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Regulation of IgE Responses to Inhaled Antigens: Cellular Mechanisms Underlying Allergic Sensitization versus Tolerance Induction

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Abstract. Previous research from our laboratory has established that the natural response of the respiratory mucosal immune system to inhaled allergens involves initial 'recognition' accompanied by transient low-level IgE production, followed by the development of protective immunological tolerance. Recent studies indicate that the crucial cellular events in this process occur at the level of the upper respiratory mucosa and in the local draining lymph nodes. Salient findings from ongoing studies, detailed below, include identification of a highly developed network of dendritic antigen-presenting cells within the airway epithelium which trap inhaled antigen, and definition of the surface phenotype of the suppressor T cells mediating tolerance as TcR $\gamma^+\delta^+$.

Introduction

We have previously demonstrated that repeated passive exposure of mice [1, 2] or rats [2, 3] to aerosols containing protein allergens such as ovalbumin, induces a profound state of T cell-mediated immunological tolerance which is preferentially expressed against the IgE-antibody isotype. This process can be mimicked to a significant degree by intranasal instillation of antigen in droplet form [1], and moreover is readily demonstrable with classical inhalant allergens such as *Der p* I from the house dust mite [4].

The sensitivity of individual animals to inhaled allergen is determined largely by their genetic background, and the threshold dosage for tolerance induction varies from the (physiological) low nanogram range for immunologically normal low-IgE responders, up to the high microgram zone for animals expressing the high-IgE-responder phenotype [5, 6].

Once established and boosted by re-exposure, this form of tolerance is long lasting [2] and provides essentially life-long protection against subsequent allergic sensitization to the inducing antigen [unpubl. observations]. The process appears similar to oral tolerance in the gut [7] which is proposed to provide protection against tissue-damaging immunoinflammatory responses to dietary antigens. Accordingly, we have proposed a comparable protective function for

tolerance in the respiratory tract in relation to inert environmental antigens [8, 9].

It is noteworthy that the induction of tolerance to inhaled antigens can be prevented by co-exposure of the respiratory epithelium to stimuli such as viral infections [10], air pollutants such as NO₂ or inflammatory mediators such as aerosolised histamine [6], or by stimulation of the regional lymph nodes draining the respiratory tract with bacterial or chemical irritants [6]. In these experimental situations it has been possible to demonstrate the development of clinical hypersensitivity in aerosol-exposed animals characterized by prolonged allergen-specific IgE production, and these exposure protocols are being studied as possible models for allergic sensitization in humans. Of particular interest are recent observations on the inefficiency of tolerance mechanisms in the respiratory tract of young animals [11] which parallels earlier reports on the failure of oral tolerance in newborns [12] - these findings provide a plausible mechanism for the enhanced susceptibility of infants to primary allergic sensitization [9].

As noted above, tolerance induction to nonpathogenic (and hence noninflammatory) environmental antigens appears to constitute the normal response of healthy immunocompetent animals to newly encountered inhaled antigens. We rationalize that a comprehensive understanding of this phenomenon will

provide fresh insight into the aetiology of allergic respiratory disease(s), and may supply fresh options for therapy.

In the ensuing report we detail recent experiments from our laboratory, which probe salient features of the overall tolerance induction process.

Localization of Site(s) of Initial 'Recognition' of Inhaled Allergen by the T Cell System

In previous experiments we employed the ELISA-plaque assay [13] to determine the distribution of allergen-specific IgE and IgG plasma cells in high-IgE-responder rats following exposure to large droplet (3–7 μ m) allergen aerosols, and these results indicated that the earliest IgG responses to inhaled allergen occurred in the regional lymph nodes draining the upper respiratory tract, whereas transient IgE production was localised in the posterior mediastinal nodes draining the lower lung [13].

