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The B-cell system of human mucosae and exocrine glands

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Summary: The mucosae and exocrine glands harbour the largest activated B-cell system of the body, amounting to some 80–90% of all immunoglobulin (Ig)-producing cells. The major product of these immunocytes is polymeric (p)IgA (mainly dimers) with associated J chain. Both pIgA and pentameric IgM contain a binding site for the polymeric Ig receptor (pIgR), or secretory component (SC), which is a requirement for their active external transport through secretory epithelia. The pIgR/SC binding site depends on covalent incorporation of the J chain into the quaternary structure of the polymers when they are produced by the local immunocytes. This important differentiation characteristic appears to be sufficient functional justification for the J chain to be expressed also by most B cells terminating at secretory effector sites with IgD or IgG production; they probably represent a "spin-off" from sequential downstream C_H switching on its way to pIgA expression, thus apparently reflecting a maturational stage of effector B-cell clones compatible with homing to these sites. Observations in IgA-deficient individuals suggest that the magnitude of this homing is fairly well maintained even when the differentiation pathway to IgA is blocked. Certain microenvironmental elements such as specific cytokines and dendritic cells appear to be required for induction of IgA synthesis, but it remains virtually unknown why this isotype normally is such a dominating product of local immunocytes and why they have such a high level of J chain expression. Also, despite the recent identification of some important requirements in terms of adhesion molecules (e.g. integrin $\alpha 4\beta 7$ and MAdCAM-1) that explain the "gut-seeking" properties of enterically induced B cells, the origin of regionalized homing of B cells to secretory effector sites outside the gut remains elusive. Moreover, little is known about immune regulation underlying the striking disparity of both the class (IgD, IgM) and subclass (IgA1, IgA2, IgG1, IgG2) production patterns shown by local immunocytes in various regions of the body, although the topical microbiota and other environmental stimuli might be important. Rational design of local vaccines will depend on better knowledge of both inductive and migratory properties of human mucosal B cells.

Introduction

Secretory immunity is the best characterized part of the mucosal immune system, although the regulation of B cells forming its basis is poorly understood. The interest in adaptive local immunity increased in the 1960s when it was reported by several laboratories that the predominant immunoglobulin (Ig) class in external body fluids is IgA rather than IgG (1). The

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secretory IgA (SIgA) molecule was found to be unique (2), both because of its polymeric structure and association with the secretory component (SC). This epithelial glycoprotein was initially termed "transport piece" to suggest that it might facilitate the entry of IgA into epithelial cells (3) – an interesting suggestion that would turn out to be true in an even more intricate manner than anticipated.

At about the same time, Crabbé et al. (4) demonstrated an isotype distribution of intestinal Ig-producing immunocytes (B-cell blasts and plasma cells) strikingly different from that in peripheral lymphoid organs; mucosal IgA cells were reported to be at least 20 times more frequent than IgG cells. Despite the absence of a satisfactory immunoregulatory explanation, a general dominance of IgA immunocytes at all secretory effector sites has now been well established for a couple of decades (5). Moreover, the first direct evidence showing that these cells are peculiar in that they produce polymeric (p)IgA (mainly dimers) rather than monomeric IgA, was provided by our laboratory in the early 1970s (6); but again the mechanism(s) driving this additional characteristic of mucosal B cells remains elusive.

The J chain plays a key role in secretory immunity

In the late 1960s we found that not only pIgA but also pentameric IgM is enriched in exocrine body fluids because of active external transport (7). This secretory IgM (SIgM) was subsequently shown to be associated with SC and to follow the same intracellular route through secretory epithelia as SIgA (8, 9). Therefore, an epithelial transport model common for pIgA and pentameric IgM was proposed by our laboratory in 1973–74 (6, 10, 11). A few years earlier, the "joining" or J chain had been identified as a unique polypeptide of approximately 15 kDa shared by the two Ig polymers (12, 13). Only the heavy chains of IgA and IgM have the extra 18 amino-acid tailpiece containing a penultimate cysteine residue employed for covalent bonding of the J chain (14). Over the next decade, we obtained evidence suggesting that the J chain and transmembrane SC represent the "lock and key" in receptor-mediated external translocation of pIgA as well as pentameric IgM (15), a notion that has recently been firmly established (16–18). Because of its affinity to both ligands, transmembrane SC is now often referred to as the polymeric Ig receptor (pIgR).

A key role for J chain in secretory immunity accords well with the fact that B cells subjected to terminal differentiation at secretory effector sites show prominent J chain expression regardless of concurrent Ig class production (19, 20). In teleological terms, one would like to think that the intricate co-operation between the mucosal B-cell system and secretory epi-

thelia has developed during phylogeny and is preserved in mammals because secretory antibodies are necessary for survival of the species.

The latter view is challenged by the fact that approximately two-thirds of subjects with selective deficiency of IgA remain healthy – apparently even when living under poor hygienic conditions (21) – and gastrointestinal infections are more common in patients with generalized B-cell deficiency than in those with a selective IgA defect (22, 23). Therefore, non-specific innate defence mechanisms together with T-cell-mediated immunity and serum antibodies often afford sufficient mucosal protection (24). In addition, a compensatory SIgM response is regularly seen in the gut when IgA is lacking (22, 25). However, the absence of SIgA antibodies does predispose to recurrent upper airway infections, allergic disorders, autoimmunity, certain gastrointestinal infections and coeliac disease (22, 23, 26). Also, a strikingly increased frequency of Crohn's disease has recently been observed in selective IgA deficiency (L. Hammarström, personal communication).

Altogether, it is difficult to evaluate the clinical role of secretory antibodies at mucosal surfaces. This is true also in subjects with a completely normal immune system, because a superimposed protective effect of concurrent systemic cellular and humoral immunity must always be considered (27, 28). We have now generated a pIgR/SC knockout mouse that completely lacks secretory immunity to better evaluate its overall role in mucosal defence (29).

Induction of secretory immunity and its critical role in infancy
Mucosa-associated lymphoid tissue (MALT) appears to be of central importance to the induction, unique regulation and special dissemination that take place for mucosal B cells (30). In the upper aerodigestive tract, the best known such organized lymphoepithelial structures are the tonsils. In the gut, MALT includes particularly the aggregated lymphoid follicles of the ileal Peyer's patches (Fig. 1) but also solitary follicles which are especially numerous in the appendix and distal large bowel (31). The mesenteric lymph nodes are sometimes considered to be part of the gut-associated lymph tissue (GALT) as well, and this may be justified because they do to some extent mirror the B-cell biology of Peyer's patches as discussed below.

The appearance of secretory antibodies in breast milk is a reflection of the MALT-mammary gland axis of B-cell migration (32, 33), and the protective value of breast-feeding is highlighted in relation to infections in the newborn period, particularly in the developing countries. Mucosal pathogens are now a major killer of children below the age of 5 years, being responsible for more than 14 million deaths of children annu-

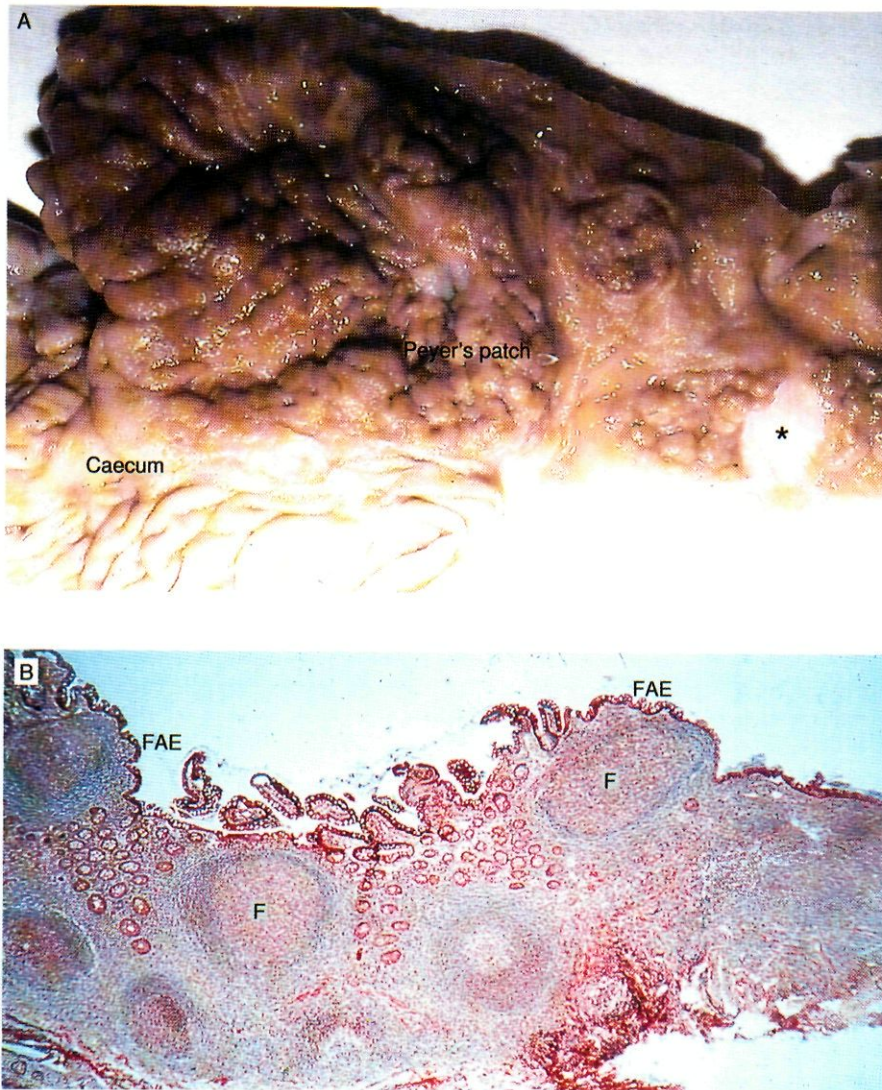


Fig. 1. Human Peyer's patches. A. Patches in the terminal ileum of a 10-year-old girl. A specimen has been excised from one of the patches (*). B. Histology of normal human Peyer's patch (haematoxylin-eosin staining) containing several activated lymphoid follicles (F) with germinal centres beneath the specialized follicle-associated epithelium (FAE) that lacks villi. Magnification: $\times 22$.

ally. Diarrhoeal disease alone claims a toll of 5 million children per year, or about 500 deaths every hour. These sad figures document the need for mucosal vaccines to enhance surface defence against common infectious agents, in addition to advocating breast-feeding. Convincing epidemiological documentation suggest that the risk of dying from diarrhoea is reduced 14–24 times in nursed children (34, 35). Indeed, exclusively breast-fed infants are better protected against a variety of infections (34, 36), atopic allergy (34, 37) and coeliac disease (38). Moreover, recent experiments in neonatal rabbits strongly suggest that SIgA is a crucial protective component of breast milk (39).

This review will focus on the functional characteristics of B cells induced in human MALT, and on the role they play in human secretory immunity by co-operating with pIgR/SC-expressing epithelia. A brief outline of the complex interac-

tions that take place between the various elements of the adaptive mucosal defence system will first be given as a basis for subsequent in-depth discussions.

Humoral defence of mucosae

Surface protection by immune exclusion

Immune exclusion is a term coined for non-inflammatory surface protection mediated by antibodies in co-operation with innate non-specific factors and thus refers to the "first line" of mucosal defence (Fig. 2). This mechanism has a formidable task because the mucosal surface area is approximately 400 m² in an adult human, and is mostly covered by a vulnerable monolayered epithelium. Antibody activities in the epithelial mucus layer and serous secretions are mainly provided by SIgA and SIgM, but there may be some contribution by serum-derived or

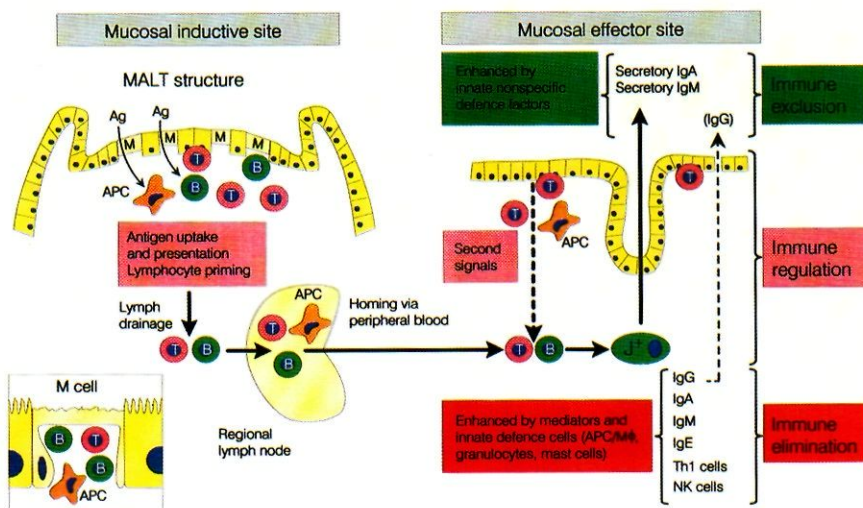


Fig. 2. Schematic representation of the three main components of mucosal immune defence: immune exclusion, immune regulation and immune elimination. Antigen stimulation in mucosa-associated lymphoid tissue (MALT) provides "first signals" to B cells which migrate ("home") to secretory effector sites via lymph and peripheral blood. "Second signals" (dashed heavy arrow) induce local proliferation and

terminal differentiation of the extravasated B cells. Most plasma cells generated in this way produce J chain-containing dimeric IgA which is translocated to the lumen as secretory IgA. Also, some IgM is actively transported through the secretory epithelial cells, whereas small amounts of IgG normally reach the lumen by passive diffusion (dashed thin arrow).

locally produced monomeric IgA and IgG (40), which can reach the surface quite rapidly by paracellular diffusion through the epithelium, particularly after some sort of mucosal irritation (41). In fact, this passive external antibody transfer most likely plays an important protective role at surfaces where there is little proteolytic degradation of antibodies, such as in the respiratory (32, 42) and female genital (43) tracts.

In addition, secretory antibodies might reinforce immune exclusion by capturing antigen during their pIgR/SC-mediated transport through epithelial cells. Thus, while continuously monitoring the vulnerable surface monolayer, pIgA and pentameric IgM could neutralize viruses intracellularly and carry the pathogens and their products back to the lumen, thus avoiding any damage to the epithelium through cytolysis (44–47).

The role of MALT in immune regulation

Regulation of mucosal immunity takes place both in the organized inductive MALT structures (Fig. 2) and at the diffuse secretory effector sites of the mucosae such as the intestinal lamina propria and epithelium (on the right in Fig. 2). Particulate and infectious antigens appear to be primarily taken up by MALT through the follicle-associated epithelium (FAE) which contains "membrane" (M) cells particularly designed for sampling and inward transport of luminal material (27, 48). By cognate interactions with antigen-presenting cells (APCs),

naïve T and B lymphocytes are primed to become memory and effector cells for subsequent dissemination ("homing") to effector sites (30, 33).

After intracellular processing to provide immunostimulatory peptides, antigen is usually presented to CD4⁺ T helper (Th) lymphocytes by professional APCs, either macrophages or more specialized dendritic cells (DCs). In addition, luminal immunogenic peptides might be presented directly to sub- and intraepithelial T lymphocytes by epithelial cells (49). In the human gut, mucosal APCs, the FAE of GALT outside the M cells, and the small intestinal villous epithelium express surface determinants encoded by loci present in the class II region of the major histocompatibility complex (MHC), particularly HLA-DR (27, 30), and also classical and non-classical MHC class I molecules (49). To elicit specific immune responses, these gene products are required for appropriate antigen presentation. Class II-positive B lymphocytes that abound close to the M cells, may also present antigens efficiently to T cells in cognate immunostimulatory or downregulatory interactions (30).

