## Mechanisms of allergic diseases

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# Mechanisms of occupational asthma

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**Overall Purpose/Goal:** To provide excellent reviews on key aspects of allergic disease to those who research, treat, or manage allergic disease.

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**Activity Objectives**

1. To identify the structure-activity relationship for causal agents of occupational asthma (OA).

2. To understand the role of environmental and genetic factors in OA development.

3. To understand the pathophysiology of immunologic and nonimmunologic OA.

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**Inhalation of agents in the workplace can induce asthma in a relatively small proportion of exposed workers. Like nonoccupational asthma, occupational asthma is probably the result of multiple genetic, environmental, and behavioral influences. It is important that occupational asthma be recognized clinically because it has serious medical and socioeconomic consequences. Environmental factors that can affect the initiation of occupational asthma include the intrinsic characteristics of causative agents as well as the influence of the level and route of exposure at the workplace. The identification of host factors, polymorphisms, and candidate genes associated with occupational asthma may improve our understanding of mechanisms involved in asthma. High-molecular-weight compounds from biological sources and low-molecular-weight chemicals cause occupational asthma after a latent period of exposure. Although the clinical, functional, and pathologic features of occupational asthma caused by low-molecular-weight agents resemble those of allergic asthma, the failure to detect specific IgE antibodies against most low-molecular-weight agents has resulted in a search for alternative or complementary physiopathologic mechanisms leading to airway sensitization. Recent advances have been made in the characterization of the immune response to low-molecular-weight agents. In contrast, the mechanism of the type of occupational asthma that occurs without latency after high-level exposure to irritants remains undetermined. (J Allergy Clin Immunol 2009;123:531-42.)**

***Key words:*** *Workplace exposure, asthma, airway hyperresponsiveness, antigen, chemical, genetics, mechanisms, inflammation*

Occupational asthma (OA) is a type of asthma due to causes and conditions attributable to a particular work environment rather than to stimuli encountered outside the workplace.[1] OA has become one of the most common forms of occupational lung disease in many industrialized countries, having been implicated in 9% to 15% of cases of adult asthma. There is general agreement that 2 types of OA can be distinguished. First, immunologic OA appears after a latency period of exposure necessary for acquiring immunologic sensitization to the causal agent. It

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Terms in boldface and italics are defined in the glossary on page 532.

*Abbreviations used*

GST: Glutathione *S*-transferase
HDI: Hexamethylene diisocyanate
HMW: High molecular weight
LMW: Low molecular weight
NAT: *N*-acetyltransferase
NK2R: Neurokinin 2 receptor
OA: Occupational asthma
TDI: Toluene diisocyanate
WTC: World Trade Center

## GLOSSARY

**ACID ANHYDRIDES, COLOPHONY, DIISOCYANATES, PERSULFATE SALTS:** Low-molecular-weight substances involved in occupational asthma. Diisocyanates and acid anhydrides are in vapor form. Isocyanates are found in paints, plastics, adhesives; acid anhydrides are found in epoxy resins; colophony (abietic and resin acids) is obtained from conifers and used in the electronic industry; and persulfate exposure occurs in hair dressers.

**APOPTOSIS:** Apoptosis, also referred to as *programmed cell death*, typically induces very little inflammation because of a lack of cell lysis and discharge of intracellular constituents. Apoptosis can occur because of intrinsic damage signals such as reactive oxygen species that activate "apoptosome" complexes, which in turn activate caspases, or because of extrinsic death signals such the binding of Fas-ligand and TNF to their receptors with subsequent activation of caspase-8. Assays such as a terminal deoxynucleotide transferase-mediated dUTP nick end labeling can detect the presence of DNA strand breaks typical of apoptotic cell death.

**α-HELIX:** The α-helix is the most common helical secondary structure found in proteins. Properties include a radius that allows for favorable van der Waals interactions and side chains that are staggered to minimize steric interference.

**CHITINASE:** Chitinases are digestive enzymes that break down glycosidic bonds in chitin. Despite the absence of chitin, mammals synthesize chitinases. Murine asthma models initially demonstrated the role of chitinases in $T_H2$ airway inflammation. Subsequent human studies have demonstrated increased pulmonary and peripheral expression of chitinase 3-like1, in patients with severe asthma and airway remodeling.

**EPIGENETICS:** Used to describe studies of stable and heritable (or potentially heritable) changes in gene expression that do not involve changes in DNA sequence. Epigenetic mechanisms such as DNA methylation, histone deacetylation, and other modes of chromatine remodeling, ensure that genes are expressed or silenced with respect to developmental stage and cell type. The changes may remain through cell divisions for the remainder of the cell's life and may also last for multiple generations. Epigenetic traits exist on top of or in addition to the traditional molecular basis for inheritance.

**γ/δ T CELL:** γ/δ T cells represent a small subset of T cells that possess a distinct T-cell receptor, made up of 1 γ-chain and one δ-chain, on their surfaces. γ/δ T cells are less common than α/β T cells, recognize antigens such as heat shock proteins and mycobacterial lipids without requiring MHC I or II presentation, and are found as intraepithelial lymphocytes in the skin and gut mucosa. γ/δ T cells are capable of producing $T_H2$-associated or $T_H1$-associated cytokines and ILs.

**GLUTATHIONE *S*-TRANSFERASE (GST):** The GST is a family of enzymes that comprises a long list of cytosolic, mitochondrial, and microsomal proteins capable of multiple reactions with a multitude of substances, both endogenous and xenobiotic. GSTs catalyse the conjugation of reduced glutathione via the sulfhydryl group, to electrophilic centers of a wide variety of substances. This activity is involved in the detoxification of endogenous compounds as well as in the metabolism of xenobiotics. Polymorphisms within genes of the GST family are associated with risk of developing both nonoccupational and occupational asthma.

**$IgG_4$:** IgG subclass that is essentially equivalent in concentration to $IgG_3$ in human serum, can be decreased in conjunction with IgA in patients with IgA deficiency, and does not activate complement. Successful immunotherapy can be associated with decreasing levels of IgE and increasing levels of $IgG_4$.