We have recently repeated and extended the IgE component of this study, employing respirable (1 μ m) allergen aerosols. The data shown in summary form in table 1 pertain to high IgE-responder BN rats undergoing long-term aeroallergen exposure, sampled at the peak of the phase of transient IgE production which precedes tolerance induction. Active allergen-specific IgE plasma cells are essentially restricted to the parathymic/posterior mediastinal lymph nodes draining the lower respiratory tract, and to the mesenteric lymph nodes (MLN), the latter presumably reflecting seeding from the respiratory tract arm of the common mucosal immune system. It is interesting to note the magnitude of the 'bystander' IgE response in various organs, demonstrable by the increase in total IgE plasma cells following aerosol challenge with a single allergen. This is particularly noteworthy in the lung parenchyma and the associated draining lymph nodes, and identifies these sites as focal points of allergen-driven T-helper cell activation and resultant cytokine secretion; in contrast, the MLN exhibit no bystander response, consistent with the suggestion above that allergen-specific IgE production here in aerosol-exposed animals resulted from seeding of preactivated IgE-B cells from the respiratory tract.

In addition to these experiments, duplicate samples of tissues were processed for RNA extraction, and examined for the presence of IgE-specific mRNA employing a ^{32} P-labelled cDNA probe for the con-

Table 1. Distribution of total and aeroallergen-specific IgE plasma cells and IgE-mRNA in high IgE-responder rats following inhalation of allergen

Tissue	IgE plasma cells and IgE-mRNA	
	control animals	post-OA-aerosol exposure
Upper respiratory lymph nodes	410 (0) [+]	647 (18) [+++]
Lower respiratory lymph nodes	195 (0) [+]	1761 (144) [+++++]
Tracheal mucosa	ND ND [$\pm$]	ND ND [+++]
Lung parenchyma	49 (0) [$\pm$]	735 (10) [+++]
Peyers patches	ND ND [+]	5 (1) [$\pm$]
Mesenteric lymph nodes	950 (0) [$\pm$]	840 (200) [+]
Spleen	1320 (0) [$\pm$]	936 (0) [+]
Bone marrow	0 (0) [+]	0 (0) [+++]

Data shown are per whole organ, and indicate total number of IgE plasma cells (OA-specific IgE plasma cells), and [relative levels of IgE-mRNA] in control animals, and in animals sampled after 4 once-weekly exposures to aerosolized OA.

ND = Not done.

stant region of the epsilon chain, by dot-blot hybridization. The relative intensity of hybridization achieved with a standard amount of RNA is shown in table 1 on a 0–5⁺ scale. It can be seen that the high levels of IgE-specific mRNA in the aerosol-exposed animals are present in the lung parenchyma and the lymph nodes draining the lower respiratory tract, consistent with the large 'bystander' IgE responses

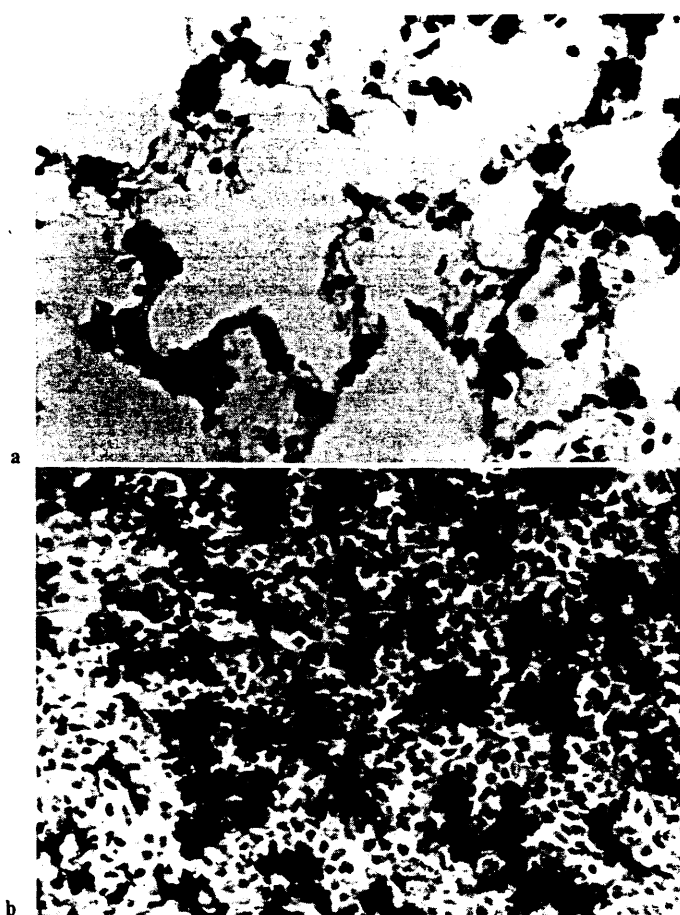


Fig. 1. Immunoperoxidase staining of Ia⁺ dendritic cells in frozen sections from rat respiratory tract. a Alveolar region. b Rat tracheal epithelium sectioned parallel to underlying basement membrane.

