Mechanisms of occupational asthma caused by low-molecular-weight chemicals

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Abstract

Understanding the pathogenesis and working mechanisms of occupational asthma (OA) is crucial towards optimizing prevention and management of the disease. The study of the sensitizing and asthma-inducing properties of low-molecular-weight (LMW) agents is evolving quickly. So far, experimental research has shown that OA caused by sensitization to LMW agents does not completely fit the pathways of the traditional allergic model, in which there is a central role for immunoglobulin E. Furthermore, recent evidence indicates that chemical respiratory allergens may induce respiratory tract sensitization by routes other than inhalation, such as dermal exposure. Knowledge on OA induced by LMW is increasing, but the pathogenesis remains largely vague. Dendritic cells, T cells, eosinophils, and several cytokines and chemokines are likely involved as in atopic asthma. However, through subtle differences in T cell subpopulations, cytokine balances and effector cells involved chemical-induced OA may well depend on processes that might differ substantially from those of atopic asthma. Furthermore, the involvement of the transient receptor potential channels in chemical-induced OA and irritant-induced asthma is intriguing. Further research in both humans and animals remains necessary to clarify the process of sensitization by LMW allergens and the mode of action inducing the OA phenotype.

Introduction

The lungs are the primary target for a diverse spectrum or work-related dusts, gases, fumes and vapors. Depending on the amount inhaled and on their physical-chemical properties, these agents have the capacity to cause annoyance, irritation, corrosive changes and/or sensitization in the respiratory tract. Occupational asthma (OA) is a type of asthma due to causes and conditions attributable to a particular work environment, rather than stimuli encountered outside the workplace [1]. It is characterized by a reversible airway obstruction of the airways associated with bronchial hyperresponsiveness upon inhalation of workplace-related agents [2]. OA has been implicated (directly or indirectly) in 9–15% of the cases of adult asthma, making OA one of the most common presentations of occupational lung diseases

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in many industrialized countries [3]. More than 350 agents have been reported to cause OA [4].

Traditionally, OA is divided into two types. The first type is immunologically mediated (or allergic) OA, in which sensitization against a workplace agent occurs after a "latency period". Immunologically mediated OA can be further divided into the well-known classical IgE-mediated form, and the more elusive "poly-immunological" cellular form (non-IgE-mediated). The second type, non-allergic OA or "irritant-induced" OA, is caused by exposure to irritant chemicals to which the host does not become sensitized. In its most typical presentation, irritant-induced asthma (IIA) is characterized by the absence of a latency period, because it is initiated by a sudden, acute exposure to high concentrations of an irritant. This form of IIA is often called reactive airways dysfunction syndrome (RADS). Other forms of IIA, caused by repeated exposures to irritants, are more controversial. Besides these forms of OA, some exposures at work may also lead to pharmacological bronchoconstriction and reflex bronchospasms [1, 5], but these reactions will not be discussed further.

The prevalence of OA depends mainly on the causative agent and the intensity of exposure [6, 7], and to some extent also on the distribution of individual-dependent factors, such as atopy and smoking status [8]. The highest prevalence of immunologically mediated OA has been reported in the detergent industry (up to 50%), in which workers are exposed to proteolytic enzymes. In cohorts of laboratory animal workers, prevalences of 30% of OA have been described. However, the prevalence of OA is generally much lower. In most occupational cohorts, prevalence varies between 9 and 15% [1]. Non-immunological OA is generally considered to occur less frequently than immunologically mediated asthma. The proportion of IIA among patients referred to an occupational lung disease clinic has been reported to be 2–3% [9, 10]. When criteria were expanded to one or more exposures to high levels of irritant, the prevalence of IIA doubled to 6%, accounting for 17% of all OA patients participating in a study of Tarlo and Broder [10]. One of the largest epidemiological studies published on IIA, concerns workers of the New York City Fire Department who were exposed to a variety of airway irritants during the rescue mission after the collapse of the World Trade Center on 11 September 2001: 16% of a sample of these workers met the criteria for IIA [11].

Depending on their molecular mass, agents causing OA can be divided into two categories: (a) biological agents of high molecular mass (HMW) (>5 kDa), such as proteins, glycoproteins and polysaccharides, and (b) chemicals of low molecular mass (LMW) (<5 kDa), such as synthetic chemicals, natural compounds, drugs and metals. HMW compounds generally induce OA *via* IgE-dependent mechanisms comparable with asthma induced by pollen or house dust mite allergens [12, 13], whereas many (although not all) LMW compounds appear to induce OA *via* pathways that do not involve IgE-dependent mechanisms.