**IL-5, IL-13, IFN-γ:** IL-5 promotes the survival, activation, and chemotaxis of eosinophils. IL-5 receptor shares a common β-chain with the IL-3 receptor. IL-13 promotes the production of IgE and induces the production of IL-5. IFN-γ is produced by $T_H1$ cells stimulated by IL-12. It decreases the production of $T_H2$ ILs.

**IL-10:** IL-10 is an anti-inflammatory cytokine. It can be produced by CD25-positive regulatory T cells; can be elevated in viral infections such as rhinovirus, respiratory syncytial virus, enterovirus, and influenza; and is associated with dampening immune response. IL-10 suppresses eosinophilia by inhibiting IL-5 and GM-CSF and is elevated after successful immunotherapy.

**KERATIN 18:** Keratin 18 is a type 1 cytokeratin. Cytokeratins are structural proteins found in epithelial cells. Autoantibodies to cytokeratin-8, cytokeratin-18, and cytokeratin-19 can be found in patients with toluene diisocyanate–induced asthma.

**LEUKOTRIENE (LT) $C_4$, $B_4$:** Leukotrienes are naturally produced eicosanoid lipid mediators. In cells that express $LTC_4$ synthase, such as mast cells and eosinophils, $LTA_4$ forms the cysteinyl LT, $LTC_4$, which can be converted by ubiquitous enzymes to $LTD_4$ and $LTE_4$. In cells equipped with $LTA_4$ hydrolase, such as neutrophils and monocytes, $LTA_4$ is converted to $LTB_4$, which is a powerful chemoattractant for neutrophils acting at $BLT_1$ and $BLT_2$ receptors on the plasma membrane of these cells. Both $LTC_4$ and $LTB_4$ are potent bronchoconstricting agents. The variability in clinical response to cysteinyl LT receptor antagonists is caused, in part, by to differences in promoters in genes such as 5-lipoxygenase.

**N-ACETYLTRANSFERASE (*NAT*): A**n enzyme that catalyses the transfer of acetyl groups from acetyl-Co enzyme A to arylamines. *N*-acetylation polymorphisms could be used as genetic markers because slow *N*-acetylators are susceptible to develop asthma.

**NEUROKININ RECEPTOR:** A class of cell surface receptors for tachykinins that prefers neurokinin A (NKA, substance K, neurokinin alpha, neuromedin L); neuropeptide K (NPK); or neuropeptide gamma over other tachykinins. Neurokinin-2 (NK-2) receptors have been cloned and are similar to other G-protein–coupled receptors.

**SUBSTANCE P:** Substance P is a neuropeptide, an undecapeptide that functions as a neurotransmitter and as a neuromodulator. It belongs to the tachykinin family. Tachykinins such as substance P and calcitonin gene-related peptide can be released by mast cells and act mediators for vascular dilation and permeability. Calcitonin gene-related peptide is elevated in bronchoalveolar lavage and bronchial biopsies in patients with allergic asthma after challenge with Fel d 1.

**VASCULAR ENDOTHELIAL GROWTH FACTOR:** Vascular endothelial growth factor is a subfamily of growth factors. It is a proangiogenic factor, is increased compared with antiangiogenic factors such as endostatin in patients with asthma, can be produced by chymase-positive mast cells in the airway, and is associated with airway remodeling that can lead to airways dysfunction.

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**TABLE I.** Summary of characteristics of the more relevant workplace agents associated with OA

| Agent | MW* | Mechanisms | | | Genetic susceptibility | Risk factors† | Exposure-response relationship |
|---|---|---|---|---|---|---|---|
| | | IgE | Non-IgE | Innate immune responses | | | |
| Polyisocyanates | Low | + | + | + | HLA, IL-4RA (I50V), IL-13 (R110Q), GST, NAT, NK2R, lysosomal genes, chitinase-1, Pi-alleles | + | + |
| Acid anhydrides | Low | + | | | HLA§ | + | + |
| Laboratory animal, allergens (rats, mice, guinea pigs, rabbits, locusts, cockroaches) | High | + | | | HLA, Toll-like receptor 4 | + | +‡ |
| Natural rubber latex | High | + | | | HLA | + | + |
| Plicatic acid (western red cedar) | Low | + | + | | HLA | + | + |
| Other wood dusts (oak, obeche, samba, iroko) | Low | + | + | | | | |
| Flour dust (wheat and rye) | High | + | | | | + | + |
| Complex platinum salts | Low | + | | | HLA§ | + | + |
| Detergent enzymes | High | + | | | HLA‖ | + | + |
| Cleaning agents | Low | | + | | | + | ? |

*High molecular weight (MW): MW greater or equal 5000 d.
†The factors recognized to increase the risk of OA are both external and host-related: exposure level, atopy for HMW agents, rhinoconjunctivitis, having a measurable $PC_{20}$ histamine, cigarette smoking for platinum salts and anhydride compounds, sex; for exposure to cleaning agents, the excess of risk is predominantly attributable to private home cleaning (kitchen and sanitary cleaning, polishing furniture, use of oven sprays and polishes).
‡A reduction of risk was observed among those with the highest measured exposures (immune tolerance? or undetected survival effect?).
§The relative risk associated with HLA phenotype was greater at lower levels of exposure.
‖In mice.

should be noted that an immunologic mechanism has not yet been proven for some agents that induce asthma after a latency period, such as ***colophony***. Second, nonimmunologic OA is far less frequent and is characterized by the absence of a latency period. Nonimmunologic OA occurs after acute exposure to high concentrations of irritants and has been termed *irritant-induced asthma*.[1,2] This term includes reactive airways dysfunction syndrome, traditionally described by Brooks et al[3] after a single massive exposure to inhaled irritants. Work-exacerbated asthma, defined by the American College of Chest Physicians' recent consensus statement on "Diagnosis and management of work-related asthma" as a condition in which asthma is pre-existing or concurrent but exacerbated by occupational exposure, is not considered in the current review.[4]

Occupational asthma is probably the result of multiple genetic, environmental, and behavioral influences and has serious medical and socioeconomic consequences. The several hundred causes of OA can be classified conveniently into high-molecular-weight (HMW) and low-molecular-weight (LMW) compounds. HMW compounds, which are often from biological sources, generally induce asthma through an IgE-dependent mechanism, whereas many LMW compounds induce asthma through non–IgE-dependent mechanisms.

