

Antigen presentation and stimulation of the immune system in human airways

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Abbreviations: APC, antigen-presenting cell; BALT, bronchus-associated lymphoid tissue; C_H, Ig heavy chain constant region; DC, dendritic cell; GALT, gut-associated lymphoid tissue; HEV, high endothelial venule; ICAM, intercellular adhesion molecule; IDG, interdigitating dendritic cell; IFN, interferon; HEV, high endothelial venule; LALT, larynx-associated lymphoid tissue; MadCAM, mucosal addressin cell adhesion molecule; MALT, mucosa-associated lymphoid tissue; MHC, major histocompatibility complex; NALT, nasal-associated lymphoid tissue; pIgA, polymers of IgA; pIgM, pentameric IgM; pIg, polymeric immunoglobulin; pIgR, polymeric Ig receptor; SC, secretory component; TALT, eustachian tube-associated lymphoid tissue; Th, T helper cell.

Introduction

Airway mucosae have to defend the body interior against a variety of microbial pathogens and foreign proteins; successful protection depends on intimate cooperation between innate defence mechanisms and the specific adaptive immune system that develops in response to antigenic stimulation.

Secretory immunity is the best defined effector mechanism of the adaptive mucosal immune system (1). This first line of specific humoral defence depends on a complex cooperation between mucosal B cells and a 100-kD transmembrane epithelial glycoprotein called secretory component (SC); it is also called the polymeric Ig receptor (pIgR) and mediates external transport of dimers and larger polymers of IgA (pIgA) as well as pentameric IgM (pIgM) to form secretory IgA (SIgA) and SIgM. Initial antigen priming of B cells responsible for local production of pIg molecules is believed to take place mainly in organized mucosa-

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associated lymphoid tissue (MALT), such as the lympho-epithelial structures constituting Waldeyer's pharyngeal ring (2, 3). From such inductive sites, primed B and T cells migrate through afferent lymph and peripheral blood to sinonasal and bronchial mucosae, thereby ensuring that these effector sites are furnished with a wide spectrum of secretory antibodies.

Effective mucosal immunity depends on antigen-priming of both T and B cells. In contrast to B lymphocytes that directly recognize native antigen through their B-cell receptors (surface Ig molecules, sIg), the T-cell receptor can only respond to antigen that has been processed and presented to the T lymphocytes by antigen-presenting cells (APCs). The T-cell receptor recognizes fragments of antigens bound to molecules of the major histocompatibility complex (MHC) expressed on the surface of APCs. The peptide-binding MHC molecules are of two types, class I and class II; the former stimulate CD8⁺ suppressor T cells or cytotoxic T cells, and the latter helper T cells (Th). Intracellular antigens, cut into peptides in the cytosol of APCs, bind to MHC class I molecules and are recognized by T suppressor cells or cytotoxic T cells which, when primed, can actively suppress or directly kill target cells, respectively. Extracellular antigens that have entered the endocytic pathway of APCs are processed (degraded) there and generally presented by MHC class II molecules to Th cells, which when primed show crucial profound immune-regulatory effects via secreted cytokines. Such primary immune responses are followed by long-lived memory of antigen-specific T and B cells that during subsequent contact with antigen leads to a more rapid response and effective rejection of invading pathogens.

This review will first discuss the induction of immune responses and distribution of immunocompetent cells involved in immunity of the airways, and thereafter discuss some mechanisms relevant for allergy in this region.

Induction of immune cells in MALT structures of the airways

Primary mucosal immune responses are believed to be elicited mainly in defined MALT structures that lack afferent lymphatics but, instead, can sample antigens directly from the epithelial surface. The various MALT structures show principally a similar lymphoid histology dominated by secondary (or activated) B-cell follicles with germinal centres and are covered by a specialized follicle-associated epithelium (3). Large collections of T lympho-

cytes, along with APCs constituting various types of dendritic cells (DCs), are found between the follicles as well as underneath the follicle-associated epithelium. This specialized epithelium contains foci of epithelial so-called "membrane" (M) cells that transport actively antigens inwards from the epithelial surface (1). Antigens endocytosed by HLA class II⁺ extrafollicular APCs are next processed and presented as immunogenic peptides to T cells, whereas native antigens are presented in immune complexes to B cells within the germinal centres by follicular dendritic cells (3). In this way memory B cells with high receptor affinity for specific antigens are generated for subsequent distribution through lymph and peripheral blood to various exocrine tissues (Fig. 1) (4).

Experimental animal work on MALT of the upper airways has focused on the paired lymphocytic cell aggregates that occur in rodents at the entrance of the nasopharyngeal duct (5). The inductive function of this nasal-associated lymphoid tissue (NALT) may be crucial for immunity of the upper aerodigestive tract. In humans, MALT structures of this region are constituted mainly by the palatine tonsils, the nasopharyngeal tonsil (adenoids) and the lingual tonsils that together constitute most of Waldeyer's ring, while the lateral pharyngeal bands are less prominent components (6). In addition, similar lymphoid structures are often present associated with the larynx (LALT) (7), and also at the entrance of the eustachian tube (TALT), the latter (so-called tubal tonsil) to some extent being inflammation-dependent (8). Although it remains uncertain whether all these organized lymphoepithelial structures are functionally comparable to NALT in rodents (5), they are indeed strategically located to orchestrate regional immune functions against both airborne and alimentary antigens.

Similar to MALT structures in the upper airways, bronchus-associated lymphoid tissue (BALT) is found in the lower airways, although variably present among species (9). In humans, BALT is present in about 40% of patients younger than 20 years of age, and in older patients only in exceptional cases. The functional role of this inductive tissue in humans remains uncertain, and it is possible that upper airway MALT structures contribute to mucosal immunity also in the lower airways (Fig. 1).

