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Animal Models of Occupational Asthma: Tools for Understanding Disease Pathogenesis

Victor J. Johnson and Michael I. Luster
Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, NIOSH/CDC, Morgantown, West Virginia, U.S.A.

INTRODUCTION

Occupational asthma is the most frequently reported occupational respiratory disease in industrialized nations, and numerous low-molecular-weight chemicals and high-molecular-weight proteins encountered in the workplace are known or suspected causative agents (1). Classical and novel animal models are being used to investigate exposure determinants, epitope identity, and the role played by the immune system in occupational asthma in order to recapitulate disease phenotype and further current understanding of its pathogenesis. Important aspects of exposure determinants that can be addressed and controlled using animal models include (*i*) relevant routes of exposure including respiratory and/or skin, (*ii*) exposure dose, (*iii*) exposure duration and frequency, and (*iv*) determination of relevant epitopes. In addition, well-defined genetics of animal models present unique opportunities for detailed investigations into pathogenic mechanisms through the use of transgenic models and antibody neutralization strategies. The data generated through the use of animal models is instrumental for risk assessment used to refine workplace exposure limits. Numerous guinea pig, rat, and mouse models of occupational asthma have been developed to address these issues. It is likely that key molecular and cellular events will be identified through the use of these models, potentially leading to new treatment modalities that may be specific for these classes of asthmogen and will aid in establishing more protective workplace exposure limits.

OCCUPATIONAL ASTHMA PRODUCED BY LOW-MOLECULAR-WEIGHT CHEMICAL HAPTENS

Among the major low-molecular-weight chemical classes known to induce occupational asthma, diisocyanates and organic acid anhydrides have been studied the most.

Occupational asthma has also been reported in workers exposed to chemicals from wood products such as plicatic acid found in western red cedar resin. Low-molecular-weight chemicals are usually too small to be recognized by the immune system in their native form. As such, low-molecular-weight chemicals act as haptens that bind to host proteins, and result in new antigenic determinants capable of eliciting immune responses. Although some progress has been made, the nature of these conjugates in humans and the mechanisms underlying occupational asthma caused by low-molecular-weight chemicals, for the most part, remain to be elucidated. The following section will focus on the use of animal models of occupational asthma caused by low-molecular-weight chemicals, to further the understanding of disease pathogenesis.

Diisocyanate Asthma

The Clinical Condition

Diisocyanates are highly reactive, low-molecular-weight chemicals that represent the leading cause of occupational asthma, a disease that accounts for nearly 10% of all adult-onset asthma (2). The three major diisocyanates encountered in the workplace include toluene diisocyanate (TDI), diphenyl-methane diisocyanate (MDI), and hexamethylene diisocyanate (HDI), and all are used in a variety of industries including polyurethane foam manufacturing, auto body painting and repair, and plastics manufacturing. It is estimated that as many as 5% of workers exposed to diisocyanates develop asthma and the disease may persist indefinitely, even in the absence of continued exposure.

Occupational asthma to diisocyanates is usually characterized by a variable lag period consisting of months to years of exposure prior to the development of symptoms (3). Once sensitized to diisocyanates, low-level exposures, even those below permissible workplace limits, can induce clinical onset of disease (4). Clinical expression of diisocyanate asthma displays characteristics similar to those present in allergic asthma, suggesting common immunopathogenesis. These include development of immediate, late, or dual asthmatic responses following chemical exposure. In addition, patients often develop persistent airway hyperresponsiveness (AHR) to nonspecific stimuli that can last for years, even in the absence of continued exposure, and complete recovery of lung function may never be achieved (5–7). Recent evidence shows a correlation between recovery of AHR and persistence of eosinophilic inflammation in diisocyanate asthma, persistent inflammation being associated with poor or no recovery (8). Pathological features of diisocyanate asthma also include goblet cell metaplasia, mucus hypersecretion, upper and lower respiratory tract inflammation consisting of leukocytic infiltration of the airway mucosa, and leukocyte extravasation leading to luminal eosinophilia and neutrophilia. There is also evidence that humans demonstrate airway remodeling in diisocyanate asthma, which is characterized by subepithelial thickening (collagen deposition) and fibrosis (9). Airway remodeling may be an important determinant for persistence of diisocyanate-induced asthma.

Despite the clinical similarities to allergic asthma, human data on the immune mechanisms of diisocyanate asthma highlight important differences between the two diseases. The immunopathogenesis of allergic asthma involves recruitment of $CD4^+$ T lymphocytes to the lung and production of T-helper 2 (T_H2) type cytokines including interleukin (IL) -4, -5, and -13. $CD4^+$ lymphocytes and T_H2 cytokines are integral to the development and severity of diisocyanate asthma, but recent evidence also points to an important role for $CD8^+$ lymphocytes and T_H1 cytokines, including

IFNγ (10–12). Another difference between the etiopathogenesis of diisocyanate asthma and allergic asthma is the low prevalence of specific IgE antibodies in diisocyanate asthma. Specific IgE antibodies are only detectable in 5% to 30% of diisocyanate asthmatics, suggesting that other immune mechanisms may be responsible for disease progression (13,14).