Many aspects of the pathophysiology of OA are controversial. Recent reviews identify some of the key issues[2,5]; of particular relevance is a report that highlights 100 key questions and needs in OA.[6] Issues that frequently stimulate debate are the characteristics of immune responses induced by chemical respiratory allergens and the nature of the important immunologic effector mechanisms.

This review examines the current understanding of the role of environmental and genetic factors in the development of OA and recent advances in the pathophysiology of immunologic and nonimmunologic OA (Table I). Because the majority of the recent studies examined the mechanisms involved in LMW-induced OA, this review focuses on this type of OA.

## ROLE OF EXPOSURE IN PATHOPHYSIOLOGIC MECHANISMS

### Characteristics of the allergen

More than 350 agents have been reported to cause OA. Although there are fewer LMW chemicals than HMW agents in the lists of occupational respiratory sensitizers, LMW chemicals still represent an important subset of etiologic agents, including approximately 100 separate chemical entities.[7] The most important chemicals that cause OA include ***acid anhydrides***, polyisocyanates and their prepolymers, plicatic acid (from Western red cedar), colophony fume, and metals such as chlorinated platinum salts, ***persulfate salts***, and some acrylates. Recent data indicate that LMW chemicals account for more new cases of OA caused by sensitization than HMW agents.[7,8] Because there are about 30,000 registered chemicals, it can be estimated that 1 in 300 is asthmagenic. It is therefore reasonable to believe that asthmagenic chemicals have some important features in common. Quantitative structure-activity relationships have been used to correlate chemical function with chemical structure, on the assumption that chemical structure is a determinant of reactive properties and hence biological activity. Because of the uncertainties regarding the molecular mechanism for LMW respiratory sensitizers, quantitative structure-activity relationships seem particularly appropriate because they make no *a priori* mechanistic assumptions.[9,10]

Using a large data set of respiratory sensitizers and inactive controls, Cunningham et al[11] identified biophores and developed a categorical structure-activity model whose predictive power gave a sensitivity of 89% and a specificity of 95%. Jarvis et al[9] demonstrated that the odds ratios that indicate a chemical substructure is present in an asthmagenic compound compared with a control are elevated for a range of fragments, including reactive groups such as isocyanate, anhydride, acrylate, nitrogen, carbonyl, or amine. A logistic regression model using only statistically significant independent variables for OA hazard correctly assigned 90% of the chemicals identified in the

literature as causes of OA and gave a sensitivity of 86% and a specificity of 99%. However, important asthmagenic chemicals were misclassified, including ethylene oxide, sulfonechloramide, plicatic acid, phenylglycine acid chloride, and phthalate compounds. These models are applicable only to organic chemicals, and their reliability is strongly dependent on the data base of asthmagenic chemicals used. These limitations and inconsistencies make structure function models not suitable for use as single predictors for sensitizing properties. However, an understanding of structure-activity relationships may provide insight into the molecular mechanisms involved in the initial chemical reaction between environmental chemicals and the human host.[10] For some reactive groups, like carbonyl and amine, the presence of 2 or more fragments is associated with more significant odds ratios, supporting the hypothesis that bifunctional or polyfunctional chemicals—that is, those with cross-linking potential—are more likely to be asthmagens. Similarly, multiple chlorine atoms are required to confer allergenicity of hexachloroplatinate salts. A more sophisticated approach should include not only structural chemical formulae but also the relative position of atoms in 3-dimensional space. Although there is evidence that chemical respiratory allergens differ from contact allergens in the quality of immune response—that is, the ability to provoke the preferential development of $T_H2$-type immune responses—the physicochemical properties of chemical allergens that dictate the type of sensitization they will cause remain largely unknown.[12,13]

## Intensity of exposure

Intensity of exposure to a respiratory sensitizer is the most important determinant of OA. Studies during the past decade have shown dose-response relationships for several agents, including flour, fungal α-amylase, laboratory animal proteins, detergent enzymes, Western red cedar, colophony, complex halogenated platinum salts, and acid anhydrides.[2] However, there is still a lack of information regarding the existence of "no effect" levels and whether peak exposures are of greater relevance than cumulative doses. Although a positive linear exposure-response relationship has been reported for several HMW and LMW allergens, there are some exceptions.[14] In those who work with laboratory animals, the relative risk for sensitization does not show a linear association with increasing exposures.[14,15] Increasing risk of sensitization and work-related respiratory symptoms were observed with increasing exposures to rats except at the highest exposure levels, when the risk of both outcomes was lower. In addition, at high exposures, an attenuation of specific IgE antibody response was observed[16] that was associated with an increased prevalence of individuals producing high levels of specific ***IgG4*** antibodies. Ratios of specific $IgG_4$:IgE antibodies were found to be significantly increased in those handling the highest number of rats. In a longitudinal study, high exposure to rats was associated with higher $IgG_4$ response, but the workers with the highest cumulative exposures had the lowest prevalence of skin prick test positivity to rat antigens and work-related respiratory symptoms. A similar exposure-response relationship has been reported for workers exposed to laboratory mice.[17-19] Mouse-specific $IgG_4$ levels were significantly associated with mouse allergen exposure, but at the highest levels of mouse-specific $IgG_4$, there was a reduction in mouse skin test sensitivity. The evidence that specific $IgG_4$ production is driven by increasing allergen exposures and is associated with a reduction in sensitization rate suggests a modified $T_H2$ response, as described in children with high exposure to cat allergens.[20] This natural form of immunotolerance mimics that induced during specific immunotherapy, when the ratio of specific $IgG_4$:IgE increases by 1 to 2 log.[21] Because the development of natural tolerance at high-dose exposure is not observed with other occupational allergens, such as flour or enzymes,[22,23] it has been hypothesized that the route of allergen delivery is important for the type of immune $T_H2$ response.[14] Thus, workers who are at higher risk to be bitten or scratched by laboratory animals may be exposed by the intradermal route, as occurs in immunotherapy or in beekeepers who are exposed to stings and may became tolerant. Upregulation of regulatory T cells producing TGF-β or ***IL-10*** is a mechanism implicated in the tolerance induced by immunotherapy, but it has not been evaluated to any significant degree in occupational asthma.