All categories of airway inductive tissue contain four lymphoid cell compartments, namely the reticular crypt epithelium, the extrafollicular area, the mantle zones of lymphoid follicles and the follicular germinal centres (10, 11). Primary follicles are present as early as 16 weeks' gestation, but formation of germinal centres reflecting development of B-cell memory by exogenous antigens (4)

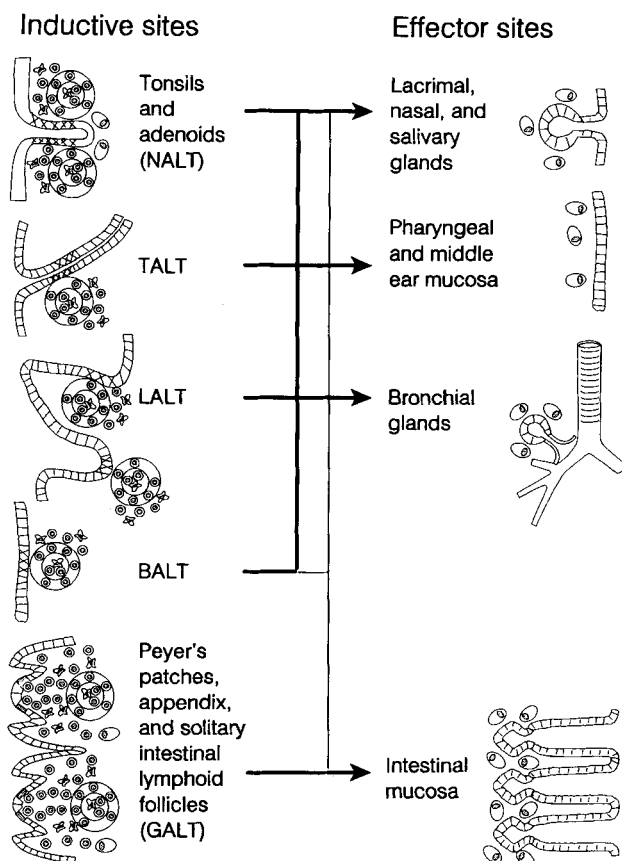


Figure 1. Model for putative homing pathways of primed B and T cells from organized lymphoepithelial tissue (inductive sites) to various effector sites in the integrated mucosal immune system. Presumed compartmentalization is indicated, the heavier arrows representing preferential migration routes. Homing from gut-associated lymphoid tissue (GALT) appears to be determined mainly by leucointegrin $\alpha 4\beta 7$ interacting with MADCAM-1 expressed on the microvasculature in the intestinal lamina propria (see text for details). Other as yet unknown interactions direct primed immune cells from nasal-associated lymphoid tissue (NALT), eustacian tube-associated lymphoid tissue (TALT), larynx-associated lymphoid tissue (LALT) and bronchus-associated lymphoid tissue (BALT) to regional effector sites as indicated. Among these organized lympho-epithelial structures, the palatine tonsils and adenoids (NALT) have obvious advantages as inductive sites because of antigen-retaining crypts and absence of protein-degrading digestive enzymes. Modified from Brandtzaeg and Farstad [91].

does not take place until shortly after birth; terminal differentiation of B cells to extrafollicular plasma cells can be seen about 2 weeks postnatally in the palatine tonsils [10]. It is believed that naive B cells ("germinal centre founder cells") are initially stimulated in the extrafollicular area (Fig. 2) through cognate help by activated $CD4^+$ T cells to which interdigitating dendritic cells (IDCs) have presented processed foreign antigen in the context of MHC class II molecules. The activated virgin B cells produce unmutated IgM (and some IgG) antibody of low affinity that is available to bind circulating antigen; the resulting soluble

immune complexes are subsequently deposited on follicular DCs in the germinal centres where native antigen may be retained for prolonged periods to maintain B-cell memory [4].

The tonsillar germinal centre reaction normally generates a variable number of intrafollicular Ig-producing immunocytes (plasmablasts and plasma cells) predominated by the IgG (55–72%) and IgA (13–18%) isotypes [3, 10]. Both these germinal centre immunocyte classes, and those producing IgM or IgD, often express J-chain in normal palatine tonsils and adenoids of children, but in recurrent tonsillitis this expression is considerably reduced [3, 10]. The cytokine profiles and other microenvironmental factors determining this B-cell differentiation process remain obscure, but it appears that clonal maturation by massive or repeated antigen stimulation downregulates the J-chain, thus promoting monomer production by tonsillar IgA immunocytes [1, 4, 12]. Nevertheless, Ig heavy chain constant region (C_H)-gene switching in tonsillar germinal centres normally gives rise to considerable percentage of extrafollicular IgA immunocytes with J-chain expression [3, 10]. The fact that their isotype is mainly IgA1 (approximately 90%), together with the parallel occurrence of many IgG and some IgD immunocytes, supports the notion that tonsillar B-cell differentiation takes place mainly in a sequential downstream C_H -gene switching fashion [3, 4, 10, 13].

Because the J-chain is a key peptide in the formation of pIgA with pIgR-binding properties [1], the tonsillar B-cell differentiation process exhibits features compatible with precursor generation for the SIgA system [3]. It may be visualized that only some of the germinal centre-derived pIgA-producing B-cell blasts give rise to extrafollicular plasma cells (Fig. 2), whereas an unknown fraction migrate to regional secretory effector sites for terminal differentiation there (Fig. 1). The nasopharyngeal tonsil, in addition, has its own secretory immune system, because patches of its surface and crypt epithelium express SC, but this is not true for the palatine tonsils [10].

