

IgE and mucosal immunity: Studies on the role of intraepithelial Ia + Dendritic Cells and γ/δ T-Lymphocytes in regulation of T-cell activation in the lung

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The primary function of the mucosal immune system is to maintain the sterility of the fragile epithelial surfaces in contact with the outside environment. In order to do so, it must recognise foreign antigens present on the microbial surface and respond via sensitisation of T-helper cells, a process which potentially leads to the activation of a cascade of humoral and cellular immunoinflammatory mechanisms.

However, T-cell immunity represents a double edged sword, because many aspects of the immunoinflammatory cascade (in particular Type I Immediate and Type IV Delayed type hypersensitivity responses) carry the attendant risk of damage to host tissues, and thus there is a clear necessity to limit the magnitude of immune responses at mucosal surfaces to the minimum required for elimination of pathogenic antigen.

The mucosal immune system, however, is faced with an even more fundamental problem: in both the GIT and the respiratory tract, relatively high levels of exposure to inert antigens present in food and environmental antigens in the air (pollens, animal danders etc.) occur virtually continuously, and the T-cell system must accurately discriminate between these ubiquitous non-pathogenic antigens and those associated with microbial pathogens. Failure to discriminate between these classes of antigen places the host at risk of chronic T-cell-mediated tissue damage due to prolonged low-grade immune responses at mucosal surfaces, and such inappropriate T-cell responses represents the basis for chronic allergic disease [1].

While physical and immune exclusion barriers (such as secretory IgA) provide an effective first line of defence on the outer surface of the epithelium, the high solubility and low molecular weight of many of the common non-pathogenic environmental and dietary antigens dictates that low-level penetration into the epithelium must be a frequent occurrence. Within the respiratory tract, contact between the T-cell system and incoming environmental antigen is potentially immediate, due to the presence of significant numbers of T-cells within epithelial lining fluids [2], with additional (and much larger) populations being found directly below the alveolar epithelial surface [3,4], while in the GIT large T-cell populations also occur within the intestinal wall. Thus it can be argued teleologically that specific control mechanisms must exist to limit the responses of these mucosal T-cell populations to ubiquitous non-pathogenic antigens.

While it is accepted that there is a fundamental commonality between immune mechanisms in different organs, it is becoming increasingly apparent that the immune system has evolved strategies for fine regulation of T-cell activation which are tailored to the specific requirements of individual tissue microenvironments, and this realisation has spawned an upsurge of interest in "regional" immunology. Our group's particular interest relates to regulation of T-cell responses to antigen impinging upon surfaces in the respiratory tract, and in this brief review we summarise some recent major findings in this area.

General constraints on T-cell activation in the lung

The T-cell population in the alveolar lining fluids, which is in the order of $1-5 \times 10^8$ in the normal human lung [2], is in direct contact with inhaled antigen. The capacity of these T-cells to respond to antigenic challenge is directly related to the local availability of antigen presenting cells (APC).

The most obvious candidate for this role is the pulmonary alveolar macrophage (PAM). However, the weight of available evidence indicates that this cell population functions poorly in antigen presentation due to inadequacies in processing, the instability of physical clustering with T-cells, and insufficient provision of "second" (IL1-like) activation signals [reviewed in 5]. In addition, PAM also appear to actively suppress T-cell activation via a combination of mechanisms which include constitutive secretion of lymphocytostatic factors, together with acquired suppressive activity following interaction with certain T-cell blasts [5].

However, this suppressive milieu appears reversible in the face of local inflammation of the type which triggers an influx of mononuclear cells into the alveolar spaces. The resulting alveolar cell population recoverable by lavage then includes accessory cells which are permissive for T-cell activation [6], and recent evidence (K. Havenith & E. Hoefsmit ... personal communication) suggests that the active cell in alveolar exudates may be an immature Dendritic Cell (DC).

Recent studies from our laboratory [3,4] and indicate the presence of a much larger T-cell population within alveolar septal walls, many of which represent transient immigrants from the peripheral blood [7]. The lung wall contains a population of active APC which we have identified as Dendritic Cells (DC), and their capacity to present antigen-specific activating signals to T-cells appears to be negatively controlled by endogenous lung tissue macrophages [1].