## Route of exposure

The respiratory tract is believed to be the main route of exposure and site of initiation of the immune response toward occupational-chemical allergens. Despite a reduction in respiratory exposure in the workplace, OA continues to occur. There is reason to suppose that chemical respiratory allergens can induce sensitization of the respiratory tract by routes other than inhalation; dermal exposure may be of particular relevance from an occupational health perspective.[24] Phthalic anhydride, a human respiratory allergen never implicated as a skin sensitizer, elicits positive responses in guinea pig and mouse skin sensitization assays.[13] Isocyanate skin exposure in various animal models induces systemic $T_H2$-like sensitization that leads to asthmatic-like responses of the lung on subsequent specific inhalation challenge.[25-27] Interestingly, lower skin exposures may result in a greater lung inflammatory response after inhalation challenge in mice.[28] Data supporting the hypothesis that skin may be a relevant site for systemic sensitization to certain occupational allergens in human beings are largely indirect. OA induced by methylene diphenyl ***diisocyanate*** has been described in human beings, who appear to develop IgE-mediated asthma after dermal exposure to spot use of methylene diphenyl diisocyanate.[29]

Assessment methodology for skin exposure is not as well developed as it is for exposure by inhalation, and skin exposure has rarely been included in studies of risk assessment for OA. Isocyanate skin exposure has been documented in auto body shops and among spray painters using polyurethane products, despite the use of standard personal protective equipment, and also in a setting where airborne concentrations are minimal.[30-32] Further studies are needed to establish the role of skin exposure at work in the mechanisms of sensitization and the development of OA.

# GENETIC ASPECTS

Occupational asthma, a phenotype of adult-onset asthma, might provide a means to understand better the interaction between a susceptible genotype and multiple environmental factors. The expanding evidence for gene-environmental interactions in asthma highlights the importance of measuring environmental exposure in genetic studies of occupational and nonoccupational asthma.[33] It is often hard to define environmental exposures, but among subjects with OA there is the advantage

that exposure, sensitizer, and phenotype can be identified, or at least suspected.

Several models of interactions have been proposed.[34] Not only gene-environment interactions but also gene-gene, gene-gene-environment, and gene-environment-environment interactions may contribute to the risk of developing asthma.

Genetic susceptibility is both context-dependent and developmentally regulated.[34] ***Epigenetics*** refers to stable and heritable changes in gene expression that do not involve changes in DNA sequence.[35] Epigenetic mechanisms include DNA methylation, histone deacetylation, and others; they ensure that genes are expressed or silenced with respect to developmental stage and cell type. Environmental factors may modify the epigenome, suggesting that gene expression can be changed through environmental exposures.[35]

Asthma susceptibility genes fall into 4 groups: (1) genes associated with innate immunity and immunoregulation, (2) genes associated with $T_H2$-cell differentiation and effector functions, (3) genes associated with epithelial biology and mucosal immunity, and (4) genes associated with lung function, airway remodeling, and disease severity.[36]

The genes of group 1 are involved in triggering the immune response. These genes are located on the HLA complex on chromosome 6, which contains more than 200 genes, more than 40 of which encode leukocyte antigens. HLA class II molecules are cell surface glycoproteins expressed principally by immune cells, including B cells, activated T cells, macrophages, dendritic cells, and thymic epithelial cells, which present processed antigens (in the form of peptides) to antigen-specific T lymphocytes, leading to their specific activation and proliferation.[37] Thymic epithelial cells have this ability in the context of positive or negative selection of thymocytes. Cells that survive to both positive and negative selection leave the thymus and enter the periphery as naive T cells. The central role of HLA class II molecules in antigen presentation has been confirmed by studies showing a strong association between specific HLA-DR, HLA-DQ, and HLA-DP alleles and allergen-specific IgE responses.[38] Data obtained in occupational studies identify HLA class II molecules as prominent factors for the specificity of the response to occupational agents having either LMW or HMW, including polyisocyanates and prepolymers, Western red cedar, acid anhydrides, chlorinated platinum salts, natural rubber latex, detergent enzymes, and animal proteins.[39-48]

In a study of diisocyanate-induced asthma in northern Italy, the frequencies of DQA1*0104 and DQB1*0503 were significantly higher in subjects with asthma than in asymptomatic exposed subjects, whereas DQA1*0101 and DQB1*0501 were significantly higher in asymptomatic exposed subjects.[40] The 2 DQB1 alleles, DQB1*0503 and DQB1*0501, differ in a single position, amino acid 57, with aspartic acid in the first and valine in the second. The majority of subjects with asthma were homozygous for the presence of an aspartic acid residue at position 57, indicating a role for this residue in the etiology of diisocyanate-induced asthma. Position 57 is located on the ***α-helix*** with the side chains pointing in toward the peptide-binding groove, so it is potentially a peptide and/or T-cell receptor contact residue. Substitutions at the equivalent position 57 of the HLA-DQβ1 have been found in other types of OA, such as HLA-DRβ1 in laboratory animal–induced allergy.[47] It is also of interest that the DQB1*0501 allele is particularly frequent in subjects sensitized to organic acid anhydrides, suggesting that the DQB1*05 gene confers susceptibility to developing specific IgE antibodies against organic acid anhydrides.[43] However, the same allele is protective for other LMW agents, such as diisocyanates and plicatic acid,[40,44] indicating that different affinities for the corresponding specific class II molecules may exist.

