Progress report

Lymphoepithelial interactions in the mucosal immune system

The efferent limb of secretory immunity depends on local production and selective epithelial transport of polymeric immunoglobulins (pIg). To this end there is a fascinating cooperation of the B-cell system and the epithelium with their production of two key factors, the J chain and the secretory component (SC), respectively. Interactions between activated leucocytes – for example, T cells and macrophages, and the secretory epithelium may contribute to modulation of the SC-dependent pIg transport. Indirect evidence is accumulating, moreover, that lymphoepithelial interactions may also be involved in regulation of the mucosal B-cell system and in T cell-mediated mucosal induction of systemic tolerance to luminal antigens.

Nature of mucosal immunity

The exocrine secretory tissues constitute the most important mediator organ of humoral immunity – with the gut as the major contributor. There are about $10^{10}$ Ig-producing cells per metre of human small bowel. Most of these immunocytes form J chain-containing dimers or larger polymers of IgA (pIgA) which can be transported through the glandular epithelium along with pentameric IgM by combining with a pIg receptor called the secretory component or SC. More IgA is translocated to the gut lumen every day than the total daily production of IgG, which is approximately 30 mg/kg. It is still obscure how the particular immunoregulatory requirements of local immunity are normally fulfilled. Although the concept of a 'common mucosal immune system' has gained wide acceptance, there are several indications that regulatory mechanisms operating in gut associated lymphoid tissue and in organised lymphoepithelial structures of the respiratory tract, such as tonsils and bronchus associated lymphoid tissue, differ in several ways.

In this review we will discuss established and putative lymphoepithelial interactions of regulatory importance in the afferent and efferent limbs of the mucosal immune system. When possible, reference will be made primarily to studies carried out on human material.

Distribution and particular features of mucosal lymphocytes

The basis for local immunity is the migration of B and T cells from gut associated lymphoid tissue and bronchus associated lymphoid tissue to various secretory tissues such as the gut mucosa. Local extravasation of lymphoid cells probably depends primarily on organ specific endothelial recognition mechanisms. In addition, epithelial factors may be involved in the attraction of B cells; the selective migration of T cells into epithelia may...
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Fig. 1 Immunofluorescence (green) localisation of T4 cells (left panel) and T8 cells (right panel) in adjacent sections of normal human small intestinal mucosa. The villous epithelium is visualized by costaining for cytokeratin (red). Note that most T4 cells are found in the lamina propria whereas most T8 cells are seen in the epithelium.

to some extent be explained by a surface molecule preferentially expressed on intraepithelial lymphocytes.11

Migration of T cells into the gut epithelium is partly antigen independent because intraepithelial lymphocytes are observed in the fetus;12 but luminal antigens clearly determine the magnitude of this migration.13 14 The follicle associated epithelium covering the domes of human Peyer’s patches contains particularly many T cells, especially near the antigen transporting M (‘membrane’) cells.15

Intraepithelial lymphocytes appearing in the gut epithelium are at least in
part derived from Peyers patches and they show a striking predominance of T8 (CD8+) cells (cytotoxic/suppressor phenotype), whereas the lamina propria contains mainly the T4 (CD4+) (helper/inducer) subset (Fig. 1). Nasal and bronchial mucosae contain fewer intraepithelial lymphocytes than the gut, and T4 cells are remarkably predominant (Fig. 2). In the epithelium of the normal jejunum, a ‘blast marker’ (CD7) is expressed mainly on the T8 cells, indicating that they are stimulated, which is in line with morphological features. Most intraepithelial lymphocytes, however, seem to be negative for other activation markers such as major histocompatibility complex class II and Tac antigens. Both in the jejunum and colon the intraepithelial lymphocytes are also mainly negative for the H366 antigen of cytotoxic cells, indicating that the intraepithelial T8 cells are functional suppressor cells.

