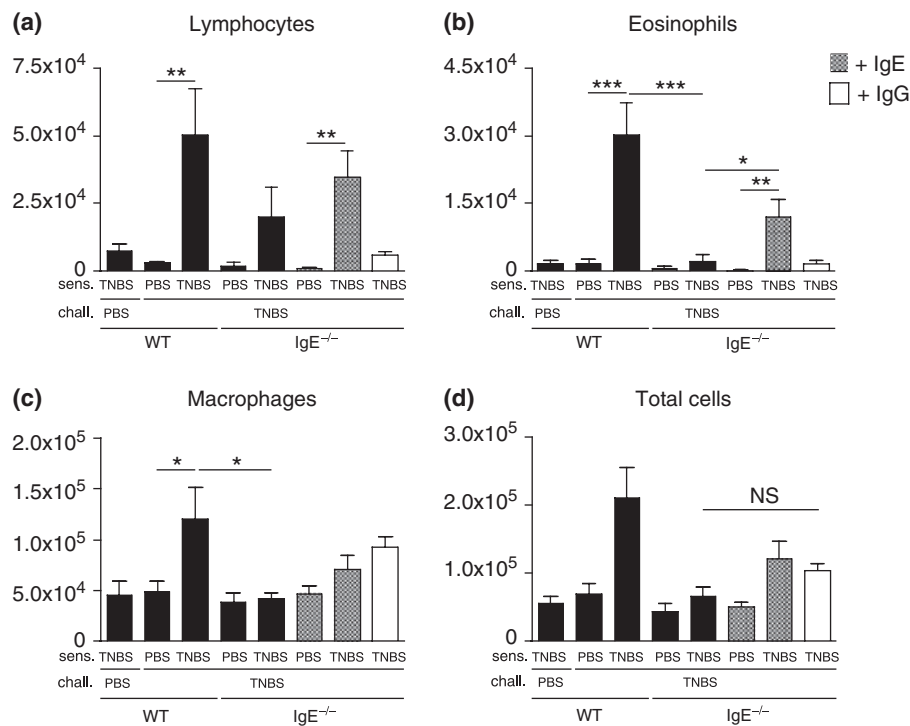


Fig. 2. Inflammatory cell influx into the airways following hapten challenge is enhanced by the presence of IgE antibodies. Cytologic analysis of BAL was performed after sensitization (Sens.) with TNBS or PBS and challenge of WT and $IgE^{-/-}$ mice with TNBS. Absolute numbers of (a) lymphocytes, (b) eosinophils, (c) macrophages and (d) total cells are presented. Data are representative of two experiments. $n = 5$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ by two-tailed Student's t test. TNBS, trinitrobenzene sulphonic acid; WT, wild-type; PBS, phosphate-buffered saline; BAL, bronchoalveolar lavage.