In contrast with the study in Italy, a study in Germany did not find involvement of HLA class II alleles in isocyanate-induced asthma.[46] The difference in conclusions between the 2 studies may be a result of small sample size, the phenotyping methods used, and/or geographic differences between the study populations.[49] Other possible causes for the lack of replication of results in genetic studies include ethnic variations, gene-gene interactions, or haploid specificity, which likely occur in a polygenic disease such as asthma.

Another explanation could be polymorphisms. It has been suggested that the most important gene variants for asthma are polymorphisms that exert their influence on the network system controlling biological responses to asthma-related exposures.[50] Martinez[50] emphasized that the expression of the genotype is dependent on the context in which it acts: the same allele that increases risk in one context may decrease it in another.

Genetic studies performed in platinum refinery workers suggested that the relative risk associated with the HLA phenotype was greater at lower levels of exposure, indicating that the strength of this association varies inversely with intensity of exposure to the sensitizing agent.[51,52] This finding implies that as exposure-control measures are implemented to prevent sensitization and asthma, the importance of genetic susceptibility will likely increase.[52]

Among agricultural farmers, environmental exposure to endotoxin, a cell wall component of gram-negative bacteria, is an important occupational hazard for the development of wheeze and impairment of lung function. The prevalence of wheeze was higher among farmers homozygous for CD14/-159T or CD14/-1619G than among farmers with the CC or AA genotype.[53] Nonsmoking male farmers with the CD14/-159TT genotype or the CD14/-1619GG genotype had lower lung function as measured by $FEV_1$. The authors speculated that the CD14/-159 TT farmers may have had increased levels of serum s CD14. Genetically determined higher levels of sCD14 in the lung may result in increased responsiveness to environmental endotoxin.[53]

Among researchers at work with laboratory animals, the presence of minor G alleles of Toll-like receptor 4 bears a positive association with atopy and sensitization to laboratory animals and a negative association with chest symptoms caused by exposure to laboratory animals.[54]

Group 2 asthma susceptibility genes include genes that regulate the differentiation of naive $CD4^+$ $T_H$ cells into a $T_H2$-cell polarized effector phenotype. A role for genes of this group has been shown in diisocyanate-induced asthma: both IL-4Rα and the combination of IL-4RA (I50V) and ***IL-13*** (R110Q) were significantly associated with asthma.[55]

For group 3 susceptibility genes expressed in epithelial cells, at the interface between innate and adaptive immunity, there are no available studies of subjects with OA. However, in C57BL/gJ mouse lung exposed to hexamethylene diisocyanate (HDI), expression of a large number of genes involved in stress response, growth arrest, ***apoptosis***, signal transduction, and inflammation was increased after exposure to HDI.[56] Among the gene products expressed were ***keratin 18*** and other keratins. These cytoskeletal elements are present in epithelial cells, which—because of their location lining the respiratory tract—will sustain the most injury

from inhaled HDI. The authors suggested that the increase in keratin gene expression may occur in response to the inactivation of these proteins on conjugation to HDI. This suggestion fits well with the identification of keratin 18 in lung biopsy specimens as a protein conjugated to HDI after inhalation of HDI monomer by human subjects.[57]

Finally, for group 4, the more heterogeneous genes associated with lung function and airway remodeling, some studies have been performed in OA. The role of genes such as ***glutathione* S-*transferase* (GSTP1 *and* GSTM1)** and **N-*acetyltransferase* (NAT)** has been explored in isocyanate-induced asthma.[58-60] The polymorphic GSTs, especially the mu class, have an important role in asthma induced by diisocyanates.[58] In this type of OA, homozygosity for the *GSTP1*Val* allele confers protection against the development of asthma and airway hyperresponsiveness. Moreover, the protective effect increases in proportion to the duration of exposure to toluene diisocyanate (TDI).[61] *GSTP1* is located on a chromosomal region of particular interest for asthma—that is, the 11q13—and is the major isoform expressed in the lung.

With regard to the role of *NAT* genotypes, the effect of the *NAT1* genotype was especially relevant for workers exposed to TDI, among whom the *NAT1* slow acetylator genotypes posed a 7.77-fold risk of asthma (95% CI, 1.18-51.6).[59] A Swiss longitudinal study confirmed that slow *N*-acetylators are susceptible to asthma induced by exposure to diisocyanates in the workplace.[60] Both rapid and slow *NAT* are expressed in the respiratory epithelium and are able to react directly with the hydrolyzed amines from the parent diisocyanate.[62] In addition, among workers with OA induced by diisocyanates, antitrypsin phenotypes, although not specific for isocyanate-induced asthma, are modifiers of pulmonary diseases.[60] Specifically, heterozygous antitrypsin-phenotype carriers are susceptible to isocyanate-induced asthma. The authors suggested that antitrypsin activity may play a role in the balance between ***substance P*** and its inactive propeptide. The study showed that 93.6% of the responders exhibited at least 1 risk factor for asthma.

A study performed among Danes living in rural areas showed that there is an association between farm workers and the rare α1-antitrypsin-alleles, leading to a higher proportion of airway hyperresponsiveness among these subjects.[63]

A role for neurogenic inflammation in isocyanate asthma has been suggested by a Korean study.[64] The authors investigated whether neurokinin 2 receptor (NK2R) gene polymorphisms are associated with the clinical phenotype of TDI asthma. No significant differences in allele, genotype, or haplotype frequencies of the NK2R polymorphisms were present among the groups of subjects with asthma, asymptomatic exposed subjects, and healthy controls. However, subjects with the NK2R 7853GG genotype had higher serum ***vascular endothelial growth factor*** levels than subjects with GA or AA among TDI-exposed subjects, which may contribute to perpetuating airway inflammation.