Animal Models of Diisocyanate Asthma

Guinea Pig Models. The first animal studies of diisocyanate asthma were conducted in guinea pigs and identified this class of chemicals as respiratory toxins that are capable of producing acute airway irritation as well as sensitization. Guinea pig models display many of the clinical features of human diisocyanate asthma including AHR, epithelial injury, and neutrophilic inflammation (15–17). Guinea pig models also demonstrate airway eosinophilia and a late phase asthmatic reaction characteristic of human disease (18,19). The guinea pig has been used as a model to demonstrate that isolated airway exposure to TDI results in skin sensitization and that dermal exposure results in pulmonary hypersensitivity (20,21).

The classic guinea pig model developed by Karol involves inhalation exposure to vapor phase TDI (0–7.6 ppm) for three hours per day for five consecutive days followed by a three week–rest period prior to inhalation challenge with aerosolized TDI–guinea pig serum albumin (GPSA) conjugate (22,23). Additional animals were exposed to 20 ppb TDI for six hours per day for 70 days followed by TDI–GPSA challenge three weeks later. Subacute inhalation exposure (five days) at levels ≥120 ppb TDI were required for specific antibody production, the titer of which increased progressively with exposure concentrations above 120 ppb. Changes in pulmonary function were observed at ≥360 ppb TDI. Subchronic inhalation to low concentrations of TDI (20 ppb for 70 days) did not result in immune sensitization or altered lung function (22). Interestingly, higher TDI antibody titers in guinea pigs did not always correlate with changes in pulmonary function, suggesting that different mechanistic pathways are involved. Aoyama et al. (17) showed a similar relationship between immune sensitization and changes in pulmonary function following TDI vapor challenge. Their model also demonstrated that nonsensitizing concentrations of TDI were capable of eliciting pulmonary reactivity in previously sensitized guinea pigs. This is an important discovery and suggests that acceptable workplace atmospheres (<5 ppb for TDI) may result in potential life-threatening reactions in sensitized workers. In fact, workplace levels of TDI as low as 1 ppb have been shown to invoke respiratory distress in sensitized workers (24). Using a similar sensitization protocol, Raulf et al. (25) demonstrated elevated numbers of eosinophils and increased leukotrienes in the bronchoalveolar lavage fluid (BALF). Huang et al. (26) used the same model to demonstrate that exposure to TDI–GPSA conjugates stimulated histamine release from lung mast cells in guinea pigs when sensitized with TDI concentrations sufficient to elicit respiratory responses. The authors suggested that mast cell stimulation might underlie the different sensitivity of antibody and pulmonary responses.

Additional studies using guinea pigs investigated the effects of combined inhalation and intradermal exposure to diisocyanates. Pauluhn exposed guinea pigs to TDI by inhalation (0–7 ppm, three hours per day for five days), with or without intradermal injection of 0.3% TDI (27). Challenge consisted of TDI vapor or TDI–GPSA conjugates three weeks following initial exposure. Lower incidence and reduced intensity of respiratory responses were observed in guinea pigs

challenged with TDI vapor compared to that observed after exposure to TDI–GPSA conjugate with an approximately equal TDI concentration. This suggests that only a portion of the reactions between TDI and biological molecules resulted in haptens capable of being recognized by the immune system. This notion is supported by the observation that TDI–GPSA was only one of several adducted proteins present in the BALF of guinea pigs following inhalation exposure (28). The TDI–albumin conjugates are known to cause sensitization, but the identity and immune reactivity of other conjugates formed in the lungs of guinea pigs are presently unknown (29). Exposure of guinea pigs to HDI following a similar sensitization/challenge paradigm demonstrated that both inhalation and intradermal exposure result in sensitization, although antibody and respiratory responses were greater following intradermal injection (30). Challenge with free HDI did not result in appreciable changes in respiratory parameters in contrast to the marked respiratory responses elicited by HDI–GPSA conjugates, similar to the observation made in experiments involving TDI and TDI–albumin conjugate. The authors suggested that the reaction of free HDI with the nasal mucosa may have effectively reduced the bronchial concentration below a threshold level required to provoke pulmonary responses in sensitized animals.

Guinea pigs have also been used to investigate the role of dermal and intradermal exposure in the development of diisocyanate asthma (18,21,27,30–32). In a model developed by Karol et al. (21) a single epicutaneous exposure to 50 μL of TDI (1% to 100%; 25 μL applied to two shaved dorsal sites) was followed two weeks later by a bronchial provocation challenge with TDI vapor and/or TDI–protein conjugates. Contact hypersensitivity and antibody production were consistently detected in animals sensitized with >10% TDI. Consistent with this finding, a single dermal exposure to MDI at concentrations as low as 10% resulted in a dose-dependent increase in specific antibody production (32). These models were able to demonstrate respiratory hypersensitivity following skin exposure in approximately 25% to 30% of the guinea pigs topically exposed to TDI or MDI, following inhalation challenge with the respective chemical.