T cells subjected to stimulation in MALT express co-stimulatory molecules and release mediator substances (cytokines) which act on other lymphocytes in the microenvironment. Most adjacent B cells primed by such "first signals" migrate rapidly via lymphatics to regional lymph nodes where they may be subjected to further stimulation; they then mostly reach

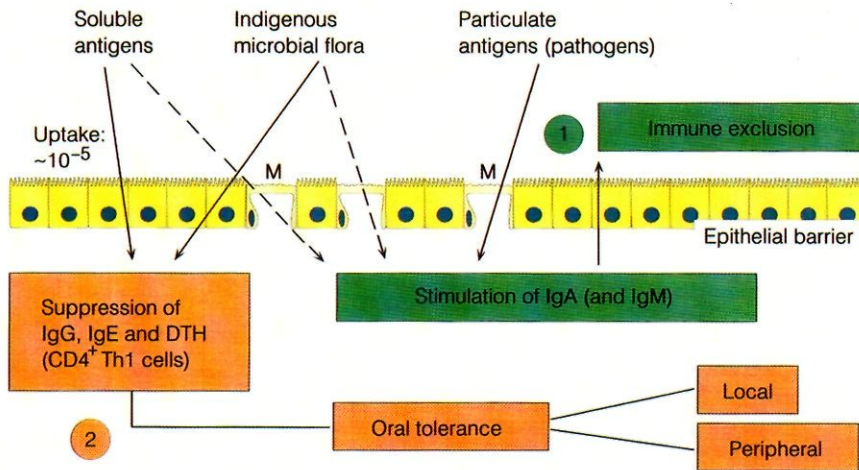


Fig. 3. Schematic depiction of two major adaptive immune mechanisms induced in the gut. (1) Immune exclusion limits epithelial colonization of pathogens and inhibits penetration of harmful foreign material. This first line of defence is principally mediated by secretory antibodies of the IgA (and IgM) class in co-operation with various non-specific innate protective factors (not shown). Secretory immunity is preferentially stimulated by particulate antigens and pathogenic infectious agents taken up through M cells as indicated (see Fig. 2).

(2) Penetrating soluble dietary antigens (magnitude of uptake indicated) and the normal microbial flora are less stimulatory for secretory immunity (broken arrows) but induce, instead, suppression of proinflammatory humoral immune responses (IgG and IgE antibodies) as well as delayed-type hypersensitivity (DTH) mediated by activated T helper cells (CD4⁺) of the interferon- γ -producing Th1 subset. This complex and poorly defined phenomenon is called oral tolerance; it may exert down-regulatory effects both locally and in the periphery.

peripheral blood and are finally seeded into distant secretory effector sites such as the intestinal lamina propria where they develop into Ig-producing immunocytes (30, 33). This terminal differentiation requires "second signals" that are modulated by available cognate antigen, various local cell types expressing MHC class II molecules and regulatory T lymphocytes (Fig. 2). Most B cells that home from MALT, apparently belong to memory or effector clones of an early maturation stage; this is indicated by their proneness to express cytoplasmic J chain regardless of concomitant isotype production, although the IgA class is normally predominant (30). J chain-containing pIgA and pentameric IgM are finally translocated to the lumen as SIgA and SIgM by a regulated receptor-mediated (pIgR/SC-dependent) epithelial transport mechanism (see below).

Immune elimination and mucosal tolerance

Multiple mechanisms are involved in removal of foreign material that has penetrated the epithelial barrier. Such immune elimination represents a "second line" of mucosal defence and depends partly on serum-derived or locally produced antibodies of various isotypes, probably often operating in combination with antibody-dependent cell-mediated cytotoxicity (ADCC), T cells, natural killer (NK) cells, and various phagocytes and DCs (Fig. 2). As discussed below, clearance of antigens from the lamina propria may be performed by a non-inflammatory pIgR/SC-dependent mechanism, but is likely to be

enhanced by a variety of non-specific biological amplification systems of innate immunity. Thus, if satisfactory immune elimination is not rapidly achieved, inflammation and immunopathology may develop, thereby giving rise to clinically overt mucosal disease (27). Such "frustrated" immunological antigen clearance is apparently part of the evolving pathogenesis of various chronic disorders, including coeliac disease and inflammatory bowel disease (IBD). The purpose of the secretory immune system is to avoid this adverse development, particularly by excluding infectious agents (Fig. 3).

Mucosally induced tolerance is another strategy employed by the immune system to avoid hypersensitivity reactions against innocuous soluble antigens that gain access to the sub-epithelial compartment (50, 51). In the gut, this suppressive regulatory mechanism is best known as "oral tolerance" against dietary proteins, which are probably mainly taken up through the extensive epithelial surface covering the mucosal effector tissue (Fig. 3). Mucosally induced tolerance is poorly understood and might involve several immunological events, although rapid removal of penetrating antigens from the lamina propria by poorly stimulatory DCs appears to be one important facet (50, 52–55). Chronic mucosal disorders such as coeliac disease and IBD are considered to reflect abrogation of oral tolerance to gluten and certain components of the indigenous microbiota, respectively (56, 57). Excessive local production of IgA and IgG antibodies to innocuous luminal antigens occurs

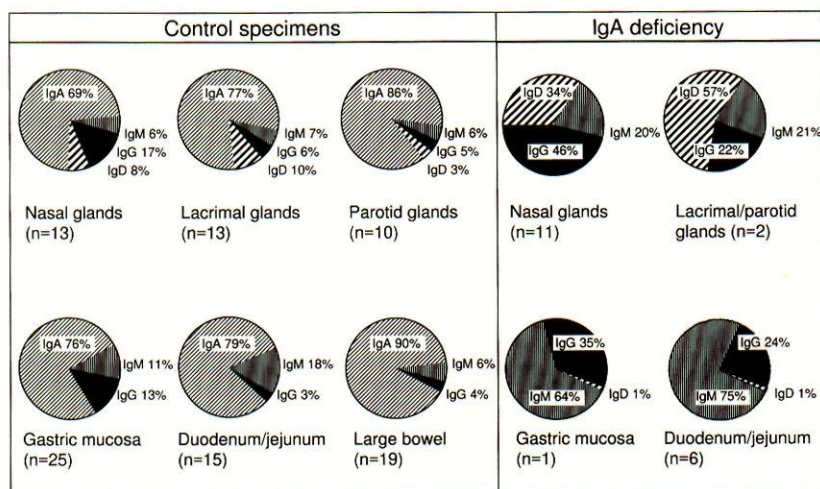


Fig. 4. Average percentage distribution of immunocytes producing different Ig classes in various secretory tissues from controls and subjects with IgA deficiency.

[Based on published data from the authors' laboratory]

in both conditions, but the relationship between secretory immunity and mucosal tolerance remains elusive (50).

B cells in normal secretory effector tissues

Distribution of B lymphocytes and Ig-producing immunocytes
All normal secretory effector sites in adult humans contain a remarkable preponderance of IgA-producing immunocytes, including B-cell blasts and plasma cells (Fig. 4). This is particularly true for the intestinal mucosa as first reported on the basis of immunohistochemical studies by Crabbé et al. (4) and Rubin et al. (58). There are approximately 10^{10} such cells per metre of adult bowel (5). Absolute figures are difficult to obtain for other secretory tissues where the cells are more heterogeneously distributed throughout the stroma than they are in the intestinal lamina propria; however, it has been convincingly established that at least 80% of all Ig-producing cells of the body are located in the gut, both in the murine (59) and human (60) species. Moreover, in view of the length of the adult human gut (7–9 m), the size of the intestinal immunocyte population must be quite impressive compared with the number of Ig-producing cells present in human bone marrow, spleen and lymph nodes – altogether estimated to 2.5×10^{10} (61).

Phenotypes of intestinal B cells

Analyses of B cells isolated from the gut lamina propria of mice (62) indicated a fairly similar content of Ig-producing immunocytes and small B lymphocytes (22% vs 18%). Most of the latter expressed IgD and IgM on their surface (sIgD⁺IgM⁺) or only

IgM (sIgD⁺IgM⁺), thus representing the naive and memory/effector phenotype, respectively (63). The total fraction of B cells was found to equal that of T lymphocytes (62). However, such studies are prone to underestimate the relative number of Ig-producing immunocytes because it is difficult to exclude circulating small lymphocytes from the tissue suspensions and there may, in addition, be a selective loss of large (stimulated) lymphoid cells during the isolation steps (64). Indeed, earlier analyses of B cells isolated from human intestinal mucosa have provided highly discrepant results and are inconclusive in quantitative terms (65). Moreover, preparation of mucosal mononuclear cell suspensions is jeopardized by the fact that various lymphoid cells have different compartmental distributions, and it is difficult if not impossible to exclude inclusion of small GALT structures (solitary lymphoid follicles) when studying dispersed lamina propria cells.

Distribution of B cells in GALT vs intestinal lamina propria

In our recent studies of human GALT (represented by Peyer's patches and appendix), in comparison with normal small intestinal lamina propria, we found striking differences in the phenotypic distribution of lymphoid cells (66). Suspensions of GALT structures contained approximately equal proportions of small B lymphocytes (CD19⁺) and T lymphocytes (CD3⁺), but very few terminally differentiated (CD38⁺) B cells (Fig. 5). Paired immunofluorescence in situ staining showed that the small naive B lymphocytes were mainly derived from the follicular mantle-zone population (CD19⁺CD20⁺sIgD⁺IgM⁺) surrounding the germinal centres (Fig. 6). Conversely, most small lymphoid cells in the lamina propria were T lymphocytes, and

Table 1. Flow-cytometric analysis of the phenotypic distribution of B and T cells in two human gut compartments^a

| Phenotype proportion ^b | Co-expression pattern ^c | |
|---|---|---|
| | Small cells ^b | Large cells ^b |
| Organized GALT | | |
| B cells | | |
| 50% CD19 ⁺ CD38 ⁺ α4β7 ^{int} | 25% L-selectin ⁺ 50% sIgD ⁺ (40% L-selectin ⁺) 30% sIgA ⁺ (15% L-selectin ⁺) 14% sIgG ⁺ (25% L-selectin ⁺) | |
| <5% CD19 ⁺ CD38 ^{hi} α4β7 ^{int/hi} | | L-selectin ⁻ |
| T cells | | |
| 45% CD3 ⁺ α4β7 ^{int/hi} | 40% L-selectin ⁺ 70% CD4 ⁺ αEβ7 ⁻ 20% CD8 ⁺ αEβ7 ^{-/+} | Only few cells |
| Mucosal lamina propria | | |
| B cells | | |
| 10% CD19 ⁺ CD38 ⁺ α4β7 ^{int/hi} | L-selectin ⁻ >80% sIgA ⁺ | |
| 25% CD19 ⁺ CD38 ^{hi} α4β7 ^{hi} | | L-selectin ⁻ >90% sIgA ⁺ ^d |
| T cells | | |
| 60% CD3 ⁺ α4β7 ^{int} | L-selectin ⁻ 65% CD4 ⁺ αEβ7 ^{-/+} 30% CD8 ⁺ αEβ7 ⁺ | |
| 4% CD3 ⁺ α4β7 ^{hi} | | L-selectin ⁺ αEβ7 ⁻ 90% CD4 ⁺ <5% CD8 ⁺ |

^a Adapted from (66, 202, 203) and unpublished data from the authors' laboratory.^b Dispersed mononuclear cells gated according to size and analyzed for surface markers and fluorescence intensity (+, positive; -, negative; lo, low; int, intermediate; hi, high).^c Calculated from all B or T cells.^d Cytoplasmic IgA (cIgA) determined by immunohistochemistry.

most large cells expressed B-cell markers (Fig. 5) and were mainly of the terminally differentiated phenotype (CD38⁺) with IgA on the surface and/or in the cytoplasm (Table 1). Importantly, this flow-cytometric distribution was confirmed by *in situ* studies (66).

Animal experiments have indicated that T lymphocytes become preferentially localized in the villi (67). This is also where most IgA-producing cells are found in the mouse (unpublished observations), whereas in the rat they accumulate mainly around the crypt regions (68). Likewise, in human proximal small intestinal mucosa approximately 65% of all Ig-producing immunocytes are normally located in a zone including the luminal 100 μm of the crypt layer and the basal 100 μm of the villi (5, 69). In this zone, which constitutes less than

one-third of the total mucosal height, Ig-producing cells are intermingled with relatively few T lymphocytes, whereas the latter often abound more apically in the villi. However, this *in situ* distribution of memory/effector cells does not necessarily indicate the level where they immigrate into the mucosa (68).

Disparate distribution of IgA subclass-producing cells

A relatively large fraction of the IgA2 subclass (17–64%) has been reported for IgA immunocytes in gastrointestinal mucosa compared with the proportion (7–25%) seen in peripheral lymphoid tissue, tonsils and the airway mucosae (70–74). However, the IgA2 immunocytes predominate only in the large bowel mucosa (Fig. 7). The concentration ratios of the two SIgA subclasses in various exocrine secretions (72, 75, 76) are quite similar to the relative distribution of IgA1 and IgA2 immunocytes at the corresponding effector sites (Fig. 8), attesting to the fact that pIgA of both isotypes are equally well transported externally, as also suggested by their binding affinity to SC (77). The relative increase of pIgA2 in secretions may be important for the stability of secretory antibodies because SIgA2, in contrast to SIgA1, is resistant to several IgA1-specific proteases which are produced by a variety of potentially pathogenic bacterial species (78).

The molecular events underlying preferential IgA1 or IgA2 responses remain elusive. Secretory antibodies to lipopolysaccharide (LPS) from Gram-negative bacteria are generally of the SIgA2 subclass, whereas protein antigens stimulate predominantly SIgA1 (79, 80). The fact that jejunal IgA immunocytes are mainly of the IgA1 subclass (~77%), in contrast to the IgA2 dominance (~64%) in the colon (73), may hence reflect the disparate distribution of food antigens vs Gram-negative bacteria in the normal gut. We have observed that bacterial overgrowth in bypassed jejunal segments alters the composition of the local immunocyte population with an increase of IgA2 and a decrease of IgM (81), suggesting LPS-induced direct isotype switching from Cμ (IgM) to Cα2 (IgA2), or progressive sequential downstream switching of the Ig heavy-chain constant region (C_H) genes (30).

IgM-producing cells

IgM immunocytes constitute a substantial but variable fraction of the normal gastrointestinal B-cell population in adults (Fig. 4). The reason for the relatively high proportion of this isotype (~18%) in the proximal small intestine remains unknown; it may be related to the absence of LPS (see above), and is in striking contrast to the situation in the upper aerodigestive tract (25, 82). This disparity between the two regions is remarkably accentuated in patients with selective IgA deficiency (Fig. 4).