Induction of immune cells in peripheral lymph nodes draining the airways

Studies in rats have revealed a dense network of intra-epithelial DCs in normal tracheal mucosa [14]. This cell population has a rapid turnover with a half-life of less than 2 days. Intra-epithelial DCs have also been identified in human airway mucosa [15–17]. Data from our laboratory (Gran et al., manuscript in preparation) obtained in normal

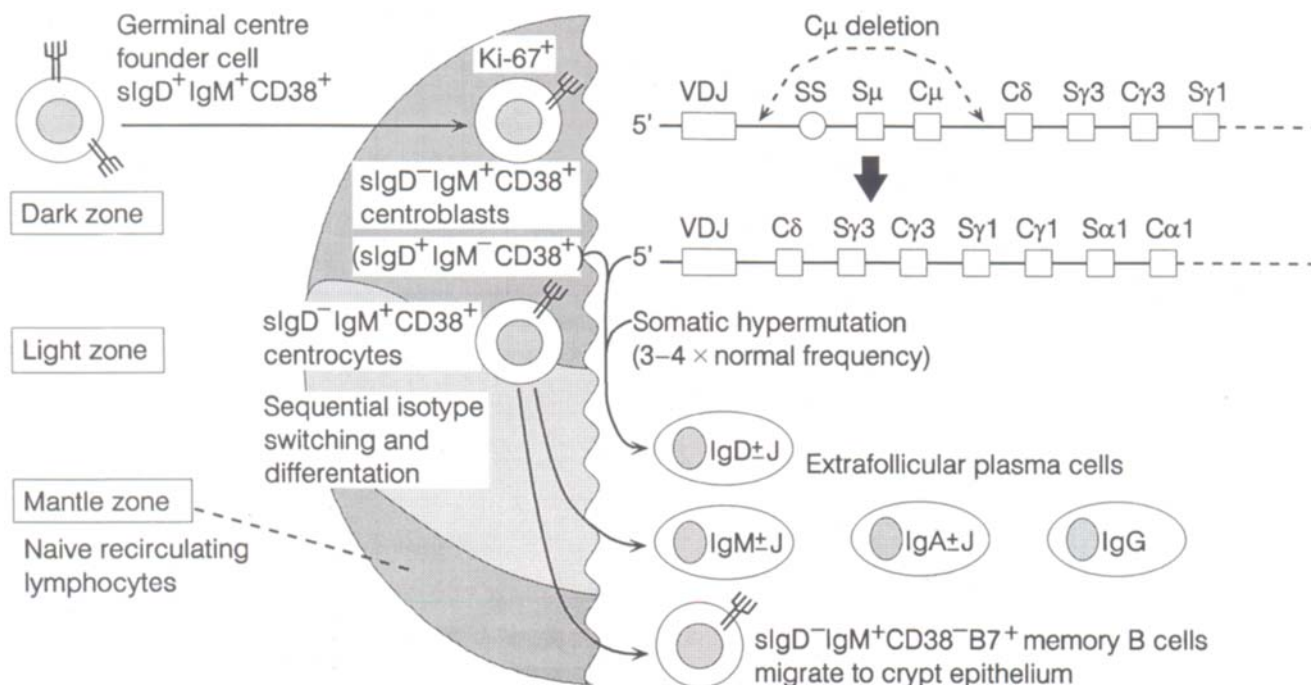


Figure 2. B-cell developmental events presumed to take place in the dark and light zones of tonsillar germinal centres, leading to the generation of extrafollicular plasma cells of various isotypes with or without J-chain expression ($\pm \text{J}$). Precursor cells with activated J-chain gene may also migrate from the germinal centres to regional mucosal effector sites as a basis for secretory immunity (see text for details). In the dark zone, a subset of B cells with surface IgD ($\text{slgD}^+ \text{IgM}^+ \text{CD38}^+$) exists that undergoes C_μ deletion (broken curved arrows at the top), including loss of the somatic mutation silencer (SS) and the switch region (S_μ) required for further downstream C_H -gene switching. These cells show extremely high frequency of somatic mutations and probably represent an important precursor subset for IgD-producing plasma cells in the upper airways. Adapted from Brandtzaeg et al. (4).

nasal mucosa, show that 150–200 intra-epithelial MHC class II $^+$ (HLA-DR $^+$) cells/mm ^{2} epithelial section area (4 mm thick) express the putative DC marker CD1c (Fig. 3); this suggests that an intra-epithelial network of DCs exists in humans as in rat airways.

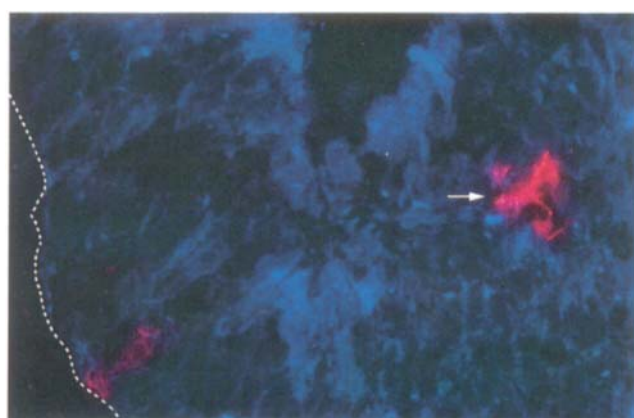


Figure 3. Two-colour immunofluorescence staining for CD1c (red) and cytokeratin (blue) in cryosection from normal nasal mucosa. There are two CD1c $^+$ cells within the cytokeratin-positive epithelium, one of them with distinct dendritic appearance (arrow). Basement membrane indicated by broken line.

This highly dynamic system of “immature” DCs that migrate through the airway mucosa capture and process antigens that penetrate the epithelial barrier. In the normal state they appear locally inert while continuously transporting antigens from the mucosa to the regional lymph nodes, where they end up as IDCs in the T-cell areas (18). Here they may complete their maturation and become extremely efficient APCs, with capacity for activation of both naive CD4 $^+$ and CD8 $^+$ T cells by means of MHC class II- and I-peptide complexes, respectively. The primed T cells then extend the immune response by interacting with other cells, such as B cells to stimulate antibody formation, macrophages to provide cytokines, and targets to induce cell lysis.

Homing of primed immune cells to regional mucosal effector sites

Tissue recruitment of leucocytes

Extravascular recruitment of immune and inflammatory cells is controlled by dynamically regulated complementary adhesion molecules expressed on the surface of circulating

leucocytes and on the endothelium of venules. Such molecules regulate the trafficking (recirculation) of naive lymphocytes through organized lymphoid tissue, and particularly the dissemination (homing) of primed B and T cells to immunological effector sites such as the mucosae and exocrine glands, as well as the local extravasation of different leucocyte subsets in inflammation (19).

