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ADVANCES IN MOLECULAR IMMUNOTOXICOLOGY OF OCCUPATIONAL ASTHMA INDUCED BY LOW MOLECULAR WEIGHT CHEMICALS

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Abstract. The paper reviews the literature reports on low molecular weight (LMW) sensitizers that are commonly encountered in the work environment as well as on the major mechanisms responsible for their effect on the immune cells of the respiratory tract. Current studies have focused on: LMW-antigens; the role of airway epithelial and dendritic cells (DCs); activation of naive helper T (Th) cells by DCs; naive B cell-effector Th2 cell interactions; and activation of mast cells by LMW asthmogens. A better understanding of the pathogenesis of occupational asthma due to LMW asthmogens should facilitate the development of better diagnostics and the improvement of strategies for disease surveillance and intervention.

Key words:
Low molecular weight asthmogens, Occupational asthma, Dendritic cells, Epithelial cells, Macrophages, T cells, Mast cells

INTRODUCTION

It is generally accepted that occupational asthma is a disease in which exposure to chemical and biological agents such as allergens and irritants plays an important role [1,2]. In occupational asthma, one needs to distinguish two different toxicant effects. First, a toxicant acting as an antigen producing antibodies specific for this antigen. Second, a subsequent exposure to this antigen results in the production of the immediate and/or delayed asthmatic reaction, and the development of airway hyperresponsiveness. This is observed when secondary effects of toxicants are brought into play. An enhanced airway hyperresponsiveness means that the airways, which usually do not respond to substances, like ozone or sulfur dioxide, are now adversely affected by these stimuli, and the frequency of attacks of acute airway narrowing increases. Thus, commonly innocuous substances now evoke a bronchoconstrictor response in a person with asthma (Fig. 1).

In the context of immunotoxicology, occupational asthma may be defined operationally as adverse health effects that result from the stimulation of specific immune responses by chemicals. Sensitization to occupational allergens is an important underlying mechanism for developing bronchial hypersensitivity. This serves to distinguish chemical allergens, the chemicals that cause adverse health effects, secondary to the stimulation of an immune (allergic) response, from the agents that may provoke similar symptoms, but via irritant, pharmacological or other non-immune mechanisms [3,4]. Clinical irritant and allergic lung inflammation appear to have more features in common

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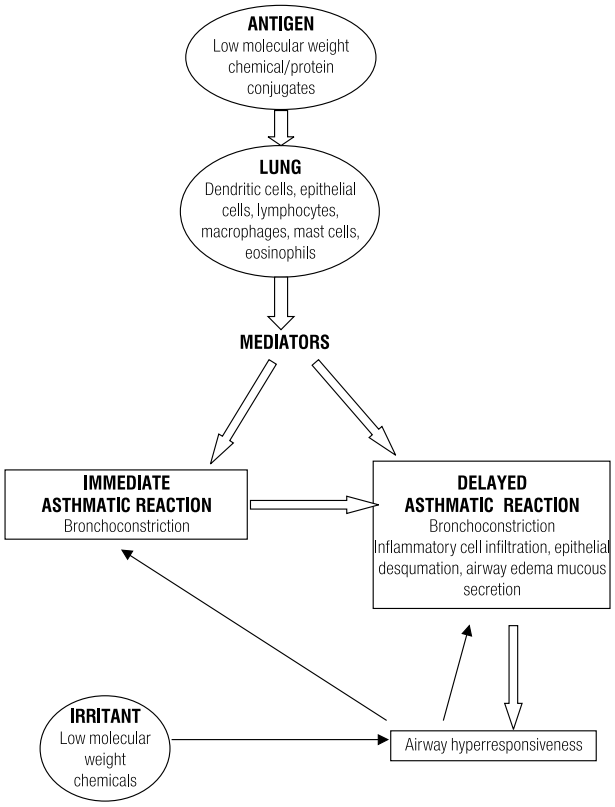

Fig. 1. Pathways in the pathogenesis of occupational asthma caused by low molecular weight (LMW) chemicals.

than differences. Studies of the histology and immunochemistry of allergic and irritant lung inflammation have failed to show any significant differences between them. The patterns of T-cell infiltration and cytokine release are similar [5]. This article is primarily concerned with occupational asthma of immunologic origin caused by chemical sensitizers (asthmogens) with low molecular weight (LMW).

LMW ASTHMOGENS

Different classes of occupational allergens may cause diverse immune responses. A distinction is usually made between chemical sensitizers with high molecular weight, proteins or glycoproteins that can provoke a specific IgE response in humans exposed to these agents and LMW chemicals. Several thousand LMW agents, i.e., chemicals with a molecular weight of less than 1000 Da, are known. Yet only about 100 of these are recognized as causes of occupational asthma and named LMW asthmogens. Their biological behavior in this respect is determined by their chemical structure and interaction reactive groups with proteins [6]. Many LMW asthmogens that cause occupational asthma are polyfunctional, including aliphatic or cyclic diamines, dicarboxylic acid anhydrides, and dialdehydes [7,8]. Some of them possess a unique inherent ability to react directly (or indirectly, after metabolic activation) with functional groups present in human proteins [9]. The bivalency of LMW asthmogens may contribute to their allergenicity by forming "neo-epitopes" that arise from cross-linking of individual proteins, which may lead to their aggregation [10,11]. The evidence strongly supports a hypothesis that cross-linking properties brought about by the presence of at least two functional chemical groups increases the lung hypersensitivity risk even for chemicals that do not behave as antigens, either directly or by acting as haptens. This is particularly true if one excludes from consideration irritants causing reactive airway dysfunction syndrome (RADS). Quantitative analyses show that the hazard odds ratio for lung hypersensitivity reaction rises significantly not with the mere presence or absence of a single chemical substructure fragment (biophore), but with the presence of two or more reactive groups. Even groups, which on their own do not appear to pose an appreciable lung hypersensitivity risk such as carboxyl and alcohol groups, become likely to exhibit the lung hypersensitivity risk when other reactive groups are present on the same molecule. Thus aliphatic (mono) alcohols or (mono) amines do not appear to be lung sensitizers, but in the presence of both these groups (e.g., ethanolamine) lung hypersensitivity has been reported. Examples of the chemical structure of varied low molecular occupational asthmogens are shown in Fig. 2.