Functional analysis of these resident lung T-cells in both human [8] and rat [7] additionally indicates reduced cloning frequencies relative to their counterparts in peripheral blood, and this suggests that they are under active suppression while resident in the lung wall. Additionally, the 20-35% of T-cells which maintain immunocompetence during their residence within the lung wall contain a disproportionately high ratio of high Interferon-gamma/low IL-2 producers compared to blood T-cells, which suggest either selection or functional modification by lung-tissue-derived signals. Work is in progress to determine the origin of the T-cell suppressive signal in the lung wall which is responsible for depression of cloning capacity, and preliminary data suggest an important contribution from local macrophages (D. Strickland ... to be published).

Selective suppression of IgE-T-helper cell activation

A series of studies from our lab have defined a phenomenon in the respiratory tract which parallels Oral Tolerance in the GIT. Thus, the natural immune response to initial exposure to aerosols containing non-pathogenic antigen involves selective suppression of antigen-specific IgE antibody production [reviewed in 1,9]. Under appropriate conditions of exposure, repeated inhalation of nanogram amounts of antigen by immunologically naive animals can lead to a profound state of IgE-isotype-specific tolerance, which confers long-term protection against subsequent allergic sensitisation to the antigen.

An essential feature of this phenomenon is the presence of a transient phase of antibody production during early exposure, which indicates active "recognition" of the antigen by the T-cell system.

The mechanisms underlying this form of tolerance involves activation of IgE-isotype-specific CD8⁺ suppressor T-cells (Ts) in the regional lymph nodes draining the upper respiratory tract [10], which is followed by clonal expansion of these Ts and eventual seeding to the periphery. The mechanism(s) by which antigen impinging on the respiratory tract epithelium is transported to T-cell zones in the draining lymph nodes is under study in our lab, and again attention has been focussed on the role of local DC populations.

We have recently reported the presence of an intraepithelial network of DC in the airways in man [11] and rat [12] which is virtually identical to the Langerhans cell network in the skin. These cells express very high levels of Class II MHC (Ia), and function *in vivo* as highly efficient "trappers" of inhaled antigen [13] and as potent "presenters" of the antigen to T-cells *in vitro* [13]. By analogy with related experiments involving migration of antigen-bearing cells from the gut wall, we have proposed that these airway DC shunt inhaled antigen from the airway epithelium to T-cells in the draining lymph nodes, and we are currently examining their role in tolerance induction.

Specialised immunoregulatory mechanisms within the airway epithelium - role of TcR $\gamma\delta$ lymphocytes

Recent reports from a number of labs [reviewed in 14] have described a specialised lineage of predominantly mucosal T-cells which do not contain conventional $\alpha\beta$ chains in their T-cell receptors (TcR), but instead possess $\gamma\delta$ chains. Their precise biological role and their antigen specificity has not been defined, but it appears that their receptors may have a broader specificity than conventional T-cells, and they may respond to certain classes of microbial (especially mycobacterial) antigens as "superantigens" i.e. in an essentially polyclonal fashion. Attention has also been drawn to the cross-reactivity between these mycobacterial "superantigens" and stress proteins which are expressed on the surface of mammalian cells following infection with viruses or intracellular parasites [14,15]. Taken together with their apparent tropism for epithelial tissue microenvironments [14], this has led to the suggestion that T $\gamma\delta$ cells have a primary role as the "first line of defence" of epithelial surfaces against pathogens i.e. they function by rapidly eliminating infected epithelial cells **before** the intervention of the adaptive T-cell immune system, thus protecting the epithelium both from infection and from the tissue damage which often accompanies conventional T-cell responses [14].

Ongoing experiments in our lab suggest that T $\gamma\delta$ cells may also protect the respiratory epithelium from inflammatory damage by selectively suppressing IgE responses, and detailed below is the data which supports this suggestion.