observed here with the ELISA plaque plasma cell assay.

However, it is interesting to note that high message levels also appeared at sites such as bone marrow and upper respiratory lymph nodes, which did not demonstrate increases in IgE plasma cells. The possibility that these may represent nonproductive mRNA is currently being examined employing Northern blot analyses. It is also intriguing to note the decrease in IgE-mRNA at sites classically associated with IgE-B cell maturation (gut wall and Peyer's patches), which may represent recruitment of committed IgE-B cell precursors to other sites as a result of stimulation of the respiratory mucosa.

Complementing these studies on early antibody production preceding tolerance induction, we have also sought to identify the site(s) of initial activation

of the suppressor T cells (Ts) mediating tolerance in this system. These experiments [3] indicated that Ts first appear in the superficial cervical lymph nodes draining the tongue and nasolabial lymphatic plexus, and spread thereafter to other respiratory lymph node groups and to the periphery.

Collectively, these observations indicate that active 'recognition' of inhaled allergen occurs within lymphoid structures throughout the respiratory system. However, the sequelae of the interactions between inhaled allergen and the T cell system vary as a function of level of the respiratory tract; sites associated with the lower lung being characterized by initial allergen-specific and accompanying 'bystander' IgE responses, in contrast to rapid IgE-Ts activation in lymph nodes draining the nasopharynx and large airways.

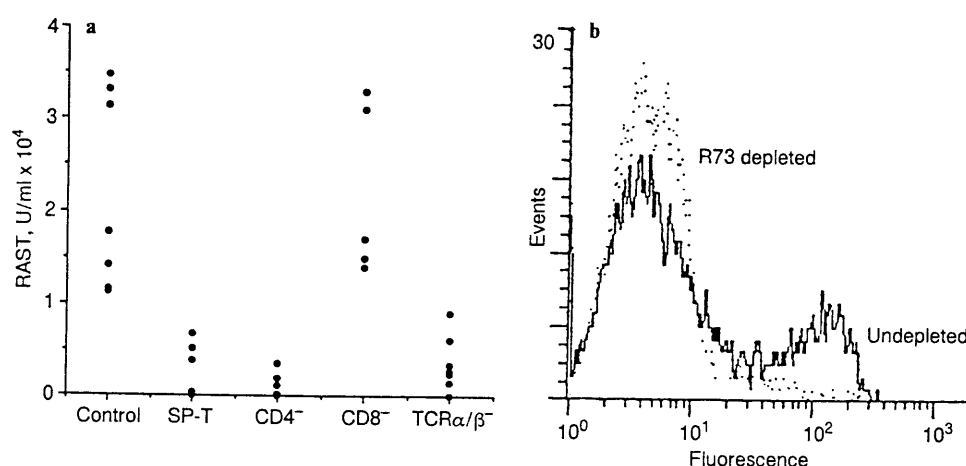
These variations may reflect differences in patterns of antigen deposition throughout the respiratory tree, and/or local differences in antigen processing/presentation. The latter possibility is under intensive study in our laboratory.

Antigen-Presenting Cell Populations in the Lung and Airway Wall

Our initial studies in this area focussed on the parenchyma of the peripheral lung in rat, and identified the principal endogenous APC population as non-phagocytic, nonadherent, IgG-Fc-receptor negative and of ultra-low density on Percoll gradients. These observations constituted the first reported evidence of a major role for dendritic cells (DC) in local antigen presentation in the lung wall [14], conclusions which have subsequently been substantiated by further studies in rats [15, 16], mice [17], and humans [17, 18]. We are currently defining the distribution and surface phenotype of these cells in frozen sections of lung (example in fig. 1a), preparatory to studies on their population dynamics.