A group of some transition metal compounds are marked lung sensitizers. They comprise platinum, nickel, cobalt, chromium and vanadium. Atoms of transition metals in the ground state tend to have partially filled *d* electron orbitals and they may combine with a wide range of ligands (ions or neutral molecules with an electron pair available to share with the metal) through a coordination bond. Moreover, ligands (basic amino acids from protein) can

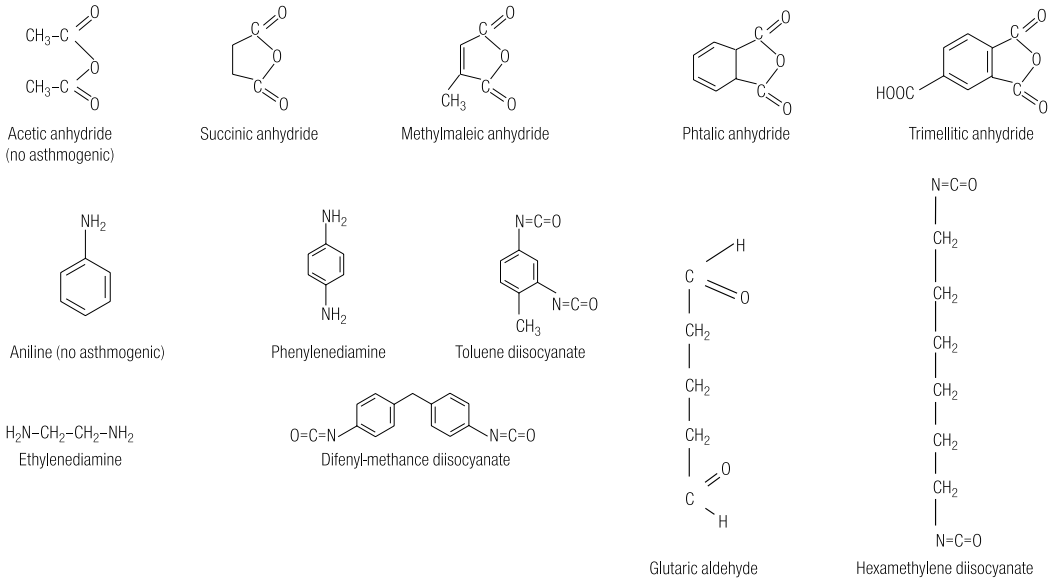

Fig. 2. Low molecular weight (LMW) chemicals in relation to risk of asthma. Acetic anhydride is polyfunctional, but does not form cross bonds in proteins, and aniline is monofunctional and does not form cross bonds in proteins. Other chemicals are polyfunctional and form cross bonds with proteins. They have been reported as occupational asthmogens.

form chelate rings by holding the transition metal atom in a pincer-like grip at more than one binding site [6].

Many (if not all) LMW asthmogens exhibit dose-dependent toxicity and direct or indirect (after metabolic activation) toxic effects on the lung cells. In most cases, both antigenic and irritant signals come from the hapten. Typically, the irritant signal tends to be more concentration-dependent and thus it is an overriding factor in the determination of effective sensitizing and eliciting concentrations of the hapten. The same properties are found in LMW asthmogens that are known to cause sensitiztaion of the respiratory tract and in LMW agents that induce skin contact sensitization [12,13].

LMW ASTHMOGENS AS HAPTENS AND IRRITANTS

It is widely believed that LMW asthmogens not only act as effective immunogens but they are also involved in the lung epithelial cell death-inducing mechanisms. To be an effective immunogen, inhaled LMW asthmogens are thought to bind covalently to epithelial cell proteins [9]. Wisnewski et al. [14] hypothesize that the protein microenvironment may be important in LMW asthmogen conjugation. Membrane proteins may be more likely to react with LMW asthmogen than intracellular proteins, which might be protected from LMW asthmogen by the cell membrane. Another characteristics likely to influence protein's susceptibility to LMW asthmogen conjugation is amino acid composition. LMW asthmogens have the following order of reactivity: primary amines, secondary amines, sulfhydryls and hydroxyls. It remains unclear whether lung epithelial cell proteins with a higher percentage of aminoacids that contain primary amine group (i.e., lysine) or free thiols (i.e., cysteine) are more susceptible to reactivity with isocyanates. LMW asthmogen conjugation to epithelial cell proteins may permit presentation of LMW asthmogen to the immune system in a hapten-like manner. LMW asthmogen might directly cross-link normal epithelial cell proteins in a way that alters their conformation and makes them immunogenic. Although the majority of such LMW asthmogen-protein complexes released by death epithelial cells will be degraded by lung macrophages, some will be processed by resident lung antigen-presenting cells (APCs) for presentation to T cells. Lung dendritic cells (DCs) are the true professional APCs because they may stimulate a virgin cell (that has not met antigen) and experienced CD4+ T cells (that have responded to antigen at least once). It is not entirely

sure about macrophages. Although they can stimulate experienced cells, it is not clear if they can also truly activate virgin T cells. B cells, type II pneumocytes and cells from bronchial epithelium and endothelium are not able to stimulate virgin CD4+ T cells. The primary immune response, occurring at the first encounter with a particular antigen, can only be activated by DCs [15].

Dendritic cells exist in lung tissue in an immature form. Maturation of DCs may be initiated by their direct contact with some chemicals or some biological agents (cytokines). LMW asthmogens with hapten and irritant properties, may induce or inhibit DCs maturation by stimulation of reactive oxygen species (ROS) production [16]. The supressive or stimulating effect of ROS on DCs is limited to the earliest events related to the activation of these cells [17]. Higher levels of ROS inhibit these cells and lead to DCs apoptosis or necrosis, whereas low ROS concentrations are necessary to activate these cells. Some authors suggests that apoptotic cell death is mediated by extensive loss of reduced glutathione (GSH). The reduced tripeptide is not oxidized, but instead exreted into the medium of the cells.