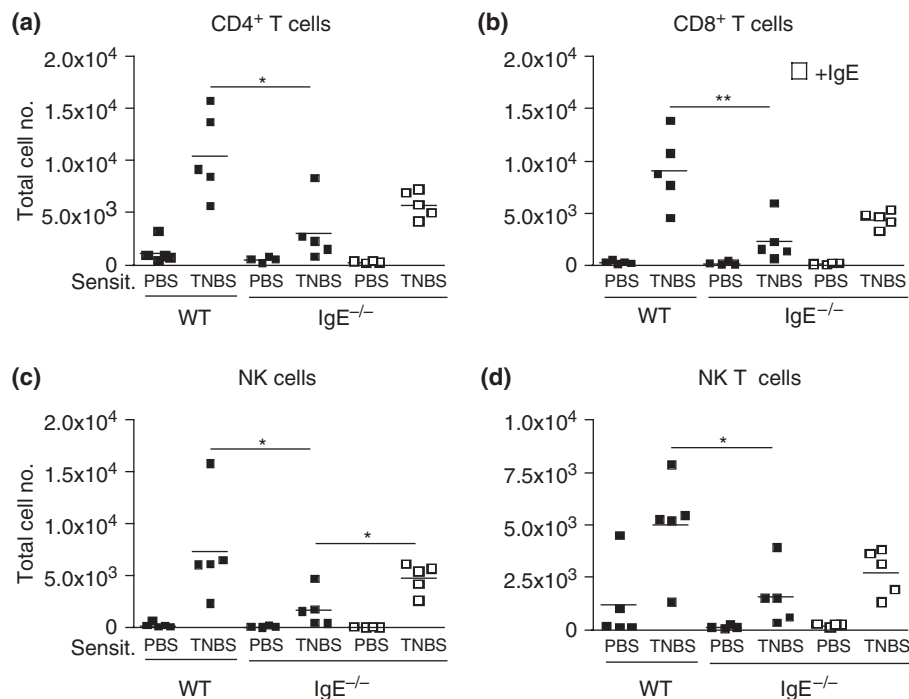


Fig. 3. Modulation of airway lymphocyte recruitment by IgE antibodies. WT, $IgE^{-/-}$ mice and $IgE^{-/-}$ mice reconstituted with IgE (+IgE) were sensitized (Sensit.) with either PBS or TNBS and challenged with TNBS on day 5. BAL was obtained 48 h later and analysed by flow cytometry. Absolute numbers of (a) CD4⁺ T cells, (b) CD8⁺ T cells, (c) NK cells and (d) NKT cells are shown. $n = 5$. * $P < 0.05$, ** $P < 0.01$ by two-tailed Student's t test. TNBS, trinitrobenzene sulphonic acid; WT, wild-type; PBS, phosphate-buffered saline; BAL, bronchoalveolar lavage; NK, natural killer.

Discussion

Allergic sensitization to chemicals is a significant cause of asthma in industrialized nations [13, 14]. Recent reports estimate that up to 15% of asthmatic patients in the United States develop the disease as a result of occupational exposure to highly reactive low-molecular-weight compounds such as toluene diisocyanate (TDI) [15]. Although Th2 responses have been implicated in the pathogenesis of occupational asthma, the role of IgE antibodies has been unclear [1]. Prior observations implicating IgE in cutaneous responses to contact sensitizing chemicals prompted us to hypothesize that IgE might also play a role in immune sensitization to inhaled chemical haptens. In order to test the hypothesis, we exposed WT and IgE^{-/-} mice to TNBS, which is water soluble, allowing inhaled sensitization and challenge without exposure to the irritating or toxic solvents required for some skin sensitizers. We observed that intranasal sensitization and challenge with TNBS elicited several key features of asthma, including a mixed inflammatory infiltrate (Figs 1–3), mucus hypersecretion (Fig. 5), BHR (Fig. 6) and mast cell activation (Fig. 7).

The mechanisms of immune sensitization and effector responses to chemical haptens have been most extensively studied using cutaneous CS reactions. It is known that these delayed type hypersensitivity (DTH) responses are T cell dependent (reviewed in [16]). Optimal sensitization

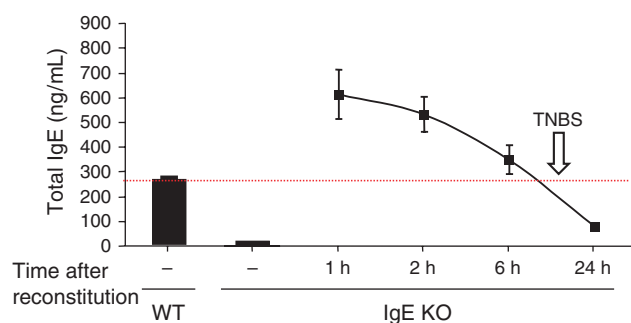


Fig. 4. Kinetics of IgE reconstitution to physiological levels in IgE^{-/-} recipients. IgE^{-/-} mice were injected i.v. with 10 µg IgE and serum was collected at the indicated intervals. IgE levels in the sera of wild-type (WT) and IgE^{-/-} + IgE mice are shown.

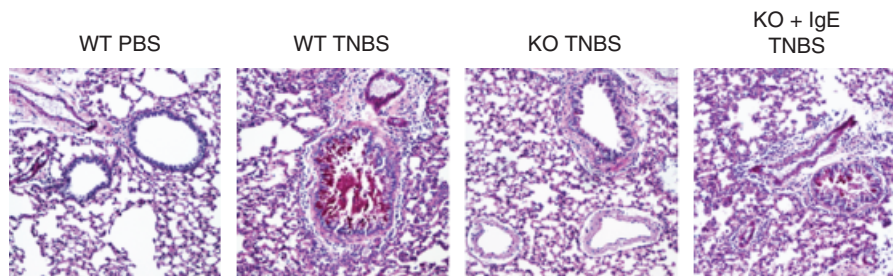


Fig. 5. Increased airway mucus metaplasia in the presence of IgE following inhaled hapten challenge. Sections prepared from mice treated as in Fig. 1 were stained with periodic acid-Schiff (PAS) to assess epithelial mucus metaplasia. Representative sections are presented.