The immunopathologic response in the lung to an inhalation challenge has been characterized in guinea pigs following intradermal injection of TDI (18). In this model, animals received intradermal injections of neat TDI (50 μL for every two dorsal sites) once a week for three weeks, followed seven days later by inhalation challenge with 5 to 20 ppb of TDI vapor. Challenged guinea pigs showed elevated TDI-specific antibodies and TDI challenge increased blood eosinophil numbers. Increased numbers of eosinophils and mast cells were observed in the submucosa and lamina propria of the airways as early as six hours postchallenge in sensitized animals. Pulmonary infiltration of $CD4^+$ lymphocytes was also evident and administration of anti–IL-5 antibody reduced eosinophil accumulation, supporting a role for T_H2 immunity in TDI asthma. Huang et al. (33) demonstrated that intradermal injection of neat TDI on days one and six resulted in TDI–GPSA specific and nonspecific AHR to methacholine in guinea pigs challenged with TDI–GPSA on day 21. In contrast to the intradermal model developed by Mapp et al. (18), increased eosinophils were not observed in this model. This may be related to slight variations in the frequency and timing of intradermal injection or the use of TDI vapor versus TDI–GPSA conjugates for challenge.

The guinea pig has also been used to study potential neurogenic mechanisms related to, or initiated by diisocyanate-induced inflammation in the lung. Intranasal application of 5% TDI for several weeks resulted in increased immunoreactivity of

substance P (SP) and calcitonin gene–related peptide (CGRP) in the nasal mucosa concomitant with nasal allergy symptoms (34). Pretreatment of guinea pigs with capsaicin abrogated nasal allergy symptoms and pulmonary hyperreactivity to TDI (34,35). More recently, Mapp et al. (36) demonstrated decreased SP and CGRP immunoreactivity in the central airways of sensitized guinea pigs following TDI challenge. Together, these findings support a role for the interaction of neurogenic peptides and inflammatory mediators in TDI asthma.

Rat Models. Rat models have not received as much attention as guinea pig or murine models for studying the mechanisms of diisocyanate asthma, although rats have been used extensively to study the irritant effects of these chemicals. A model of phenyl isocyanate–induced asthma has been developed and displays airway inflammation characteristic of asthma (37). Wistar rats were exposed to nonirritating concentrations of phenyl isocyanate (0–10 mg/m^3) for six hours per day, five days a week, for two weeks. Although the rats did not receive a specific challenge, histopathological analysis revealed goblet cell metaplasia at concentrations >4 mg/m^3 in addition to smooth muscle hypertrophy, epithelial desquamation, mucus hypersecretion, and eosinophil infiltration at concentrations >7 mg/m^3. These characteristic features of asthmatic inflammation were accompanied by decreased forced expiratory flow and quasistatic lung compliance. The pathological changes in response to phenyl isocyanate were reversed within two months following the cessation of exposure. Because rats were not challenged in this study, phenyl isocyanate challenge following a rest period will be important to demonstrate the utility of this model for studying occupational asthma.

Wistar rats have also been used to study TDI asthma following intranasal exposure. Rats were exposed to 5 μL of 10% TDI applied to each nostril for seven consecutive days followed by nasal provocation challenge with 5 μL of 5% TDI one week later. Marked increases in eosinophils, lymphocytes, macrophages, and neutrophils were observed in the BALF of sensitized rats following challenge. TDI challenge also increased IL-4 and -6 levels in the BALF as well as eosinophil infiltration in the peripheral airways. This model lends support to the involvement of T_H2 immunity in the development of airway inflammation in TDI asthma. A similar nasal sensitization and challenge study design in Brown Norway rats showed increased histamine receptor and histamine decarboxylase expression in the nasal mucosa, suggesting that the histamine pathway may be important in the observed nasal allergy symptoms following TDI exposure (38,39).

Mouse Models. Mice offer the distinct advantage of a better-defined genome than guinea pigs or rats and the availability of antibody reagents and transgenic strains to investigate detailed mechanistic pathways. Early studies using murine models demonstrated the involvement of the immune system in immunological reactivity to diisocyanates, as topical exposure to TDI induced production of TDI-specific IgE antibodies and contact hypersensitivity (40–43). It was later shown that CD4$^+$ and CD8$^+$ T lymphocytes were important effector cells in these responses (44). However, these early models lacked evidence of respiratory inflammation. Recently, Scheerens et al. demonstrated that epicutaneous sensitization to TDI led to increased tracheal hyperreactivity to carbachol (45). Male BALB/c mice were exposed to 1% TDI epicutaneously on days 0 and 1, followed by intranasal challenge eight days later with 20 μL of 1% TDI. Importantly, lymphocyte involvement in the tracheal hyperreactivity was demonstrated by adoptive transfer experiments. Changing the sensitization schedule to six weeks, with skin exposure to TDI on days 0, 7, 14, 21, 28, and 35 followed by intranasal challenge, resulted in a more robust

respiratory response (46). Necrosis was evident in nonsensitized mice, indicating that the airway pathology was likely owing to the irritancy of 1% TDI intranasal challenge. Lung inflammation, characteristic of diisocyanate occupational asthma in humans, was not evident. A significant contribution of this model was the demonstration that altering the length of sensitization and cumulative dose resulted in different immunological processes, which may help explain the diversity of symptoms exhibited in humans.