All kinds of cells express an array of adhesion molecules and some of them appear more important than others in directing different leucocyte types to various tissue sites. Receptors on the luminal face of the endothelial cells communicate via corresponding ligands or counter-receptors expressed by circulating leucocytes to initiate their extravasation (20). This multistep process involves not only several integral surface molecules but also a variety of chemokines ("chemoattractant cytokines") derived from endothelial cells and various extravascular cells (21, 22). The initial attachment of free-flowing leucocytes (mediated by the selectin family of adhesion molecules) is relatively loose and allows "rolling" on the endothelium (high "on-off" rate). Chemoattractants deposited on the luminal surface of endothelial cells can then bind to G-protein-coupled chemokine receptors that mediate activation of the integrin family of adhesion molecules on rolling the leucocytes. This step enables strong adhesion of leucocytes to the vessel wall via integrin-counter-receptor interaction. Finally, tissue gradients of chemoattractants direct the extravasation of arrested leucocytes in concert with adhesion molecules concentrated along the borders between endothelial cells (21, 23, 24).

Distribution of immune cells to mucosal effector sites

Experimental data have shown that certain leucocyte adhesion molecules are more strongly expressed on unprimed ("naive") than on primed ("memory") lymphocytes, and *vice versa*, and some of them are relatively tissue-specific in their function (19). Such molecular mechanisms apparently direct the trafficking of lymphoid cells between inductive sites and effector sites of the mucosal immune system. After antigen-induced priming, proliferation and partial differentiation in MALT, lymphoid memory and effector cells migrate rapidly to regional lymph nodes, from which (probably after some further differentiation) they pass via lymph into the peripheral blood circulation (Fig. 4). Such primed cells express adhesion molecules or "homing receptors" specific for corresponding determinants ("addressins") on endothelial cells in mucosal and exocrine glandular tissues. In the gut, mucosal addressin cell

adhesion molecule-1 (MAdCAM-1) present on the specialized high endothelial venules (HEVs) in organized gut-associated lymphoid tissue (GALT) such as the Peyer's patches, as well as on ordinary venules in the lamina propria, is believed to play a major role in the trafficking of mucosal immune cells in the abdominal region (25).

Homing of both B and T cells to the human gut lamina propria appears to depend mainly on their high levels of $\alpha 4\beta 7$ (26–28), because this integrin binds avidly to MAdCAM-1 in the mucosal microvasculature (19). It has been suggested that MAdCAM-1 is confined to inductive and effector sites in the gut (29); therefore, one may postulate that other regional endothelial determinants are shared between inductive sites and exocrine effector tissues in the airways (11, 13). Some evidence does suggest that $\alpha 4\beta 7$ is not an important homing molecule for lymphoid cells in the airways of humans (30), as well as mice (31) and sheep (32).

Evidence for homing of primed immune cells to airway mucosa

The finding that nasal and bronchial mucosae, as well as salivary and lacrimal glands, contain an IgA⁺ and IgD⁺ immunocyte distribution similar to that of tonsils (2, 10, 13), suggests that these effector sites are seeded mainly by B cells primed in Waldeyer's ring and perhaps in BALT. Indeed, tonsillar germinal centres contain a subset of B cells (IgD⁺ IgM⁺) in the dark zone that undergoes excessive somatic hypermutation (Fig. 2) and apparently gives rise preferentially to IgD-producing plasma cells of this region (4). By contrast, the intestinal lamina propria probably receives most stimulated B cells from GALT (Fig. 1). Indeed, IgD-producing plasma cells are virtually absent from the gut, even in selective IgA deficiency (33). A similar B-cell homing dichotomy within the mucosal immune system has been suggested by studies in the rat, as reviewed elsewhere (3).

Additional support for an inductive MALT role of Waldeyer's ring comes from the preferential appearance of IgA antibodies in parotid sections, recently shown after antigen exposure of the palatine tonsils in rabbit (34). Furthermore, occurrence of IgA-producing plasma cells in the middle-ear mucosa of guinea pigs has been induced both by BALT and GALT immunization (35). It is also of great interest that activated human tonsillar B cells transferred to mice with severe combined immunodeficiency, migrated to the lung of these animals but not to their gut mucosa (36). In keeping with this observation, direct immunization of human palatine tonsils, and particularly nasal vaccination, gave rise to local B-cell responses in palatine tonsils and