TNF is a proinflammatory cytokine. One study found that the polymorphism at position -308 of the TNF-α gene promoter is associated with increased risk of asthma.[65] However, nonsignificant associations were found between polymorphisms at position -308 of the TNF-α gene promoter and isocyanate asthma.[66]

The responses of lysosomal genes were reported for *in vitro* experiments on human PBMCs and clinical studies of subjects before and after a specific inhalation challenge with HDI.[67] Significant changes in microarray gene expression were noted in lysosomal genes, especially peptidases and proton pumps involved in antigen processing. Another interesting finding was the exposure-dependent decrease in serum concentrations of ***chitinase*** 3–like—1 in subjects who lack the major (type 1) human chitinase (because of genetic polymorphism), but not in individuals possessing at least 1 functional chitinase-1 allele.

In conclusion, genetic susceptibility, probably in combination with occupational and environmental exposures, can affect the development of OA by modifying the impact of a given gene on complex phenotypes. It is still unknown whether complex diseases such as asthma are influenced by a few polymorphisms that have large effects or by synergism of several variants individually associated with low risk.[68,69] In animal models, gene-environment interactions explain phenotype variance better than the 2 components, genes and environment considered separately.[70] However, classic genetic mechanisms may be inadequate to explain complex diseases such as asthma.[71] It has been suggested that many environmental factors interact with our genes through epigenetic mechanisms, and that these interactions act primarily during early life.[34] In addition, a link has been shown between certain maternal occupations during pregnancy and atopic diseases, confirming the complexity of asthma susceptibility and the potentially large number of factors that could influence risk.[72]

## IMMUNOLOGIC OCCUPATIONAL ASTHMA

### Specific IgE antibodies

The pathophysiology of immunologic OA usually involves an IgE-dependent mechanism. OA induced by IgE-dependent agents is similar to allergic asthma that is unrelated to work.[2-5,73-76] Many occupational sensitizers, particularly HMW agents (eg, flour and animal proteins), induce asthma by producing specific IgE antibodies. For some LMW agents (eg, chlorinated platinum salts, sulfonechloramide, trimellitic anhydride, and other acid anhydrides), the development of OA is accompanied by the production of specific IgE antibodies.[77,78] HMW agents act as complete antigens, whereas LMW chemicals must first react with autologous or heterologous proteins to produce a functional allergen. Cross-linking of allergens with a specific IgE antibody on the surface of mast cells, basophils, and possibly macrophages, dendritic cells, eosinophils, and platelets gives rise to a cascade of events that is responsible for inflammatory cell activation. There is subsequent synthesis and/or release of preformed and newly formed inflammatory mediators that then orchestrate the inflammatory reaction.

For most other LMW agents, the presence of specific IgE has been documented in only a small subset of affected workers without showing a consistent correlation with clinical symptoms. Specific IgE antibodies against plicatic acid have been detected in workers with and without asthma exposed to red cedar. Because the prevalence of detectable specific IgE did not differ between the 2 cohorts, it was concluded that the presence of these antibodies may be a manifestation of work exposure, but it did not necessarily prove a cause-and-effect relationship with the subsequent development of OA.[79]

Polyisocyanates and prepolymers cause OA, which has the clinical and pathologic features of atopic asthma, but do not consistently induce specific IgE antibodies.[2,80,81] Specific IgE antibodies to TDI in a range of 0% to 40% of workers have been reported.[82-85] There is a consensus about when IgE antibody is highly diagnostic,[86-89] but it has no sensitivity in detecting OA

induced by isocyanates. One possibility is that sensitization of the respiratory tract by isocyanates can be achieved via more than 1 immunologic mechanism, including those that are IgE antibody–independent. Another interpretation is that IgE antibody goes undetected for largely technical or methodologic reasons. Preparing suitable hapten-protein conjugates for detection of antibodies raised against chemical allergens presents major technical hurdles, and the use of inappropriate conjugates may cause the specific antibody to be missed.[84,90,91] Conjugates formed with isocyanate under reaction conditions that deviate substantially from those *in vivo* may be immunologically different and could lead to artifacts in serology studies. Thus, exposure of fluid-phase albumin (ie, in the epithelial lining fluid) to occupational levels of TDI vapors (approximately 20 ppb) results in different and more specific chemical conjugates. Recent studies have suggested that serum IgE, which recognizes such isocyanate vapor–exposed albumin, can be found in 40% to 50% of subjects with TDI and HDI asthma, but in only 1% to 4% of exposed asymptomatic individuals. In contrast, isocyanate-albumin conjugates made by reacting fluid-phase albumin with more concentrated liquid-phase isocyanate are less sensitive in detecting IgE and are more likely to be recognized by IgG from unexposed individuals.[92] It must also be emphasized here that monofunctional substitution of diisocyanate hapten appears to eliminate many false-positive reactions that occur after bifunctional substitution of the protein or even over substitution.

Moreover, it is possible that in some instances analyses have been conducted at times when induced IgE antibody is no longer detectable. It has been estimated that, after cessation of workplace exposure, the plasma half-life of detectable IgE antibody specific for the chemical allergen is in the range of 4 months to 2 years.[85,93] Likewise, it was observed that measurements of specific IgE antibody were more likely to be positive if blood samples were drawn from subjects within 30 days of the last exposure to the chemical allergen. Finally, the significance of IgE antibody in isocyanate-induced asthma has been challenged by the work of Jones et al.[94] The authors found a striking absence, within the bronchial mucosa, of RNA message for Cϵ, ϵ heavy chain, and IL-4, the cytokine that promotes the B lymphocyte switch to IgE synthesis, in patients sensitized to diisocyanates after specific inhalation challenge.