Pathophysiology

Immunologically mediated OA

LMW chemicals comprise an important subset of etiological agents of OA, including approximately 100 chemical entities [4]. Isocyanates, acid anhydrides, plicatic acid from western red cedar, colophony fume, metals, complex platinum salts, persulfate salts, and some acrylates are just a few examples of important chemicals causing OA.

Since LMW agents are non-immunogenic in their native state, it is assumed that they must form a stable association with proteins to initiate an immune response. These protein-hapten conjugates can be recognized and internalized by professional antigen-presenting cells (APC) such as dendritic (DC) or Langerhans cells. Like most HMW agents, these conjugates are presented to T cells, which initiate an immune response and, possibly, asthma *via* an IgE-mediated mechanism or another mechanism. Complex platinum salts and trimellitic anhydride (TMA) are LMW asthmagens that are generally considered to induce asthma *via* specific IgE antibodies. These agents most likely possess a unique inherent ability to react directly (or indirectly, after metabolic activation) with functional groups present on human proteins [14, 15]. Not only albumin, but also other proteins such as keratine and tubuline can serve as carriers to render LMW agents immunogenic [15, 16].

Wisnewski et al. [17, 18] showed that LMW asthmagens can conjugate with proteins present on the surface of epithelial cells, thereby permitting presentation of LMW asthmagens to the immune system in a hapten-like manner. This may facilitate the uptake of the protein-hapten conjugates by professional APC to initiate the T cell response. If this is true for these LMW agents, then overall, the mechanism by which LMW antigens are presented to T cells and the following cascade of B cell activation plus IgE class switching, cross-linking of antigen and IgE on mast cells and attraction of inflammatory cells would be relatively similar between LMW and HMW compounds. Nevertheless, there are some questions and differences. For example, it is not exactly known in which form LMW asthmagens are displayed to responsive T cells. The way an antigen or hapten is processed, is dependent on where the hapten-protein conjugate is produced. Endogenous antigens are processed inside the cell, while exogenous antigens are processed through the endosomal pathway in DC. The binding of an LMW asthmagen (or its metabolite) to some lung intracellular protein may give rise to an endogenous antigenic determinant, and this may, therefore, be presented to $CD8^+$ T cells by major histocompatibility complex (MHC) class I [19]. However, the hapten may escape endogenous processing by cells in the lung and enter the peripheral circulation to bind proteins in the circulation. Such a chemical-modified antigenic protein is then processed by professional APC, e.g., B cells, macrophages and DC, and presented to $CD4^+$ T cells on MHC class II [20].

Depending on the pathway of LMW antigen presentation (MHC class I or MHC class II), different types of immune responses might develop ($CD4^+$ or $CD8^+$), which are categorized by their dominant cytokine secretion profile into T helper (Th) type 1 (IL-2, IL-12, IFN-γ), Th2 (IL-4, IL-13, IL-5), T regulatory (Treg) (TGF-β, IL-10) and Th17 (IL-17). While previously it was suggested that Th1 and Th2 cytokines counterbalanced each other, it has become clear that both Th1 and Th2 cytokines are involved in OA caused by LMW antigens [21–23]. Th17 cells, producing IL-17 – a potent attractant of neutrophils – are the latest T cells suggested to play a role in the proinflammatory pathway of OA [24]. While Th1, Th2 and Th17 cells are involved in proinflammatory pathways, Treg cells are thought to dampen the immune (asthmatic) response, possibly explaining why the majority of individuals do not develop adverse reactions to LMW asthmagen exposure [23, 25].

Besides LMW agents that initiate an IgE-mediated asthmatic response, there are also LMW agents, such as diisocyanates and plicatic acid that do not act *via* specific IgE antibodies, even though they lead to the same phenotypical characteristics as IgE-mediated OA [26–29]. In humans, the airway inflammation process is indeed similar in both IgE- and non-IgE-dependent asthma [13, 30, 31], and is characterized by the presence of eosinophils, lymphocytes, neutrophils, mast cells, and typical features of airway remodeling [6, 31, 32]. Airway inflammation is accompanied by a wide range of proinflammatory mediators and proteins. An influx of inflammatory cells, along with proinflammatory mediators can lead to a broad variety of adverse effects, such as toxic damage, increased oxidative stress, and loss of barrier integrity, contributing to long-term airway remodeling. Although in OA to LMW asthmagens, $CD4^+$ cells are associated with eosinophilia and airway inflammation [32, 33], a role has been suggested for $CD8^+$ cells in non-IgE-dependent OA [4, 23]. Interestingly, a small but significant proportion of T lymphocytes from the peripheral blood of subjects with OA induced by red cedar produce IL-5 and IFN-γ after stimulation with the conjugate of plicatic acid and human serum albumin, which is indicative of a mixed Th1/Th2 response [34].