The experiments detailed in Figs. 1 and 2 involve the *in vivo* adoptive transfer of tolerance, employing individual T-cell subsets, and represent an extension of our earlier studies [10]. The strategy was as follows: firstly, BN rats were tolerised to the antigen ovalbumin (OVA) by aerosol exposure, spleen cells were prepared from the animals, and different aliquots were selectively depleted of either CD3⁺, CD5⁺, CD8⁺, Ox52⁺ or TcR α^+/β^+ lymphocytes employing rat T-cell-specific monoclonal antibodies, followed by immunomagnetic sorting. 10⁶ of each cell population was then injected i.p. into naive BN rats, and together with controls these animals were challenged with the OVA antigen in adjuvant. Undepleted spleen cells mediated a 3 fold decrease in IgE anti-OVA responses (Fig. 1), whereas corresponding IgG responses were unaffected (not shown). In contrast, depletion of conventional T-cells bearing

either CD3, CD5, CD8, or Ox52 abrogated suppression. Removal of CD4⁺ cells [10], or T-cells bearing conventional TcR α/β receptors (Fig. 1) did not affect suppression.

Purity data for the TcR α^{-}/β^{-} spleen cell group is shown in Fig. 2, employing single and double staining via FACscan analysis. The frequency of TcR α^{+}/β^{+} lymphocytes remaining in the depleted population which elicited suppression of IgE was <1% of the overall population. It was also noted (bottom of Fig. 2) that 70% of the CD3⁺ T-cells surviving TcR α/β depletion exhibited strong staining with the anti-CD8 MoAb, indicating high expression of this surface marker.

We, therefore, deduce that the functional phenotype of the IgE-Ts in this system is: CD3⁺, CD4⁻, CD5⁺, CD8^{high}, TcR α^{-}/β^{-} . Spleen cells expressing this phenotype in rat have recently been demonstrated to be part of the TcR γ/δ lineage [16], and our data suggest that a subset of these cells may be specialised to protect the respiratory epithelium from damage resulting from IgE-mediated responses to inhaled antigens. Whether these cells are initially activated within the airway epithelium, or are derived from APC/T-cell interaction in regional or peripheral lymphoid organs, is currently under investigation.

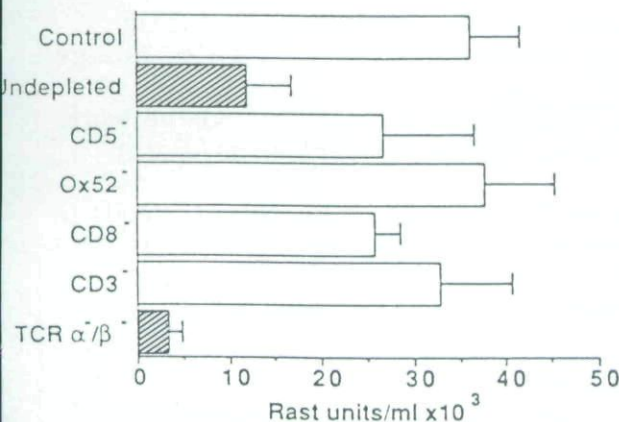


Fig 1: Suppression of IgE responses by adoptive transfer of splenic T-cells after selective depletion for individual surface markers.

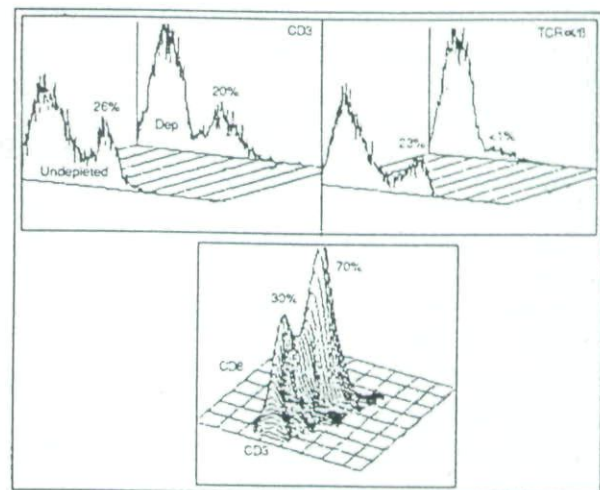


Fig 2: FACscan analysis of spleen cells after selective depletion of T-cells bearing receptors containing alpha/beta chains.

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