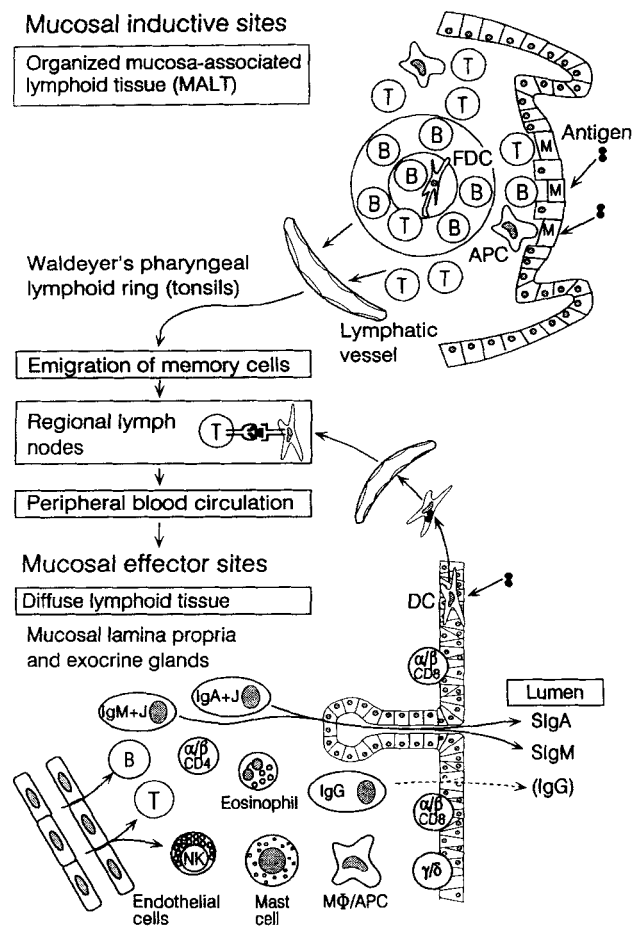


Figure 4. Schematic depiction of the mucosal immune system in human airways. *Inductive sites* are constituted by regional mucosa-associated lymphoid tissue (see Fig. 1) with their B-cell (B) follicles and M-cell (M)-containing follicle-associated epithelium through which exogenous antigens are actively transported. Alternatively, dendritic cells (DC) capture antigen and migrate from airway epithelium via afferent lymph to regional lymph nodes where they become activated and stimulate T cells. Primed B and T cells migrate further via efferent lymph and peripheral blood to become seeded by selective extravasation at regional mucosal *effector sites* as indicated. The local extravasation of other types of leucocytes is also directed by the profile of adhesion molecules expressed on the microvasculature of the effector site.

adenoids as well as specific circulating B cells that apparently did not enter the intestinal mucosa (37).

Together these observations provide indirect evidence for the notion that human tonsils, and perhaps particularly the adenoids, function as NALT and supply secretory effector sites of the upper aerodigestive tract with stimulated pIgA precursor cells within an integrated mucosal immune system (Fig. 1). The reason for the observed dichotomy in B-cell dissemination between this region and the gut may be differences in homing receptors and vascular addressins as alluded to above, but a regional chemokine repertoire may also exist.

To explore the NALT concept in humans, it is important to evaluate the effect of adenotonsillectomy on the regional SIgA levels. The pioneer report by Ogra (38) in 1971 showed that combined tonsillectomy and adenoidectomy in children reduced the level of IgA antibody to poliovirus three- to four-fold in their nasopharyngeal secretions and delayed or abrogated their local immune response to subsequent live poliovaccine. D'Amelio et al. (39), however, observed no salivary IgA reduction (but decreased serum IgA) in previously tonsillectomized adults (16–24 years old). Finally, Cantani et al. (40) observed more recently that salivary IgA as well as serum IgA (and less so IgG and IgM) were significantly reduced 4 months after combined tonsillectomy and adenoidectomy in children. More recently, however, studies in tonsillectomized children showed instead elevated salivary Ig levels after 3–4 years (41), whereas no effect was found in tonsillectomized young adults after 6 months except for a slight reduction of total IgM and salivary IgG antibodies to *Streptococcus mutans* and Epstein–Barr virus (42).

More extensive clinical studies are clearly needed to obtain conclusive information. Altogether, the current concept of lymphocyte homing is based on the notion that lymphocytes and endothelial cells are equipped with several surface receptors that may be modulated by antigen priming (naive versus memory cells) as well as by the microenvironment (mainly by local cytokines and chemokines); pairs of such adhesion molecules operate in concert to direct memory cells to their “home” (the relevant effector site) within the mucosal immune system.

The apparent dichotomy between the airways and the gut with regard to humoral immunity may depend on different profiles of adhesion molecules on the regional B cells and/or on the local endothelial cells; differential immunoregulatory effects of the local microbiota may also be involved (13). It is reasonable to believe that T cells primed at inductive sites in the airways follow the same route of trafficking as proposed for primed B cells. However, little direct evidence is available to support this possibility.

Local immune regulation

After extravasation at effector sites, the primed B cells are subjected to immunoregulatory second signals governed by local APCs (mainly DCs or macrophages) and Th cells that cause local proliferation and particularly terminal differentiation to Ig-producing plasmablasts and plasma cells (collectively called immunocytes).

T cells (dominated by the memory phenotype) accumulate throughout the respiratory mucosal lamina propria (43–45), but a substantial number enter the surface epithelium (43, 46). These intraepithelial lymphocytes upregulate integrin $\alpha E\beta 7$, which is the counter-receptor to E-cadherin expressed on epithelial cells (47). As discussed above, Th cells can only be stimulated by processed antigen presented to them in the context of MHC class II molecules. Such molecules are present not only on ordinary APCs, but also on certain epithelial cells at mucosal sites, such as in the tonsils (10) and nasal mucosa (2) (Fig. 5). It is interesting that class II⁺ epithelial cells can act as APCs under certain *in vitro* experimental conditions. The main effect obtained with gut epithelial cells in such test systems has been activation of CD8⁺ T suppressor cells, as reviewed elsewhere (48, 49). The intestinal epithelium might thus be an important immunoregulatory element for the induction of suppressive mechanisms, explaining peripheral and local tolerance or hyporesponsiveness to foreign soluble antigens encountered at mucosal surfaces. This phenomenon is best known in relation to dietary antigens as so-called “oral tolerance” and has been defined particularly by feeding experiments in rodents (48).

Because intra-epithelial lymphocytes are mainly CD8⁺ T cells, they may play an important role as T suppressor cells by downregulating systemic types of proinflammatory humoral immune responses (IgG and IgE) and T-cell mediated (Th1-dependent) delayed-type hypersensitivity against harmless environmental antigens. However, evidence obtained until now rather points to a role of GALT-

derived CD4⁺ regulatory T cells in the induction and maintenance of oral tolerance in the periphery (50), although CD8⁺ cells may be crucial for local hyporesponsiveness or normal homeostasis in the mucosa itself (48, 51).

Little direct evidence exists for tolerance mechanisms operating in human mucosae. Nevertheless, such a homeostatic control function is strongly suggested by the fact that the vulnerable intestinal mucosa normally exhibits no substantial IgG response (49), and few T cells with signs of activation (52). The same is true for the normal human airways, although nasal and bronchial mucosae usually contain relatively more IgG-producing cells, particularly in the connective tissue stroma beneath the surface epithelium (2, 53). Immunosuppressive mechanisms similar to those in the gut therefore appear to operate in the airways, and this includes normal control of IgE responses to inhaled antigens (54). Nevertheless, hypersensitivity reactions to allergens and microbial antigens are much more frequent and persistent in the airways than in the gut (55).