In contrast with the variability of immune responsiveness in diisocyanate-exposed workers, investigation of acid anhydride-induced asthma has revealed that IgE-mediated immune response may play a major role in the development of asthma. This may be because various compounds in this class of chemicals (eg, phthalic anhydride, trimellitic anhydride, himic anhydride) readily form highly allergenic epitopes after conjugation with autologous human proteins. Cutaneous puncture tests with these reagents have exhibited good diagnostic sensitivity.[95] RAST cross-inhibition studies using sera of anhydride-sensitized subjects have demonstrated that in some workers, specific IgEs are directed primarily against the haptenic ligand (eg, phthalic anhydride, himic anhydride), whereas in others, IgE antibodies are directed against new antigenic determinants with no evidence of hapten specificity.[96] A systematic immunologic investigation of acid anhydride chemicals demonstrated that ring structure, position of double bonds, and methyl group substitution may be critical determinants of IgE-mediated sensitization.[97]

A special role for IgE-mediated immunopathogenesis has been demonstrated in workers exposed to chlorinated platinum salts. These workers exhibit positive prick tests to very dilute concentrations of these salts.[98-101] Specific IgE tests also correlate with clinical respiratory symptoms.[102] It has been shown that skin test–positive platinum workers have higher serum total IgE levels than skin test–negative platinum workers. Moreover, it was observed that both high levels of IgE and cutaneous reactivity to chlorinated platinum salts persisted 4 years after cessation of exposure.[101]

If the role of specific IgE responses in asthma induced by LMW agents looks uncertain, the role of specific IgG responses to occupational agents seems even more complex. Serum-specific IgG may persist for many years after the last exposure to TDI.[103] The sensitivity of specific IgG in the diagnosis of TDI-induced asthma based on the results of specific inhalation challenge is higher than that of specific IgE, but the specificity is poor. Therefore, it has been suggested that IgG could be used to monitor the effect of exposure to diisocyanates before clinical disease appears.[103] For other occupational agents, conflicting results have been reported, showing that the prevalence of specific IgG is significantly higher in symptomatic workers, with no correlation with the level and duration of exposure,[104] or that the presence of specific IgG may be a response to high levels of exposure but is unrelated to the development of respiratory symptoms.[105,106]

From the previous discussion, it is apparent that the precise role of humoral antibodies in the pathogenesis of occupational asthma depends on the intrinsic nature of the sensitizing agent or immunogen, the conditions of exposure, and the susceptibility of the exposed workers. The presence of specific IgE antibodies may be highly diagnostic and prognostic in the case of HMW allergens and some LMW chemicals such as acid anhydrides and platinum salts. Under other circumstances, either specific IgE or IgG antibodies may be useful adjuncts in association with pulmonary function tests in following the evolutionary course of immunologically mediated asthma. Finally, when either specific IgE or IgG antibodies are present in both symptomatic and asymptomatic workers, their sole value may be as biological markers of exposure.

## IgE-independent immune response

Many LMW agents, such as plicatic acid and polyisocyanates or their prepolymers, cause asthma that has the clinical and pathologic features of atopic disease but, in most of the cases, is not consistently associated with the production of either specific IgE antibodies or upregulation of IgE receptors.[2,8,94] These features include similar symptoms and an excessive reaction to bronchoconstricting stimuli—the functional hallmark of both occupational and nonoccupational asthma. The histopathologic features of OA include airway infiltration of inflammatory cells (particularly eosinophils), activation of lymphocytes, and airway remodeling characteristically represented by increased thickness of subepithelial collagen, again similar to those of allergic asthma.[5]

The mechanisms by which chemicals induce OA are believed to be mainly related to a specific immune response. This does not necessarily imply an IgE-associated immunity, but possibly includes cell-mediated and mixed reactions. There is evidence that immune responses provoked by respiratory sensitizing chemicals are mainly of selective $T_H2$ type, and therefore are comparable to immune responses induced by protein respiratory allergens.[107,108] In addition, chemicals known to cause respiratory

allergy and OA in human beings elicit selective $T_H2$-type immune responses in mice.[109-113] Although the predominant immune response to chemical respiratory allergens may be of $T_H2$ type, it is important to acknowledge that other cells may play important support or regulatory roles. In an animal model, female BALB/c mice sensitized epicutaneously with HDI followed by intranasal challenge with HDI–mouse serum albumin conjugate developed HDI-specific IgG antibodies as well as lymphocyte and eosinophil lung infiltrates.[113] Inflammatory cells from the lung digest produced elevated levels of ***IL-5***, IL-13, and ***IFN-***$\gamma$ on restimulation with HDI–mouse serum albumin, indicating a mixed $T_H1$/$T_H2$ immune response. Interestingly, the optimal sensitizing dose for antibody production was higher than that for airway inflammation and cytokine production. This finding is in accordance with that of Matheson et al,[114] suggesting that a different mechanism may dominate for these outcomes. Herrick et al[112] reported that the respiratory inflammatory responses provoked in mice by an inhaled diisocyanate (HDI) are mediated by $CD4^+$ $T_H2$ cells, but that $CD8^+$ T lymphocytes are also induced; it is conceivable that these provide counterregulatory activity. Recently, the persistence of the respiratory responsiveness to TDI was examined in a mouse model.[115] Ventilatory and lung inflammatory responses decreased with increasing delay between dermal sensitization and challenge, despite persistent humoral signs of sensitization.

In human beings, Del Prete et al[116] and Maestrelli et al[117] observed that the majority of T-cell clones derived from patients with TDI-induced asthma were $CD8^+$ and capable of producing IL-5. These T lymphocytes may represent a $CD3^+/CD4^-/CD8^+$ population expressing the $\gamma/\delta$ ***T-cell*** receptor.[118,119] 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-$\gamma$ after stimulation with the conjugate of plicatic acid and human serum albumin.[120] This pattern of mixed $T_H1$/$T_H2$ cytokine production is compatible with both the nonatopic status and the airway mucosa eosinophilia present in these subjects. In agreement with the finding of eosinophilia and nonatopic status, an increase in both $T_H1$ and $T_H2$ cells in blood and sputum was observed in OA induced by specific inhalation challenges with HMW and LMW agents. Moreover, in this instance, peripheral blood regulatory T cells decreased in subjects with OA and increased in nonsensitized control subjects.[121] The speculation is that, in common with other forms of allergic disease, immune responses to sensitizing agents in healthy subjects and subjects with allergy are finely balanced between allergen-specific effector cells and regulatory cells.[122]