As intraepithelial lymphocytes are usually found along the basement membrane, apparently crossing it in either direction, most of them probably leave the epithelium quite rapidly by re-entering the lamina propria. It is possible that they do to some extent perform their immunoregulatory function(s) in the latter microenvironment. Suppressor and helper activities have been indicated from in vitro studies of human intestinal intraepithelial lymphocytes in cocultures with peripheral blood mononuclear cells, apparently depending on the T- to B-cell ratios. It seems likely that some of the immunoregulatory effects observed with crude cell preparations were caused by interferon-γ or other lymphokines produced by stimulated intraepithelial lymphocytes (Fig. 3). It is of particular interest that interferon-γ – especially along with interleukin 2 (IL2) – has been shown to induce preferentially IgA antibody production to a bacterial polysaccharide.
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Interactions between lymphocytes and major histocompatibility complex positive epithelium

Major histocompatibility complex subregion products act as restriction elements in T-cell-dependent immune responses. Class I determinants exhibit a general epithelial distribution and class II determinants (particularly HLA-DR) are normally expressed by small intestinal villous epithelium, human follicle associated epithelium (excepting the M cells), parts of respiratory tract epithelium, lactating mammary gland epithelium and certain salivary duct cells. Immunofluorescence staining of the human small intestine shows striking apical expression of HLA-DR related to the brush border and less intense fluorescence along the basolateral borders and in the cytoplasm of the enterocytes (Fig. 4); immunoelectronmicroscopic observations have confirmed that HLA-DR is expressed on the microvilli. This distribution has raised the interesting possibility that epithelial cells may perform class II-dependent genetically restricted transport and presentation of luminal antigens to intraepithelial lymphocytes (Fig. 3).

Epithelial class II expression is probably dynamically modulated by lymphokines. Intraepithelial lymphocytes isolated from the rat gut were

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**Fig. 3** Schematic depiction of putative local immune regulation at secretory site. Antigen penetrates the epithelial lining and becomes processed by macrophage (M) before presentation to T4 lymphocyte by major histocompatibility complex (MHC) class II-positive dendritic cell. Antigen can also be processed in the lumen or on passage through epithelial cell (E). After activation, the T4 cell migrates into the epithelium and receives secondary signals by epithelial presentation of antigen or by mere autoreactive stimulation by MHC class II determinants. Interferon-γ (IFN-γ) or other cytokines stimulates epithelium to enhanced expression of class II determinants and secretory component, and trigger B cells and intraepithelial T8 lymphocytes. B cells are, in addition, influenced directly by various epithelial factors. Stimulation of intraepithelial T8 cells leads to nonspecific or antigen-specific suppression. The possibility exists that not only T4 cells, but also T8 cells (?) are stimulated after MHC class II-mediated transport and presentation of gut-processed antigen.
found to mediate epithelial class II expression, and recombinant interferon-γ induced dose-dependent differential expression of HLA-DR, -DP and -DQ in the HT-29 cell line derived from a colonic carcinoma. Increased epithelial positivity for these three subregion products has been observed in jejunal epithelium of patients with untreated coeliac disease depending on the number of intraepithelial lymphocytes. Moreover, colonic epithelium, which normally is virtually DR-negative, has been found to turn partially positive in inflammatory bowel disease. Enhanced expression has likewise been seen in salivary gland epithelium in sialadenitis.

It has been suggested that particular regulatory T cells are conducive to the switch of B cells from IgM to IgA expression in murine Peyers patches. These switch T helper cells are autoreactive and are apparently triggered directly by major histocompatibility complex class II determinants. There is evidence, moreover, that lymphokine(s) from autoreactive T cells are important for the promotion of IgA, and activated T cells may in addition induce transcription of the J-chain gene – perhaps via IL2 production.
Recent experiments with human appendiceal lymphoid cells have supported an important role of HLA-DR molecules in the induction of IgA and IgM production. As HLA-DR determinants are normally expressed on villus epithelium and follicle associated epithelium, immunoregulatory epithelial interactions with intraepithelial lymphocytes is a likely possibility (Fig. 3). It is interesting in this context that the follicle associated epithelium of human Peyers patches contains a much higher proportion (~40%) of T4 cells than the villous epithelium (~6%).