It is possible that the airways show less tight control of mucosal immune responses than the gut because of a relative scarcity of CD8⁺ intraepithelial lymphocytes (48, 53). A comparative study in our laboratory showed that while only 10% CD4⁺ intraepithelial lymphocytes are present in the jejunum, this subset averages 30% in the surface epithelium of normal nasal mucosa (43). Interestingly in this respect, class II⁺ airway epithelial cells have been shown to stimulate CD4⁺ Th cells *in vitro* (56). Moreover, ICAM-1 is readily induced *in vivo* basally on the airway epithelium (Fig. 5) but not on the gut epithelium (53). Because this adhesion molecule may provide a costimulatory signal for T-cell activation, it seems justified to conclude that the epithelium of the respiratory tract, in contrast to the gut, appears to possess properties favouring immunological help instead of suppression (Fig. 6). This difference could be conducive to the preferential development of airway allergy through break of immunological tolerance.

Holt et al. (55) have emphasized the putative central role of intra-epithelial DCs as an immunoregulatory element in the airways. An increasing functional complexity of DCs is emerging (18). As discussed above, these cells transport antigens from the mucosa to regional lymph nodes where they present antigenic peptides for T cells. In rodents, it has been shown that leakage from the MHC class II to the class I pathway might provide stimulation of CD8⁺ regulatory T cells of the T cell receptor g/d phenotype (Fig. 7) (57). The cytokine pattern resulting from this immune deviation apparently causes downregulation of Th2 cells necessary for

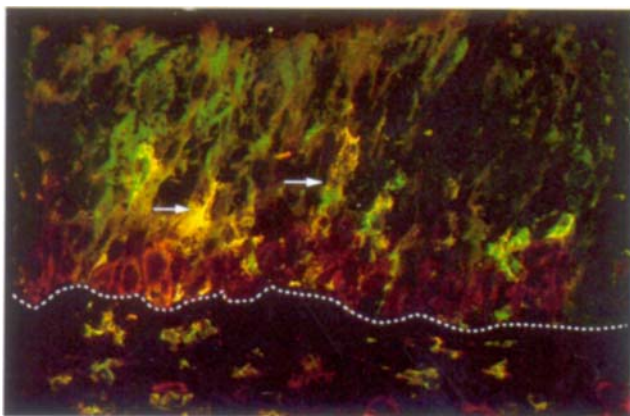


Figure 5. Two-colour immunofluorescence staining for ICAM-1 (red) and HLA-DR (green) in cryosection from normal nasal mucosa. Some of the intra-epithelial HLA-DR⁺ICAM-1⁺ cells (yellow) are arrowed. Note that basal epithelial cells are positive for ICAM-1 (red), whereas HLA-DR is weakly expressed by patches of the remaining epithelium (green). Basement membrane of surface epithelium indicated by broken line. The two markers are also variably expressed by elements in the lamina propria.

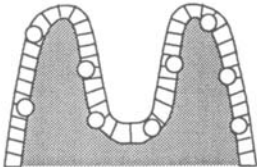
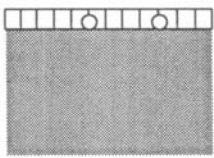
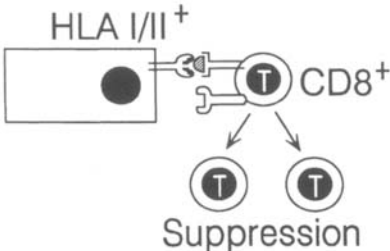
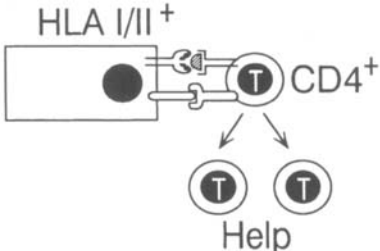
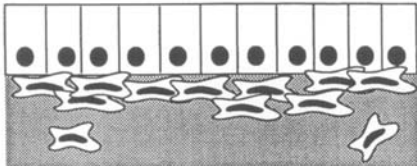
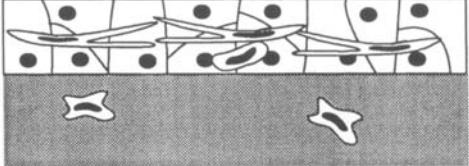
Variable	Gut	Airway
IELs	 Many $CD8^+$ $\gg$ $CD4^+$	 Few $CD8^+$ $\gg$ $CD4^+$
Epithelial cells	 HLA I/II ⁺ CD8 ⁺ Suppression	 HLA I/II ⁺ CD4 ⁺ Help
Professional HLA class I/II-positive APCs		

Figure 6. Schematic representation of three biological variables that may contribute to the apparent disparity in immunoresponsiveness between the gut and the airways. Upper panels: numbers and phenotypic distribution of intra-epithelial lymphocytes (IELs). Middle panels: capacity for antigen presentation, including expression of costimulatory signals to T cells by epithelial cells (e.g. ICAM-1), leading to preferential suppression or help. Bottom panels: distribution of subepithelial and intra-epithelial professional antigen-presenting cells (APCs). See text for details. Adapted from Brandtzaeg et al. [92].

IgE responses [54, 55]. However, other types of immune modulation may also tolerize Th2 cells [58].

In the lungs, resident alveolar macrophages are also believed to play an important part in regulating immune responses. Experimental animal studies and *in vitro* studies with human leucocytes have shown that alveolar macrophages are able to produce soluble factors that inhibited both T-cell proliferation and antigen-presenting functions of DCs [59, 60]. Moreover, these resident phagocytic cells presented antigen ineffectively to T cells due to defective expression of the costimulatory molecules B7.1 (CD80) and B7.2 (CD86) [61]. Together, these findings suggest that alveolar macrophages may have important immunosuppressive functions, but whether similar regulatory APCs exist in the upper airways remains elusive.