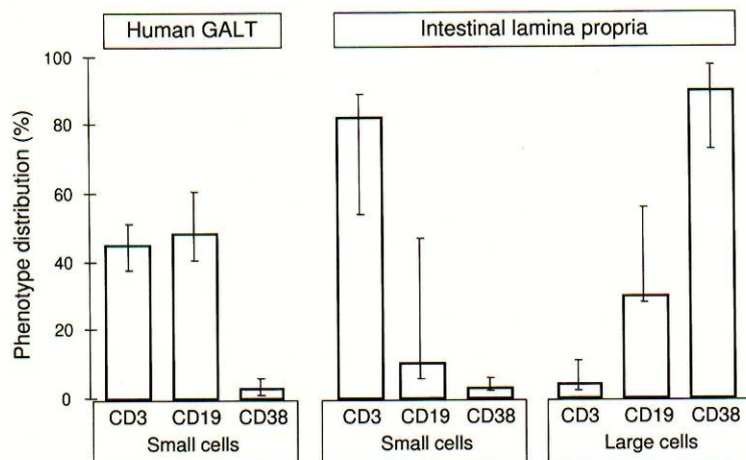
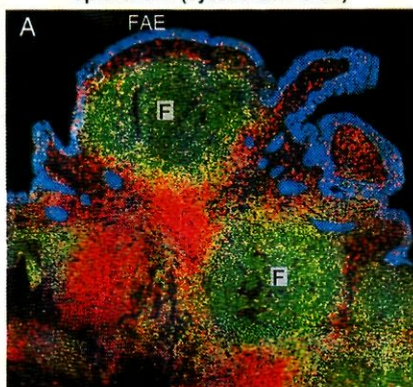


Fig. 5. Phenotypic proportions (%) of T cells (CD3), B cells (CD19), and B-cell blasts or plasma cells (CD38) obtained by flow cytometry with small mononuclear cells obtained from human gut-associated lymphoid tissue (GALT) represented by Peyer's patches and appendix, or with normal human intestinal lamina propria small or large mononuclear cells as indicated. Medians (columns) and observed ranges (vertical lines) are depicted. [Adapted from (66) and including unpublished data representative of 10 additional samples]

B cells (CD20 green), T cells (CD3 red), epithelium (cytokeratin blue)



B cells (CD20 green), naive B cells (sIgD red), epithelium (cytokeratin blue)

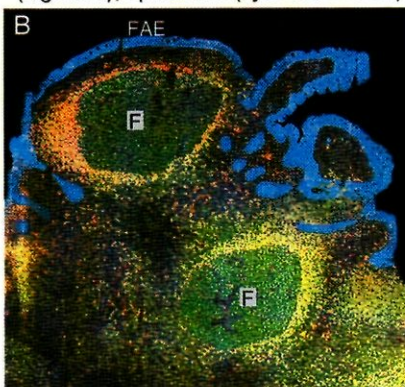


Fig. 6. Paired immunofluorescence staining for (A) B and T cells and (B) naive B cells (sIgD⁺) in parallel cryosections from human Peyer's patch. Note that IgD is mainly expressed on the surface of B cells in the mantle zones surrounding germinal centres of the lymphoid follicles (F) but is also to some extent present on B cells distributed in the extrafollicular areas. FAE, follicle-associated epithelium. Magnification: $\times 25$.

IgG-, IgD- and IgE-producing cells

IgG immunocytes constitute 3–4% in normal adult intestinal mucosa, but a considerably larger contribution is found in gastric and nasal mucosa (Fig. 4), which often shows some low-grade inflammation even in healthy subjects (82, 83). Only occasional IgD-producing cells are encountered in the gastrointestinal mucosa (5), whereas such immunocytes constitute a significant immunocyte fraction (3–10%) at secretory sites of the upper aerodigestive tract, including nasal mucosa and salivary and lacrimal glands (25, 82, 84). In IgA deficiency, the disparity between the two regions is even more striking for IgD than for IgM immunocytes (Fig. 4). According to our immunohistochemical studies, IgE-producing cells are virtually absent from human mucosae, although occasional exceptions are seen in atopic allergy (85).

Disparate distribution of IgG subclass-producing cells

Our studies of mucosal IgG immunocytes have shown a distribution of IgG1 (56–69%) that generally makes it the predominant locally produced IgG subclass (86–88). In the distal gut, IgG2 cells are more frequent (20–35%) than IgG3 cells (4–6%), whereas the reverse is often true in the upper airway mucosa. This IgG subclass disparity supports the idea that the B-cell isotype switching pathways differ in various mucosal regions (30). Interestingly, the genes for $\text{C}\gamma 2$ and $\text{C}\alpha 2$ are located on the same duplicated DNA segment (89), and many carbohydrate and bacterial antigens preferentially induce an IgG2 response in addition to IgA2, whereas proteins (T-cell-dependent antigens) primarily generate an IgG1 (and IgG3) response together with IgA1 (90), such as seen in the proximal small intestine (73, 87).

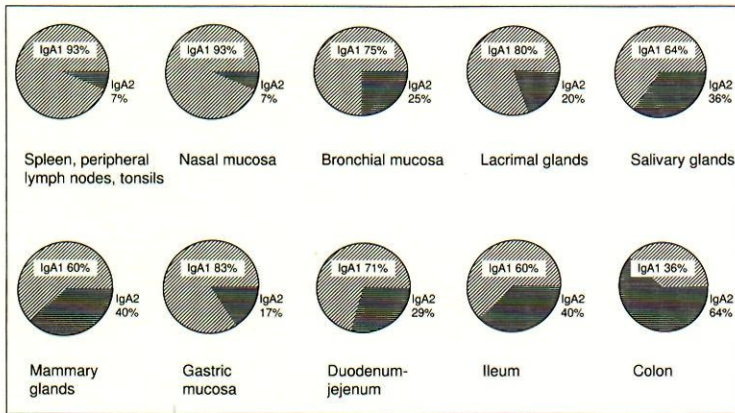


Fig. 7. Average percentage subclass distribution of IgA-producing immunocytes in human lymphoid and normal secretory tissues.

[Based on published data from the authors' laboratory, and from (74) for bronchial mucosa]

J chain and nature of locally produced IgA and IgM

To support secretory immunity, MALT must favour the development and dissemination of B cells with prominent J chain expression, thereby allowing subsequent production of pIgA and pentameric IgM that can complex with pIgR/SC and become externally transported as SIgA and SIgM at secretory effector sites (Fig. 2). The J chain gene appears to be evolutionarily conserved to a remarkable degree (91), suggesting that it has been acquired from the innate immune system. Its functional role in the adaptive immune system was an enigma until our laboratory obtained evidence that the presence of J chain in pIgA and pentameric IgM is crucial for the efficient non-covalent binding of these polymers to free SC or pIgR (15, 92–94). This notion has been supported by observations made in J chain knockout mice (95) and in transgenic cells expressing genes for Ig, J chain and pIgR/SC (18, 96). The actual pIg binding site for free SC (94) or pIgR (16, 17) can indeed be blocked by antibody to J chain. Moreover, experimental studies have suggested that although this peptide is not required for IgA and IgM polymerization, the available amount of J chain determines the production ratio between monomeric IgA and pIgA as well as between pentameric IgM and J chain-deficient hexameric IgM (94, 97–99).

Expression of J chain and cytoplasmic SC affinity

IgA immunocytes at secretory effector sites clearly differ from those found in lymph nodes, spleen and bone marrow by showing a much more prominent synthesis of pIgA than of monomeric IgA (6, 20). The presence of pIgA in their cytoplasm becomes immunohistochemically apparent by incubating tissue sections with purified free SC (Fig. 9). The SC binding site (and therefore the polymeric Ig structure) can thus be shown to be generated at the cytoplasmic level by incorpora-

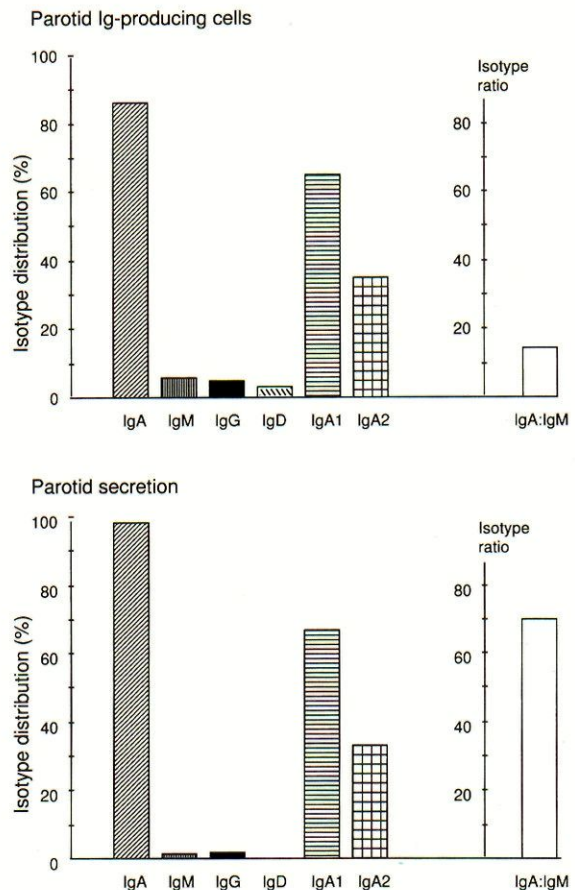


Fig. 8. Comparison of isotype proportions expressed by Ig-producing cells in normal adult parotid glands and present in stimulated parotid secretions.

[Based on data from the author's laboratory]

tion of J chain (Fig. 10A), and both IgA and IgM immunocytes at secretory effector sites produce abundant amounts of this polypeptide (20, 100).

Immunohistochemical staining of IgA-associated J chain is largely dependent on the unmasking of its antigenic determinants by acid urea treatment (Fig. 10B). Conversely, mucosal IgM immunocytes seem to contain a substantial excess of free cytoplasmic J chain as indicated by 100% positivity even in untreated tissue sections (20). This disparity could be explained by the fact that J chain is mainly incorporated into pentameric IgM late in the intracellular assembly process, at a stage when the polymers are not yet completely stabilized by covalent bonds (101, 102). Moreover, studies of murine plasmacytomas showed that J chain production was excessive in IgM cells compared with that in IgA cells (103). Likewise, a 5-fold molar excess of cytoplasmic over secreted J chain was found in IgM-producing rabbit spleen cells after mitogen stimulation (104).

Mucosal production of pIgA

On average, 88% of IgA1 and 100% of IgA2 immunocytes in normal intestinal mucosa are engaged in pIgA production as revealed by their J chain expression (105), but this fact does not exclude a variable concurrent output of monomers (20, 94). The latter possibility was indicated by the finding that the venous effluent of perfused segments of human gut contained 20–30% monomeric IgA (106). However, physicochemical analyses of the IgA spontaneously secreted by cultured mononuclear cells obtained from human gut mucosa have provided discrepant information: MacDermott et al. (107) reported 31% monomers whereas Kutteh et al. (108) concluded that about equal proportions of monomeric IgA and pIgA were produced. Contamination with epithelial cells, which release preformed SIgA, is an inherent problem in such experiments (108). It is also possible that free SC, released into the culture fluid from epithelial cells, may complex with pIgA and thereby partially mask its *in vitro* production. There is, on the other hand, evidence suggesting that the proportion of pIgA secreted from lymphoid cells may increase after prolonged culturing (109). Altogether, therefore, no conclusive information exists with regard to the proportion of monomers actually secreted by the intestinal pIgA-producing immunocytes.

Nature of intracellular IgA

There are likewise discrepant opinions as to the nature of intracellular IgA in the mucosal immunocytes. We have maintained that the diffuse cellular *in vitro* binding of free SC (Fig. 9), together with the immunohistochemical requirement for unmasking of cytoplasmic J chain (Fig. 10), constitutes direct

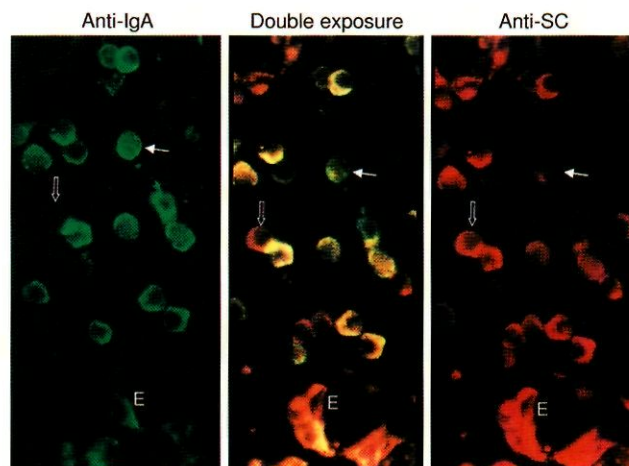


Fig. 9. Paired immunofluorescence staining to demonstrate cytoplasmic SC affinity of mucosal IgA immunocytes. Section of washed human colonic mucosa preincubated with free SC followed by green anti-IgA + red anti-SC. Note in double-exposure (centre) that most IgA cells show varying degree of mixed colour as evidence for binding of SC to pIgA, whereas a few cells virtually lack SC-binding capacity (arrow) and are therefore rather green (probably mainly monomer producers). The purely red cells (open arrow) in the double exposure are SC-binding IgM immunocytes. The columnar epithelial cells of the glands at the bottom (E) contain innate SC and transport pIgA; they therefore show mixed cytoplasmic colour. Magnification: $\times 110$.

evidence of a substantial intracellular covalent polymerization in intestinal IgA immunocytes (20, 94). Ultrastructural localization of J chain in such normal cells has in fact suggested that the polymerization process begins in the endoplasmic reticulum (110), and this notion is supported by similar studies performed on transformed normal lymphoid cells (111). Conversely, pokeweed mitogen-stimulated human peripheral blood lymphoid cells, and an Epstein–Barr virus (EBV)-transformed lymphoblastoid cell line, were reported to contain very little cytoplasmic pIgA despite secreting mainly polymers (109). These results are in agreement with previous studies on murine tumour cells (112).

It is difficult to know to what extent such cell culture results reflect the normal situation in the gut. After B-cell stimulation, there is increased intracellular expression of J chain (104, 113), and also induction of a sulphhydryl oxidase catalysing the assembly of pIg subunits (114). Delamette et al. (115) suggested that its activity is expressed only in the Golgi apparatus of Ig-producing tumour cells, whereas stimulation of normal immunocytes triggers its synthesis at the membranous elements of the rough endoplasmic reticulum. Thus, the amount and subcellular distribution of this enzyme might influence the

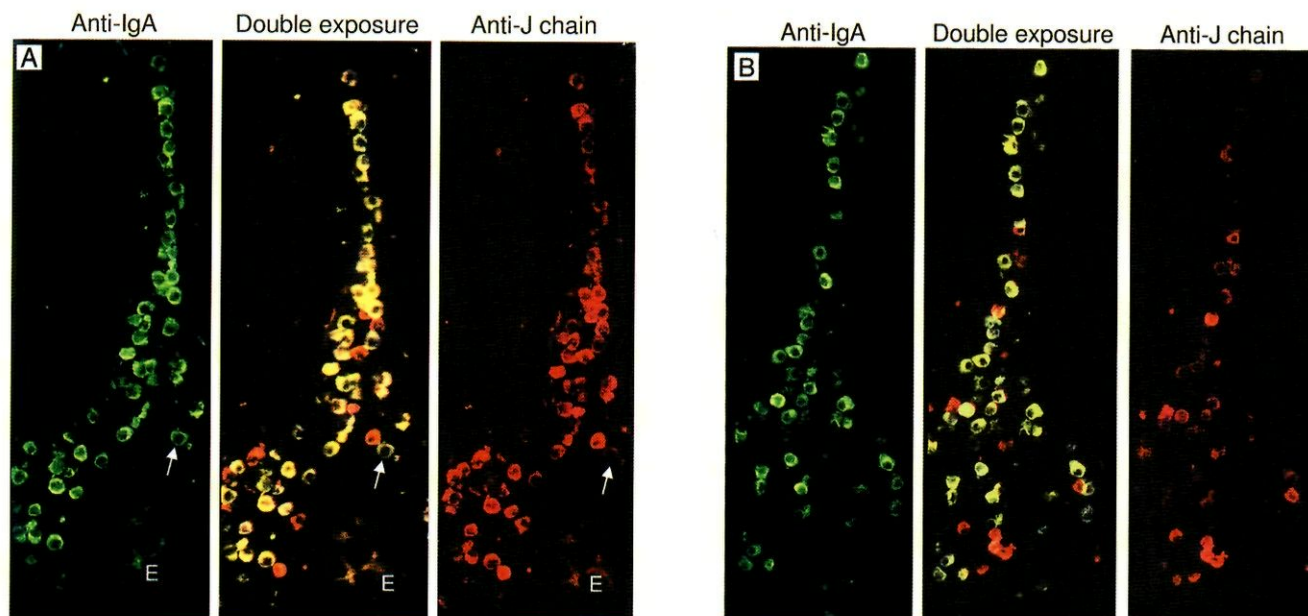


Fig. 10. J chain expression in mucosal immunocytes. A. Paired immunofluorescence staining for IgA (green) and J chain (red) in washed normal human duodenal mucosa after pretreatment of section with acid urea to unmask J chain epitopes. Double exposure (centre) shows yellow colour in most IgA immunocytes, whereas cells of another isotype (probably mostly IgM) show pure red staining. Only one IgA cell (arrow) is almost negative for J chain, therefore probably

producing mainly monomers. Crypt epithelial elements (E) are yellow as a sign of plg transport. **B.** Comparable double exposure from parallel untreated section shows numerous IgA immunocytes, most of them being rather greenish because there is only faint concurrent J chain staining. Several intensely red cells of another isotype show strong positive reactivity for cytoplasmic J chain even without acid-urea treatment. Magnification: $\times 50$.