requires that dendritic cells (DCs) deliver hapten encountered in the skin to regional lymphoid tissue for presentation to responding T cells. Our understanding regarding the subset(s) of DCs (epidermal vs. dermal) responsible for the initiation of sensitization is evolving. Epidermal Langerhans cells (LC) have been thought to be the initiating cell type. However, recent studies suggest that CS can be induced in LC-deficient mice, and that dermal mice DCs actually travel to lymph nodes much more rapidly than slower migrating LCs [17–19]. There is a complex interplay between innate and adaptive compartments in orchestrating the effector response. The role of complement in the development of DTH responses is well known [20–22]. More recently, the activation of invariant NKT cells followed by the rapid production of hapten-binding IgM antibodies by B-1 B cells in the early phase after sensitization has been shown to be critical for the recruitment of effector T cells to the inflammatory site [23, 24]. In addition, NK cells have also been shown to be a critical mediator of the CS response in the absence of T and B cells [25]. For some chemical sensitizers, mast cells have been

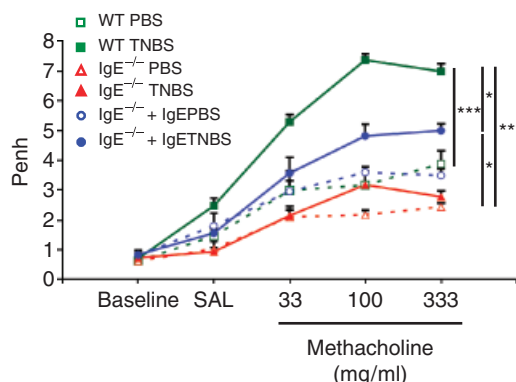


Fig. 6. Inhaled sensitization and challenge with TNBS induces airway hyperresponsiveness, which is enhanced in the presence of IgE antibodies. Wild-type (WT), IgE-deficient (IgE^{-/-}) and IgE-deficient mice reconstituted with i.v. IgE (IgE^{-/-} + IgE) were TNBS or sham (PBS) sensitized via inhalation and challenged on day 5. After 48 h, all animals were subjected to whole-body plethysmography and Penh was measured following administration of graded doses of methacholine. Significance was calculated by ANOVA. **P* < 0.05, ****P* < 0.001. *n* = 5 animals/group. TNBS, trinitrobenzene sulphonic acid; PBS, phosphate-buffered saline.

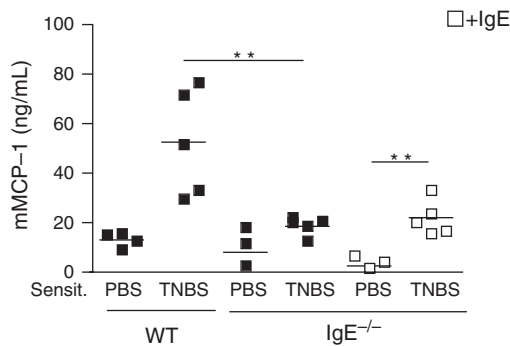


Fig. 7. Serum levels of murine mast cell protease-1 are elevated in wild-type (WT) animals sensitized and challenged with TNBS. mMCP-1 levels were assessed by ELISA in sera of PBS or TNBS-sensitized WT mice, IgE-deficient (IgE^{-/-}) mice and IgE^{-/-} mice reconstituted with IgE (IgE^{-/-} + IgE) mice 48 h after TNBS challenge. $n = 4$ or 5 . $**P < 0.01$ by two-tailed Student's t test. TNBS, trinitrobenzene sulphonic acid; PBS, phosphate-buffered saline.

shown to play a potent amplifying role [8, 26, 27]. Accumulating evidence suggests that ambient IgE levels affect mast cell homeostasis and activation state [28].

Bronchial inflammation in response to chemicals in animals has been examined by several groups and the most common approaches have involved sensitization of mice via cutaneous application followed by inhalation challenge [29–33]. Some investigators have described pulmonary inflammatory responses in mice subjected to both sensitization and challenge via inhalation [34–36]. Although most of these protocols used frequent rounds of sensitization, Lee et al. [36] reported that sensitization to TDI could be achieved by just two exposures to a 3% solution delivered by ultrasonic nebulization.