As the magnitude of immune responses is related to the density of major histocompatibility complex class II determinants, it is particularly tempting to speculate that epithelial HLA-DR determinants are crucial for terminal B-cell differentiation in secretory tissues remote from mucosal surfaces – for example, mammary and major salivary glands, where little or no foreign antigen gains access. The spatial relationship for such lymphoepithelial interactions seems to be available because IgA-producing cells tend to accumulate near DR-expressing ducts in human salivary glands and the alveolar cells of lactating mammary glands are strongly DR-positive. It is interesting in this context that murine lacrimal gland cells were shown to enhance IgA but suppress IgM and IgG production in cocultures with lymphoid cells from Peyers patches; the suppression seemed to be a direct epithelial effect whereas the enhancement depended on T cells. Involvement of autoreactive T helper cells stimulated by epithelial major histocompatibility complex class II molecules was therefore one suggested possibility.

Epithelial antigen presentation

Bland and Warren recently reported that major histocompatibility complex class II-positive columnar cells from rat intestinal villous epithelium could present ovalbumin to primed lymph node T cells which thereby were induced to proliferate. This class II-restricted immune response apparently led to antigen specific suppression. It is intriguing that although the phenotype of the inducer cell was not clearly established, it was suggested to belong to a T8 subset.

In keeping with the above findings, Mayer and Shlien applied human colonic epithelial cells in autologous or allogeneic mixed lymphocyte responses and reported preferential stimulation of T8 cells. These lymphocytes did not express the 9-3 antigen which is a marker of cytotoxic T cells and showed no cytotoxic effect; instead they performed non-specific suppression. It was possible to block the response with a rabbit antibody to human major histocompatibility complex class II determinants, indicating that such molecules in some way were involved in the induction phase (Fig. 3). The colonic epithelial cells were, moreover, able to present tetanus toxoid to T cells from subjects recently boosted with this soluble antigen.

Because T8 lymphocytes are the predominant intraepithelial lymphocytes along the normal gut (Fig. 2), the above observations suggest that these cells are responsible for epithelium-dependent suppression as part of a controlled mucosal immunoresponsiveness (Fig. 3). It should be noted, though, that several immunohistochemical studies have reported normal colonic epithelium to be virtually devoid of HLA-DR but strongly class I positive, and it has probably not been completely excluded that the
reported induction of T8-mediated suppression depended on initial activation of a small subset of intraepithelial T4 cells (Fig. 3). Colonic epithelial cells with abundant DR expression, obtained from patients with inflammatory bowel disease, were in fact found to stimulate T4 rather than T8 cells.62 Also, when Sollid et al63 tested the human HT-29 cell line after interferon-γ treatment to induce class II expression, these epithelial cells did not alone stimulate T cells in an allogeneic system. Nevertheless, the possibility exists that intraepithelial lymphocytes before or after contact with epithelial cells, receive additional critical stimulatory signals from other accessory cells (Fig. 3).

Much thus remains to be learned about interactions between epithelial cells and lymphocytes: the requirement for putative third party cells and their soluble mediators; the phenotype of the primarily stimulated T cells; and the actual restriction element(s) involved. The suggestion that a T4 helper response induced by antigen in the context of HLA-DR, may be controlled by specific T8 suppressor cells activated in the context of HLA-DQ,64 would apparently oppose the possibility of epithelium-dependent suppression because the latter restriction element does not normally seem to be expressed by gut epithelial cells.65 The existence of as yet unidentified class II subregion products, however, cannot be excluded.66 The presence of functional class II molecular hybrids67 also has to be considered. In this context the role of differential epithelial expression of various class II determinants in disorders like Sjögren’s syndrome68 and coeliac disease32 are of great interest.