Ig polymerization process. Therefore, direct analyses of single normal intestinal IgA immunocytes are required before their content of monomers and dimers can be defined in quantitative terms.

Nature of locally produced IgM

We found to our surprise that the cytoplasmic SC-binding capacity of IgM-producing cells in various secretory tissues was 17–23% lower than the J chain positivity, and in some intestinal specimens the proportion of SC-binding IgM cells was below 40% (20). Such disparity between J chain-expressing and SC-binding capacity was much smaller for the IgA immunocytes (20). The fact that J chain incorporation takes place late in the covalent polymerization of pentameric IgM (101, 102), probably explains its relative lack of SC affinity while in the cytoplasm. Human IgM immunocytes may actually secrete varying proportions of J chain-positive pentamers and J chain-negative hexamers (116), the latter being produced mainly in T-cell-independent responses, especially when LPS activates the B cells (97).

Teleologically speaking, it could be advantageous to avoid pIgR/SC-mediated external transport of hexameric antibodies

as their superior complement-activating properties (97, 98) might be useful to clear infectious agents from the mucosae, although at the risk of acute inflammation and tissue damage. To what extent this type of putative IgM hexamer-dependent second line of defence operates at secretory effector sites is as yet not known, however. Conversely, it has been reported that murine J chain-containing pentameric IgM has little or no ability to activate complement (98). A similar difference between human pentameric and hexameric IgM has recently been suggested (99). Thus, it appears that SIgM as well as SIgA antibodies are principally designed to function in a non-inflammatory fashion.

J chain expression unrelated to IgA and IgM

Almost 90% of the IgG immunocytes in normal intestinal mucosa express J chain (100, 117, 118). However, J chain cannot combine with IgG but is therefore not secreted from IgG-producing cells and becomes degraded intracellularly (119). The same is probably true for J chain in IgD immunocytes which are almost 100% positive for this polypeptide in secretory tissues (20, 25, 84, 100). We have proposed (30) that the J chain-positive mucosal IgG and IgD immunocytes probably

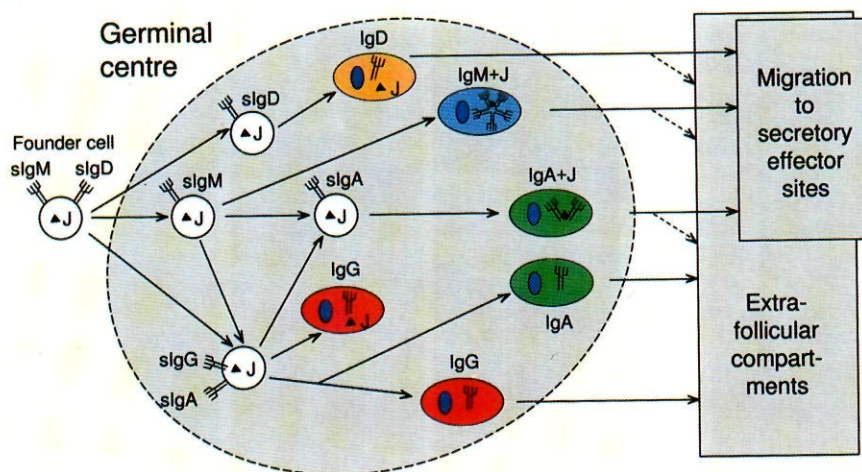


Fig. 11. Schematic representation of putative B-cell developmental stages in germinal centre (outlined by broken line) of MALT follicle. The pathways to terminal plasma-cell differentiation may include co-expression of cytoplasmic J chain and any of the four Ig classes depicted, but the J chain can only combine with cytoplasmic IgA and IgM to form polymers (IgA+J, dimeric IgA; IgM+J, pentameric IgM). The J chain-expressing B cells may to some extent terminate their differentiation topically in extrafollicular compartments (broken arrows), but they preferentially migrate to distant secretory effector sites. Further details are discussed in the text.

represent a “spin-off” from MALT-derived relatively immature B-cell effector clones on their way to pIgA expression (Fig. 11). This notion is supported by our finding that the replacement with J chain-expressing IgM, IgG and IgD immunocytes in various secretory tissues of IgA-deficient subjects (Fig. 4), approaches numerically the magnitude of the normal immunocyte populations (25, 30, 100). Thus, homing within the mucosal B-cell system is more related to J chain expression than to IgA commitment.

This notion is further in keeping with the fact that IgA immunocytes show less than 50%, and IgG immunocytes less than 10%, J chain positivity in non-secretory tissues (19, 20, 100), with the exception of the MALT germinal centres (30). Downregulation of J chain expression in extrafollicular B cells appears to be a sign of clonal maturation according to the “decreasing potential” hypothesis, involving enhanced tendency for terminal differentiation and apoptosis (120). Thus, B cells undergoing terminal differentiation in extrafollicular MALT compartments generally show reduced J chain and prominent IgG production (121–124), suggesting that they belong to relatively exhausted effector clones that have been through several rounds of stimulation (Figs 11 & 12). Local retention of such putative mature clones with downregulated J chain expression shows a progressive increase of GALT, mesenteric lymph nodes, peripheral lymph nodes and palatine tonsils from adults (30). These differences might to some extent depend on the magnitude of persistent stimulation, which is probably much higher in the tonsils with their antigen-retaining crypts than in GALT.

J chain regulation

Little is known about cytokine profiles or other microenvironmental conditions that induce and maintain so strikingly the expression of J chain in B cells giving rise to immunocytes at secretory effector sites. As discussed above, such expression is also seen, although to a lesser degree, in immunocytes associated with organized MALT structures (Figs 11 & 12), the highest levels being expressed by extrafollicular IgA- and IgD-producing cells in normal tonsils from children and by IgA-producing cells retained around GALT follicles (30, 100, 123).

Murine studies

Cytokine-induced transcriptional regulation of J chain expression has mainly been studied in mice (125), and both positive and negative regulatory elements have been defined on the promoter of the murine J-chain gene. A nuclear transcription factor, BSAP or Pax5, mediates silencing of the gene during early stages of B-cell development, but this repression is relieved by interleukin (IL)-2 during antigen-driven differentiation that downregulates BSAP RNA expression (126). BSAP apparently acts in competition with two adjacent positive control elements, JA and JB; the activity of the latter has been shown to be mediated by PU.1, a transcription factor belonging to the Ets oncoprotein family (127), whereas the activity of the former is mediated by a myocyte enhancer factor 2 (MEF2)-related nuclear factor present in B cells and therefore named B-MEF2 (128). A described zinc finger-containing putative transcription factor, Blimp-1 nuclear protein, has also been reported to be involved in murine B-cell differentiation; it apparently acts

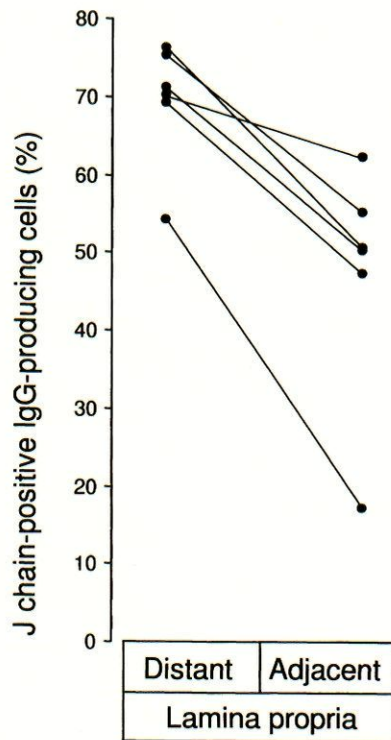


Fig. 12. Comparison of J chain expression shown by IgG-producing immunocytes in lamina propria of normal appendix mucosa distant from, and adjacent to, lymphoid follicles as indicated. Observations made in the same tissue section are connected by lines. [Adapted from (121)]

as a target for IL-2 and IL-5 regulation, thereby contributing to the induction of J chain mRNA and Ig production (129).

In addition to IL-2 and IL-5, IL-6 has also been suggested to contribute to murine J chain upregulation (130, 131), whereas IL-4 appears to have an opposing effect (132). A specific enhancer sequence was recently identified 7–8 kb upstream of the start site of the gene, and inductive interactions were shown to depend on an IL-2 signal; this event triggered opening of the enhancer chromatin, allowing binding of activated STAT5 to a specific DNA element (133).

Human studies

Our immunohistochemical observations suggest that also the human J chain production increases as a function of plasma-cell differentiation. However, molecular information concerning the regulation of J chain expression during B-cell develop-

ment in humans has been obtained mainly from leukaemic and EBV-transformed cell lines. Contrary to the situation in mice, these studies have suggested that transcription of the human J chain gene is initiated during early stages of B-lineage differentiation, even before Ig production takes place (134–137). More recent data on normal cells have supported the apparent difference in J chain regulation between the two species. Thus, J chain RNA could be detected in human foetal liver before μ chain expression (138). This result was confirmed and extended with haematopoietic subpopulations from human foetal and adult tissues (139). In the bone marrow, transcripts for J chain were detected at all B-lineage stages, including the progenitor (CD19⁺CD34⁺)- and pro-B (CD19⁺CD34⁺)-cell subsets.

Interestingly, J chain RNA was also detected during human fetal thymocyte development, including the double-negative (CD4⁺CD8[−]) and single-positive (CD4⁺ or CD8⁺) subpopulations, but the transcription was turned off in peripheral CD3⁺ T cells from both foetal and adult samples examined with the same molecular method (139). Similar studies have apparently not been performed on the various inductive MALT compartments. Altogether, therefore, much remains to be learned about the regulation of the human J chain gene.

Stimulation of B cells in MALT

Mucosal immune responses are believed to be generated primarily in organized MALT structures which lack afferent lymphatics but, instead, are designed to sample antigens from mucosal surfaces (Figs 1 & 2). In mechanistic terms, the best studied such inductive sites are the GALT structures of experimental animals, including the ileal Peyer's patches and the appendix (27, 48, 140). The chief function of GALT is to provide adaptive immune protection for the gut, but its primed effector cells also migrate to other exocrine tissues such as the upper airway mucosae and the lacrimal, salivary, and lactating mammary glands (33). Additional immune responses are presumably elicited in bronchus-associated lymphoid tissue (BALT), palatine tonsils and other parts of the Waldeyer's pharyngeal ring in humans, including nasal-associated lymphoid tissue (NALT) – particularly the nasopharyngeal tonsil, also called adenoids (124, 141, 142). Vaccine development can only to some extent be based on the functional integration of the so-called “common” mucosal immune system, because accumulating evidence suggests that regionalized regulation and homing properties of B cells must also be considered (30, 33).

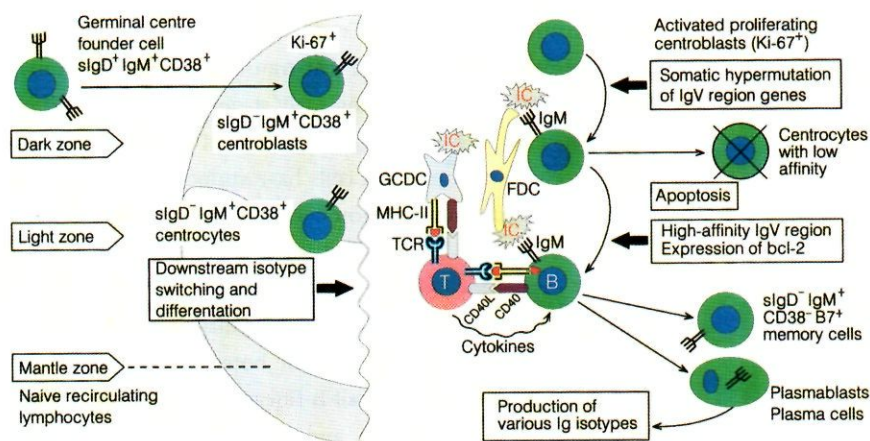


Fig. 13. B-cell developmental events believed to take place in the dark and light zones of germinal centres, leading to the generation of extrafollicular or distant plasma cells of various isotypes. The germinal centre founder cell is activated in the extrafollicular compartment and migrates to the dark zone where it proliferates (see Fig. 11). The molecular cell–cell interactions and immune events taking place in the germinal centre reaction are schematically depicted on the right. Further details are discussed in the text. B, B cell; FDC, follicular dendritic cell; GCDC, germinal centre dendritic cell; IC, immune complex; MHC-II, major histocompatibility complex class II; TCR, T-cell antigen receptor.

Generation of memory and effector B cells in germinal centres

A Peyer's patch consists by definition of five or more B-cell follicles (27). Primary intestinal follicles occur early in fetal life, but secondary follicles with germinal centres (Fig. 1B) depend on antigenic stimulation and do not appear until shortly after birth (143). The primary follicles contain recirculating CD19⁺ CD20⁺ lymphocytes with a naive phenotype (sIgD⁺ IgM⁺), both sIg isotypes contributing to the same antigen specificity of the B-cell receptor (BCR). The naive lymphocytes pass into the spaces of the network formed by the antigen-capturing follicular dendritic cells (FDCs), the origin of which remains obscure (144).

One important signal for FDC clustering and the follicle development is known to be the soluble homotrimer lymphotoxin (LT)- α , previously termed tumour necrosis factor (TNF)- β (145, 146). Experimental evidence suggests that the B-cell-derived type of this cytokine is particularly important (147). Among the known actions of LT- α is augmentation of B-cell proliferation and adhesion molecule expression. Knockout mice deficient in LT- α (LT- $\alpha^{-/-}$) virtually lack lymph nodes and have no detectable Peyer's patches. A membrane-associated form of LT moreover exists as a heterotrimeric complex containing LT- α together with a transmembrane protein designated LT- β . Knockout mice deficient in LT- β have no detectable FDCs, and they lack Peyer's patches, peripheral lymph nodes and organized splenic germinal centres (146).

The germinal centre reaction

In humans, this process has been extensively studied in the tonsils (63, 148–150), but functional information is mainly derived from lymph nodes and spleen of immunized experimental animals (151). Germinal centres are of vital importance

for T-cell-dependent generation of memory B cells, affinity maturation of the BCR, and Ig isotype switching (84). It is believed that naive B cells are initially stimulated in the extrafollicular area (on the left in Fig. 13) through cognate help by activated CD4⁺ T cells to which interdigitating APCs have presented processed foreign antigen (152). The B cells then produce unmutated IgM (and some IgG) antibody of low affinity that can bind circulating antigen; the resulting soluble immune complexes subsequently become deposited on FDCs (Fig. 13) where antigen is retained for prolonged periods to maintain B-cell memory (120, 148–153). Such a role of IgM in the induction of secondary immune responses with antibody affinity maturation, has been strongly supported by observations in knockout mice lacking the natural ("non-specific") background IgM antibodies (154).

The actual germinal centre "founder cell" has been tentatively identified in human palatine tonsils as an sIgD⁺ IgM⁺ CD38⁺ proliferative lymphocyte subset (148); murine experiments suggest that its attraction to the primary follicles partially is determined by a chemokine that interacts with the Burkitt's lymphoma receptor 1 (BLR1) on these cells (145, 155, 156). This chemokine has recently been identified and termed B-lymphocyte chemoattractant (BLC) in mice (157) and B-cell attracting chemokine 1 (BCA-1) in humans. It belongs to the CXC family and its receptor has been called CXCR5 (158). The source of BLC could be the FDCs and, interestingly, its follicular expression was much more consistent in Peyer's patches than in peripheral lymph nodes (157). Therefore, alternative B-cell-specific chemokines might operate in lymph nodes and perhaps also in tonsils. Indeed, another CXC chemokine, stromal cell-derived factor 1 α (SDF-1 α), was recently shown by an *in vitro* assay to attract naive and memory tonsillar B cells which bear the specific receptor (CXCR4) for this chemokine (159).