Low levels of ROS formed in DCs participate in the regulatory mechanisms responsible for the maintenance of protein conformation and function. This process occurs through the impact of ROS on the oxidoreduction level of -SH protein groups [18]. This applies to proteins generating intracellular pathways of the cell signal transfer, particularly protein kinases and phosphatases. The ROS-dependent level of -SH groups oxidoreduction is also essential to maintain specific protein-DNA interactions related to the induction of gene expression and mobilization of *de novo* protein synthesis. ROS play a particular role in the regulation of these interaction for transcriptionally active AP-1 and NF-κB factors, essential for the regulation of immune response of the DCs.

The chemical stress induced by LMW asthmogens, may influence not only maturation of DCs but also the type of immune response to these agents. Glutathione, owing to its ability to reduce ROS and maintain a proper state of redox-SH groups in proteins, is the first defensive line protecting DCs against effects of oxidative stress induced by exposure to some chemicals. It was found [19] that glutathione depletion was associated with DCs activation and a shift in cytokine profile that favored a Th2 rather than a Th1 response. The decreased Th1 cytokine production was due to short-term, readily reversible depletion of glutathione in DCs. Also exogenous adenosine released in excess during inflammatory and ischemic conditions or tissue injury, may polarize the Th1/Th2 balance toward Th2 dominance and to selective suppression of Th1 responses and cellular immunity [20].

DANGER THEORY OF MATZINGER

There is always a question of whether the chemical-induced lung hypersensitivity reaction represents a direct toxic reaction or whether it is truly immune-mediated. However, such arguments may become redundant if one applies the "danger theory" of Matzinger [15] to chemically-induced lung hypersensitivity [13,21,22]. The danger model is based on the principle that the signals which control an immune response are endogenous, not exogenous, with alarm signals being raised by stressed or injured tissue [23,24]. An injured cell sends signals to its local APCs – lung dendritic cells in the case of the lung. Then the cells take up the local antigen and up-regulate the co-stimulatory molecules needed to activate T cells.

The essential aspect of this theory is that simple presentation of chemical-induced antigen by a target cell (signal 1) should in fact result in tolerance to the chemical through apoptosis of the specific T cell rather than through cellular damage. According to Matzinger [15], if a foreign entity does not cause injury it does not evoke a response (no signal 2), no matter how it is distributed in the body. There is, however, one exception – an agent could theoretically accumulate in an APC and might remain dormant until activated (in both the biochemical and immunological sense) when the APC is "alarmed" by some unrelated pathogen. Therefore, an immune response should only occur in response to some form of the co-stimulatory signal (signal 2) indicative of cellular stress.

The theory of Matzinger open up useful avenues of research into chemical-induced lung hypersensitivity re-

actions. From a chemical perspective, it is possible that a chemically reactive LMW asthmogen (or its metabolite) could serve two functions. First, it could act as a hapten to provide signal 1 for recognition by specific T cells. Second, it could provide a co-stimulatory or danger signal (signal 2) by the activation of signaling pathways linked to oxidative stress or protein damage [25]. The chemical--protein conjugate provides antigenic properties (covalent binding to protein, processing and major histocompatibility complex (MHC) presentation), whereas the danger signal is provided by irritant properties of the chemical [12,26]. It is possible that a reactive LMW asthmogen may only provide the antigenic stimulus, and the danger signal could be completely independent of the chemical, and could be, for example, a host factor such as viral or bacterial infection [27]. It is also possible that a reactive LMW asthmogen may provide the antigenic stimulus through a direct noncovalent interaction with MHC [28].

No model can fit the characteristics of all airway hypersensitive reactions generated by LMW asthmogens. However, the danger model provides a new perspective and suggests avenues of research that have the potential to increase our ability to predict such reactions.

THE ROLE OF LMW ASTHMOGENS IN THE INITIATION OF AN IMMUNE RESPONSE

To begin to understand the role of the asthmogen in the initiation of an immune response, two aspects must be considered: First, the distribution of the asthmogen or the asthmogen-protein antigen. Second, the mechanism by which the asthmogen-protein antigen is recognized by specifc T cells. The exact form in which LMW asthmogens are displayed to responsive T cells is uncertain. LMW asthmogens are haptens or prohaptens (in the case of asthmogens that require metabolic activation to a protein-reactive species). In their native state they are non-immunogenic and must form stable association with proteins in order to stimulate an immune response. Professional antigen presenting cells such as DCs might internalize, process and present extracellular LMW asthmogen-protein conjugates. T cells respond to small peptide fragments of the original asthmogen-protein antigen, presented indirectly on an MHC molecule by the lung DCs [29]. Probably the MHC-restricted peptide contains the original asthmogen molecule.

The processing of an antigen (LMW astmogen-protein conjugate) can originate inside or outside the DC; exogenous antigens are presented on MHC class II molecules for recognition by CD4+ cells, whereas endogenous antigens are presented on MHC class I molecules for recognition by CD8+ cells. The binding of LMW asthmogen or its metabolite to some lung intracellular protein may give rise to a new antigenic determinant, which is endogenous and therefore presented to CD8+ T cells by MHC class I [30]. A chemical-modified antigenic protein may escape endogenous processing by cells in the lung and enter the peripheral circulation. Such a chemical-modified antigenic protein is processed by professional APCs, e.g., B cells, macrophages and DCs, and presented to CD4+ T cells on MHC class II. An active LMW asthmogen or its metabolite may also bind directly to the MHC cleft or a peptide embedded within [31]. This pathway is also MHC-restricted, but avoids the requirement of antigen processing. These findings open up the possiblity that a non-covalently bound chemical may be able to trigger immunological events. Such diverse pathways of T cell recognition of chemical-protein antigen may explain why some chemicals are known to activate both CD4+ and CD8+ T cells [13].