The fate of inhaled diisocyanates in the human body and the nature of the antigen that is eventually produced are largely unknown, as is the case for most chemicals that can induce OA [4]. Extracellular glutathione was able to prevent isocyanate induced toxicity in human epithelial cells [18]. Human monocytes exposed *in vitro* to toluene diisocyanate (TDI)-albumin conjugates, undergo activation and up-regulation of lysosomal genes, along with increased production of monocyte chemoattractant protein-1 (MCP-1), and chitinase-1 [35]. Repetitive antigenic stimulation of *in vitro* cultured PBMCs obtained from subjects with diisocyanate asthma revealed that these cells synthesized TNF-α, a non–IgE-dependent proinflammatory cytokine, and MCP-1, but not IL-4 or IL-5 [35]. These observations are consistent with the hypothesis that isocyanate-induced up-regulation of immune pattern-recognition receptors by monocytes and release of damage-associated molecular pat-

terns from injured epithelium may be a mechanism by which isocyanates stimulate the human innate immune responses and consequently influence the hypersensitivity reactions [36].

Irritant-induced OA

Besides the allergic type of OA, there is another type of OA that is caused by exposure to airway irritants and may occur without a latency period in its most typical presentation [9]. Originally, this disease entity was termed 'reactive airway dysfunction syndrome' (RADS). A case of RADS was defined as: (1) a documented absence of preceding respiratory complaints; (2) onset of symptoms after a single exposure incident or accident; (3) exposure to a gas, smoke, fume, or vapor with irritant properties present in very high concentrations; (4) onset of symptoms within 24 h after the exposure with persistence of symptoms for at least 3 months; (5) symptoms simulate asthma with cough, wheeze, and dyspnea; (6) presence of airflow obstruction on pulmonary function tests and/or presence of nonspecific bronchial hyperresponsiveness; and (7) other pulmonary diseases ruled out. In 1989 these diagnostic criteria were modified by Tarlo and Broder, in the sense that patients may have experienced 'more than one' high-level exposure to the irritant, since in many industries accidental spills are relatively common [10]. The term RADS was progressively replaced by 'irritant-induced asthma' (IIA), but this acronym remains often cited because of its high recognition value.

Only few studies are available to characterize the histopathology of the bronchial wall of patients with IIA. In general, nonspecific inflammatory infiltrates (lymphocytes, plasma cells, neutrophils) are present, often with thickening of the connective tissue [9, 37]. Gautrin et al. [38] described desquamation of bronchial epithelium and squamous cell metaplasia, as well as fibrosis of the bronchial wall and increased basement membrane thickness in five workers, 2 years after repeated exposures to high concentrations of chlorine. In biopsies from a patient exposed to chlorine, Lemière et al. [39] saw considerable epithelial desquamation with inflammatory exudates and swelling of the subepithelial space 2 weeks after the exposure; 2 months later, biopsies showed regeneration of the epithelium by basal cells and still a pronounced inflammatory infiltrate that recovered after steroid treatment [39]. Chan-Yeung et al. [40] were the first to show the presence of eosinophils in the bronchial inflammatory infiltrate of patients that suffered from 'gassings' in a pulp mill. These scarce data on histopathology suggest that inflammatory characteristics of IIA may be less extensive than in immunologically mediated OA, but this picture is nonspecific and cannot serve to make a definite diagnosis of IIA.

Data on possible mechanisms inducing IIA are only speculative. Brooks et al. [9] proposed a 'big bang' theory in which the initial irritant exposure causes significant

epithelial damage associated with activation of the non-adrenergic, non-cholinergic (NANC) nerve system *via* axon reflexes, with the onset of a neurogenic inflammation through the release of neuropeptide transmitters such as Substance P and neurokinins. This epithelial damage can lead to release of relaxing factors, along with non specific macrophage and mast cell activation, which release proinflammatory cytokines and other mediators such as leukotrienes B_4 and C_4 [41], resulting in epithelial cell desquamation, smooth muscle cell hypertrophy and matrix degranulation [1, 38].