The role of inflammatory mediators in TDI-induced asthma was recently demonstrated using a murine model employing subcutaneous sensitization and inhalation challenge (47). Female C57BL/6 mice were sensitized by multiple subcutaneous injections of TDI (20 μL neat TDI, day 1; 20 μL of 20% TDI, days 4 and 11) and then challenged by inhalation with 100 ppb TDI vapor on days 20, 22, and 24. AHR to methacholine was accompanied by mucus metaplasia, inflammation, and cytokine expression in the airways. However, inflammation was evident only in the upper airways with lymphocyte and neutrophil infiltration and inflammatory cytokine expression in the nares and trachea, respectively. Matheson et al. (47) found that histopathological changes and inflammatory cell involvement were not evident in athymic mice, supporting the hypothesis that diisocyanate occupational asthma is dependent upon specific immunity. Using tumor necrosis factor α (TNFα)–deficient mice, Matheson et al. (48) identified TNFα as an integral proinflammatory cytokine in disease development. Interestingly, however, mice developed TDI-specific IgG antibodies regardless of their TNFα status, supporting the hypothesis that IgG antibodies are better markers of exposure than disease. Similarly, diisocyanate-specific IgG levels generally correlate better with exposure than disease in humans (49).

In recent studies, female BALB/c mice were sensitized epicutaneously with 0.1% or 1% HDI on days 0 and 7 followed by intranasal challenge with HDI–mouse serum albumin (MSA) (50 μL at 2 mg/mL) conjugate on days 14, 15, 18, and 19 (50). Mice developed HDI-specific IgG antibodies as well as lymphocyte and eosinophil lung infiltrates. Inflammatory cells from the lung digest produced elevated levels of IL-5, -13, and IFNγ upon restimulation with HDI–MSA, indicating a mixed T_H1/T_H2 immune response as seen in humans (10,51). The optimal sensitizing dose for antibody production was higher than that for airway inflammation and cytokine production. This finding is in concordance with that of Matheson et al. (48) suggesting that differing mechanism may be dominant in these outcomes. In addition to the dose, frequency of dermal exposure greatly influences respiratory responses to TDI in sensitized BALB/c mice (52). The role of $CD4^+$ and $CD8^+$ T lymphocytes was also investigated by Herrick et al. (53) who showed that the presence of eosinophils in the BALF was markedly reduced in mice deficient in $CD4^+$ T lymphocytes, despite an intact contact hypersensitivity response. This finding is consistent with their previous work in which attenuated inflammatory responses were observed in IL-4 and -13 knockout mice (50). In contrast, mice deficient in $CD8^+$ T lymphocytes showed a partial reduction in contact hypersensitivity, but no change in the presence of lung inflammatory cells. Together, these findings suggest a dominant role for $CD4^+$ T lymphocytes and T_H2 immunity in HDI-induced lung inflammation. Herrick et al. (53) also showed an influence of mouse strain on the development of diisocyanate asthma in mice.

Lee et al. (54–56) developed a murine (BALB/c) model of TDI asthma using intranasal sensitization with 3% TDI given for two cycles of five daily instillations with a three week–rest period between each cycle, and inhalation challenge with

1% TDI a week later. This was the first model to demonstrate AHR following sensitization via the respiratory tract in mice. In addition, significant pathology in the lower airways was evident and was accompanied by a variety of inflammatory mediators. Administration of specific inhibitors of two of these mediators, vascular endothelial growth factor, or matrix metalloproteinase (MMP), markedly reduced the disease (55,56). Doxycycline, a tetracycline antibiotic capable of inhibiting MMPs, also reduced MMP-9 expression and disease pathogenesis following TDI challenge (57). More recently, this model was employed to demonstrate that TDI challenge decreased the lung ratio of MMP-9/tissue inhibitor of metalloproteinase (TIMP)-1, starting at seven hours postchallenge and persisting for at least 72 hours (58). A balance between MMPs and their TIMP inhibitors is essential for maintenance of homeostasis of the lung's extracellular matrix, and these studies support the notion that there may be a disruption of this balance in the pathogenesis of occupational asthma.

