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EDITORIAL

Unravelling synergistic immune interactions between respiratory virus infections and allergic airway inflammation

J. Schwarze* and S. L. Johnston†

The important role of respiratory viruses in exacerbations of asthma has increasingly been recognized since the advent of PCR diagnostics for viruses. Eighty percent of wheezing episodes in schoolchildren and 76% of asthma exacerbations in adults were associated with respiratory viruses [1–3]. Rhinoviruses (RVs) were detected most frequently, accounting for about 60% of infections associated with wheezing. Other viruses known to be associated with asthma exacerbations include enteroviruses, coronaviruses, parainfluenza viruses, adenoviruses, influenza viruses and respiratory syncytial virus (RSV), the virus detected most frequently during wheezing episodes in hospitalized children under the age of 2 years. These findings indicate that respiratory viruses are the most important triggers of asthma exacerbations and wheezing in infancy and childhood, and that they also account for a large number of such episodes in adults. The mechanisms by which respiratory viruses contribute to asthma exacerbations are only understood incompletely. They likely include direct viral effects on airway epithelial cells, pulmonary nerves and airway smooth muscle, as well as effects on pulmonary immune responses. All of these could result in increased airway inflammation, airway hyper-responsiveness (AHR) and in reduced pulmonary airflow, all hallmarks of asthma exacerbations. Interactions of respiratory viruses with allergen sensitization and allergic inflammation of the airways are of particular interest since these constitute the underlying pathomechanisms of asthma in the majority of patients.

In allergic individuals, experimental RV infection prior to allergen provocation results in increased and prolonged histamine release, greater recruitment of eosinophils to the airways, enhanced AHR and the development of late asthmatic responses in the majority of allergic individuals tested [4]. Prior to infection, only one of the same 10 allergic subjects developed a late asthmatic response upon allergen challenge. These observations indicate that respiratory viruses may indeed augment inflammation and functional airway responses to allergen, thus contributing to asthma exacerbations. Such interactions were also evident in a case–control study of patients hospitalized with asthma exacerbations. The risk to be hospitalized due to an asthma exacerbation doubled following allergen exposure, but increased eightfold if the allergen exposure was accompanied by a respiratory viral infection [5].

Due to limitations in studies with humans, animal models have been used to define immune mechanisms involved in the interplay between viral respiratory tract infections, allergic airway inflammation and the development of asthma. Different respiratory viruses have been used and are useful in this endeavour, but observations obtained with one virus may not be transferable to the other viruses. Indeed, differing immune responses to allergen have been reported recently, after infection with influenza and RSV [6]. Since the 1980s, researchers have studied animal models to test the hypothesis that infections with respiratory viruses can trigger and enhance allergic sensitization to inhaled antigens, as has been suggested in children [7, 8]. Infection of mice with influenza A followed by sensitization to ovalbumin (OVA)/aluminium hydroxide aerosol resulted in elevated serum levels of OVA-specific IgE provided that OVA-aerosol exposure occurred during acute infection (days 2–6 post-infection) [9]. Such enhancement of sensitization did not occur if mice were exposed to OVA 14 days post-infection. Sensitization to allergen without adjuvant in parallel models of influenza infection, followed by an additional allergen challenge 3–4 weeks after primary sensitization led not only to increases in allergen sensitization, but also to increased allergic airway inflammation and AHR [10, 11]. Effects of influenza infection were also studied in a model of tolerance induced by exposure to OVA via the airways prior to intraperitoneal sensitization with OVA/aluminium hydroxide. This results in inhibition of OVA-induced T cell proliferation, reduced levels of OVA-IgE and increases in OVA-IgG [12, 13]. Influenza infection at the time of initial OVA exposure prevented tolerance induction resulting in increases in OVA-specific T cell proliferation, OVA-IgE levels and Th2 cytokine production. When influenza infection preceded the initial allergen exposure by 15 or 30 days, OVA-induced T cell proliferation was not inhibited indicating that tolerance was still prevented. However, under these conditions sensitization was associated with a strong Th1 immune response, which suppressed production of OVA-IgE but resulted in enhanced OVA-IgG2a serum levels. These findings suggest that infection with influenza prevents the development of tolerance and enhances IgE-mediated allergic sensitization if primary sensitization coincides with acute infection. If primary allergen exposure occurs during the recovery phase of infection, a non-allergic Th1-driven sensitization is promoted. This may in part be due to the presence of virus-induced, IFN-γ producing CD8 T cells in the lung, which persist following influenza infection. These CD8 T cells are able to suppress allergic airway inflammation [14].

In the current issue of *Clinical Experimental Allergy*, Marsland et al. [15] report a model of influenza infection prior to primary allergen challenge in previously sensitized mice. This model represents virus-induced exacerbations of allergic asthma in that the host is sensitized prior to infection. In asthmatics, though, respiratory viruses encounter a lung previously exposed to allergen, resulting in some degree of airway inflammation at least. In the model used here, airway allergen challenge during the acute phase of infection resulted