Depending on the pathway of LMW asthmogen-protein conjugates presentation (MHC I/II) and stimulation of the antigen presenting cell, different types of immune response might develop (CD4/CD8), which are further categorized according to the cytokine pattern secreted: T helper (Th) type 1 (IFN-γ), Th2 (IL-4, IL-5) and Th3 (TGF-β, IL-10). The initiation of a Th3 response (i.e., silent immune response) may explain why the majority of individuals do not go on to develop an adverse reaction to LMW asthmogens [25].

Activation of an effective cellular immune response was originally described by a two-signal model: signal 1, the interaction between an MHC-restricted antigen and the T cell receptor; and signal 2, additional receptor-ligand in-

teractions, commonly called co-stimulatory signals [32,33]. The best defined co-stimulatory interaction involves B7-1 and B7-2 ligands (found on antigen-presenting cells) that interact with T cells expressing the CD28 or CTLA-4 receptor [34–36]. Curtsinger et al. [37] suggested that a three-signal model might describe T cell activation more accurately. Factors that determine the type of immune responses (Th1 vs. Th2) are referred to collectively as signal 3. Obviously, the factors that induce polarized T-cell differentiation are of crucial importance, because elucidation of their regulation will result in a better understanding of the immunopathology of occupational asthma. It has also become clear that the lack of clinical reactivity to these agents in the majority of individuals, considered to be "tolerance" in the context of aeroallergens, is in fact explained by immune deviation, given that T cells from virtually 100% of adults respond to them by lymphoproliferation *in vitro*. Whereas allergic individuals mount a Th2-type immune response, which results in allergic inflammation, non-allergic people are not tolerant in the sense of being non-reactive, but they rather react to the antigen with a non-pathogenic Th-type response [38,39].

ACTIVATION OF LUNG DENDRITIC CELLS BY DANGER SIGNALS

Dendritic cells are antigen-presenting cells with a unique ability to induce primary immune responses. DCs capture and transfer information from the outside world to the cells of the adaptive immune system. DCs are not only critical for the induction of primary immune responses, but may also be important for the induction of immunological tolerance as well as for the regulation of the type of T cell-mediated immune response. DCs exist in the lung tissue in an immature form, but after antigen capture, and in response to an inflammatory signal DCs switch to a T cell-stimulatory mode and migrate to lymph nodes to initiate immunity. Maturation of DCs is associated with up-regulation of co-stimulatory molecules (e.g., B7) and expression of chemokine receptors that promote migration to the nodal T-cell areas [40]. Lung DCs which exist in a resting state may be roused by different danger signals. They consist of molecules or molecular structures, released or produced by cells undergoing stress (chemical, physical, biological), or abnormal cell death (non-apoptotic death). Only a few candidates have yet been identified as extracellular danger signals induced in lung by irritant properties of LMW agents. Such danger signals may be purine nucleotides, adenosine, and stress response proteins, called heat-shock proteins [41–45]. Some danger signals (intracellular danger signal) may be released by metabolically-stressed cells, e.g., ROS activated human DCs [16]. ROS participate in the regulatory mechanisms responsible for the maintenace of protein conformation and function. This process occurs through the impact of ROS on the oxidoreduction level of -SH protein groups. The ROS-dependent level of -SH groups oxidoreduction is also essential to maintain specific protein-DNA interaction related to the induction of gene expression. ROS play a particular role in the regulation of these interactions for transcriptionally active AP-1 and NF-κB factors, essential to regulate immune response of the immune system cells [18].

In injured or inflamed lung tissues, the activation of extracellular proteases or the release of intracellular proteases can lead to the cleavage of components of the extracellular-matrix into small fragments, and some of these have been reported to activate DCs and macrophages. The matrix-proteolytic enzyme, metalloproteinase-9, induced changes in DCs, characteristic of the maturation process [46]. The degradation products of heparan sulfate have been shown to activate DCs, probably by binding to Toll-like receptor [47]. Some cellular adhesion molecules on DCs might serve dual functions: to localize DCs in the normal structure of a tissue by binding their "regular ligands", and to act as activation receptors when they bind the degradation products of those ligands [48].

Galluci and Matzinger [43] hypothesize that DCs might have a default mechanism that activate cells whenever an insult hits them. Such a mechanism might explain the ability of simple compounds, like $NiCl_2$, $MnCl_2$, $CoCl_2$ or $SnCl_2$ to induce DC maturation. These inorganic substances have been shown in other systems to block cell membrane ionic channels and interfere with the cell's energy metabolism, inducing "cell suffering". Compounds such as dinitrochlo-

robenzene (DNCB) and trinitrochlorobenzene (TNCB), well-known experimental allergens are another category of DC activators that might induce direct DC damage as well as release danger signals by other types of cells [49]. According to Galluci and Matzinger [43] the damage done by the irritants helps the development of allergy.

DANGER SIGNAL SENT BY AIRWAY EPITHELIAL CELLS

Airway epithelial cells are known to play an integral role in airway defense mechanisms via the mucociliary system and mechanical barriers. Over many years, epithelial cells were believed to act simply as a barrier, in addition to their involvement in the secretion of mucus and removal of foreign agents by their cilia. However, recent studies have shown that epithelial cells display a much wider range of activities, including release of some substances that are important in the pathogenesis of allergic airway disorders. Epithelial cells can also interact with immune cells and play a role in mucosal immunity [50].

Exposure of epithelial cells to various LMW asthmogens can result in their damage and death. Whether a cell survives or dies in the presence of a chemical insult often determined by the proliferative status, repair enzyme capacity, and the ability to induce proteins that either promote or inhibit the cell death process. Homeostasis of lung epithelial cells occurs, when a balance between cell renewal and cell death is achieved so that no net change in the cell number is present. Normal homeostatic cell deletion is controlled by apoptosis. Plasma membrane integrity is maintained during apoptosis, which prevents the leakage of cytosolic contents into the extracellular compartment therefore, normally this form of cell death is not associated with an inflammatory response, and epithelial cells, dead in apoptosis, neither evoke a danger signal nor activate DCs.