In the conducting airways, we initially defined an apparently similar population of DCs within longitudinal frozen sections of rat trachea [19] and subsequently demonstrated their ability to bind inhaled allergen *in vivo* [15]. In more recent studies employing a newly developed tissue sectioning procedure which provides a 'plan' view of the airway epithelium, we have shown that in rat [20] and human [21] these DC form part of a dense, highly developed network

Fig. 2. **a** Suppression of IgE responses by adoptive transfer of splenic T-cells after selective depletion for individual surface markers. Data show individual titres of IgE anti-OA determined by rat RAST. **b** FACSscan analysis of spleen cells stained with MoAb R73 which is specific for α/β chains in the TcR. The solid line depicts intact (undepleted) splenocytes; the dotted profile illustrates the staining pattern after selective depletion of α^+/β^+ T cells.



(fig. 1b) which is virtually identical to the Langerhans' cell network in the skin. When viewed in this plane of section, it is clear that virtually all expression of class II MHC (Ia) within the airway epithelium in the steady state can be attributed to these DC, and it is thus axiomatic that they represent the first point of contact with the T cell system, both for incoming inhaled allergens and for airborne pathogens such as viruses.

Ongoing studies [Schon-Hegrad, Oliver and Holt, to be publ.] indicate major surface phenotypic differences between rat DCs at various levels of the respiratory tree, which are consistent with recent observations on their human equivalents [22]; we are investigating the relationship of these differences to cell functions(s), particularly those associated with presentation of antigen-specific activation signals to T cells.

Characterisation of T Cells Mediating Tolerance to Inhaled Allergens

Our early studies in the mouse demonstrated that tolerance to inhaled antigen could be adoptively transferred between animals by splenocytes bearing the Thy 1.2 marker [2], and subsequent experiments in the rat [3] extended definition of the effector T cells to CD5⁺, CD4⁺, CD8⁺.

Reagents have recently become available for analysis of rat T cell receptor (TcR) peptides, in particular a monoclonal antibody (MoAb) against a common determinant on α/β chains [23]. Negative selection of splenic T cells with this antibody has been shown

to reveal a small but significant population of CD3⁺ T cells in this species which do not express α/β chains in their TcRs, but instead demonstrate γ and δ chains [24] – this population is equivalent to the T γ/δ cells which have been identified in mice and humans, particularly in association with mucosal surfaces [25].

We have recently employed this MoAb to further type the Ts which is capable of adoptively transferring immunological tolerance in the IgE isotype in high-IgE-responder rats. In the experiments illustrated in figure 2, we initially tolerized rats to inhaled ovalbumin (OA) by daily exposure for 30 min to an aerosol of 1% OA in saline for 10 days, followed by once weekly boosting for a further 2 weeks. Spleen cells were prepared from these animals and selectively depleted of B cells and macrophages (to yield >95% pure CD3⁺ T cells) and/or T cells which bound MoAbs against either CD4, CD8 or TcR α/β , by a combination of affinity rosetting and immunomagnetic sorting. 10⁶ of each of the resulting cell populations was injected into naive, syngeneic recipients, which were then challenged i.p. with OA in adjuvant. Peak primary IgE-anti-OA titres are shown for individual recipient animals and controls, determined by modified RAST. Recipients of unfractionated spleen T cells, or spleen cells depleted of the CD4⁺ subset, exhibited marked suppression of IgE production relative to controls, whereas depletion of CD8⁺ T cells abrogated suppression, and these results are consistent with our earlier observations [3]. However, depletion of TcR α^+/β^+ T cells (note relevant FACSscan profiles in fig. 2) failed to abrogate suppression. These results (to be reported in detail elsewhere) strongly suggest that the surface phenotype of the IgE-Ts in this sys-

tem is CD5⁺, CD3⁺, CD4⁺, CD8⁺, TcR α -/ β ⁺, and is thus likely to be part of the recently described γ/δ T cell system. Experiments are in progress to further characterise this T cell population, in particular to determine its origin and requirements for activation.

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