There is increasing evidence that chronic exposure to lower levels of irritants can also induce a form of OA [9, 42]. The fact that lower levels of irritant exposure could initiate asthma requires consideration of mechanisms other than airway damage alone to induce the asthma attack. It was noteworthy that 87% of the individuals that developed IIA in a less sudden way were atopic. One theory is that atopic persons elicit a different response to irritant exposure [42]. Another theory suggests an augmentation of the sensitivity to respiratory allergens by irritants, possibly through disruption of the epithelial barrier [43]. However, so far, no evidence exists to prove these theories. Moreover, the very existence of the entity of "not so sudden IIA" is currently disputed.

Acute inhalation of irritant chemicals may lead to persisting upper airway symptoms, with complaints from nose, sinuses and larynx. This entity has been described by Meggs et al. [44] as 'reactive upper airway dysfunction syndrome' (RUDS), by analogy with its asthmatic counterpart. These authors studied patients with chronic rhinitis after a chlorine dioxide exposure. Even less is known about mechanisms causing these upper airway problems after irritant exposure, but they are thought to be similar to those causing IIA, and neurogenic inflammation in response to epithelial damage is probably the key factor. Publications on RUDS or irritant-induced rhinitis are even rarer than those of RADS and irritant-induced asthma, so that the incidence and prevalence of this condition are even more obscure.

Animal models

In comparison with occupational diseases caused by inhaling mineral dusts or fibers, there has not been a lot of experimental research using laboratory animals to unravel the pathogenesis of OA. Yet, animal models can have a valuable role in gaining more information on the complex immunological and pathophysiological mechanisms involved in the development of allergies and asthma. At present a considerable part of what we know about the pathogenesis of asthma has been derived from animal experiments [45]. However, this research has been conducted mostly with HMW agents, especially ovalbumin, and only few research groups have investigated chemical-induced asthma.

Although no mouse model is currently able to mimic the full range of clinical manifestations that constitute human asthma, a number of models are available that reproduce several features that characterize its most common phenotypes. Nevertheless, important differences in airway development and morphology exist between humans and mice, thereby preventing the direct extrapolation of data between the species. Mouse airways have fewer airway generations and do not contain smooth muscle bundles. As a consequence, mouse models cannot be considered a surrogate for human asthma but they must be viewed as an opportunity to generate and test hypotheses in a relatively simple controlled system [46, 47].

The most common mouse strain used in this research area is the BALB/c mice, which exhibits a genetically determined tendency to develop Th2-biased immune responses. However, less Th2-prone mouse strains can also develop an asthma-like response. Several protocols for the induction of asthma have been developed and published employing a wide variety of antigens, application routes, doses and sequences as well as readouts [48].

Stimulation of the cholinergic and sensory nerves

The chemicals are initially recognized by APC present in the airways. Once the chemical is taken up, the APC get activated and release proinflammatory signals that not only influence the status of other cells of the immune system but also stimulate sensory pathways that activate the central nervous system. The vagus nerve has been proposed as an immune-to-brain pathway and it has been suggested that acetylcholine may modulate the airway immune response. Cholinergic mechanisms represent the predominant constrictor neural pathway, of which airway hyperresponsiveness (AHR), an important phenotype of asthma, is a good example [49].

Scheerens et al. [50] found an involvement of sensory neuropeptides in TDI-induced AHR in mouse airways. Sensory nerves are found in abundance around pulmonary blood vessels and in the epithelium of the trachea and bronchi of many species. Scheerens et al. found that tachykinins (substance P and neurokinin A) are involved in the effector phase of TDI-induced AHR when mice were sensitized (via epicutaneous application) and intranasally challenged. Furthermore, the tachykinins did not seem to act directly on the tracheal smooth muscle but *via* the activation of other cells (T lymphocytes and mast cells). Beside the effect on AHR, substance P also plays a role in the influx of inflammatory cells, particularly neutrophils [51].

It is also important to mention that many reactive chemicals, including sensitizers such as diisocyanates, have strong irritant properties when they are used in high concentrations. The airway responses to these irritants result partly also from reflexes mediated by sensory and autonomic nerve fibers in the airways [51].

Lung function measurements

A change in breathing pattern immediately after airway exposure has been documented in various mouse models of chemical-induced asthma. Pauluhn et al. [52–54] described a decrease in breathing frequency after nose-only exposure to diphenylmethane-4,4'-diisocyanate (MDI), 1,6-hexamethylene diisocyanate (HDI) and TMA in sensitized guinea pigs and rats. Vanoirbeek et al. [55–57] showed differences in enhanced pause (Penh), a parameter representing bronchoconstriction, immediately after intranasal challenge with TDI or TMA.