To examine the pathophysiological responses in occupational asthma following inhalation exposure, the predominant route of exposure in the workplace, our laboratory exposed C57BL/6J mice to 20 ppb TDI vapor via inhalation for four hours per day, five days a week for six weeks, followed 14 days later by a one-hour inhalation challenge with 20 ppb TDI. Challenge with TDI resulted in a marked increase in AHR to methacholine (59). Histopathological changes in the lung included goblet cell hyperplasia, epithelial damage, mucus hypersecretion, and eosinophilia. Serum levels of total IgE and TDI-specific IgG antibodies were elevated in sensitized mice, and serum transfer resulted in sensitivity to TDI challenge in naive animals. A role for IgE and possibly other reagenic antibodies was supported by the inability of TDI to sensitize FcErIg transgenic mice, which lack the γ-chain subunit of the FcγRI, FcγRIII and FcεRI receptors and the inability of heat-treated serum to transfer disease to naive mice (59). Elevated expression of IL-4, -5, and IFNγ was observed in the lung, consistent with a mixed T_H1/T_H2 cytokine profile as suggested by findings in other animal models and humans (10,50,51,53). Interestingly, a single inhalation exposure to 500 ppb TDI, which represents an irritating concentration, for two hours followed two weeks later by 20 ppb TDI challenge resulted in an elevated nonspecific AHR and epithelial changes in the lung consistent with asthma, although there was a lack of eosinophils (59). This exposure paradigm was examined to determine whether accidental spills in the workplace that cause respiratory irritation can lead to sensitization and the development of occupational asthma.

Adoptive transfer experiments were conducted using lymphocytes from TDI-sensitized mice to determine the role of specific immunity in the response to TDI (59). Airway reactivity was increased in naive mice that received unfractionated lymphocytes, purified B cells or purified T cells, from sensitized mice (59). That the isocyanate-induced occupational asthma involves a mixed T_H1/T_H2 phenotype was supported in studies using various transgenic mouse models sensitized to TDI subchronically, as described above. For example, IFNγ-deficient mice demonstrated a reduction in airway inflammation, serum antibody levels, and histopathological changes, but to a lesser degree than that observed in IL-4–deficient mice (60). In humans with occupational asthma, T_H2 cells comprise only a small portion of the sensitized T cells in the airways, with most cells representing a T_H1 phenotype. In fact, the majority of T cell clones derived from bronchial mucosa of patients with isocyanate-induced asthma present a $CD8^+$ phenotype, and produce IFNγ and IL-5 (12,61). In this respect, we observed that either $CD8^+$ or $CD4^+$ deficiency prevented TDI asthma following subchronic treatment (60).

Acid Anhydride Asthma

The Clinical Condition

Acid anhydrides are low-molecular-weight organic chemicals used in the production of alkyd, epoxy, and polyester resins for plastic and paint manufacturing. Acid anhydrides commonly encountered in the workplace include trimellitic anhydride (TMA), phthalic anhydride (PA), and maleic anhydride, as well as many derivatives of these compounds. Exposure to these agents can result in direct irritation, immunological respiratory hypersensitivity, or a combination of both (62). Direct irritant effects are usually associated with high exposure concentrations (62). Although workplace concentrations have been reduced in recent years, intermittent spikes in ambient levels in air can result in irritation despite the time-weighted average being within an acceptable range (62).

Respiratory hypersensitivity reactions to acid anhydrides include three clinically distinct syndromes: allergic asthma/rhinitis, late respiratory systemic syndrome, and pulmonary disease anemia (63). Sensitized workers present asthmatic symptoms including a T_H2 phenotype with early- and late-phase AHR and eosinophilic inflammation, with or without pulmonary hemorrhage. The particular syndrome presented in exposed workers is related to the nature of the immune response as well as the exposure route and concentration, with asthma resulting at lower concentrations. The immune response to acid anhydrides in humans includes the production of IgE and IgG antibodies specific for chemical–protein conjugates including haptenized serum albumin (64,65). Cell-mediated immune responses have also been reported in humans. It is thought that specific IgE antibody production and T_H2 cytokines are related to the asthma/rhinitis syndrome, whereas specific IgG antibody production and cell-mediated responses are related to the late respiratory systemic syndrome/hypersensitivity pneumonitis (63). As with other allergens, atopy and HLA genotype have been identified as important risk factors for the development of acid anhydride–induced asthma (66–68).

Animal Models of Acid Anhydride Asthma

Guinea Pig Models. Inhalation exposure to vapor and aerosol forms of acid anhydrides is a primary concern in the workplace. Guinea pigs have been shown to develop immunological sensitization following inhalation exposure. Homocytotropic antibodies were detected in the serum of guinea pigs exposed to atmospheres containing TMA dust ($1–100\,mg/m^3$) for three hours per day, for five consecutive days (69). Despite evidence of sensitization, inhalation challenge with TMA–GPSA failed to elicit changes in the respiratory rate in this model. In contrast, Pauluhn and Eben showed that challenge with TMA dust or TMA–GPSA induced an immediate change in the breathing pattern indicative of an early asthmatic response in guinea pigs sensitized to TMA via inhalation (70). The differences between the two studies may be related to the sensitization exposure paradigms employed. Exposure of guinea pigs to PA dust ($0.5–5.0\,mg/m^3$) through inhalation resulted in dose-dependent production of allergic IgG_{1a} antibodies with detectable antibodies present even at the lowest concentration tested (71). Challenge of sensitized animals with TMA–GPSA, but not TMA dust, resulted in changes in respiratory rate indicating a pulmonary hypersensitivity response. Pathological changes included hemorrhagic foci and alveolar hemorrhage, which were similar to changes observed in workers exposed to acid anhydrides.