Epithelial immunoglobulin transport

The ultimate goal of positive immune regulation in the secretory immune system, must be to favour development of B-cell clones with a prominent potential for J-chain expression; this is a prerequisite for external translocation of locally produced pIgA and pIgM.3 All normal secretory tissues of adults contain a remarkable preponderance of IgA-producing cells (Fig. 5), and about 90% of these immunocytes are J-chain-positive.13 J-chain-positive IgM-producing cells constitute a substantial fraction in the proximal small intestine,12 while IgG-producing cells are particularly numerous in respiratory mucosae – mainly in the stroma below the surface epithelium (Fig. 5).31

A common epithelial transport model for pIgA and pIgM was proposed by our laboratory in 1973–74 (reviewed in ref. 3); the model suggested that J chain and secretory component represent the ‘lock and key’ in the transport process (Fig. 6). Cell biology studies subsequently showed that rabbit secretory component was produced as a transmembrane protein family 25–30 kD larger than secreted free secretory component.69 It was proposed that the relatively large cytoplasmic extension (~15 kD) might function as an effector domain that contains information for guiding secretory component on its migration through the epithelial cell; this has recently been supported by experiments with mutant transmembrane secretory component.70 Studies of rat secretory component has shown that the endodomain is phosphorylated;70 whether this is of importance for its receptor function remains unknown.

Secretory component is synthesised as a core glycosylated trans-
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membrane glycoprotein on the rough endoplasmic reticulum and needs 30–60 minutes for maturation in the Golgi apparatus (Fig. 6); the subsequent migration to the basolateral plasma membrane and the transport of pIgA to the luminal cell face apparently take place very rapidly.71,72 Experiments with the HT-29 cell line have demonstrated that also human secretory component is produced as a transmembrane precursor (~95 kD) which, by addition of sugars, becomes about 20 kD larger than the secreted form.73 Human transmembrane secretory component depends on incorporation of J chain into pIgA and pIgM to undertake specific epithelial uptake of these polymers.74

Cytokine induced modulation of epithelial secretory component expression

When cDNA of rabbit secretory component was cloned, it was found to contain five extracellular domains with remarkable sequence similarities to each other and to Ig variable or constant regions.75 The amino acid sequence of human secretory component suggests the existence of similar homology

Fig. 5 Percentage distribution of IgA-, IgM-, and IgG-producing cells in various secretory tissues as indicated. Data from authors’ laboratory. Note that the small number of IgM-producing cells in bronchial mucosa was not included.
Fig. 6  Model for secretory component (SC)-mediated epithelial transport of dimeric IgA and pentameric IgM. [1] Synthesis and core glycosylation (−) of transmembrane SC in rough endoplasmic reticulum (RER) of epithelial cell. [2] Terminal glycosylation (●) in Golgi complex. [3] Phosphorylation (@) at some later step. [4] Complexing of SC with J-chain-containing IgA and IgM on basolateral cell membrane. [5] Endocytosis of ligand-SC complexes and excess SC. [6] Transcytosis of vesicles. [7] Cleavage and release of secretory IgA (SIgA) and free SC. The cleavage mechanism and the fate of the cytoplasmic tail of transmembrane SC are unknown (?). During the external translocation, covalent stabilization of the IgA-SC complexes regularly occurs (two disulphide bridges indicated in SIgA), whereas an excess of free SC in the secretion serves to stabilise the non-covalent IgM-SC complexes (dynamic equilibrium indicated for S1gM).

regions. Secretory component thus seems to belong to the ‘Ig-superfamily’ which includes a variety of cell surface recognition structures.

Several members of the Ig superfamily show striking responsiveness to immunoregulatory cytokines, and this was recently found to be true also for secretory component. Recombinant interferon-γ and tumour necrosis factor α upregulated the intracellular pool and epithelial membrane expression of functional secretory component in a dose-dependent manner. Interferon-γ is secreted by T cells during immune responses whereas activated macrophages are the best recognised source of tumour necrosis factor α. Our observations suggest that both these cell types may promote the external transport of pIgA and pIgM and thereby enhance the efferent limb of the secretory immune system (Fig. 3).