A danger signal released by chemically-damaged epithelial cells has not as yet been identified. In our opinion, adenosine (ADO), which is utilized in selective extracellular signaling may be such a danger signal. ADO, together with ADP and ATP, belong to a class of endogenous purine nucleotides produced by many cells during normal metabolic activity. Substantial amounts of adenosine may be formed from the breakdown of ATP and ADP. These adenine nucleotides are rapidly converted to adenosine by a family of ecto-ATP/ADPases and ecto-5 nucleotidases. ADO is converted to inosine and then further degraded to uric acid [51]. ADO appears to express identical modulatory effects on the balance of pro-inflammatory/anti-inflammatory cytokines such as PGE_2, catecholamines and histamine [52–54].

Local lung extracellular ADO levels increase dramatically during severe inflammation or tissue injury, and represent pathologic states that are also associated with high extracellular ADO concentrations. A high ADO concentration exerts potent anti-inflammatory and immunosuppressive effects [55,56]. Low or only slightly higher ADO concentrations may rather exert pro-inflammatory and immunostimulative effects. This phenomenon was repeatedly linked to A-2 receptor activation at a high ADO concentration, and A-1 receptor activation at slightly higher ADO concentrations. Activation of A2 receptors simultaneously inhibits IL-12 and stimulates IL-10 production by DCs and macrophages/monocytes [20,57]. Owing to this mechanism, ADO released in excess during inflammatory conditions or tissue injury may polarize the Th1/Th2 balance toward Th2 dominance and contribute to selective suppression of Th1 responses and cellular immunity.

Epithelial cells undergoing apoptosis are recognized and removed by lung macrophages. Uptake of apoptotic cells by macrophages inhibits release of inflammatory cytokines by mechanisms that invole anti-inflammatory mediators, including TGF-β. This cytokine also prevents the maturation of DCs [58]. Contrary to apoptosis, necrosis is a passive form of cell death associated with inflammation, often resulting from an overwhelming cellular insult that causes cell and organelle swelling, breakdown of the plasma membrane, release of lysosomal enzymes, and spillage of cell contents into the extracellular milieu. Necrotic epithelial cells are removed by lung macrophages and stimulate production of pro-inflammatory cytokines, IL-1β and TNF-α. According to Galluci and Matzinger [43], these cytokines are primal danger signals made during tissue

damage, whose elaboration does not require the previous activation of APCs.

Killed or damaged by LMW asthmogens, lung epithelial cells can release danger signals which may have some influence on immunoregulatory properties of lung macrophages. We suggest that high mobility group box 1 (HMGB1) protein is such a danger signal for macrophages. This protein is passively released by necrotic or damaged, but not apoptotic, lung epithelial cells [59]. HMGB1 is leaked rapidly into the medium when membrane integrity is lost in permeabilized or necrotic epithelial cells. Release of HMGB1 can serve as a diffusible signal of non programed death, which can be used to nearby lung macrophages to activate the appropriate responses. HMGB1 activates macrophages to release proinflammatory cytokines, including TNF-α, IL-1 α and β, IL-1Ra, IL-6, IL-8, MIP-1α and MIP1β, but not IL-10 or IL-12 [60,61]. Apoptotic epithelial cells do not release HMGB1 even after undergoing secondary necrosis and partial autolysis [62].

LMW ASTHMOGENS AND EQILIBRIUM BETWEEN LUNG DENDRITIC CELLS AND LUNG MACROPHAGES

A consequence of DCs activation by LMW asthmogens is the potentation of pulmonary immune function. Because the effectiveness of antigen presentation to T-lymphocytes in the lung is a function of a dynamic equilibrium between the potentiating properties of lung DCs and the immunosupressive characteristics of lung macrophages, it is likely that the induction of respiratory sensitization to inhaled LMW asthmogens will be then influenced markedly by factors that perturb this equilibrium. LMW asthmogens can not only cause DCs activation, but also their damage at DCs and death and thus prevent the initiation of a primary immune response.

In our opinion, the primary events of the pathogenic cascade, leading to the perturbation of a dynamic equilibrium between lung DCs and lung macrophages, are responsible for the induction of the airway epithelial barrier dysfunction by LMW asthmogens. This dysfunction may be caused by conjugation of these agents with selected airway epithelial cell proteins and matabolic perturbation (high level of oxidative stress) that lead to the damage or to necrosis of these cells [9]. After damaging these cells, LMW asthmogen-epithelial protein conjugates are released and taken up by lung DCs and macrophages. Lung macrophages have rather immunoregulatory properties and due to their "scavenging" function, the ingested proteins are often completely digested to amino acids and thus peptides suitable for loading into MHC class II may not be available.

LMW asthmogen-epithelial protein conjugates, phagocytosed by DCs, can be presented via MHC class II to CD4+ lymphocytes. DCs might have a default mechanism that activate cells whenever an insult hits them. For example, such insult may be released by LMW asthmogen, which by changing the intracellular glutathione/glutathione disulfide ratio and low level of oxidative stress (low level of ROS) may in turn stimulate DCs maturation. These metabolic perturbations may act as an intracellular danger signal [43]. The process of DCs maturation is likely to include, in addition to up-regulation of MHC class II, the expression of B7 molecules. B7-2 (CD86) on DCs is so far the most critical molecule for the amplification of naive T cell responses [40]. Migratory flux of mature DCs, with LMW asthmogen-peptide/MHC class II complex and B7-2 expression, from lung to lymph nodes and antigen presentation by such DCs might induce the differentiation of naive T cells toward an immune reactive phenotype. Immature DCs, with only MHC class II expression and lacking B7-2 expression, may block naive T-cell activation during antigen presentation. Naive T-cell recognition of LMW asthmogen-peptide/MHC class II complexes on non-fully mature DCs induces tolerance. Naive T cells are converted in weakly proliferative CTLA4+ T cells that produce IL-10 [45].