It has been shown that synthesis of IL-10, a key regulator of immune and inflammatory responses produced by peripheral blood regulatory T cells, is attenuated in persistent asthma compared with mild asthma.[123] A specific deficit of IL-10 secretion explains the reduced production of soluble human leukocyte antigen G (sHLA-G) molecules by PBMCs from subjects with moderate-severe asthma.[124] HLA-G antigens are nonclassic HLA class I molecules that exhibit tolerogenic and anti-inflammatory functions and have been associated with asthma susceptibility.[125-127] A recentstudy indicated that occupational exposure to diisocyanates was associated with high baseline levels of secretion of IL-10 by PBMCs *in vitro*, whether or not the exposed subjects had asthmatic symptoms. However, spontaneous production of sHLA-G by PBMCs was significantly higher in subjects with isocyanate asthma compared with asymptomatic exposed controls and subjects with nonoccupational allergic asthma.[128] These findings show that sHLA-G production and IL-10 secretion are influenced by workplace exposure to isocyanates and by development of asthma, and suggest further differences in the pathophysiology of OA compared with nonoccupational allergic asthma. Thus, the common view, though controversial, of an association between asthma phenotype and reduced IL-10 production[123,124,129,130] does not seem to be true in isocyanate-induced asthma.[128]

As for most chemicals that can induce OA, the fate of inhaled diisocyanates in the human body and the nature of the antigen that is eventually produced are largely unknown.[131] Isocyanate toxicity to human epithelial cells was prevented by physiologic levels of extracellular glutathione.[132] Human monocytes *in vitro* take up diisocyanate-albumin conjugates and undergo activation with upregulation of lysosomal genes and increased production of monocyte chemoattractant protein-1, an autocrine C-C chemokine, and chitinase-1, a soluble pattern-recognition receptor.[133] Repetitive antigenic stimulation of diisocyanate asthmatic PBMCs in culture revealed that these cells synthesized TNF-$\alpha$, a non–IgE-dependent proinflammatory cytokine, and monocyte chemoattractant protein-1, but not IL-4 or IL-5.[133] Taken together, these observations are consistent with the hypothesis that isocyanate-induced upregulation of immune pattern-recognition receptors by monocytes and release of damage-associated molecular patterns from injured epithelium may be a mechanism by which isocyanates stimulate the human innate immune responses and consequently influence the hypersensitivity reactions.[67]

## NONIMMUNOLOGIC OA

Establishing mechanisms of irritant-induced asthma is challenging. One reason is that unintentional exposure to high concentrations of respiratory irritants, whether particulates or chemicals, at or outside the workplace can induce the new onset of asthma.[134] Respiratory health consequences of these exposures can occur from both occupational exposure and environmental disasters.[135] For example, irritant-induced asthma is one of the respiratory consequences observed in workers in the New York City Fire Department after the collapse of the World Trade Center (WTC) on September 11, 2001. One year later, 16% of these workers met the criteria for irritant-induced asthma.[136] To date, the main consequence is the "WTC cough syndrome" (chronic rhinosinusitis, asthma, and/or bronchitis, often complicated with gastroesophageal reflux dysfunction). Incidence and severity have been linked to WTC dust exposure intensity.[137]

Another challenge in establishing mechanisms of irritant-induced asthma is that the main target for the injury has not been identified.[138] The target could be bronchial epithelium, which becomes denuded and loses its protective properties. A number of consequences arise from injured epithelium, including exposure of nerve endings leading to neurogenic inflammation; loss of relaxing epithelial factors; release of inflammatory mediators such as ***leukotrienes*** $B_4$ and $C_4$ and proinflammatory cytokines; secretion of growth factors for epithelial cells, smooth muscle cells, and fibroblasts; and matrix degradation.[139-141] In 1 subject with irritant-induced asthma caused by exposure to chlorine, sequential bronchial biopsies showed considerable epithelial desquamation with an inflammatory exudate and swelling of the subepithelial space a few days after the exposure. Later, the inflammatory exudate persisted, even though the basal cells regenerated the epithelium. Three to 5 months later, the

inflammatory exudate was reduced, and the epithelium was greatly improved.[141]

Because irritants can disrupt epithelial structure, allergens could cross the epithelium more easily, leading to sensitization and asthma.[142-145]

In view of the challenges involved in clarifying the mechanisms of irritant-induced asthma, and because population-based studies have shown a high relative risk of asthma in jobs with expected low to moderate exposure to irritants,[146,147] more attention should be focused on the effects of chronic low-level and intermittent high-level exposures. In addition, by performing studies focused on host and environmental factors involved in exposure to low levels of irritants, we could significantly increase our understanding of the pathogenesis of irritant-induced asthma. Finally, by recruiting subjects exposed to low levels of irritants, we could identify representative samples of exposed subjects rather than limiting our knowledge to historical data or to case reports of irritant-induced asthma.

## CONCLUSION

Understanding the pathogenesis of OA is a crucial step toward optimal prevention and management of the disease. Identification of structure-activity relationships for causal agents of OA shows great promise for understanding induction of airway sensitization. A dose-response relationship between the level of exposure and the development of OA is well established for several sensitizing agents. Recent evidence indicates that chemical respiratory allergens may induce respiratory tract sensitization by routes different from inhalation, mainly dermal exposure.

Genetic susceptibility, probably in combination with occupational and environmental exposures, can affect the development of OA by modifying the impact of a given gene on complex phenotypes. In this respect, interactions between genes and environment, in part through epigenetic mechanisms, seem to be more effective than the 2 components considered separately.

The pathogenesis of OA caused by LMW agents remains largely uncertain. Available data suggest that T-cell subset and cytokine profile involved in OA caused by LMW agents may differ from those operating in atopic asthma. However, further research is needed to clarify the relationships between this immunologic and inflammatory scenario and the initiation and perpetuation of OA.

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