The epithelial transport capacity may thus be immunologically adjusted to
increased local pIg production during mucosal immune responses. Our immunohistochemical studies on human gastric mucosa are in keeping with this notion; signs of increased secretory component expression and enhanced uptake of IgA were observed in fundic and antral glands in gastritis specimens with dense lymphoid infiltration. Similar epithelial features have been noted in coeliac disease and in inflamed salivary glands. Conversely, dysplastic epithelium in longstanding ulcerative colitis may show reduced secretory component expression and decreased uptake of IgA.

**Induction and abrogation of systemic tolerance**

When the efferent limb of the secretory immune system is unable to undertake adequate exclusion of luminal antigens, the internal mucosal environment should preferably be protected against potentially harmful systemic type of immune reactions elicited by IgG, IgE, and T cell mediated delayed type hypersensitivity. There is experimental evidence in animals that such protection may be afforded by immunosuppressive mechanisms referred to as 'oral tolerance'. This phenomenon probably involves multiple mechanisms which in part may be different for humoral immunity and delayed type hypersensitivity. Antigen handling by an intact gut epithelium seems to be a critical induction event, and a role of intestinal antigen 'processing' has been suggested, at least for suppression of delayed type hypersensitivity. The nature of such processing and the cellular element(s) involved (epithelium or special mucosal macrophages), are still obscure. In view of the recent information about lymphoepithelial interactions discussed above, however, it is tempting to propose that suppression mediated by intraepithelial T8 cells is an important aspect of 'oral tolerance' (Fig. 3).

It is interesting that suppression of systemic IgE responses and delayed type hypersensitivity can likewise be obtained by exposing the upper respiratory tract mucosa – as opposed to the lower lung – to antigen-containing aerosols. Nevertheless, hypersensitivity reactions are much commoner in the upper respiratory tract than in the gut, which may be because of the scarcity of T8 cells in respiratory epithelia (Fig. 2). This disparity in the epithelial distribution of putative suppressor cells may likewise explain the relative preponderance of IgG-producing cells in respiratory mucosae (Fig. 5).

Abrogation of systemic tolerance to luminal antigens is probably involved in the pathogenesis of a variety of mucosal diseases. In experimental animals oral antigen feeding combined with some sort of damage to the gut epithelium or direct injection of antigen into the Peyers patches seems to be incompatible with induction of suppression. The same is true when the antigen-presenting cells are excessively activated by stimuli such as muramyl dipeptide, oestrogen or a graft-versus-host reaction. Both situations apparently favour overstimulation of T helper cells. Similar mechanisms seem to cause abrogation of tolerance in the respiratory tract.

Also aberrant epithelial major histocompatibility complex class II expression may be involved, as indicated by the preferential activation of T4 lymphocytes by colonic epithelial cells obtained from patients with inflammatory bowel disease. Enhanced and differential epithelial class II
expression may likewise be involved in exaggerated immune responses to gluten and other dietary antigens in coeliac disease and to autoantigens in Sjögren's syndrome. The epithelium may in this way contribute to the class II-restricted predisposition seen in the latter diseases.  

Conclusions

The secretory immune system is unquestionably the most important mediator organ of humoral immunity. Several components of this system have been well characterised, particularly those involved in the local production and epithelial transport of plgA and plgM. Understanding of mucosal regulation of local and systemic immunity, however, requires extensive exploration. The role of lymphoepithelial interactions is of great interest; and exciting information to this end has recently become available, from in situ studies of the spatial relationship between various cell types identified by functional markers and from in vitro experiments.

In the future the patient will hopefully benefit from rational manipulation of the mucosal immune system. Problems that have to be solved relate mainly to long-term induction of local immunological memory without concurrent generation of T cells that decrease the local IgA responses. New avenues based on epithelial targeting of immunogens by genetic manipulation of non-pathogenic microbes are currently being explored in several laboratories.

Supported by grants from the Norwegian Research Council for Science and the Humanities and the Norwegian Cancer Society.

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