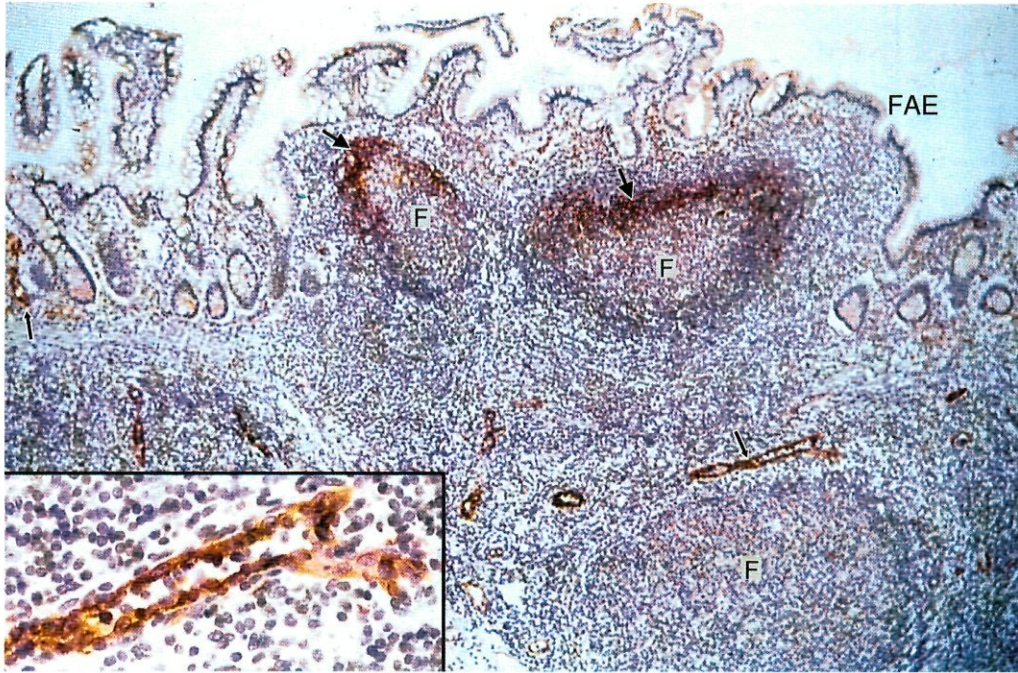


Fig. 14. Immunoperoxidase staining (brown) of MAdCAM-1 in section from human Peyer's patch counterstained with haematoxylin. Note diffuse expression of this addressin on dendritic elements in parts of lymphoid follicles (heavy arrows), on ordinary venule in mucosal lamina propria (thin arrow on the left), and on parafollicular high endothelial venules (thin arrow on the right; shown at higher magnification in insert). F, lymphoid follicle; FAE, follicle-associated epithelium. Magnification: $\times 30$.

SDF-1 appeared to be expressed by stromal cells lining the germinal centres of tonsils (159), but other MALT structures have to our knowledge not been examined.

Molecular cell-cell interactions in germinal centres

A variety of adhesion molecules and other receptor proteins mediate the cellular interactions that lead to the germinal centre reaction (Fig. 13). Tonsillar FDCs express both intercellular adhesion molecule (ICAM)-1 (CD54) and vascular cell adhesion molecule (VCAM)-1 (CD106) that can bind B cells through the leukointegrins lymphocyte function-associated antigen (LFA)-1 (α L β 2 or CD11a/CD18) and very late antigen (VLA)-4 (α 4 β 1 or CD49d/CD29), respectively (153). This appears to be a general feature of germinal centres present in peripheral lymphoid tissue, whereas relatively high levels of mucosal addressin-cell adhesion molecule (MAdCAM)-1 occur on dendritic GALT elements as observed in normal Peyer's patches of mice (160) and humans (Fig. 14). This regional difference might be involved in GALT recruitment of memory B and T cells with high levels of the counter-receptor integrin α 4 β 7 (the so-called "mucosal homing receptor", or LPAM-1), whereas a dominance of VCAM-1 in normal tonsils and peripheral lymph nodes could favour localization of α 4 β 1^{hi} (predominantly α 4 β 7^{lo}) cells (Fig. 15). Alternatively, such differential dendritic expression of MAdCAM-1 might, instead, induce the high levels of α 4 β 7 expressed by memory/effector cells destined for the intestinal mucosa (see below).

The complement receptors CR2/CR1 (CD21/CD35) are considered among the cell surface molecules that play a crucial role in the germinal centre reaction. CD21 is expressed abundantly on both FDCs and B cells, thereby having a critical function either by localizing antigen to the FDC network and/or by lowering the threshold of B-cell activation via recruitment of CD19 into the BCR (145). Activation of complement occurs on FDCs when they retain immune complexes (on the right in Fig. 13) but generally with no apparent harm to the germinal centres. Inhibition of C9 polymerization by associated S-protein (vitronectin), protectin (CD59), and decay accelerating factor (DAF or CD55) most likely dampens the lytic activity (161, 162). Nevertheless, release of inflammatory mediators may cause local oedema that facilitates dispersion of FDC-derived "immune complex-coated bodies", or iccosomes, thereby enhancing the BCR-mediated uptake of their contained antigens by B cells (161).

Positive and negative B-cell selection in germinal centres

Secondary follicles resulting from the germinal centre reaction can be divided into different compartments in which antigen-dependent selection of B cells takes place (149). Stimulation in the dark zone produces exponential growth of B-cell blasts positive for the Ki-67 nuclear proliferation marker (161). The resulting centroblasts somatically hypermutate their Ig variable (V)-region genes and give rise to sIgD-IgM⁺CD38⁺ centrocytes (Fig. 13). This process changes the affinity as well as specificity of the BCR and will likely induce some self-reactivity. However,

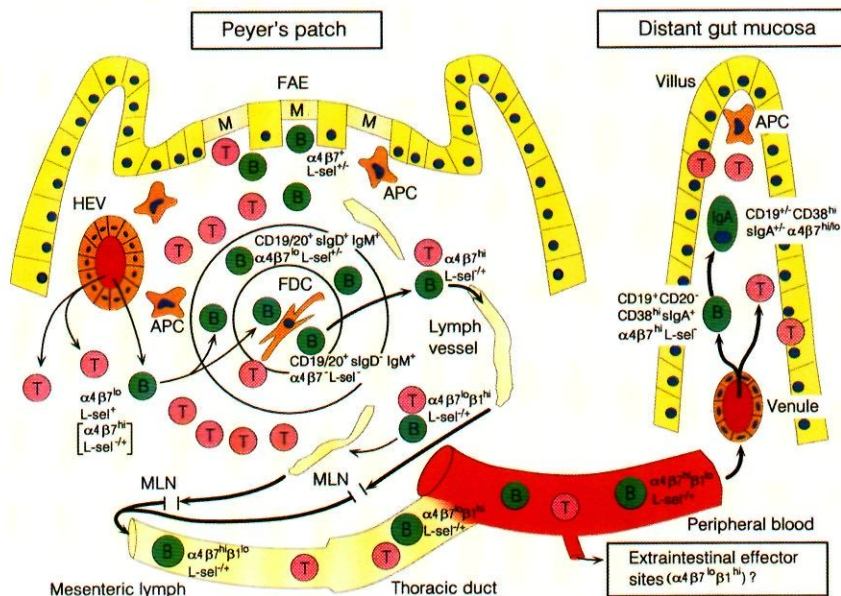


Fig. 15. Schematic distribution of B-cell phenotypes and associated adhesion molecules in various human intestinal tissue compartments, mesenteric and thoracic-duct lymph, and peripheral blood. Predominant expression patterns indicated (+, positive; -, negative; lo, low; int, intermediate; hi, high). Phenotype given in bracket indicates low priority. The migration routes of B and T cells are marked by arrows, the heavy ones representing the gut-associated lymphoid tissue (GALT)-lamina propria axis preferentially followed by the primed (memory and effector) $\alpha 4 \beta 7^{\text{hi}}$ L-selectin-subsets, most B cells terminating as IgA-producing mucosal plasma cells in distant gut mucosa. MLN arbitrarily marks location of mesenteric lymph nodes which receive cells from the parafollicular draining lymph vessels; FDC represents follicular dendritic cells in germinal centre of secondary lymphoid follicle; and HEV depicts high endothelial venule located in the T-cell zone of Peyer's patch. Other abbreviations: APC, antigen-presenting cell; B, B cell; FAE, follicle-associated epithelium; L-sel, L selectin; M, M cell; T, T cell.

mechanisms exist to eliminate autoreactive B-cell clones (150, 153, 163). Also, centrocytes with specificities for exogenous antigens die by apoptosis unless selected by high affinity binding to FDCs via their sIgM (Fig. 13). The centrocytes may actually pick up antigen from iccosomes, process it and present foreign peptide to CD4⁺ Th cells that are always found scattered within the follicles (148, 151, 153).

In addition to B-cell adhesion to FDCs and antigen cross-linking of BCR, cognate costimulatory interaction takes place between the activated intrafollicular T cells, which express the CD40 ligand (CD40L, gp 39 or CD154), and memory B cells, which express CD40 (Fig. 13); this appears to be an important event in rescuing centrocytes from CD95 (APO-1/Fas)-induced apoptosis and a key signal for their maintained survival (150, 151, 153, 164, 165). The interaction may be enhanced by sustained activation of the Th cells via specialized CD4⁺CD11c⁺ germinal centre DCs which also can pick up foreign antigens by binding immune complexes (166). Following immune activation, it is furthermore crucial that the positively selected centrocytes express Bcl-2, a protein implicated in the prevention of apoptosis (Fig. 13).

No germinal centres are formed when CD40-CD40L ligation is experimentally blocked, documenting the importance of cognate interaction between B and T cells (153). Moreover, this ligation promotes switching of the C_H genes from C_μ (IgM) to downstream isotypes, while apparently representing a nega-

tive signal for terminal B-cell differentiation within the follicles (148, 151, 167). Resistance to the effect of CD40L, or other elusive signals, probably contribute to the important decision as to whether primed B cells should continue to leave the memory pathway and differentiate along the effector pathway (120, 168) - either locally to become extrafollicular plasma cells, or after migration to distant secretory effector sites (Fig. 11).

Regionalized isotype development and emigration of primed B cells

Emigration of activated B cells from germinal centres is most likely directed by chemokines such as EBV-induced molecule 1 (EBI-1) ligand chemokine (ELC). In mice, this CCR7 ligand was shown to be constitutively expressed in extrafollicular areas of Peyer's patches and lymph nodes (169). In fact, most MALT-induced B cells (sIgD-IgM⁺CD38⁻) appear to migrate rapidly out of the germinal centres into extrafollicular compartments such as the tonsillar crypt epithelium (170) or Peyer's patch M-cell areas where they may continue to present antigen (30). Also most of the precursors for plasma cells (CD20-CD38^{hi}) rapidly exit to terminate at local (extrafollicular) or distant effector sites (Fig. 11), after which their half-life varies from a few days to several months (120). Those ending up in gut mucosa may be particularly short-lived, which may explain why mucosal immune responses often are deemed to have a relatively limited memory (171).

Local stimuli for isotype differentiation

As alluded to above, the cytokine profiles and other microenvironmental factors determining isotype differentiation and co-expression of J chain in B cells remain obscure. The fact that the IgA immunocytes of the tonsils and regional secretory effector sites are mainly of the IgA1 subclass (Fig. 7), supports the notion that effector-cell differentiation in this mucosal region takes place mainly from sIgD-IgM⁺CD38⁺ centrocytes by sequential downstream C_H gene switching (30, 124, 141). Conversely, the enhanced IgA2 development in the Peyer's patches and the distal gut altogether, including the mesenteric lymph nodes (73, 86, 117), might reflect direct switching from C_μ to Cα2 expression with looping out of intervening C_H gene segments (143). There is much evidence to suggest that B cells from murine Peyer's patches can undergo a direct switch from C_μ to Cα (172), and in human B cells this pathway may preferentially lead to IgA2 production (173). Molecular evidence for autocrine transforming growth factor (TGF)-β-induced direct S_μ→Sα as well as equential S_μ→S_γ, S_γ→Sα DNA recombination, was recently obtained in naive human B cells after engagement of CD40 (174).

The germinal centre reaction of human GALT structures (Peyer's patches and appendix) generates relatively more intrafollicular IgA immunocytes with J chain than seen in the tonsillar follicles; and in the adjacent intestinal lamina propria and dome zones, immunocytes producing IgA equal, or dominate over, those producing IgG (27, 117). This is in contrast to the more than 2-fold extrafollicular dominance of IgG immunocytes in the tonsils (124). Thus, the drive for switching to IgA and expression of J chain is clearly much more pronounced in GALT than in tonsils and peripheral lymph nodes. Many explanations for this remarkable intestinal B-cell regulation have been offered as discussed elsewhere (172). Perhaps the continuous superimposition of new environmental and microbial stimuli in the gut enhances the development of early effector B-cell clones with increased potential for IgA and J-chain expression (see above). Alternatively, the GALT microenvironment may be at least partially distinct from that of other immune-inductive sites because of special accessory cells or a particular cytokine profile. Altogether, it is possible that molecular events in the GALT germinal centres promote the generation of a particular (and as yet incompletely characterized) B-cell phenotype.

In this context it is interesting that a unique sIgM-negative subset (sIgD⁺IgM-CD38⁺) of centroblasts has been identified in the dark zone of palatine tonsillar germinal centres; they show C_H-gene deletion of the C_μ and switch region (S_μ), therefore apparently giving rise to immunocytes only of the IgD class

(175). We have recently obtained molecular evidence for the frequent presence of B cells with the same C_H-gene deletion, also in the adenoids and secretory effector tissues of the upper aerodigestive tract, but virtually never in GALT and intestinal mucosa (176). Our observations strongly support the notion that preferential B-cell homing takes place from nasopharyngeal MALT structures to the upper aerodigestive tract (30, 33, 141, 142); such regionalized migration of sIgD⁺IgM-CD38⁺ centroblasts probably explains the relatively high frequency of the IgD isotype among mucosal plasma cells in this region normally, and particularly in selective IgA deficiency (Fig. 4). It is possible that the upper airway microbiota contributes to this regional B-cell regulation. Thus, most strains of *Haemophilus influenzae* and *Moraxella* (*Branhamella*) *catarrhalis*, which are frequent colonizers of this region, produce an IgD-binding factor (protein D) that can crosslink sIgD (177, 178). In this way, the sIgD⁺sIgM-CD38⁺ tonsillar B-cell subset could be stimulated to proliferate and differentiate in a polyclonal manner. On the other hand, LPS that is abundantly present in the distal gut, may inhibit selective expression of IgD (179).

Identified IgA-promoting stimuli

DCs from murine Peyer's patches and spleen were initially suggested to enhance IgA production in a microculture system based on cognate interactions between B and T cells (180). A similar role for DCs was recently observed in a human *in vitro* test system not including T cells but employing CD40-activated naive B cells (181). As reviewed elsewhere (182), TGF-β has previously been reported to be a crucial IgA "switch factor", while IL-2, IL-5, and IL-10 are important cytokines for clonal expansion of activated B cells and their preferential IgA expression (on the left in Fig. 16). Terminal differentiation to IgA-producing plasma cells may additionally involve IL-6, IL-10 and possibly interferon (IFN)-γ (on the right in Fig. 16), all cytokines known to be produced by antigen-activated CD4⁺ T cells cloned from human intestinal mucosa (183). IL-6 preferentially enhances IgA production (IgA2>IgA1) by human appendix B cells (184), and in mice this cytokine appears to be essential for terminal B-cell maturation (182). In humans, a central role for IL-10 is supported by the fact that this cytokine can release the differentiation block of IgA-committed B cells from IgA-deficient patients (185). Human naive B cells activated through CD40 can be pushed towards IgA secretion by TGF-β and IL-10 (186), but recent studies suggest that TGF-β is the more active IgA-inducing cytokine (174). Interestingly, DCs enhance synergistically the effect of both TGF-β and IL-10 on IgA expression, and may indeed, via unknown signals, be essential for IgA2 production *in vitro* (181).