The intradermal sensitization route was used in a number of guinea pig studies of acid anhydride respiratory effects. Hayes et al. (72) sensitized guinea pigs using intradermal injection of 0.1, 0.3, or 30% TMA in corn oil as an acute exposure or with four injections over two weeks. TMA-specific IgG_1 antibodies were detected in the serum of all animals receiving TMA, while no specific antibodies were detected in the control animals. Anaphylactic IgE antibodies, specific for TMA, were also present in the sera from low-dose sensitized guinea pigs (73). Challenge consisted of intravenous injection of TMA–GPSA, which resulted in elevated pulmonary inflation pressure in half of the sensitized guinea pigs. This study showed that guinea pigs exposed to either single or repeated low doses of TMA during sensitization had greater pulmonary responses to challenge than those sensitized with a high dose despite high antibody titers in the later group. Challenge with TMA–GPSA directly into the trachea resulted in immediate increases in lung resistance and significant microvascular leakage in low-dose sensitized animals (73). Inhalation challenge with TMA dust induced AHR to acetylcholine in high-dose sensitized animals, which was accompanied by eosinophil infiltration into the lung tissue and increased mucus secretion (74,75). A strong correlation between eosinophilia and AHR was shown after inhalation of TMA dust in guinea pigs that were sensitized via intramuscular injection of TMA–BSA (bovine serum albumin) in complete Freund adjuvant (76). Decreased respiratory rates were also evident following challenge with TMA or PA dust in animals sensitized to the respective chemical (31). Challenge with TMA dust, but not TMA–GPSA, induced mild changes in airway resistance and eosinophilia in lungs of nonsensitized guinea pigs, suggesting that challenge with protein-conjugated TMA may not accurately reflect occupational exposures (77).

Using a similar intradermal sensitization protocol including two doses of 0.3% TMA administered 24 hours apart, Arakawa et al. (78) showed that TMA-specific IgG_1 antibodies were elevated within two weeks following sensitization and remained elevated for more than eight weeks. Intratracheal challenge with TMA–GPSA induced plasma extravasation, evidenced by Evans blue dye leakage into the lung, which occurred as early as one week after sensitization and increased until eight weeks. In contrast, the challenge increased lung resistance as early as one week after sensitization, although this response declined with time following sensitization. Systemic antibody production correlated with changes in microvascular permeability, but not with airway resistance. These findings do not preclude the involvement of TMA-specific IgG in AHR because the administration of a combination of TMA-specific IgG_1 and IgG_2 antibodies to naive guinea pigs resulted in significant elevation of AHR following the TMA–GPSA challenge (79).

The role of inflammatory mediators and the complement system in acid anhydride–induced respiratory disease was studied using similar guinea pig models. Intravenous administration of antagonists of histamine, cyclooxygenase, platelet activating factor, lipoxygenase, or thromboxane synthase significantly attenuated changes in lung resistance following TMA–GPSA, whereas only inhibitors of histamine, cyclooxygenase, and lipoxygenase attenuated plasma extravasation. These results suggest that arachidonic acid metabolism may play different roles in the observed changes in lung resistance versus changes in microvascular permeability following acid anhydride exposure (80–82). Treatment with cyclosporine A during sensitization also attenuated changes in lung resistance and microvascular leakage (83). A similar model was employed to demonstrate a critical role for complement activation in disease pathogenesis. Elevated levels of the C3a complement activation product were evident in the lungs of sensitized guinea pigs following challenge with

TMA (84). Administration of cobra venom factor, a potent inhibitor of complement activation, markedly attenuated bronchoconstriction and microvascular leakage (85). Together, these observations suggest that complement activation is an important pathway involved in acid anhydride–induced respiratory disease.

Rat Models of Acid Anhydride Asthma. The pathogeneses of TMA-induced pulmonary hemorrhage and hypersensitivity pneumonitis have been investigated in Sprague-Dawley rats (86). Rats were exposed to dry TMA powder (0–300 mg/m^3) by inhalation, six hours per day, five days a week for two weeks. This exposure regimen resulted in elevated lung weights, grossly observable external hemorrhagic foci, alveolar hemorrhage, and pulmonary macrophage and leukocyte accumulation, all of which were dose-dependent and undetectable 12 days after exposure (86–88). Challenge on day 26 with TMA dust at the same concentration as the original exposure induced significant lung pathology although the severity was less than that observed after 10 days of exposure (86). IgG, IgA, and IgM antibodies specific for TMA–rat serum albumin (RSA) could be detected in the serum and BALF (88,89). Subsequent studies showed that specific antibody production was dependent on exposure dose and correlated with increases in lung weight and the number of hemorrhagic foci in the lung (90). Passive transfer of serum from sensitized rats into naive recipients resulted in lung injury following a single challenge with TMA (91). These studies suggest that lung pathology observed in this model is likely immunological in origin. It is important to note that chronic, low-dose inhalation exposure to naive rats can also lead to changes in respiratory physiology accompanied by eosinophilia and other asthma-associated pathology (92).