Development of T-cell immunity to environmental antigens

The mucosal immune system should preferably discriminate between harmless environmental antigens (such as

inhalant allergens) and microbial pathogens; only the latter merit a vigorous response aiming at rapid elimination. Until recently it was believed that hyporesponsiveness to inhalant allergens could be explained either by tolerance against or ignorance of such antigens. However, it has become clear that active T-cell immunity to inhalant allergens is essentially universal among adults, and that clinical outcome (allergy or unresponsiveness) depends on the cytokine profiles of the dominating $CD4^+$ Th cells constituting the relevant memory populations [55].

Epidemiological information about the pattern of allergen-specific $CD4^+$ T-cell reactivity in humans, together with available data from the literature on exposure of experimental animals to mucosal allergens, suggests that the initial immune response to inhaled allergens occurs early in life [55]. As discussed previously, soluble antigens impinging on the airway mucosa are captured by DCs that migrate to regional lymph nodes where they present antigens for naive T cells. This primary response involves Th0-like, Th1-like and Th2-like subsets, and leads to heterogeneous allergen-specific antibody responses that

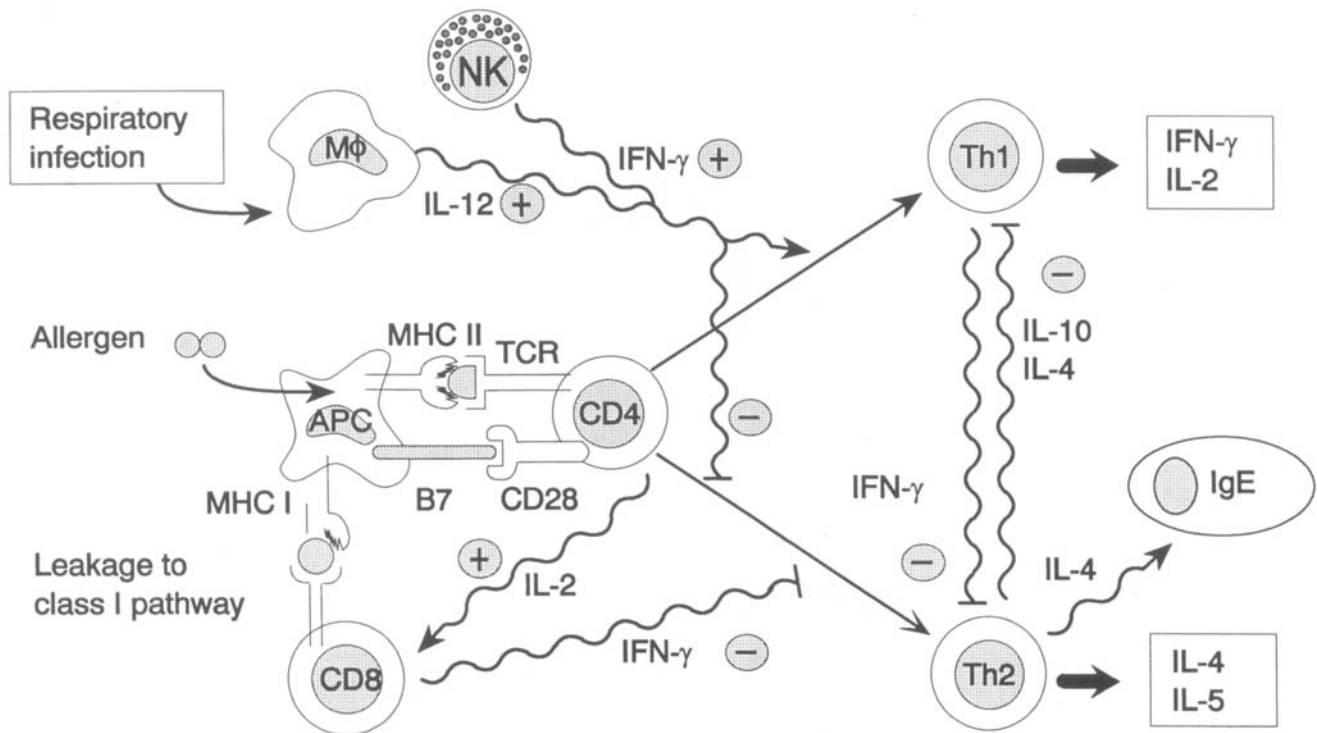


Figure 7. Schematic representation of putative mechanisms for polarized profiles of cytokine secretion by activated T-helper (Th) cells. Animal models suggest that inhaled allergen is captured by professional antigen-presenting cell (APC) which, after migration to regional lymph node, presents allergenic peptide to both MHC-class II-restricted $CD4^+$ Th0 and class I-restricted $CD8^+$ T cells. During the resulting primary immune response, mutually antagonistic subsets of allergen-specific $CD4^+$ T cells compete for dominance within the developing memory population under the influence of the local cytokine milieu. Cytokines secreted by allergen-specific $CD8^+$ T cells (require IL-2 from Th0 cells), as well as IFN- γ from natural killer (NK) cells and particularly IL-12 from infection-activated macrophages (M ϕ) favour a Th1 memory response. Modified from Holt (57).

include only low levels of IgE production (Fig. 7). As a result of continued stimulation from these ubiquitous environmental antigens a selection process is believed to take place within each immune response, involving cross-regulation of competition between Th1 and Th2 cells. In non-atopic individuals, this will lead to the eventual emergence of a dominant Th1 memory population and concomitant decline of Th2-dependent IgE production. However, for unclear reasons, this normal antigen-driven selection process (immune deviation) occasionally fails and the result is the emergence of stable Th2 reactivity and subsequently atopic allergy. The apparent restriction of propensity for $CD4^+$ Th2 "hyperreactivity" in atopic individuals to environmental (especially inhaled) allergens, as opposed to antigens in general (62), suggests that their failure to downregulate IgE responses adequately is explained by dysregulation of mucosal immunity, as opposed to a primary defect in T-cell function.

Interestingly, IgE-mediated food allergy is rare in adults, as reflected in the high efficiency with which IgE responses to dietary antigens are downregulated during early childhood.