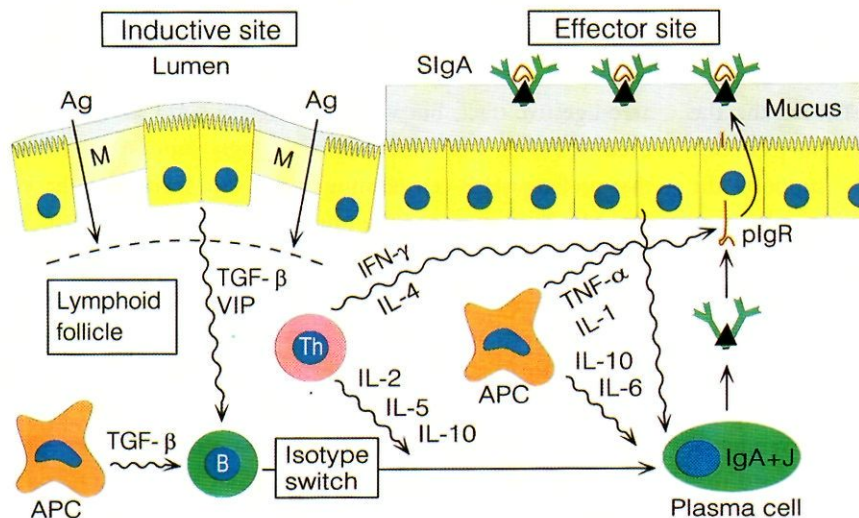


Fig. 16. Schematic representation of regulatory aspects of secretory immunity that take place in inductive compartment (on the left) and at mucosal effector site (on the right). Various cytokines are apparently involved in isotype switching and terminal differentiation of mucosal B cells to promote the striking generation of plasma cells that produce dimeric IgA with J chain (IgA+J) at secretory effector sites. Some of these cytokines may be derived from antigen-presenting cells (APC), T helper cells (Th) or the epithelium, as indicated. In the gut, both TGF- β and vasoactive intestinal peptide (VIP) may act as IgA switch factors. Certain Th- and APC-derived cytokines also upregulate epithelial expression of transmembrane secretory component (or pIgR), thereby providing a regulatory link between the immunological activity at the effector site and the magnitude of local external transport of secretory IgA (SIgA).

Gut hormones and other putative region-specific B-cell stimuli

Human fetal B cells activated *in vitro* through CD40 were selectively induced by vasoactive intestinal peptide (VIP) to IgA1 and IgA2 production (187). Similarly treated sIgM-CD19⁺ pre-B cells from human fetal bone marrow were induced to produce IgM and both IgA subclasses (187). These results suggested that VIP can act as a true switch factor (Fig. 16), which is interesting in view of its relatively high concentrations in gut mucosa. VIP was furthermore reported to promote the number of IgA precursors, together with increased IgA and decreased IgG synthesis, in cultures of intestinal mononuclear cells (188). Finally, substance P has been shown to enhance IgA and IgM production by murine B-cell lines, the latter isotype particularly in the presence of LPS (189).

In mice, intestinal "contrasuppressor cells" have been implicated as an additional mechanism selectively releasing IgA responses from T-cell-mediated suppression (oral tolerance). This IgA-enhancing effect (probably cytokine-mediated) was ascribed to TCR γ/δ ⁺ intraepithelial lymphocytes (IELs). Support for this notion was obtained in TCR δ ^{-/-} knockout mice which showed a significantly reduced number of intestinal IgA-producing cells and a poor IgA antibody response after oral immunization, while IgM and IgG were intact (190). If γ/δ T cells exert a similar effect in humans, this might contribute to the relatively large IgA production in the gut where TCR γ/δ ⁺ IELs are considerably more frequent than in the upper respiratory tract (191). Also, the increased number of γ/δ IELs in coeliac disease (56) might explain the significantly enlarged population of pIgA-producing immunocytes in the lesion (192). Additional region-specific differences could exist, for example

microenvironmental levels of IL-7, a cytokine mainly produced by goblet cells in the gut and known to be a growth factor for human intestinal lymphocytes (193).

Homing mechanisms guiding mucosal B cells

Migration of B cells through organized MALT structures

Role of adhesion molecules

As discussed above, the various MALT structures are considered to be the chief inductive sites of the secretory immune system in which recirculating virgin B lymphocytes arriving from the bloodstream are initially stimulated (on the left in Fig. 15). Certain adhesion molecules are more strongly expressed on the naive than on the primed (memory/effector) subsets, and vice-versa, and some are relatively tissue specific in their function. Counter-receptors expressed on endothelial cells may likewise show tissue specificity (194). Thus, in GALT and mesenteric lymph nodes, but not in peripheral lymph nodes, high endothelial venules (HEVs) abundantly express MAdCAM-1 (Fig. 14), and the same is true for the ordinary flat venules in the intestinal lamina propria (195). Indeed, this complex multidomain adhesion molecule appears to play a major role in intestinal extravasation of immune cells (196). The human counterpart of MAdCAM-1 has recently been cloned and characterized (197), but the microenvironmental factor(s) that explain its preferential expression on endothelial cells in the gut remains elusive (33, 198).

It has been reported in rodents that when MAdCAM-1 is expressed by HEVs in organized GALT structures, the glycosylation of its mucin-like domain promotes binding of L-selec-

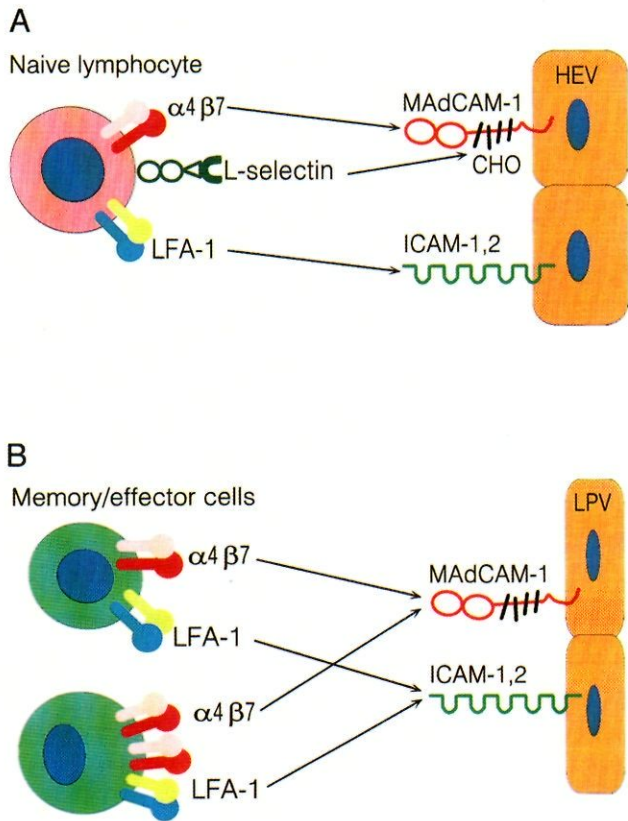


Fig. 17. Schematic depiction of adhesion molecules involved in lymphocyte-endothelial recognition in the human gut. (A) Homing of naive lymphocytes to organized gut-associated lymphoid tissue (GALT), and (B) of primed (memory/effector) B (and T) cells to the intestinal lamina propria. In GALT, high endothelial venules (HEV) in the parafollicular zone express mucosal addressin cell adhesion molecule (MAdCAM)-1, in which the membrane-near mucin-like domain (bottle brush symbol) contains L-selectin-binding O-linked carbohydrates (CHO); this interaction contributes to immigration of naive lymphocytes into the inductive GALT structures. Interaction between unmodified MAdCAM-1 expressed by ordinary flat lamina propria venules (LPV) is most important for targeting primed lymphoid cells to this mucosal effector site. Abbreviations: ICAM-1,2, intercellular adhesion molecule 1 or 2; LFA-1, leukocyte function-associated molecule 1.

tin (CD62L) that is strongly expressed on naive lymphocytes (199). This initial adherence (tethering), together with binding of leukointegrin $\alpha 4 \beta 7$ to the two N-terminal Ig-like domains of MAdCAM-1 (Fig. 17A), apparently explains the preferential emigration of naive T and B cells into inductive GALT sites such as the Peyer's patches (on the left in Fig. 15). The less prominent GALT endowment with primed counterparts ($\alpha 4 \beta 7^{\text{hi}}$ -L-selectin^{lo}) is probably mediated selectively by MAdCAM-1, because its interaction with $\alpha 4 \beta 7$ also supports tethering (200). Interestingly, it was recently shown that the chemokine 6CK/SLC (see below) stimulates $\alpha 4 \beta 7$ -mediated

human lymphocyte adhesion to MAdCAM-1 under flow, in contrast to other tested CC chemokines (201). Regardless of tissue site, additional contribution to the emigration of both naive and memory cells is provided by other more generalized adhesion molecules such as leukointegrin LFA-1 ($\alpha \text{L} \beta 2$) or CD11a/CD18) that binds to ICAM-1 and ICAM-2 (CD120) on the endothelium (Fig. 17).

We have recently analyzed the phenotypic distribution of lymphoid cells and their adhesion molecule profiles in human Peyer's patches and appendix (66, 202, 203). As discussed above (Fig. 5), approximately equal numbers of small T and B cells were present in the mononuclear cell suspensions, and in situ studies showed that the naive B cells of the follicular mantle zones mostly expressed abundant L-selectin but variable levels of $\alpha 4 \beta 7$ and usually no $\beta 1$ (CD29). Most lymphocytes expressing L-selectin around or within the parafollicular HEVs showed the naive T-cell phenotype (CD3⁺CD45RA⁺) but some were B cells (Fig. 6), again mainly of the naive phenotype (sIgD⁺). The lymphocytes expressing L-selectin were usually only weakly positive or negative for $\alpha 4 \beta 7$ (203). Altogether, our in situ data accorded well with the flow-cytometric characteristics of dispersed human GALT cells (Table 1) and were compatible with the adhesion molecule profile expected for lymphocytes at their site of entrance and accumulation in GALT (on the left in Fig. 15).

Role of chemokines

The initial adhesion molecule-mediated attachment (tethering) of the free-flowing leukocytes to the endothelium is relatively loose until they are stopped by a signalling event linked to GTP-binding proteins, probably in the main provided by chemokines that interact with corresponding surface membrane receptors (204). This triggers rapid activation of leukointegrins, leading to firm adherence (arrest) and subsequent leukocyte emigration (205). An exception to this paradigm is the binding of primed cells ($\alpha 4 \beta 7^{\text{hi}}$) to MAdCAM-1; as mentioned above, this event can take place independently of L-selectin (200), thus suggesting that memory/effector cells extravasate in the gut without initial tethering mediated by L-selectin (Fig. 17B).

Several chemokines that may activate rapid adhesion of lymphocytes to HEVs have recently been identified. Very compelling evidence is available for a secondary lymphoid-organ chemokine (SLC), which is a CC chemokine also known as 6-C-kine, TCA4 or Exodus-2 (herein referred to as 6CK/SLC). Transcripts for 6CK/SLC are strongly expressed in HEVs and non-lymphoid cells in the T-cell areas of murine peripheral lymph nodes as well as Peyer's patches (157). The migration of

naive T cells through HEVs is strongly reduced in mice lacking this chemokine (206, 207). Interestingly, 6CK/SLC can trigger rapid integrin-dependent arrest of rolling lymphocytes *in vitro* (208). The chemokines macrophage inflammatory protein (MIP)-3 α (also called LARC or Exodus-1), MIP-3 β (also called ELC or Exodus-3) and SDF-1 (also called PBSF) can likewise trigger such rapid lymphocyte adhesion (208); in the case of CD4⁺ T cells, MIP-3 α acts mainly on the memory phenotype (CD45RA⁺R0⁺) whereas SDF-1 exerts its strongest effect on the naive phenotype (CD45RA⁺R0⁻) (208). Although expression of these chemokines by HEV endothelium has not been described, endothelial transcytosis could still enable their presentation on the HEV surface if they are produced by surrounding tissue elements (209).

Microenvironmental migration

Very little knowledge exists about how immune cells, after emigration, are directed to, and retained within, the various microcompartments of organized lymphoid tissue. It is assumed that chemokines (145, 156) as well as fibronectin and other extracellular matrix components play an essential role. As mentioned above, the chemokine BLC/BCA-1 that interacts with BLR1/CXC5 on B cells appears to contribute to their migration into lymphoid follicles. Gene targeting that abolishes this receptor generates mice whose B cells, after transfer to wild-type littermates, fail to enter the follicles of spleen and Peyer's patches despite normal immigration into the T-cell areas (155). Therefore, BLC-BLR1 interactions do not seem to be crucial for the emigration of B cells through HEVs.

Other chemokines with a microcompartmental expression pattern have also been identified. Thus, it appears that 6CK/SLC (see above), which is preferentially expressed in the parafollicular areas of murine peripheral lymph nodes and GALT, attracts T cells more strongly than B cells (210–212). Moreover, interactions between leukointegrins ($\alpha 4\beta 1$ and $\alpha 4\beta 7$) and fibronectin (213) or MAdCAM-1 (160) may be particularly involved in GALT (30). Consistent with this notion, reticular fibres with fibronectin, collagen and laminin are oriented parallel to the presumed migration pathway from HEVs to the domes of murine Peyer's patches (214). In addition, critical architectural features such as channels or "corridors", as well as proteolytic activities, probably play an essential role in guiding microcompartmental cell migration (215, 216).

Exit of primed B cells from GALT

The interfollicular zones of GALT are not only the site of entrance for lymphoid cells, but also where they exit through draining microlymphatics (on the left in Fig. 15). We identified these vessels in human Peyer's patches and appendix as thin-

walled spaces lacking endothelial expression of von Willebrand factor (203, 217). Similar parafollicular microlymphatics have been described in human tonsils (218). Draining lymph vessels are believed to start blindly with a fenestrated endothelium, and it is possible that lymphoid cells enter them by a passive process, although more selective mechanisms should be considered. Thus, lymphatics of human skin possess endothelial binding sites for the chemokines RANTES, monocyte chemoattractant protein (MCP)-1 and MCP-3, but not for MIP-1 α and IL-8 (219). Moreover, lymphatics of multiple organs, including the murine intestinal lamina propria, contain transcripts for 6CK/SLC (210).

Within human GALT, memory B (sIgD⁻) and T (CD45R0⁺) cells with high expression of $\alpha 4\beta 7$ were often found to be situated near the draining microlymphatics, and were also present inside these spaces together with some CD19⁺CD38^{hi} $\alpha 4\beta 7^{hi}$ B-cell blasts (203, 217). However, the lymph vessels contained mainly naive lymphocytes with low expression of $\alpha 4\beta 7$. Cytochemical and flow-cytometric analyses of human mesenteric lymph provided similar marker profiles; notably, the small fraction of identified B-cell blasts (2–6%) contained cytoplasmic IgA, IgM and IgG in the proportions 5:1:<0.5 (217).

Our studies suggested that the $\alpha 4\beta 7^{hi}$ subsets identified at exit in GALT, reflect the first homing step to furnish mucosal effector sites with primed lymphoid cells (Fig. 15). Relatively few memory cells expressed high levels of L-selectin in intestinal and mesenteric lymph (217); those that did might likely either re-enter GALT or extravasate in non-mucosal lymphoid tissue or tonsils from the bloodstream together with naive cells by binding to mucin-like peripheral lymph node addressin (PNAd) expressed on HEVs there (220). A recent flow-cytometric study of circulating human lymphocytes supported such a fundamental subdivision according to vascular adhesion properties (221).