Nonspecific AHR is generally measured 1 or 2 days after challenging the mice with a specific antigen. Many research groups focusing on chemical-induced asthma have found an increase in AHR to methacholine [52, 55–62]. Scheerens et al. [50] were the first to find *in vitro* AHR after carbachol exposure. As mentioned above, they found that sensory neuropeptides played an important role. Furthermore, Matheson et al. [63] and Tarkowski et al. [56] showed the absence of AHR in athymic mice and severe combined immunodeficiency (SCID) mice, respectively, suggesting an important function for T-lymphocytes in these models. Herrick et al. [64] and Matheson et al. [59] showed that both $CD4^+$ and $CD8^+$ lymphocytes are crucial in mouse models of asthma caused by HDI and MDI, respectively.

Airway inflammation

In comparison with asthma induced by HMW agents, where eosinophils and lymphocytes are the characteristic cell types present in the bronchoalveolar lavage (BAL) fluid, asthma induced by LMW agents has been associated with an influx of mainly neutrophils and eosinophils [50, 55, 56, 62, 63, 65]. The type of inflammation is also highly dependent on the duration of exposure and the route of challenge. For example, Vanoirbeek et al. [55, 60] found mainly an influx of neutrophils when TDI-dermally sensitized mice received a single intranasal challenge with TDI, whereas De Vooght et al. [62] found an influx of neutrophils as well as eosinophils, using the same dermal sensitization protocol, but altering the challenge route from intranasal instillation to oropharyngeal aspiration.

Herrick et al. [66] used HDI conjugated to mouse serum albumin (MSA) to challenge their mice. Using this complex they found an inflammation in the BAL that correlated with the phenotype of atopic asthma, i.e., an influx of eosinophils and lymphocytes.

The influx of inflammatory cells is mediated by several cytokines and chemokines. Increases of Th2 (IL-4, IL-5, IL-13) and Th1 (IFN-γ) cytokines was found in homogenates of lung tissue [59, 66]. Macrophage inflammatory protein 2 (MIP-2), a chemokine for neutrophils in mice, was found to be increased [56]. IL-1 also seems to be an important mediator in chemical-induced asthma. IL-1 stimulates the release

of IL-5, which is important for the recruitment and activation of eosinophils, and induces the production of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), important for leukocyte recruitment [67]. Matrix metalloproteinase 9 (MMP-9) is the major proteinase that induces bronchial remodeling in asthma. In addition, MMP-9 as well as vascular endothelial growth factor (VEGF) induce the migration of eosinophils and neutrophils [65, 68]. Tumor necrosis factor α (TNF-α) is a major initiator and propagator of airway inflammation and promotes the migration of DC [69]. In addition, through their effect on airway inflammation, IL-1, MMP-9, VEGF and TNF-α, lead to AHR.

So far, most mouse models are based on an "acute" form of OA. The main focus has been on the inflammation found in the BAL, while structural changes of the lung and airways tissue have not often been investigated. Some degree of peribronchial and perivascular inflammation, epithelial shedding, mucus hypersecretion by proliferation of the goblet cells and some perivascular remodeling have been described in the lungs of mice with diisocyanate-induced OA [62, 66, 70].

Immunoglobulins

Both increases in specific antibodies, as well as total serum immunoglobulins (IgE and IgG) have been described in diisocyanate-treated mice. However, a consistent observation in isocyanate-induced OA is the absence of any meaningful association between these serological findings and the presence or absence of airway responses, or with airway inflammation [71].

Scheerens et al. [72] found that by altering the exposure time and/or cumulative dosage, TDI is capable of inducing different immunological reactions. When sensitizing the animals longer they were able to find specific IgE and IgG in serum, compared to a shorter protocol. Matheson et al. [58] and Herrick et al. [64] also found specific immunoglobulins (IgG) after a low-level subchronic exposure to TDI and exposure to HDI-MSA, respectively. Vanoirbeek et al. found increases in total serum IgE, IgG1 and IgG2a in TDI and TMA asthmatic mice. In isocyanate-induced OA it is known that immune responses can emerge from IgE-dependent or non-IgE-dependent mechanisms, but the functional meaning of this in animal models remains unclear and probably non-essential. It is known that immunological sensitization to LMW asthmagens is often lifelong. The only 'remedy' to avoid the symptoms of OA is removal from the exposure place [73]. If asthmatic workers can avoid contact with the causal asthmagen, improvement of AHR can occur [74]. This was confirmed in the TDI mouse model of Vanoirbeek et al. [60]. In this set of experiments, the researchers increased the time between sensitization and intranasal challenge, resulting in a decrease of the AHR and airway inflammation, regardless of the high concentrations of IgE, IgG1 and IgG2a in the serum of TDI-treated mice. This is further confirmation that immunoglobulins are present

in chemical-induced asthma; however, their role in the pathophysiology of OA is uncertain at best.