Indeed, almost all atopic children lose their IgE reactivity to foods by the age of 4 years, whereas at least 30% maintain significant titres to one or more aeroallergens (63, 64). This disparity raises the question as to why the downregulation of IgE is more rapid and effective for responses to ingested allergens than to inhaled allergens, and several possible explanations exist. A critical step for the outcome of Th1/Th2 memory-cell selection is the antigen-presenting event (65). Therefore, the differences between the respiratory tract and the gut with respect to how intrinsic APC populations function could be of considerable importance. However, animal studies have shown that these populations display similarities rather than differences (66), although it is largely undetermined how migratory APCs act in humans. Another possibility would appear to be the relative intensity of exposure to local allergens at the two sites.

The gut is confronted with much higher concentrations of dietary antigen (micrograms to grams) compared to airborne allergens (nanograms). Such differences are consistent with a dominant role for T-cell anergy (and/or deletion) in the regulation of intestinal immunity to dietary antigens. Moreover, the human gut mucosa appears to have a

propensity to develop a Th1 cytokine profile with high levels of IFN- γ production, both in virus infection (67), gluten-sensitive enteropathy (68) and also in food allergy (69). This tendency might, by cross-regulation, counteract the development of Th2 cells that are necessary for IgE-mediated allergy. Conversely, in the respiratory tract the ordinary low-level inhalant allergen exposure may only be sufficient to trigger immune deviation mechanisms, which seems less efficient to select against allergen-specific Th2 immunity (50, 55).

Local activation of T cells in allergic airway reactions

The early phase of an atopic allergic reaction to inhaled allergens is thought to reflect mainly IgE-dependent triggering of mast cells. However, the chronic phase of the disease is more complex with infiltration of several proinflammatory cells, particularly eosinophils (70, 71). Recent information suggests that this latter phase of an allergic reaction depends in large part on local activation of CD4⁺ Th2 memory cells. Activated T cells (CD25⁺) have been demonstrated in bronchial biopsy specimens (72) and bronchoalveolar lavage (73) from patients with atopic asthma, and are present in nasal mucosa from allergen challenged allergic rhinitis patients (74). Furthermore, it has been shown in a series of studies that both in allergic rhinitis and allergic asthma these T cells express both mRNA and protein consistent with a Th2 profile (73, 75–83). This cytokine profile may explain both the initial IgE response and the subsequent preferential recruitment and activation of eosinophils.

A crucial role for T cells in airway allergy is further strengthened by several experimental animal studies. Blocking the CD28/B7 costimulatory pathway abrogated completely antigen-induced airway hyperresponsiveness and lung eosinophilia in sensitized mice (84, 85). Furthermore, it was shown that selective blocking of CD28/B7-1 interaction prevented antigen-induced accumulation of eosinophils and to a lesser extent lymphocytes locally, but had no effect on systemic blood eosinophilia or IgE antibody production (86). This suggests that costimulatory signals delivered by APCs to T cells through B7.1 is not necessary for the induction of Th2 responses but rather for the maintenance or amplification of lung inflammatory responses.

In another series of experiments (87, 88) brown Norway rats were actively sensitized to ovalbumin; CD4⁺ T cells isolated from cervical lymph nodes of these animals were

then injected into naive rats. Upon challenge with ovalbumin these recipient rats slowly developed airway bronchoconstriction and eosinophilia that was dependent on antigen-primed CD4⁺ T cells with a Th2-type cytokine profile. Interestingly, there were no signs of an early-phase response in this experimental model. This finding was in agreement with a study by Korsgren and coworkers (89) which showed that antigen-induced airway eosinophilia could develop in B cell-deficient mice without any signs of mast cell degranulation. Together, these studies strongly suggest that T cells play a pivotal role in the maintenance of an allergic airway inflammation, and that this chronic phase of an allergic reaction can develop without any preceding IgE-mediated mast cell activation.

Conclusions

Lymphoid cells involved in mucosal immunity of the airways appears to be primed in organized mucosa-associated lymphoid tissue (MALT), such as the tonsils and other regional lymphoepithelial structures, as well as in the regional lymph nodes. MALT structures can sample inhaled antigens directly from the epithelial surface. B cells recognize intact cognate antigens, whereas T cells only recognize processed antigen as MHC-peptide complexes on the surface of antigen-presenting cells (APCs). In addition to stimulating mucosal immune responses in MALT, antigens that penetrate the epithelial barrier can be captured by local dendritic cells (DCs), which subsequently migrate to regional lymph nodes where they mature into extremely efficient APCs for naive T cells that may become involved in regional mucosal immunity.

Primed lymphoid cells either differentiate locally or leave the inductive sites (MALT or lymph nodes) via efferent lymph to enter peripheral blood. The current paradigm of lymphoid trafficking implies that the expression of adhesion molecules on these primed cells determines the preferential homing to specific effector sites. In humans, such mucosal homing molecules has as yet not been defined outside the gut, but there is evidence to suggest that lymphocytes primed in inductive sites of the airways preferentially home to mucosa and exocrine glands of this region.

The mucosal immune system is constantly bombarded by harmless environmental antigens (such as inhalant allergens) that should not induce an aggressive immunity because their elimination is not essential for survival of the host. How a state of hyporesponsiveness is normally achieved remains poorly understood, but substantial evi-

dence is accumulating to suggest that immunoregulatory T cells are critical in this process. Unwanted immune reactions to inhaled allergens initiated by IgE-mediated allergy appears to depend on the maintenance of a Th2 cytokine profile of CD4⁺ Th cells in the relevant memory population. Reports from studies in humans and experimental animals suggest that immune deviation away from a

Th2 to a Th1 response against a given allergen is necessary for development of mucosal tolerance. If the Th2 response is allowed to develop without cross-regulation from a Th1 response the result is first of all IL-4/IL-13-mediated stimulation of IgE production and later IL-5-mediated activation of eosinophils. Such an adverse development is governed both by environmental and genetic variables.

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