Using the TNBS system, in which both sensitization and challenge occur via the airway, we have shown that the presence of IgE antibodies enhances inflammatory responses to chemical sensitization. Compared with WT controls, IgE^{-/-} mice displayed diminished recruitment of mononuclear cells (including macrophages and CD4⁺ and CD8⁺ T cells as well as NK and NKT cells) (Figs 1–3), suppressed mucus secretion (Fig. 5), attenuated induction of BHR (Fig. 6) and reduced mast cell activation (Fig. 7).

Hapten responsiveness in IgE^{-/-} mice was partially restored by administration of IgE antibodies of irrelevant specificity before sensitization, indicating a non-specific pro-sensitizing role for IgE in the development of asthma to chemical antigens. IgE^{-/-} mice have a normal life span and, with the exception of helminthic parasites, are not susceptible to infections. They have normal T and B cell numbers, and we have reported that they mount normal cellular and humoral responses to i.p. injected protein antigens such as ovalbumin and to antigens encountered at mucosal surfaces [8, 9, 37]. However, we have found that IgE^{-/-} mice have alterations in mast cell homeostasis [38].

The enhanced response of WT mice vs. IgE^{-/-} animals following inhaled TNBS sensitization and challenge suggests that IgE antibodies might affect either (1) immune sensitization during initial antigen exposure or (2) the induction of an inflammatory response following antigen challenge or both sensitization and effector phases of the immune response. In the cutaneous CS system, which exhibits a similar IgE requirement, we were able to use adoptive transfer strategies to identify a key role for IgE antibodies at the sensitization but *not* effector phase. Splenocytes adoptively transferred from WT mice that had been sensitized with oxazolone into either WT or IgE^{-/-} naïve recipients conferred CS. In contrast, splenocytes from oxazolone-sensitized IgE^{-/-} mice were not able to transfer responses [8]. To test whether a similar requirement for IgE might be operative for immune sensitization in the lungs in the TNBS model, we performed similar experiments using mice sensitized and challenged by inhalation of TNBS. Although it appeared that splenocytes from TNBS-exposed WT but not IgE^{-/-} mice were capable of transferring the hapten-specific response to naïve recipients, the intensity of the adoptively transferred response was not as robust as in the skin model resulting in a lack of statistical significance among groups, which prohibited definitive conclusions from being reached (unpublished data).

The immunological effector mechanisms whereby IgE contributes to pathogenesis in responses to standard allergens vs. contact sensitizing chemicals are likely to be quite different. Immune sensitization to protein antigens occurs over weeks or longer, a time frame sufficient for the production of IgE antibodies directed against the immunizing antigen. These specific antibodies can bind to mucosal mast cells via the high-affinity IgE receptor, FcεRI, and then interact with specific antigen following inhalation, leading to mast cell activation with release of mediators of immediate hypersensitivity as well as *de novo* production of lipid, cytokine and chemokine factors orchestrating the recruitment of inflammatory cells to the airway. In contrast, chemical sensitizers elicit specific responses in days. IgE antibody responses are slow to arise, often requiring repeated immunizations; hence, the production of hapten-specific IgE antibodies in the course of a typical 5-day chemical sensitization protocol would be unlikely and has never been documented.

Our present understanding of the role of IgE in studies of occupational asthma is very limited. Although a few studies exist that report changes in IgE levels after exposure to chemicals in animal models [39–42], the induction of occupational asthma has not generally been correlated with atopy or high IgE levels. Recently, a novel observation on the role of IgE in occupational asthma to epoxy resin has been described [43]. Our data both in the oxazolone-induced cutaneous CS system [8] and in the TNBS lung model reported here reveal that the specificity

of the IgE antibodies is not important. Furthermore, we have shown that skin responses to contact sensitizers are restored by administration of IgE antibodies only if injection is performed before sensitization. These observations suggest that physiologic IgE levels present at the time of initial chemical exposure support an environment conducive to immunological sensitization.

Recent evidence showing that IgE levels affect mast cell homeostasis as well as reports that mast cell products regulate the trafficking of DCs lead us to speculate that IgE antibodies might exert their pro-sensitizing effect at the level of airway mucosal mast cells and DC activation. Alternatively (or in addition) mast cell mediators might be important in the elicitation of pulmonary inflammatory responses to chemicals. In this scenario, if IgE levels affect the homeostasis and activation state of mast cells in the airway, then specific immune responses to chemical haptens might be enhanced in the presence of IgE. Such potential mechanisms are speculative at this point and formal evaluation of these hypotheses will be the subject of future investigation.

Acknowledgements

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