Although DCs are undoubtedly the major APCs in the lung, interactions with other types of cells may regulate their functions. Evidence implicates tissue macrophages in the active suppression of DC functions during their period of residence in the lung. A significant part of the inhibitory effects of macrophages *in vivo* may be due to their down-regulation of the APC function of pulmonary DCs. Such

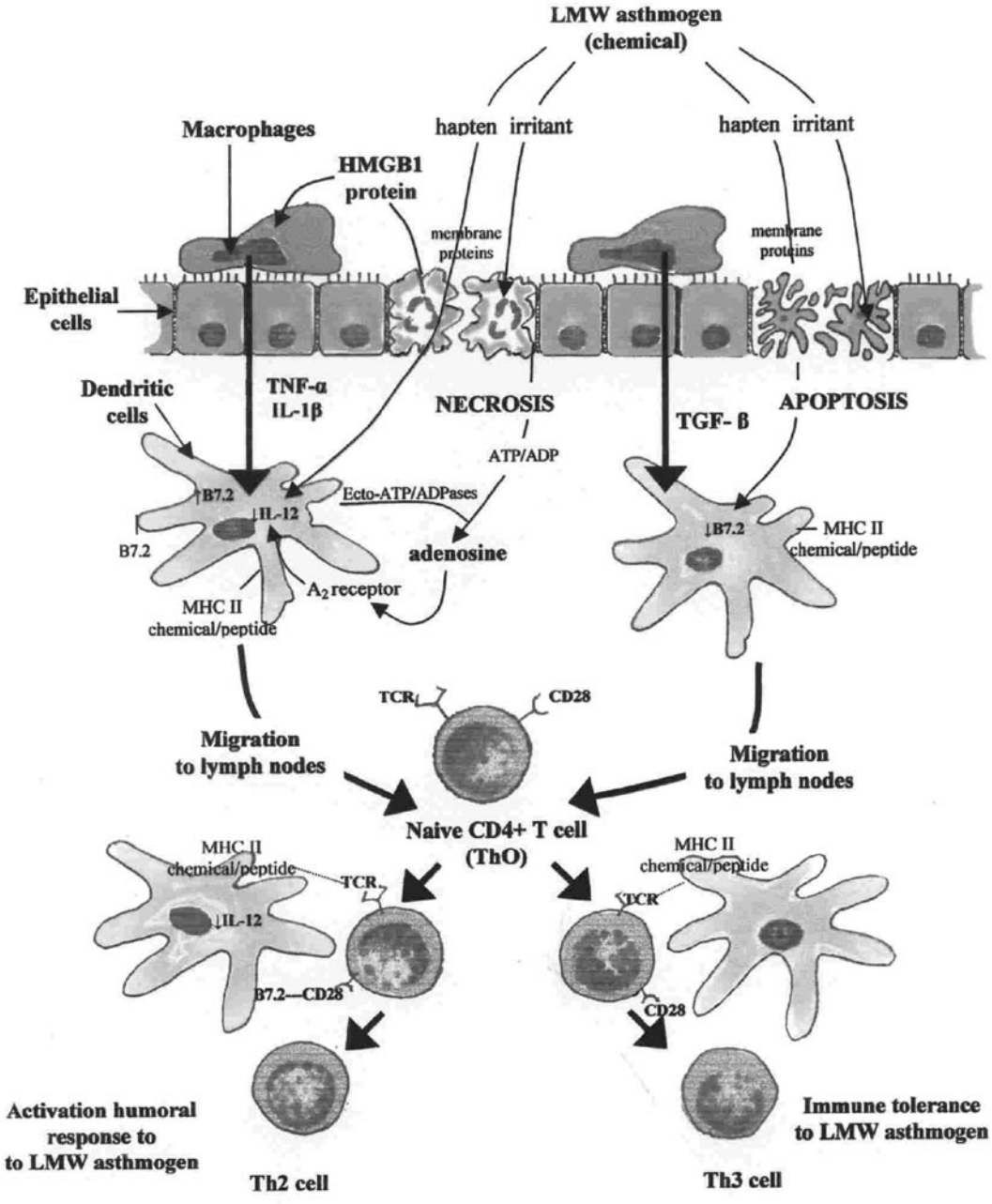

Fig. 3. Interaction between dendritic cells, macrophages and epithelial cells in the lung tissue exposed to low molecular weight (LMW) asthmogens and polarized T-cell differentiation.

down-regulation of the APC activity of DCs may represent a major pathway by which resident lung macrophages regulate the immunological milieu of the lung. It is of interset to note in this context that macrophages on the surface of surgically removed human lungs were closely juxtaposed to alveolar septal junctions. The majority of DCs are located within the same alveolar septal junctional zones, i.e., separated from adjacent macrophages by the width of single type I alveolar epithelial cells. A similar juxtaposition occurs in the airway mucosa, where DCs and mature tissue macrophages are aligned with opposite sides of the epithelial basement membrane [39]. Tight epithelial cells junctions may be disrupted during injury to these cells caused by LMW asthmogens. A greater epithelial fragil-

ity has been observed in bronchial biopsies of asthmatics as compared to those of non-asthmatic individuals [63]. Injury to the respiratory epithelium can result in disruption or removal of this cellular layer, resulting in inreased responsiveness of DCs to regulatory properties of lung macrophages and easier penetration of LMW asthmogens and LMW asthmogen/protein conjugates into dendritic cells.

Emigration of DCs from the lung to the lymph nodes, after the capture of antigen (LMW asthmogen/protein conjugates), is induced by cytokines produced by activated lung macrophages or epithelial cells [58,64]. Thus it appears that the "immature" function of DCs in the lung, i.e., moderate expression of MHC class II and modest T-cell stimulatory ability, may be at least in part accounted for active suppression of maturation processes by macrophages that uptake apoptotic epithelial cells. Such macrophages produce TGF-β that prevent DCs maturation [58]. This may be a protective mechanism to limit naive T-cell activation by DCs, but it also may serve a second important function in "locking" local incoming DC precursors into the phase of their life cycle in which their endocytic activity is maximal, thus optimizing local antigen uptake/surveillance [39]. Activation of lung macrophages by necrotic epithelial cells may disturb this *status quo* and thus release suppression of DC maturation and allow DCs to migrate to lymph nodes to initiate new primary immune responses.