The Brown Norway rat, which is genetically predisposed to favor T_H2 responses, has also been used to study acid anhydride–related respiratory effects. A single intradermal injection of 0.3% TMA in corn oil resulted in immunological sensitization, as evidenced by elevated levels of TMA-specific IgG and IgE antibodies (93). Challenge of sensitized rats with aerosolized TMA–RSA resulted in eosinophil infiltration into the bronchial wall. Lungs from TMA sensitized rats also show epithelial lesions and a trend toward increased nonspecific AHR following a single challenge. Multiple challenges over a period of seven days resulted in increased levels of TMA-specific IgG antibodies in serum and a significant increase in nonspecific AHR. In fact, multiple challenges with 0.003% TMA–RSA produced a much greater pulmonary response than a single 0.03% challenge dose (94). Extending the challenge exposure to five days a week for nine weeks resulted in considerable airway remodeling characterized by airway wall thickening and luminal narrowing, goblet cell metaplasia, and smooth muscle thickening (95). This model reproduces many of the pathological features of human asthmatic response to TMA, making it suitable for the investigation of underlying mechanisms.

Direct contact with skin represents a substantial concern for workers exposed to acid anhydrides. As such, dermal application of dry TMA powder has been used to sensitize Brown Norway rats to acid anhydrides. Zhang et al. (96) applied dry TMA powder (0–20 mg) to the shaved back of rats followed by occlusion overnight on days 0, 7, 14, and 21. TMA–RSA specific IgG and IgE were detected in the serum of half of the rats exposed to 0.3 mg of TMA. All rats developed antibodies in a dose-dependent manner at exposures above this dose. The production of TMA-specific antibodies was also time dependent, appearing by day 14 and peaking by day 28. This model successfully demonstrated immunologic sensitization following dermal exposure to TMA, suggesting that this may be a potential route for human sensitization. More importantly, rats sensitized by the dermal route developed significant

TMA-specific early- and late-phase obstruction following a challenge with dry TMA powder via inhalation (97). A similar biphasic airway response can be observed in sensitized workers upon reexposure. Dermal sensitization and inhalation challenge has also been shown to induce eosinophil infiltration and goblet metaplasia in sensitized rats (92,98).

Mouse Models. Numerous studies in mice show that dermal exposure results in immunologic sensitization characterized by IgG and IgE antibody production and predominantly T_H2 cytokine production in the draining lymph node, following dermal challenge (99–101). Nevertheless, little effort has been applied to developing murine models for acid anhydride–induced asthma. Inhalation exposure to 5 mg/m^3 of TMA for one hour per day for three days resulted in the production of specific IgE and IgG antibodies in BALB/c mice, although no measures of asthma were examined (102). Subsequently, Regal et al. (103) developed a model in which BALB/c mice were sensitized by intradermal injection of 3% TMA in corn oil on days 1 and 3, followed by an intratracheal boost with TMA–MSA (30 or 400 μg) on day 12 and subsequently challenged on days 19, 22, and 23 via the intratracheal route. This exposure paradigm resulted in lung inflammation dominated by eosinophils. With further characterization, this model may prove useful for determining mechanisms and mediators responsible for causing eosinophilia, characteristic of human occupational asthma induced by acid anhydride exposure.

Animal Models of Occupational Asthma to Other Low-Molecular-Weight Chemicals

Low-Molecular-Weight Chemicals Encountered in Lumber and Woodworking Industries

Occupational asthma from western red cedar is caused by plicatic acid present in the wood. Disease in workers is characterized by early, late, or biphasic asthmatic reactions that are associated with infiltration of eosinophils into the lung, although the mechanisms responsible remain to be elucidated (104). Chan et al. (105) immunized neonatal rabbits with plicatic acid–ovalbumin conjugates mixed with $Al(OH)_3$ using a vigorous regimen to produce specific IgE antibodies. In addition, immunized rabbits reacted to intravenous challenge with plicatic acid–protein conjugates with increases in respiratory frequency and pulmonary resistance, although these rabbits failed to respond to plicatic acid challenge by inhalation. In follow-up studies, guinea pigs were immunized to the plicatic acid–ovalbumin conjugate by intraperitoneal injection of 10 μg adsorbed to $Al(OH)_3$ once a month for six months (106). Sensitization allowed for the development of specific IgG_1 antibodies to plicatic acid in the serum and plicatic acid–mediated release of histamine and eicosanoids from mast cells obtained from the lungs. In addition, trachea isolated from sensitized guinea pigs demonstrated an IgG-dependent increase in contraction following treatment with plicatic acid.

