

EDITORIAL

The continuing enigma of non-atopic asthma

A. Jayaratnam, C. J. Corrigan and T. H. Lee

The terms 'intrinsic' and 'extrinsic' asthma derive from Rackeman's original recognition [1] that, between individuals, external environmental factors may play a variable visible role in disease exacerbation. This distinction has become equated with the presence or absence of co-existing atopy, which is the tendency, inappropriately, to produce IgE antibodies against antigens encountered at mucosal surfaces ('allergens'). Atopy is conventionally defined by the presence of specific, circulating IgE to one or more of the panel of common aeroallergens as detected by skin prick testing or RAST. In non-atopic or 'intrinsic' asthma, such antibodies are by definition not detectable by these tests.

Rackeman's observations on the clinical distinctions between atopic and non-atopic asthma have been supported by more modern epidemiological studies [2]. Such studies have shown that, in random samples of patients with asthma identified by validated questionnaires, increased age, female gender, chronic rhinosinusitis and more severe impairment of lung function are associated with an elevated chance of the patient being non-atopic [3, 4]. On the other hand, the frequency of rhinitic symptoms is equivalent in both groups [3], suggesting a common underlying abnormality of the respiratory mucosa predisposing to both asthma and rhinitis independent of conventional atopy. This same study showed that seasonal exacerbations of disease are features of both atopic and non-atopic asthma, in the former case in the summer months and in the latter case during the winter months. In non-atopic asthmatics, there is less likely to be a family history of asthma to allergy and development of symptoms is often preceded by an upper respiratory illness [5].

The fact that allergen exposure exacerbates, or 'triggers' asthma in atopic individuals is hardly surprising, as mast cell degranulation results in the release of histamine and leukotrienes onto hyper-responsive airways. To go further and imply that this process *causes* asthma is more of a leap of faith: the simple observation that not all atopic subjects develop asthma, whereas some non-atopic subjects do, implies that IgE-mediated mast cell degranulation is neither necessary nor sufficient for the development of asthma. Nevertheless, the possibility of a role for IgE in both atopic and non-atopic asthma cannot be ignored. In addition to mast cell and basophil degranulation, IgE is also involved in allergen presentation via IgE receptors on B cells and dendritic cells, perpetuating its own synthesis by the generation of allergen-specific T and B cells. It has long been known from epidemiological studies [6] that serum total IgE concentrations remain a predictor of lung function in more elderly patients, regardless of whether or not they have asthma. A more recent study [7] showed that, in a large, unselected cohort of patients defined as atopic or non-atopic on the basis of skin prick testing, asthma was fivefold more

prevalent and airways obstruction more severe in non-atopic patients with serum IgE concentrations in excess of the 'normal' upper limit of 150 IU/mL. These data support the hypothesis that inappropriate IgE production is a risk factor for asthma independent of IgE-mediated responses to allergens. This IgE might be directed against exogenous or endogenous antigens other than conventional aeroallergens. Promising candidates include the enterotoxins of *Staphylococcus aureus* [8]. Non-atopic as well as atopic asthmatics also show up-regulation of the high-affinity IgE receptor FcεRI in the bronchial mucosa [9]. As IgE up-regulates expression of its own receptor, this is indirect evidence of IgE synthesis. More compelling evidence for local IgE synthesis has come from the demonstration of elevated expression of the IgE ε-heavy-chain germline gene transcript and mature mRNA in the bronchial mucosa of both non-atopic as well as atopic asthmatics compared with controls, in the absence of elevated numbers of B cells [10]. There is also indirect evidence that IgE directed against staphylococcal enterotoxins is synthesized locally in the respiratory mucosa [8, 11]. In summary, although IgE synthesis in the bronchial mucosa of non-atopic asthmatics has yet to be demonstrated directly, it is likely that IgE plays an important role in asthma regardless of conventionally defined atopic status.