Controversial issues

Neutrophils and OA

In the pathophysiology of OA due to LMW asthmagens, discussion often occurs concerning the presence and the role of neutrophils, as the main BAL inflammatory cell. In non-OA the presence of neutrophils is considered a marker of disease severity [75]; however, this is not yet established in OA. Moscato et al. [31] found that a positive response to the specific inhalation challenge (SIC) of persulfate salts was correlated with an increased sputum eosinophilia, while in symptomatic workers with a negative response to the SIC a neutrophilic inflammation was predominant. Park et al. [76] found a predominant neutrophilic inflammation in TDI-induced asthmatics, which was linked to IL-8, a chemokine involved in neutrophil attraction. This dichotomy between a predominant BAL neutrophil or eosinophil inflammation is also found in animal model of OA [52, 58, 60, 62, 66, 70]. Probably, the nature of the pulmonary inflammation in asthma is heavily dependent on the time course of the disease, the pattern of the exposure and individual susceptibility factors [28, 31, 77].

The skin and OA

In OA it is generally assumed that exposure to the respiratory tract is the key route and site for the initiation of the immune responses. Accordingly, research, regulation and prevention focus almost exclusively on airborne exposures. However, despite reductions in workplace respiratory exposures, isocyanate asthma continues to occur, and this has prompted a focus on skin as a route of exposure [78, 79]. Evidence that skin exposure may increase risk for isocyanate sensitization and asthma in humans is mainly derived from case reports and limited cross-sectional studies [78, 80, 81]. Recently, isocyanate skin exposure has been documented using newly developed qualitative and quantitative methodologies in car body shop workers and painters. The authors found substantial skin exposure to isocyanates, while these workers were occupied in a setting where airborne exposure was minimal, and despite the use of standard personal protective equipment such as gloves [81–83]. Several animal models have shown convincingly that skin exposure to chemical sensitizers (predominantly isocyanates, but also anhydrides) can induce systemic sensitization, which may result in asthma-like respiratory responses when the animal is later challenged *via* the airways [56, 64, 70]. These murine studies suggest that the

occurrence of respiratory responses depend on several factors related to both the nature and timing of the sensitization and that of the challenge [55, 60, 61].

Specific antibodies and OA

Controversy still exists regarding the role of specific antibodies in asthma induced by LMW asthmagens. While some LMW asthmagens (e.g., complex platinum salts and TMA) consistently produce specific IgE antibodies, other LMW asthmagens, most notably TDI and western red cedar (plicatic acid), do not. For example, in TDI-asthmatics specific IgE antibodies are only found in 0–50% of exposed workers [84]. It has been suggested that sensitization to isocyanates can be achieved *via* other immunological mechanisms, such as direct T cell activation [20]. On the other hand, it has been suggested that IgE antibodies go undetected for largely technical and methodological reasons [4]. A technical limitation was shown by Son et al. [85], who showed that the variable results in the presence of specific IgE antibodies to TDI in serum of exposed workers depended on the heterogeneous binding of specific IgE of a TDI-asthmatic to an antigenic determinant of TDI-human serum albumin conjugate *in vitro*. This binding can differ between one individual and another. So, it remains unclear whether IgE-mediated responses contribute to the development of asthmatic symptoms in workers exposed to TDI. Not only the role of specific IgE responses, but also the role of specific IgG is under debate. After TDI exposure, specific serum IgG can persist for many years [26]. Although the sensitivity for measuring specific IgG in serum of TDI-induced asthmatics is higher than specific IgE, the sensitivity is still poor. Therefore, it is rather suggested that IgG could be used to monitor exposure to diisocyanates, rather than act as a marker of sensitization [26, 86].