The heterogeneity of GALT-derived B cells might explain systemic dissemination of IgG as well as IgA responses after oral immunization. As discussed elsewhere, convincing evidence exists that the systemic and mucosal immune systems are not totally segregated (27, 28); it is quite possible to prime and boost local immune responses by parenteral immunization, and vice-versa. Interestingly, we observed both B and T memory cells with high levels of $\alpha 4\beta 1$ in GALT (203). These subsets probably corresponded to the $\alpha 4\beta 7^{lo}$ cells appearing in the draining lymphatics (217), perhaps being destined to extravasate at extraintestinal mucosal effector sites by means of VCAM-1 which is the chief ligand for $\alpha 4\beta 1$ (see below). Conversely, regardless of origin, the $\alpha 4\beta 7^{hi}$ subsets with little L-selectin would not be expected to adhere to PNAd and only subsidiarily

to VCAM-1; they were therefore most likely destined primarily for the intestinal lamina propria (Fig. 15).

Migration of B cells from GALT to distant intestinal lamina propria

Characteristics of lamina propria effector cells

After antigen-induced activation, proliferation and partial differentiation in GALT, it is assumed that primed lymphoid cells go rapidly to mesenteric lymph nodes, from which (after some further differentiation) they follow the lymph into the bloodstream (Fig. 2). In experimental animals, it has been directly shown that IgA-expressing plasmablasts mature as they migrate from Peyer's patches via mesenteric lymph nodes and the thoracic duct to the intestinal lamina propria (222); the relative fraction of cells containing cytoplasmic IgA was shown to increase from initially around 2% through 50% and 75% to 90%, respectively (223).

In mice, peritoneal B cells are composed of a special B-1 (CD5⁺) subset that can repopulate the gut with IgA-producing immunocytes (224). Kroese and co-workers have determined that approximately 50% of the murine intestinal lamina propria plasma cells are derived from this subset. It is generally believed that B-1 cells produce so-called natural or polyreactive antibodies encoded by unmutated (germline) IgV region genes, but murine CD5⁺ B cells often show hypermutation as a sign of selection (224). Human intestinal plasma cells from the duodenum, ileum and colon have highly mutated IgV region genes, suggesting that their BCR is shaped by persistent antigenic challenge in germinal centres (225). This is true of both the IgA and IgM immunocytes (226), thus documenting that SIgM antibodies may be part of a secondary intestinal immune response. Also interestingly, sequences of IgV_H region genes from Peyer's patch germinal centre B cells are reported to be clonally related to those of ileal plasma cells (227), which substantiates the presumed intestinal homing pattern of GALT-derived primed B cells (Fig. 15). Altogether, despite the presence of polyreactive SIgA antibodies in human exocrine secretions (see below), there is no evidence that these antibodies are produced by plasma cells originating from the peritoneal cavity (228).

Extravasation of primed B cells

As mentioned above, homing of primed lymphoid cells to the intestinal lamina propria appears to be determined mainly by their high levels of $\alpha 4\beta 7$ in the absence of L-selectin (Fig. 17B). This phenotype can bind to unmodified MAdCAM-1 expressed on the lamina propria microvasculature (33, 194), and fits

Anti- $\alpha 4\beta 7$ (red), anti-CD38 (green), anti-cytokeratin (blue)

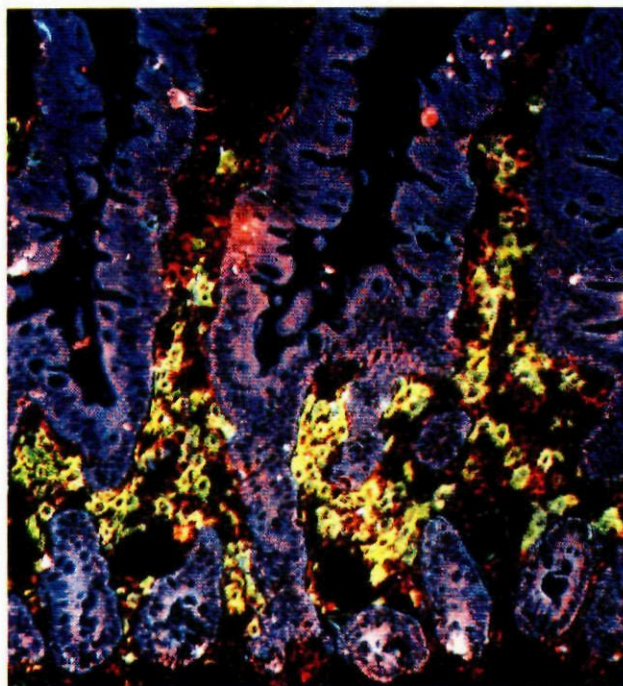


Fig. 18. Paired immunofluorescence staining of adhesion molecules on leukocytes in cryosection of normal duodenal mucosa. Most terminally differentiated large B cells (CD38⁺) co-express $\alpha 4\beta 7$ and are therefore yellow, whereas most small cells (mainly T lymphocytes) in the lamina propria are only positive for $\alpha 4\beta 7$ and are purely red. Magnification: $\times 60$.

with the predominant adhesion molecule profile of specific antibody-producing cells present in human peripheral blood after intestinal stimulation (229–231). Conversely, circulating specific B cells generated by systemic immunization show preferential expression of L-selectin but relatively low $\alpha 4\beta 7$ levels (229–231).

At present, interactions of human MAdCAM-1 with L-selectin have apparently not been explored to the same extent as for the murine counterpart. Nevertheless, the virtual absence of lymphoid cells bearing the latter adhesion molecule in human intestinal lamina propria (Table 1), strongly suggests that MAdCAM-1, when expressed outside organized GALT structures, does not bind L-selectin (33). Many large B cells retain high levels of $\alpha 4\beta 7$ after extravasation in the human intestinal lamina propria, despite abundant coexpression of CD38 (Fig. 18) and cytoplasmic IgA as signs of terminal maturation (Fig. 15) (Table 1). It is possible that $\alpha 4\beta 7$, in addition to mediating homing of primed cells, contributes together with CD44 to retention of effector cells (see below).

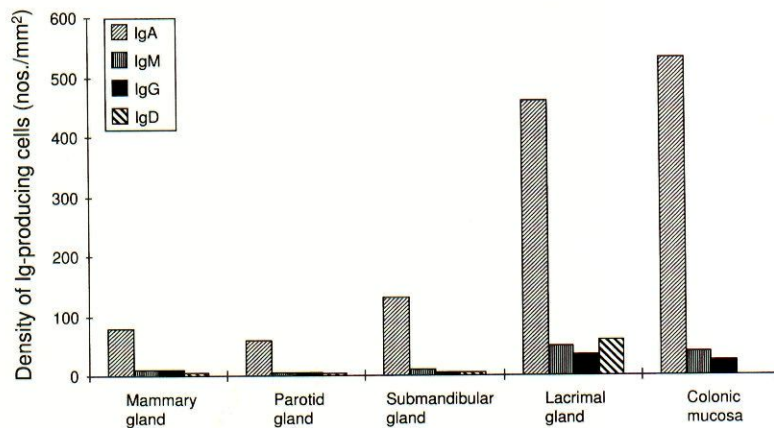


Fig. 19. Average densities of immunocytes producing different Ig classes in various normal human secretory tissues as indicated.

Observations obtained by paired immunofluorescence staining in 5- μ m thick tissue sections.
[Based on published data from the authors' laboratory]

Accumulation of primed immune cells at secretory effector sites

Second signals for proliferation,
terminal differentiation and retention

Little is known about factors triggering terminal B-cell differentiation at various secretory sites (Fig. 2), although IL-5, IL-6 and IL-10 have been suggested to be important as discussed above (Fig. 16). Also the local antigen repertoire appears to play an important role in site-specific accumulation of effector B cells, but without imposing any selectivity on their initial emigration. Thus, in experimental animals GALT-derived blasts home to presumably antigen-free neonatal intestinal mucosa (232), and to foetal gut grafted under the adult kidney capsule (222, 233). However, after some hours, antigen deposition clearly influences the observed homing pattern by causing specific accumulation (retention, proliferation and terminal differentiation) of IgA-producing cells (see below). Therefore, it appears that primed immune cells generally tend to home preferentially to the effector site that corresponds to the inductive site where they were initially stimulated.

The observed impact of local antigen on the IgA system is most likely mediated largely via "second signals" from activated T cells. In addition to cognate exogenous antigens, several other factors probably contribute to site-specific retention of memory T cells and their restimulation as well as rescue from apoptosis (234). Such regional variables are not well defined but one of them could be the MHC expression level since the magnitude of immune responses might be related to the density of such "self-antigens" (235); thus, their abundant epithelial expression – in combination with only trace amounts of foreign antigens or anti-idiotypic antibodies – could elicit sufficient "second signals" for some terminal B-cell differentiation to take place. Interestingly, in salivary and lactating mam-

mary glands, the IgA immunocytes notably accumulate preferentially adjacent to HLA-DR-expressing ducts (236–238).

The epithelium might also mediate more non-specific stimulatory effects on the B cells. For instance, a soluble factor from bursal epithelial cells has been reported to induce IgA production (239), perhaps representing one of the cytokines mentioned above (Fig. 16). Furthermore, local DC subsets and matrix components, including adhesion molecules such as $\alpha 4\beta 7$ in the gut (see above), probably contribute, together with high CD44 post-germinal-centre levels (240), to local retention of primed immune cells by virtue of affinity for extracellular matrix components. Site-specific survival differences of Ig-producing cells might directly or indirectly (via activated T cells) be influenced by similar variables (171), including rescue from apoptosis by contact with stromal cells (241).

The role of topical antigen exposure

Substantial antigen-driven proliferation of IgA precursor cells has been revealed in the intestinal lamina propria of various experimental animals (242–244), especially in the crypt regions (68) where most IgA immunocytes are also found in the human small intestinal mucosa (5, 69). Altogether, the role of topical antigen outside the Peyer's patches has been clearly demonstrated in terms of localization, magnitude and persistence of induced human SIgA antibody responses (245). Likewise, rectal immunization elicits particularly high levels of IgA antibodies in colorectal secretions and feces both in experimental animals (246) and humans (247), apparently reflecting an enhancing effect of exposing the local mucosa to the same antigen as the inductive tissue (solitary follicles) of the large bowel. Repeated vaginal or rectal immunizations in monkeys have likewise demonstrated local accumulation of effector B cells at these sites (248). Moreover, an immunological rela-

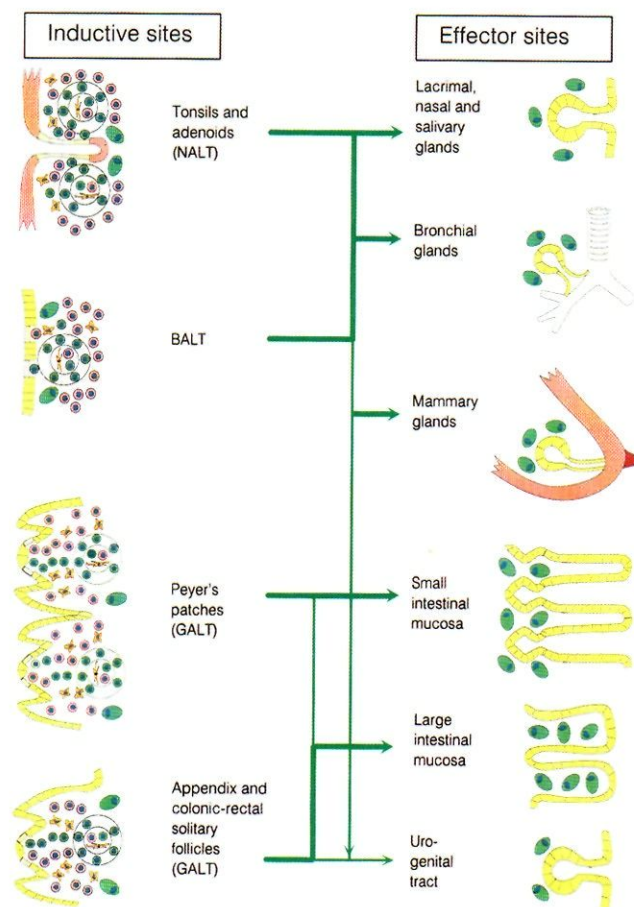


Fig. 20. Model for homing of primed lymphoid cells from inductive sites with their activated lymphoid follicles, to secretory effector sites in the integrated human mucosal immune system. The specialized follicle-associated epithelium contains thin M ("membrane") cells with antigen-transporting properties (see Fig. 2). Putative regionalization in communication between inductive and effector sites is indicated, the heavier arrows representing preferential B-cell migration pathways. Homing from gut-associated lymphoid tissue (GALT) is believed to be determined mainly by integrin $\alpha 4 \beta 7$ on primed cells, interacting with mucosal addressin cell adhesion molecule-1 expressed on the microvascular endothelium in the intestinal lamina propria (see Fig. 17B). As discussed in the text, other adhesion molecules appear to be employed by immune cells primed in bronchus-associated lymphoid tissue (BALT) and nasal-associated lymphoid tissue (NALT), which in humans appears to be constituted of the various lymphoepithelial structures in the Waldeyer's ring, including the palatine tonsils and the nasopharyngeal tonsil (adenoids). The urogenital tract might employ similar molecular homing mechanisms as the upper aerodigestive tract and therefore appears to receive primed immune cells from NALT, although probably to some extent from GALT as well. Also lactating mammary glands appear to receive primed cells from both types of inductive tissue. The model is based on both human and animal data as discussed in the text. Colour key: green, B cells and IgA-producing plasma cells; violet, T cells; orange, antigen-presenting cells; bright yellow, epithelium; faint yellow, antigen-transporting epithelium, including M cells.

tion between the appendix and the large bowel mucosa is suggested both in experimental murine colitis (249) and in human ulcerative colitis, as discussed elsewhere (57).

Notably, the immediate natural availability of environmental antigens at various secretory effector sites also appears to be reflected in the local density of Ig-producing immunocytes (238); thus, this is about seven times higher in the mucosa of the human colon (which has an enormous microbial load) than in the parotid and lactating mammary glands (Fig. 19). Although MALT-related antigens theoretically should be required to provide secondary B-cell stimulation throughout the mucosal immune system, it is not likely that such live or dead foreign material normally gains direct access to the parotid and mammary gland, particularly not to the latter organ. Interestingly, the lacrimal gland, which is connected by many short ducts to an excessively aeroantigen-exposed mucosa (the conjunctiva), shows an IgA immunocyte density approaching that of the colon (Fig. 19). In this context it is of further interest that rapid and widespread dissemination of fed antigen, presumably carried by DCs, and sequestration of circulating specific T cells as well as their activation at sites of cognate antigen presentation, were recently observed in TCR transgenic mice (54). That study, together with previous results of adoptive B-cell transfer in syngeneic rats (250), suggests that local antigen-driven T-cell activation might provide important "second signals" for retention, expansion and terminal differentiation of mucosal Ig-producing immunocytes at secretory effector sites, even at a long distance from mucosal surfaces.

Extraintestinal homing of MALT-derived B cells

Heterogeneity in mucosal homing mechanisms

The molecular mechanisms that coordinate migration of primed B cells from GALT to secretory tissues beyond the gut are poorly defined, although they would form the functional basis for several desired oral vaccines. In contrast to such GALT-derived dissemination of secretory immunity to extraintestinal effector sites, migration to the gut lamina propria of B cells induced in NALT and BALT appears to be negligible in terms of generating intestinal SIgA antibodies. This notion is based on homing results obtained by local immunization or infection experiments in rodents (251, 252) and pigs (253). On the other hand, considerable indirect evidence summarized elsewhere (142), suggests that dissemination of primed pIgA precursor cells takes place from Waldeyer's pharyngeal lymphoid ring, or NALT, to regional secretory effector sites both in humans and various experimental animals (Fig. 20). In humans, such putative homing dichotomy between the aerodigestive

tract and the gut, is furthermore strongly supported by the apparent disparate distribution of sIgD⁺IgM-CD38⁺ B cells, as identified by tracing of their heavy-chain C μ - and S μ -gene deletion (30, 176). As discussed above, this unique tonsillar centroblast subset most likely contributes significantly to the relatively frequent occurrence of IgD-producing immunocytes not only in the palatine tonsils and adenoids, but also in the salivary and lacrimal glands as well as nasal mucosa, especially in IgA-deficient individuals (Fig. 4). Furthermore, activated human tonsillar B cells transferred intraperitoneally to mice with severe combined immunodeficiency, migrated to the lung but not to the gut mucosa (254).