Analogous to the role of IgE, many studies over the past decade have revealed striking similarities in the cellular and molecular immunopathology of atopic and non-atopic asthma. The bronchial mucosal inflammatory cellular infiltrate is identical save for an excess of tissue macrophages in non-atopic patients [11, 12]. Furthermore, there appears to be equivalent, elevated expression not only of the key eosinophil-active cytokine IL-5 but also of the two B cell IgE-switching cytokines IL-4 and IL-13 in the bronchial mucosa of both atopic and non-atopic asthmatics as compared with atopy-matched controls [13–15], in the case of IL-5 and IL-4 at the mRNA and protein levels, and with a similar cellular distribution in T cells, mast cells and eosinophils. Furthermore, submucosal expression of mRNA encoding the α-subunits of the IL-4 [16] and IL-5 receptors [17] was elevated, compared with controls, in both patient groups. Mucosal expression of the eosinophil chemotactic chemokines eotaxin, eotaxin-2, RANTES, MCP-3 and MCP-4, as well as expression of the chemokine receptor CCR3, a ligand for these chemokines expressed on eosinophils and Th2-type T cells, was also similarly elevated, at least at the level of mRNA, compared with controls, in both atopic and non-atopic asthmatics [18, 19] and these molecules again showed a similar cellular distribution (predominantly epithelial cells, endothelial cells and macrophages in contrast to the cytokines).

The only described differences between the groups in terms of molecular pathology include the following: increased

numbers of cells expressing the α -subunit of the GM-CSF receptor in non-atopic, as compared with atopic asthmatics, reflecting the increased numbers of tissue macrophages in the form of patients [20, 21]; elevated numbers of induced sputum cells (mainly epithelial cells, eosinophils and macrophages) expressing mRNA and immunoreactivity for eotaxin in patients with non-atopic, but not atopic asthma as compared with controls [22]; and elevated concentrations of RANTES in bronchoalveolar lavage fluid (BALF) from non-atopics [20, 23].

However, expression of cytokines, even at the level of both mRNA and protein in the bronchial mucosa, may not always equate with their release, and at least one group of researchers have produced data suggesting that stimulated T cells from the peripheral blood and BALF of non-atopic, as compared with atopic asthmatics are deficient in IL-4 release when stimulated *ex vivo* [24, 25]. There also exists evidence that STAT-6 mRNA expression may be deficient in the bronchial mucosa of non-atopic asthmatics [26], suggesting that IL-4 signalling may be deficient in these patients. Despite these reservations, the balance of evidence would seem to indicate that the cellular and molecular features of atopic and non-atopic asthma are strikingly similar.

In this month's issue, Tedeschi et al. [27] present a study based on the hypothesis that, if allergen-induced, IgE-dependent degranulation of mast cells 'triggers' atopic asthma, there may be an analogous process causing mast cell degranulation in non-atopic asthma which is allergen and IgE independent. They have used chronic urticaria as a model. Chronic urticaria is often labelled 'idiopathic' because of ignorance of its mechanism, but is now thought to be more likely an autoimmune disease at least in some patients [28]. Intradermal injection of autologous serum produces a weal and flare reaction in up to 60% of patients with chronic urticaria. In addition, sera from a proportion of these patients induce histamine release from basophils *in vitro*. In some of these patients, the serum histamine-releasing factor has been identified as IgG autoantibody specific either for the high-affinity IgE receptor Fc ϵ RI or for IgE itself. Tedeschi and colleagues have applied these observations to non-atopic asthma.