Although LMW asthmogens have the potential to provide signal 1, i.e., a LMW asthmogen-protein conjugate, this is probably formed in many, if not all, patients with no adverese effects. The ability of signal 1 to elicit an immune response of any kind depends on both signal 2 and signal 3, the baseline cytokine profile of the individual, and perturbation induced by the LMW asthmogen. It is likely that signal 2 can be extremely powerful for weak immunogenic LMW asthmogens and in general sense provides the immune system with information about an impending "danger" of a possible development of the airway hypersensitivity reaction. It is possible that LMW asthmogens can provide signal 2 through chemical stress in the lung DCs and, in the extreme, through necrosis of epithelial cells. The danger signal (HMGB1 protein) sent by necrotic epithelial cells to lung macrophages stimulate pro-inflammatory cytokine synthesis [65]. Cytokines (IL-1β and TNF-α) induce migration and maturation of DCs. However, it is possible that down-regulation of DCs by TGF-β may normally prevent these processes, for example, if lung epithelial cells undergoing apoptosis (physiological or stimulated by LMW asthmogen) are removed by macrophages (Fig. 3). It is very important that a danger signal may also be provided from lung epithelial cells that are stressed or killed necrotically either by viral or bacterial infection.

ACTIVATION OF NAIVE Th CELLS BY DENDRITIC CELLS

The activation of naive Th cells requires signaling through the T cell receptor (TCR) for antigen (LMW asthmogen/peptide) and delivery of a series of signals commonly referred to as costimulation. DCs provide naive Th cells not only with an antigen-specific stimulatory signal (signal 1, ligation to the T cell receptor) and co-stimulatory signals (such as cell surface molecules stimulated by signal 2), but also with a polarizing signal (signal 3), which determines the nature of the immune response, Th1 or Th2 [66]. Mature DCs, bearing LMW asthmogen/peptide-MHC class II complex and expressing costimulatory molecules such as members of the B7 family, migrate through secondary lymphoid tissues and form low affinity contact with naive Th cells. The important point is the transient nature of these interactions [33]. As the cells form temporary conjugates, they can survey their partner for expression of surface-expressed molecules capable of transforming the interaction into one of a more long-lasting nature. This occurs, for example, if a naive Th cell expresses TCR, which recognizes the right LMW asthmogen/peptide complex on the DC. Several things contribute to the higher affinity binding between the two cells. First, signals from the occupied TCR help to increase the affinity of LFA-1 with naive Th cell for ICAM molecules on the DC. Second, the CD4 molecules enter into the TCR/LMW asthmogen/peptide MHC class complex. Third, costimulatory molecules such as DC ex-

pressed B7 family members and naive Th cell expressed CD28, engage between the two opposed cells eliciting important growth and/or differentiation signals [45].

It is not exactly known how LMW asthmogens may polarize the Th1/Th2 balance toward Th2 dominance and selective suppression of Th1 responses. The concept of polarized maturation is now widely confronted by a growing list of factors that allow the generation of mature IL-12-nonsecreting DCs such as PGE2, β2-agonists, TGF-β, IL-1, TNF-α, and Fas engagement. Moreover, certain DC costimulatory molecules, like OX40L, can deliver a Th2 polarizing signal to CD4+T cells [30,33]. As suggested earlier in this article, adenosine might be such a Th2 polarizing signal. ADO, released form lung epithelial cells damaged by LMW asthmogen, participates in the generation of IL-12-nonsecreting DCs. Such DCs contribute to selective suppression of Th1 responses and skew the balance toward Th2 dominance (Fig. 3).

Naive Th cell, appropriately activated and converted in Th2 cell, secretes IL-2 that subsequently promotes early stages of Th2 cell proliferation. Some of the expanded daughter cells differentiate into effector Th2 cells and return to the resting state to await for encounter with another antigen presenting cell, for example, B cell.

NAIVE B CELL-EFFECTOR Th2 CELL INTERACTIONS

Naive B cells, leaving the bone marrow to populate peripheral lymphoid organs, express both surface IgM and IgD as receptors for antigen. The occupancy of the naive B cell antigen receptor (BAR) by antigen (LMW asthmogen/protein conjugate) elicits several biochemical processes such as activation of protein tyrosine kinases, phosphatidylinositol (PI) metabolism and PI_3-kinase activity. However, these BAR-induced biochemical changes are not sufficient to elicit B cell cycle progression.

The most important consequence of BAR occupancy for immune response to LMW asthmogen may be LMW asthmogen/protein conjugate uptake [67]. Naive B cells, migrating through lung tissue, can bind LMW asthmogen/protein conjugate to their BAR. Such antigen bound BAR are internalized, processed and some peptides (also LMW asthmogen/peptide) re-expressed on the surface of the naive B cell in association with MHC class II. Since antigen processing and MHC class II association of peptides are similar to those expressed by DC, the naive B cell can express LMW asthmogen/peptide identical to those expressed by DC (which activated specific naive Th cell and converted it in specific effector Th cell) [68].

The resting effector Th2 cells, migrating through the lung secondary lymphoid tissue, can form low-affinity contacts with cells in the same environment, including antigen presenting B cells. This allows the resting effector Th2 cells to survey the surface of the naive B cells and to determine if their TCR recognizes any of the expressed B cells, LMW asthmogen/peptide-loaded MHC class II molecules. If the TCR is engaged, the low-affinity interaction between the opposed cells is changed into a higher affinity interaction. A microtubule-organizing center is established adjacent to the contact site of the effector Th2 cell with the B cell so that newly synthesized lymphokines can be secreted directly at the opposed B cell. The signals delivered to the effector Th2 cell during these cognate interactions determine the array of lymphokines ultimately produced and secreted by the effector Th2 cell. Signals delivered to the cognate B cells initiate the B cell cycle progression, and along with signals from the effector T cell-derived lymphokines, result in the expansion of B cells and differentiation of the daughter cells into IgE (after the process of class switching) secreting cells specific to asthmogen [68,69].