in increases in eosinophils and OVA-specific Th2 cells in the airways and in enhanced airway responsiveness, while allergen exposure during the recovery phase of infection suppressed eosinophil influx. These findings confirm that the timing of allergen exposure after influenza infection is critical for the outcome of sensitization. Interestingly, in RSV infection, enhancement of allergic inflammation and development of AHR can be observed when mice are exposed to the allergen during the recovery phase of infection [16]. Employing their model in IL-4-deficient mice, Marsland et al. show that the eosinophilia induced by infection and allergen challenge was largely dependent on IL-4. A small non-IL-4-dependent influx of eosinophils into the airways could be mediated by other Th2 cytokines like IL-5 or IL-13, as the authors discuss. In addition, it could be a consequence of increased production of the growth factor granulocyte macrophage-colony stimulating factor and of chemokines like eotaxin by virus-infected epithelial cells, which are exposed to antigen (most likely containing lipopolysaccharide (LPS)). These mediators can directly promote eosinophil differentiation and migration. When Marsland et al. assessed Th2 cell recruitment they found increased numbers of adoptively transferred OVA-specific Th2 cells in the airways if transfer was followed by influenza infection. This seems to be in contrast to findings in a very similar model, in which numbers of Th2 cells and eosinophils were markedly reduced in the airways if allergen challenge was preceded by influenza infection and recruitment of transferred OVA-specific Th2 cells to the airways was diminished following influenza infection [17]. In contrast to Marsland's experiment, OVA-specific Th2 cells were transferred 7 days after and not prior to infection in this study. The different outcomes could indicate that allergen-specific T cells are recruited as bystanders during the initial phase of influenza infection, but not anymore once strong Th1 responses and inflammation are established. Impaired homing of Th2 cells to sites of Th1-mediated inflammation has been reported previously [18, 19]. Bystander recruitment of T cells is well documented for cytotoxic CD8 T cells in viral infections including influenza [20] and RSV [21], and has also been demonstrated for CD4 T cells. Vaccinia virus infection can lead to proliferation of CD4 memory T cells specific for lymphocytic choriomeningitis virus [22] and enhanced recruitment of OVA-specific Th2 cells to the lung has been observed following RSV infection [23]. Increased numbers of such cells in the lung likely enhance local Th2 responses and aggravate allergic inflammation.

Further, Marsland et al. assessed the role of lung dendritic cells (DCs) in the interaction between influenza infection and allergic inflammation. In naïve lungs, these professional antigen-presenting cells are mostly immature. Uptake of antigen and tissue inflammation induce maturation of DC, which then migrate to the regional lymph nodes to present antigen to T cells. Marsland et al. traced DC migration from the lung to the draining mediastinal lymph nodes. Administration of fluorescent dextran particles during acute influenza infection induced a threefold increase in numbers of fluorescent DC in the mediastinal lymph nodes compared with non-infected mice, indicating that acute influenza infection is associated with increased migration of DC and increased transport of antigen to the draining lymph nodes, as has been suggested previously [24]. Unfortunately, Marsland et al. do not provide the data of antigen uptake and DC migration during the recovery phase of infection when suppression of allergic inflammation was observed. Increased migration is thought to be a consequence of DC maturation. A transient increase in numbers of airway DC from day 2 to day 5 after influenza infection has been reported in mice [11]. Sensitization of these animals to OVA-aerosol at the time of infection resulted in sustained increases in numbers of mature DC for up to 5 weeks. This observation led to the hypothesis that recruitment of DC to the airways during influenza infection may contribute to enhanced sensitization to aeroallergens. Recently, direct evidence for this hypothesis has been provided by data demonstrating a close association between the appearance of increased numbers of mature DC in mediastinal lymph nodes following influenza infection and strong immune responses to inhaled LPS-free OVA, a normally non-immunogenic antigen [25]. DC isolated from mediastinal lymph nodes following influenza infection and OVA inhalation were potent inducers of proliferation of naïve, transgenic T cells specific for OVA. In these experiments, no OVA was added to the co-cultures, indicating that following influenza infection, mature DC in the mediastinal lymph nodes can carry and present allergen encountered in the lung and induce specific T cell responses. Maturation of pulmonary DC during influenza infection appears to be dependent on an IFN-γ dependent mechanism [26]. Interestingly, lung DC which have matured as a consequence of strong virus-induced Th1 responses are able to enhance not only Th1 but also Th2 responses to antigen as observed in virus-induced enhancement of allergic airway inflammation.

In conclusion, influenza infection can facilitate allergen sensitization via the airways and enhance allergic airway inflammation if allergen exposure coincides with the acute phase of infection. Mechanisms involved in this interaction include non-specific recruitment of T cells, including allergen-specific Th2 cells, to the lung during acute infection and importantly, infection-induced maturation of pulmonary DCs, which can take up inhaled allergen and present it to T cells following transport to the regional lymph nodes. While the latter mechanism may be responsible for facilitated *de novo* sensitization to allergen during infection, both mechanisms could induce enhanced memory responses to allergen in previously sensitized individuals. This concept is also supported by our recent finding of increases in numbers of mature pulmonary DCs during the recovery phase of RSV infection [27]. In murine models of RSV disease, it is during this late phase of infection that allergen exposure can lead to enhanced allergic airway inflammation and AHR [16]. While this knowledge of interactions between respiratory viruses and allergic inflammation of the airways has no immediate clinical implication – other than to attempt avoidance of aeroallergens during and immediately after respiratory viral infections – it suggests that the recruitment of memory T cells to inflamed tissues and the maturation, migration and antigen-presentation capacity of DC could become important therapeutic targets in virus-induced exacerbations of asthma.

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J. Schwarze* and S. L. Johnston†
**Department of Clinical Science and*
†*Department of Respiratory Medicine*
Faculty of Medicine National Heart and Lung Institute
Imperial College London
Norfolk Place
Paddington London W2 1PG
UK
E-mail: j.schwarze@ic.ac.uk