They studied 24 patients with asthma defined according to standard clinical criteria, and non-atopic as defined by negative skin prick tests or RAST to a range of (unspecified) aeroallergens. Only four of these patients had total serum IgE concentrations in excess of the 'normal' cut-off of 150 IU/mL. Comparison was with 27 subjects with 'respiratory allergy' (atopic patients with asthma, rhinitis or both) and three non-atopic normal controls. 'Fresh' serum (isolation technique not specified) and enriched basophil suspensions were isolated from the peripheral blood. All subjects underwent an autologous serum skin test (ASST) by intradermal injection of 0.05 mL of 'fresh' serum and measurement of the diameter of any induced weal reaction after 20 min. The ASST was positive (defined as a weal diameter ≥ 3 mm) in 14 of the 24 non-atopic asthmatics but none of the 30 comparison subjects. Reactions to injected saline were negative in every case. Sera from two of the non-atopic asthmatics caused significant histamine release (defined as at least 5% of the total available histamine) from autologous blood basophils, in one case directly *ex vivo* and in the second case only after

lactic acid 'stripping' of the basophils (which is said to remove surface-bound IgE without altering the functional properties of the basophils). Both of these patients had a positive ASST. The sera had no effect on basophils from normal donors, stripped or otherwise. Sera from none of a subset of seven of the 30 comparison subjects caused histamine release from autologous basophils or cells from normal donors. The authors conclude that skin reactivity to intradermal injection of autologous serum is common in non-atopic asthmatics, and that in a small proportion of these patients there is functional evidence of serum histamine-releasing factor activity on basophils, possibly at least partly reflecting the action of autoantibodies.

The authors' findings, while intriguing, raise a number of questions. None of their data implicate autologous serum-induced skin reactions in the pathogenesis of non-atopic asthma. On the contrary, the size of the skin reactions in these asthmatics did not correlate with disease severity (at least according to the GINA classification). Furthermore, as the authors point out, the prevalence of urticaria is not known to be increased in non-atopic asthma, and vice versa. The pathophysiological significance, if any, of the skin reactions is also difficult to interpret, as sera from only two of the 14 ASST-positive patients induced basophil histamine release *ex vivo* (in one case only after lactic acid stripping, which did not completely abolish histamine release induced by anti-IgE, suggesting that the stripping process may have been incomplete). The authors argue that this may reflect relative insensitivity of the histamine release assay, or that the skin weals are caused by one or more 'histamine-releasing factors' which are active on skin mast cells but not on blood basophils (many known factors such as the complement anaphylatoxins C3a and C5a are selective in this fashion). An alternative explanation is that the skin weals were caused by mechanisms other than mast cell histamine release. The authors argue that non-specific generation of vasoactive mediators during the blood-clotting process is unlikely, as ASST positivity was observed only in non-atopic asthmatic and not in atopic asthmatics. They do not speculate, however, as to how this activity, whatever its nature, could be produced in non-atopic, but not atopic patients. Finally, although at least part of the activity might comprise of autoantibodies against IgE or Fc ϵ RI, their data do not show this directly. The data are nonetheless provocative and a salutary reminder to keep an open mind when planning research. If IgE plays a role in non-atopic asthma, why should not non-IgE-mediated mast cell degranulation do so as well?

Such studies further underline the idea that, although atopic and non-atopic asthma have distinct clinical characteristics, they share many common immunopathological mechanisms. In the future, it may be possible in non-atopic asthma to identify novel allergens or antigens involved in disease pathogenesis, to clarify the importance of locally mediated IgE responses and to understand the significance of possible macrophage dysfunction. In addition, a role for autoimmune processes in the pathology of asthma, as the present study highlights, has yet to be clearly defined. These problems are not just of scientific interest. For example, firm evidence that IgE-mediated mechanisms are involved not only in the pathogenesis of atopic but also of non-atopic asthma, coupled with the imminent availability of anti-IgE therapy,

opens a major new therapeutic door for these chronic, severe patients.

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A. Jayaratnam, C. J. Corrigan and T. H. Lee
Department of Asthma, Allergy & Respiratory Science
5th Floor, Thomas Guy House
Guy's Hospital
London SE1 9RT
 UK
 E-mail: aj.jayaratnam@kcl.ac.uk
 chris.corrigan@kcl.ac.uk
 tak.lee@kcl.ac.uk