ACTIVATION OF MAST CELLS BY LMW ASTHMOGENS

In allergic occupational asthma, two different LMW asthmogen effects are distinguished. The first effect occurs when LMW asthmogen acts as an antigen (hapten) and produces antibodies specific to this asthmogen. The second effect (effector stage) occurs when a subsequent exposure to LMW asthmogen results in the production of the asthmatic reaction and the development of airway hyperresponsiveness. Increased airway hyperresponsiveness means that the airways, which normally do not respond

to substances such as ozone or sulphur dioxide are now adversely affected by these stimuli (Fig. 1), and the frequency of attacks of acute airway narrowing increases. Thus a normally innocuous substance elicits a bronchoconstrictor response in a person with allergic occupational asthma [67].

Mast cells are known to play a key role in the immediate phase allergic reaction. However, recent studies have emphasized that mast cells perform a more versatile role in prepetuating allergic inflammation [70]. The initial step in the effector stage of IgE-mediated occupational asthma requires binding of IgE specific to LMW asthmogen (secreted by B cells) to a cell surface receptor FcεRI expressed on lung mast cells and basophils [67]. Cross-linking of the FcεRI receptor-IgE complex by LMW asthmogen causes clustering of receptors, followed by signal transduction. This results in the release of preformed mediators of inflammation such as serotonin and histamine, which contribute to the bronchoconstriction [70]. The cross-linking of the FcεRI receptor-IgE complexes possess only LMW chemicals with at least two reactive groups. Having encountered LMW asthmogen, allergic individuals may either respond acutely, show late-onset symptoms or both. The acute reaction develops within few minutes of the LMW asthmogen exposure. Histamine, tryptase, PDG2 and LTC4 are among the mast cell products, which can be detected immediately after exposure to LMW asthmogens. Histamine induces vasodilation, increased vascular permeability and increased glandular secretion. Prostaglandins (e.g., PGD2) also cause edema by vasodilation and increased vascular permeability. The late-phase response begins 3–12 h following the LMW asthmogen exposure. The late-phase allergic reaction is thought to be orchestrated by activated T cells, resulting in the infiltration of eosinophils, basophils and T cells and the subsequent release of a number of soluble products such as prostaglandins, leukotrienes, platelet activating factor, eosinophilic cationic protein, or major basic protein. The identification of a variety of cytokines (TNF-α, IL-4, IL-5, IL-6, IL-13) in mast cells and demonstration of their release when activated via the IgE receptor suggest a potential role of mast cells in orchestrating the late-phase allergic reaction [69,70].

The acute asthmatic reaction (anaphylactic reaction) to LMW asthmogen (e.g., muscle relaxants) appeared to be a very useful model to study the IgE-dependent mediator release from mast cells and basophils. It is now clear that such small divalent molecules (with two ammonium groups) can induce anaphylactic shock and bridge IgE antibodies on mast cells and basophils through the ammonium ion determinants. The presence of IgE antibodies to the allergenic determinants does not appear sufficient to induce allergic reactions. The length and the flexibility of the chain bearing the haptenic determinant appear to be importanat in the elicitation of mediator release (Fig. 4). When the length of the chain linking the ammonium groups is <4Å, no significant histamine release can be obtained, whereas the optimal length for histamine release appears to be >6Å. Also compounds with a rigid backbone in the chain linking the hapten determinants (e.g., pancuronium) are less active than flexible molecules (e.g., suxamethonium) in bridging IgE molecules and in initiating mediator release [71,72].

The presence of IgE antibodies in blood serum of workers exposed to certain LMW asthmogens such as acid anhydrides (e.g., trimellitic anhydride) is well documented [67]. By analogy, it could be expected that with enough effort, IgE antibodies could be demonstrated for other LMW asthmogens. Admittedly, in some cases it may be difficult,

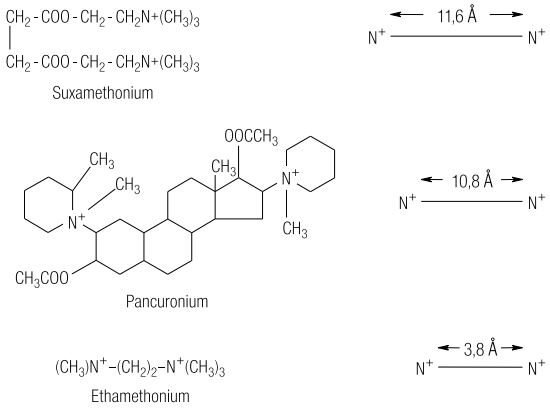

Fig. 4. Structural formulas for suxamethonium, pancuronium and ethamethonium salts with the length of the chain linking the two quaternary ammonium determinants. With ethamethonium no histamine release has been observed.

but feasible, to demonstrate sensitization to diisocyanates and resin acids, as evident in numerous reports [9].

We know that there are other non-classic, non-IgE-mediated mechanisms of great importance and interest, e.g., IgG-mediated mechanisms. Park et al. [73] demonstrated a strong association between toluene diisocyanate (TDI)-specific serum IgG and TDI-induced asthma. Non-antibody processes, and indeed non-immunologic processes can also result in the production of the same mediators, and therefore in the same inflammatory response. A number of occupational chemicals have been found to cause symptoms typical of allergic reactions, but without evidenced immunologic involvement. We agree with Agius [6] that IgE-mediated mechanisms are important pathways for the genesis of occupational allergic asthma, but they are by no means the only ones.

CONCLUSION

The study of the chemical properties of LMW asthmogens responsible for development of occupational asthma is an evolving issue. It is probable that sensitization mechanisms that do not confine to the traditional allergic model play a crucial role in inducing asthma and they need to be unravelled. Knowledge of a wide range of chemical structures and mechanisms that may generate occupational asthma should help occupational medicine physicians think more broadly, not confining the clinical diagnosis to compendiums of specific confirmed and reported causes. In so doing, the aspiration to a wider clinical awareness of the disease and better prospects of prevention are possible.

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