Mechanisms of IIA

Little or no experimental research has been conducted to clarify the mechanisms of persistent airway hyperreactivity that occurs in some victims of a single acute inhalation injury. Morris et al. [87] showed that capsaicin treatment could reverse the respiratory effects of chlorine gas inhalation in mice, suggesting an important role of sensory receptor channels on the nerve endings in the respiratory mucosa. Martin et al. [88] described histological changes in the airways and increases in bronchial reactivity to methacholine in mice after a single inhalation of chlorine gas, but their experiments did not go beyond 7 days. Further publications by the same group point to acute immunological changes and airway remodeling [89, 90], but these studies do not yet help us understanding the determinants of RADS in humans. Both Guo et al. [91] and Venglarik et al. [92] have shown a reduction in

bronchial transepithelial electric resistance in response to hypochlorite exposure. However, no phenotypic responses have been linked to this finding so far. The possible role of repeated exposures to low concentrations of occupational chemical irritants in the causation of asthma has been studied even less. An ozone-induced asthma model has been set up by the group of Pichavant et al. [93], in which iNKT cells and IL-17 seem to be important disease markers. Furthermore, findings related to co-exposures of antigens with ozone, cigarette smoke or diesel exhaust particles, involving DC priming, GM-CSF and leading to up-regulation of Th2 related cytokines, may contribute to understanding the mechanisms of IIA [94–98].

Transient receptor potential channels and IIA

As already mentioned, neural activation causes pain and irritation, neurogenic inflammation, mucus secretion, and reflex responses such as cough, sneezing, and bronchoconstriction [99, 100]. Recently, members of the transient receptor potential (TRP) superfamily of ion channels have been proposed to play a key role in the response of sensory neurons to inflammatory mediators [99, 101, 102]. The two major proinflammatory TRP ion channels in sensory neurons are TRPV1, the capsaicin receptor, and TRPA1, activated by mustard oil [99, 103]. Agonists of TRPV1 and TRPA1, such as capsaicin, acrolein, diisocyanates or chlorine, are potent tussive agents and have been associated with allergic and occupational asthma and RADS [101, 104–106]. In $TRPA1^{-/-}$ KO mice or when a TRPA1 antagonist is used in an animal model, inflammation and acute airway responses to chemical exposure are substantially decreased [104–107]. These data suggest that activation of TRPA1 and TRPV1 on airway sensory fiber terminals by hazardous irritants could evoke noxious respiratory sensation, sensitization of respiratory reflexes, and the local release of proinflammatory neuropeptides, which can lead (in the long term) to OA or IIA [99].

Putative mechanism of action

Combining all data from human and mice, sketches have been made trying to give an overview of the mechanisms of OA induced by LMW asthmagens. Keeping in mind that the skin is a relevant site for initiation of sensitization, Figure 1A gives an overview of the sequential events that presumably take place after dermal contact with LMW sensitizers that could lead to sensitization. When applied on the skin, LMW sensitizers bind to proteins (e.g., keratine) [16] and form hapten-protein complexes. Langerhans cells in the epidermis internalize these hapten-protein complexes. The activated Langerhans cells mature and migrate to the draining lymph nodes, while processing the protein complex. The processed protein complex is