OCCUPATIONAL ASTHMA TO HIGH-MOLECULAR-WEIGHT PROTEIN ALLERGENS

Unlike low-molecular-weight asthmogens, less effort has been devoted to establish and characterize animal models for high-molecular-weight occupational asthmogens,

these usually being proteins. This is despite the fact that there is a considerable incidence of asthma in certain occupations involving materials such as latex and subtilisin (proteolytic enzymes of bacterial origin found in detergents). This problem is probably as much because of the difficulties in establishing realistic exposure paradigms as to the fact that ovalbumin animal models have served well, from the point of view of mechanistic studies. In this respect, ovalbumin animal models have also been used to assess the "adjuvant" activity of many occupational and environmental pollutants such as ozone and diesel exhausts (107–112). Historically, guinea pig has been the animal of choice to identify respiratory protein allergens, although not necessarily related to asthma (113). This is because of the ease with which they become sensitized and respond to allergen challenge following inhalation or intratracheal instillation. Responses have been measured by relatively simple, nonspecific events such as monitoring labored breathing, to more complicated methods such as whole body plethysmography as well as assessment for changes in histology and biochemical mediators (21,70). Guinea pigs were used to identify the occupational allergen, subtilisin (114). In these studies, animals sensitized to $150\,\mu g/m^3$ subtilisin 15 minutes per day for five consecutive days demonstrated both immediate- and delayed-onset respiratory responses. Ritz et al. (115) and later Sarlo et al. (116) developed an intratracheal model in guinea pigs using exposure levels of subtilisin similar to the then threshold limit value. The relative ability of proteins to give rise to the formation of anaphylactic antibody in the guinea pig has been used as an indicator of its ability to produce asthma, although its positive predictive value is inconclusive (113,116,117). Recently, an alternative model has been proposed, which assesses the formation of specific IgG_1 antibody in a mouse intranasal test, the assumption being that specific IgG_1 antibody is a surrogate for anaphylactic antibody in the mouse (117).

Although latex products have been used for over a century, latex allergies have received worldwide attention only during the last decade. Latex allergies include both allergic contact dermatitis and IgE-mediated pulmonary responses, although the latter is less common (118). Aamir et al. (119) first demonstrated that the cutaneous response to latex proteins in guinea pigs is mediated by IgE and induced systemic anaphylaxis to latex proteins using a passive systemic anaphylaxis assay. Guinea pigs were also used initially to identify the most active (immunogenic) fractions from latex, which contains over 250 peptides (120). In mice, exposure to latex proteins through the respiratory tract results in elevated nonspecific AHR following nonspecific or latex-specific challenge and pulmonary histopathology consistent with the presence of eosinophils and dose-dependent increases in total IgE. The latter occurs independent of the exposure route (121–123). In a detailed study, Hardy et al. (124) observed that sensitization of mice with either Hev b 5, a major latex allergen, or latex glove protein extract by intraperitoneal injection resulted in a pattern of response similar to that which occurs following ovalbumin sensitization. This included antigen-specific IgE, eosinophilic pulmonary infiltration, elevated levels of IL-5 protein in lung lavage fluid, and epithelial cell mucus hypersecretion in the lung airways. Studies by Xia et al. (125) noted latex-specific IgE and IgG_1 antibodies, increased antigen-specific airway resistance, and histological evidence for eosinophilia, goblet cell metaplasia, and inflammatory cell infiltration, following intranasal challenge of BALB/c mice with latex proteins. Sensitization was induced by intranasal administration of $30\,\mu g$ of latex proteins twice per week for four weeks. IgE-specific antibodies and eosinophils were absent in mice deficient in IL-4 following this exposure regimen (125).

SUMMARY AND FUTURE DIRECTIONS

Controversy surrounds the nature of the antigen, role of specific antibodies, and the role of T lymphocyte–subsets in orchestrating the pathophysiological response leading to occupational asthma. Animal models of occupational asthma share common features with human disease, although no single model recapitulates the disease in its entirety. Newer murine models of occupational asthma have made progress toward a more accurate representation of human exposure and disease. For example, mouse models of diisocyanate asthma have identified a role for both T_H1 and T_H2 cells as well as $CD4^+$ and $CD8^+$ T lymphocytes, and evidence now strongly suggests that reagenic antibodies provide a major contribution to disease outcome, indicating the need for better antibody detection systems in humans. Species differences in susceptibility to sensitization and clinical disease outcome strongly support the hypothesis of genetic predisposition to occupational asthma and support recent findings in human studies. Demonstration of sensitization in animal models at exposure concentrations below current exposure limits should be used to prompt reexamination of such limits. Continued use of animal models will enable the pursuit of novel findings from patient-oriented studies and will facilitate improvement of preventive, therapeutic, and diagnostic interventions aimed at improving worker safety and health.

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ASTHMA IN THE WORKPLACE

And Related Conditions

THIRD EDITION

edited by

I. Leonard Bernstein
University of Cincinnati
Cincinnati, Ohio, U.S.A.

Moira Chan-Yeung
University of British Columbia
Vancouver, British Columbia, Canada

Jean-Luc Malo
Université de Montréal
Montreal, Quebec, Canada

David I. Bernstein
University of Cincinnati
Cincinnati, Ohio, U.S.A.

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