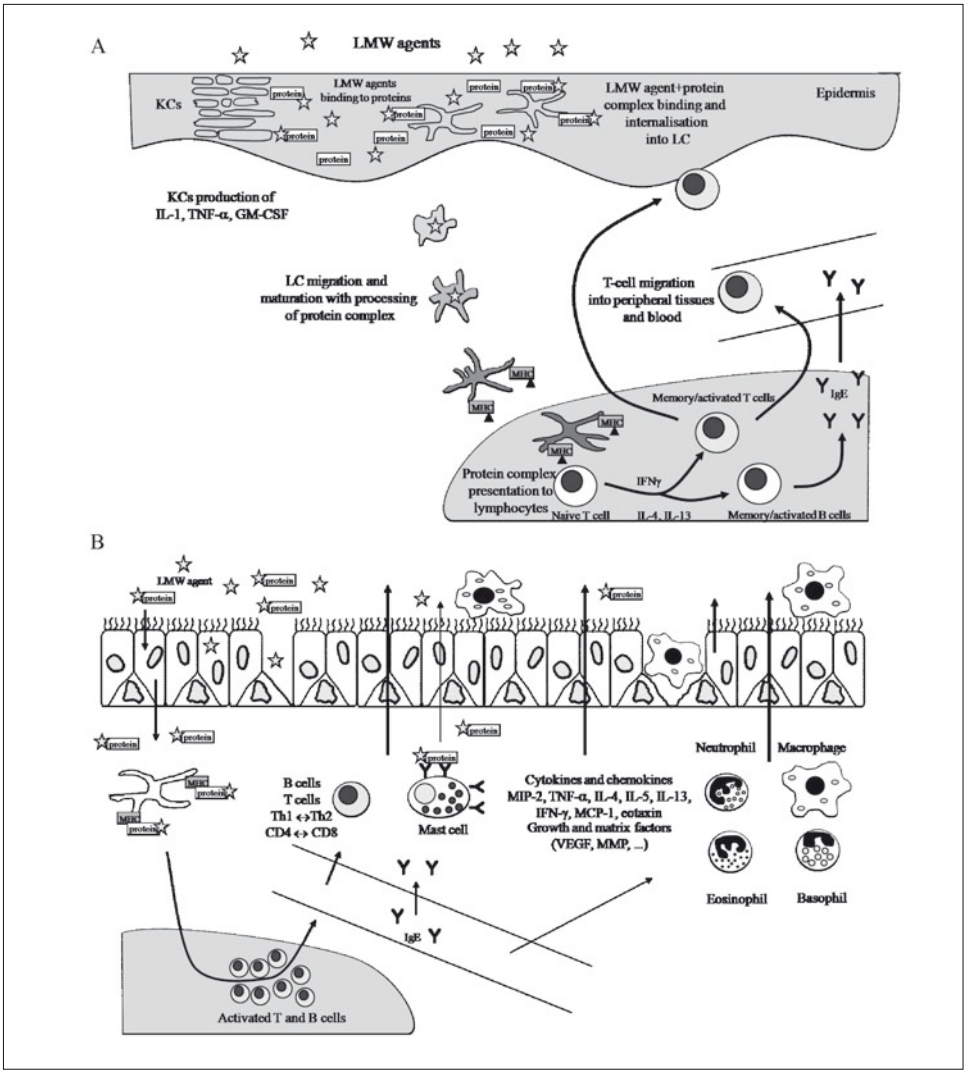

Figure 1.
(A) Hypothetical scheme to describe the dermal sensitization phase. (B) Hypothetical model of the immunopathogenesis of asthma induced by LMW agents. These two models give an overview of findings in the literature. APC, antigen presenting cell; GM-CSF, granulocyte-macrophage colony stimulating factor IFN, interferon; IgE, immunoglobulin E; IL, interleukin; KC, keratinocyte; LC, Langerhans cells; LMW, low-molecular-weight; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; MCP, monocyte chemotactic protein; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor. Figure adapted and modified from [71, 108, 109].

presented to naïve T cells *via* the MHC, hereby activating the T cells. T cells differentiate to both memory Th1 (*via* IL-12) and Th2 (*via* IL-4) cells. IL-4 and IL-13 released from Th2 cells also stimulate B cells to produce IgE, which is released into the blood. Activated and memory T cells (Th1 and Th2) and B cells migrate from the local draining lymph nodes to the peripheral tissues and the blood [108, 109].

Subsequently, Figure 1B illustrates the possible pathogenic cascade leading to LMW asthmagen-induced OA. The primary event in this process, after the LMW asthmagens have reached the respiratory mucosa, is the conjugation of asthmagens with proteins in the airways, such as albumin and possibly other epithelial cell proteins (e.g., tubulin on top of the cilia and actin) [16, 17, 110]. The antigenic epitopes resulting from the interaction of LMW asthmagens with the proteins will lead to airway inflammation, but this remains poorly characterized. Probably the antigenic epitopes of the protein-hapten complex will be presented to the Th1, Th2 and B cells by APC. IgE from the B cells will cross-link the protein-hapten complex with mast cells that release their mediators (e.g., histamine) and cause an acute asthmatic response. Moreover, *via* the T cells several cytokines and chemokines get released, which mediate several cellular responses and activate neutrophils, eosinophils and basophils, leading to a chronic state of asthma [20, 71, 79, 109, 111].

Admittedly, in this schematic overview we only focused on the well-known allergic pathway of OA and we did not included the neurogenic mechanisms of action (TRP-receptors, substance P, neurokinines), which recently have been suggested to play a (important) role in both IIA and OA. Multiple questions remain to be answered, including the determination of relevant routes of exposure, better characterization of the immune response, the inflammatory cells involved and mediators responsible for LMW asthmagen-induced sensitization and OA, along with the identification of the genetic factors that regulate airway inflammation. Although the mouse models of LMW asthmagen-induced asthma share features common with human chemical-induced OA, none of them perfectly replicates real-life human exposures or the disease in humans. Nevertheless, good models are important to protect workers from compounds that act as respiratory sensitizers, which can lead to asthma after repeated exposures.

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