Putative mucosal homing molecules operating outside the gut

Regionalization of the mucosal immune system might in part be explained by disparity in adhesion molecules expressed on the local microvascular endothelium and lymphoid cells primed in different MALT structures, and perhaps by different local chemokine profiles. Animal and human studies have both demonstrated that MAdCAM-1 is shared between GALT structures and the intestinal lamina propria (Fig. 17), and it also shows considerable expression on HEVs in mesenteric lymph nodes (194, 196). Other, as yet unidentified, regional endothelial determinants might be shared between the immune-inductive sites and exocrine effector sites in the upper aerodigestive tract (Fig. 20). Interestingly, very little or no MAdCAM-1 can be detected on HEVs in normal human tonsils (T. Yamana-ka, M. J. Brisikim, P. Brandtzaeg, unpublished results), and this molecule appears to be absent from regional exocrine tissues such as lungs and salivary glands, as well as from mammary glands and the human and murine uterus (195, 255). Moreover, some evidence has been reported suggesting that $\alpha 4\beta 7$ is not an important homing receptor for lymphoid cells in the airways of humans (256), mice (257) or sheep (258). Indeed, circulating specific antibody-producing cells, detected after nasal immunization in humans, were reported to show considerable coexpression of $\alpha 4\beta 7$ and L-selectin, in contrast to the results obtained after intestinal immunization (230). However, the $\alpha 4\beta 7$ level was apparently not sufficient to make the cells gut-seeking, whereas antibody production was evoked in both adenoids and nasal mucosa (259). Likewise, when the sIgD⁺IgM⁻ putative primed tonsillar effector B-cell subset (identified by its C μ - and S μ -gene deletion; see above) is observed in the circulation, its adhesion molecule profile shows rather little $\alpha 4\beta 7$ but a relatively high and uniform level of L-selectin expression (P. Brandtzaeg, F.-E. Johansen, E. S. Baekkevold, I. N. Farstad, unpublished results).

A non-intestinal homing receptor profile might also explain effector B-cell migration from NALT to the urogenital tract (Fig. 20). As reviewed elsewhere (43), this putative link is reflected by particularly high levels of specific IgA and IgG antibodies in cervicovaginal secretions of mice and monkeys after intranasal immunization with a variety of antigens. Also BALT could be involved as inductive sites in these experiments, especially in anesthetized mice (260). Other studies have shown that stimulation of GALT, particularly via the rectal route, can induce antibodies in the female genital tract of rodents (43) and humans (261, 262), although the human vaccination results have been variable (247, 263). This problem might be caused by uncontrolled accessibility to the inductive tissue of the human large bowel (Fig. 20), which is constituted by numerous scattered lymphoid follicles (31) and perhaps the appendix (see above). Interestingly, an immunological link has been suggested to exist between the upper respiratory tract and rectal mucosa (247), but no difference in the expression of $\alpha 4\beta 7$ or L-selectin was observed for circulating antibody-producing cells resulting from rectal compared with oral immunization (230, 247).

Regionalization of the mucosal immune system must be further evaluated and eventually taken into account in the development of effective local vaccines to protect mucosae beyond the gut, such as the airways, the eyes, the oral cavity and the urogenital tract (33). Notably, even without employing interactions between $\alpha 4\beta 7$ and MAdCAM-1, homing of putative early effector B-cell clones with preferential expression of J chain and pIgA, is just as remarkable to secretory tissues in the upper aerodigestive tract as to the intestinal lamina propria (20, 30, 238). It is difficult to understand how L-selectin and its major counter-receptor (PNAd) might contribute to selectivity in B-cell extravasation at such effector sites of the upper aerodigestive tract when the same adhesion molecules are operating in organized peripheral lymphoid tissue.

A similar problem relates to the possibility of a significant involvement of $\alpha 4\beta 1$ on primed mucosal B cells that migrate to secretory tissues outside the gut (Fig. 15). The chief counter-receptor for this integrin is VCAM-1, which is expressed on microvascular endothelium both in human bronchial and nasal mucosa (264, 265), and also in the murine uterus (255). However, no direct evidence exists to suggest that high expression of $\beta 1$ integrin directs B cells to the upper aerodigestive tract, lungs or urogenital tract. Altogether, the remarkably selective B-cell homing to various secretory effector sites, combined with the regionalization of the mucosal immune system, cannot as yet be fully explained in molecular terms.

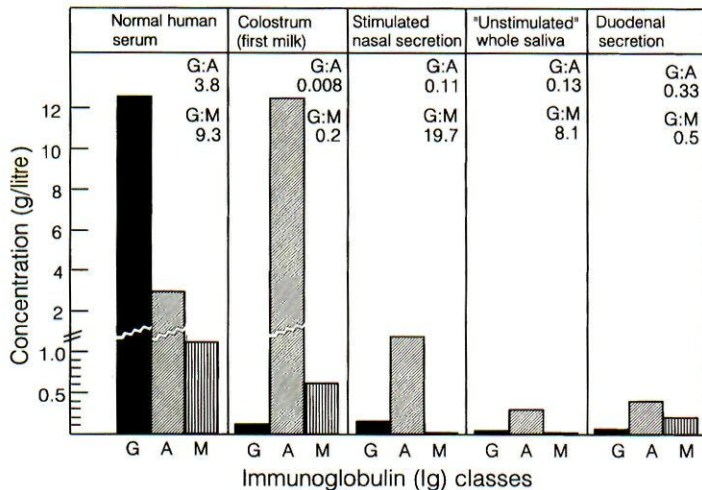


Fig. 21. Average levels of IgG (G), IgA (A) and IgM (M) in various normal human body fluids. The concentration ratios (G: A and G: M) are also indicated to show the strikingly preferential appearance of IgA and, to a lesser extent, IgM in external secretions. This selectivity is particularly remarkable for colostrum, which is obtained directly from the duct opening, thus avoiding substantial contamination of IgG from interstitial fluid by leakage through surface epithelia. The same is true for parotid secretion (see Fig. 8). [Modified from (278)]

Co-operation between local B cells and secretory epithelium

Receptor-mediated epithelial transport of polymeric immunoglobulins

Normal expression and function of pIgR/SC

As alluded to above, epithelial pIgR/SC is a key factor in the secretory immune system by mediating active and selective external transport of J chain-containing pIgA as well as pentameric IgM (15, 94, 182). Thus, in a pure glandular secretion such as colostrum and parotid fluid, with minimum contamination by paracellular epithelial leakage of tissue fluid, the concentration ratio of IgG: IgA is reduced to about 0.2% of that in serum, and the IgG: IgM ratio is reduced to 2% or less (Figs 8 & 21). This receptor function is now well established (266), and knockout mice in which the pIgR/SC is defective, show no selective epithelial transport of Ig polymers (29). The receptor is normally expressed on the basolateral surface of secretory columnar epithelial cells (267, 268): in various human exocrine glands, its expression is restricted to the serous type of acinar and the duct elements (269); in the small intestine, it is produced abundantly by the crypts of Lieberkuhn but decreases gradually in the enterocytes covering the villi (269, 270); in the large bowel, it is variably present also in the surface epithelium (269, 270); and in the normal gastric mucosa, it is produced mainly by cells of the antral glands and their isthmus zones (271).

After being formed on the basolateral surface of the secretory epithelial cell, the pIg-pIgR complexes are translocated by endocytosis and transcytosis to the lumen (Fig. 22). As a consequence, immunohistochemical staining reveals a cytoplasmic distribution of SC and the two pIg classes that is congruent

except in the Golgi region (Fig. 23) where pIgR accumulates selectively (Fig. 24) before it migrates basolaterally to meet its ligands (10). The epithelial staining for IgA is normally stronger than that for IgM (8), both because of the relatively abundant local pIgA production (Fig. 4) and because the pIgR is less accessible to pentameric IgM (see below).

Mechanism and magnitude of pIgR-mediated transport

The pIgR is an epithelial glycoprotein (transmembrane SC) of ~100 kDa which belongs to the Ig supergene family (182, 266). The first of its five extracellular Ig-like domains (D1) initiates the non-covalent ligand interaction at the basolateral epithelial-cell membrane, and a loop similar to the first complementarity determining region (CDR1) of IgV κ domains has been shown to be essential for pIgA binding (266). This region of D1 shows a remarkably high degree of sequence conservation, but the ability of pIgR to interact with pentameric IgM is nevertheless differently preserved among various species (266). Thus, human pIgR binds both pIgA and pentameric IgM, while the rabbit counterpart has virtually no binding capacity for the latter ligand. We interchanged D1 between human and rabbit pIgR, and found that human D1 was essential for pentameric IgM binding, and sufficient to confer such binding to a chimeric pIgR with rabbit D2-D5 (272). Furthermore, exchange between the human and rabbit pIgR regions comprising the CDR-like loops in D1, showed that the CDR2-like region is most important for pIgM binding, with additional contribution from the CDR1-like region. Altogether, our results demonstrated that pentameric IgM and pIgA interact differently with human pIgR: binding of the former ligand appears to depend fully on strong interactions with the D1 CDR2-like

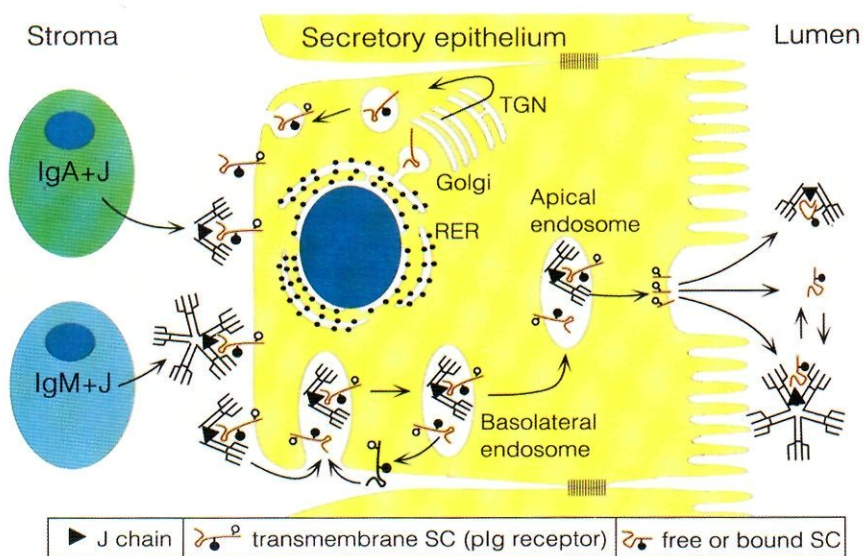


Fig. 22. Schematic representation of various steps involved in the generation of human secretory IgA (right, top) and secretory IgM (right, bottom) via pIgR-mediated epithelial transport of J chain-containing dimeric IgA (IgA+J) and pentameric IgM (IgM+J) produced by mucosal plasma cells (left). Transmembrane secretory component (SC) is synthesized in the rough endoplasmic reticulum (RER) of secretory epithelial cells and matures by terminal glycosylation (●) in the Golgi complex. After being sorted through the trans-Golgi network (TGN), it is phosphorylated (○) and expressed as pIgR at the basolateral plasma membrane. Endocytosis of ligand-complexed and unoccupied pIgR is followed by transcytosis to apical endosomes and finally by cleavage and release of secretory Ig molecules with bound SC, as well as excess of free SC, at the luminal cell face. Some basolateral recycling may initially take place for unoccupied pIgR as indicated. During the external translocation, covalent stabilization of the IgA/SC complexes regularly occurs (disulfide bridge indicated in secretory IgA), whereas free SC in the secretion apparently serves to stabilize the non-covalent IgM/SC complexes (dynamic equilibrium indicated for secretory IgM).

region, while binding of pIgA depends in addition on determinants within D2 and/or D3 to support the initial non-covalent interaction with D1 (273).

After epithelial transcytosis, SIgA and SIgM are released by cleavage of the pIgR at the apical surface, and only the C-terminal transmembrane and cytoplasmic part of the receptor remains for degradation in the epithelial cell (Fig. 22). More pIgA (~40 mg/kg body weight) is translocated to the adult gut lumen as SIgA by this mechanism every day than the total daily IgG (~30 mg/kg) production (274). Therefore, the intestinal mucosa is quantitatively the most important effector organ of adaptive humoral immunity. During the transport process, the ~80-kDa extracellular part of pIgR is incorporated into the secretory antibody molecules as so-called bound SC, thereby being "sacrificed" to confer protection against non-specific proteolytic degradation; this is particularly true for SIgA in which SC becomes covalently linked (32, 93, 275–277).

Excess of unoccupied pIgR is released apically by proteolytic cleavage in the same manner as SIgA and SIgM to form so-called free SC (Fig. 22). Variable amounts of this fragment (~80 kDa, identical to bound SC) are usually present in exocrine secretions (278). By equilibrium with the bound component, free SC exerts a stabilizing effect on the quaternary structure of SIgM in which SC remains noncovalently linked (8). In

various ways, free SC in secretions might also contribute to innate mucosal defence (see below).

Different transport efficiency for pIgA and pentameric IgM

In normal adults, external secretions contain much more SIgA than SIgM (Fig. 21); this difference cannot solely be accounted for by the striking predominance of local pIgA-producing cells (Fig. 4). Thus, in well-controlled quantitative studies of jejunal fluid and parotid saliva, the concentration ratio of IgA-to-IgM is found to be 2.4- to 4.9-fold higher than the corresponding local immunocyte class production ratio (7, 75, 76, 278, 279). This estimate is based on the observation of a fairly similar synthetic rate in IgA- and IgM-producing cells (280). Moreover, as discussed above, mucosal IgA plasma cells release variable amounts of monomeric IgA in addition to pIgA, whereas IgM-producing cells normally are virtually restricted to pentamer secretion (281). Altogether, our calculations suggest that the external transport of pIgA is favoured at least 6- to 12-fold over that of pentameric IgM on a molar basis (Fig. 8). Significant biological variables of secretory immunity other than the local immunocyte distribution therefore appear to exist. Such variables could be differences in the ligand affinity for pIgR, in the efficiency of receptor-mediated epithelial transcytosis of the

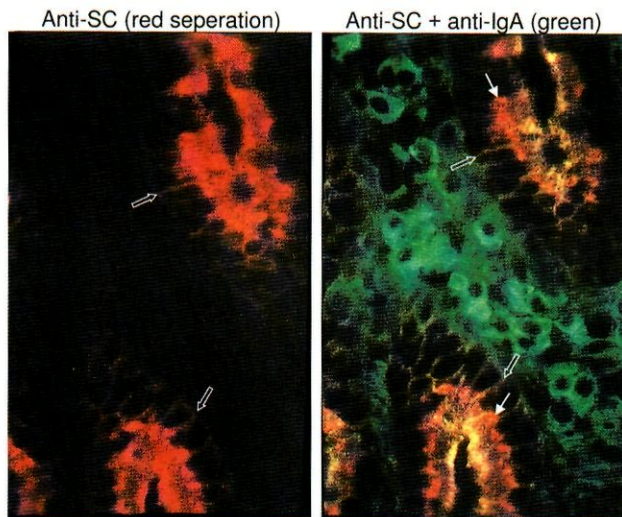


Fig. 23. Paired immunofluorescence staining for IgA and SC in tissue section from crypt region of human intestinal mucosa. The same field is shown after incubation with red anti-SC + green anti-IgA. Note in double exposure on the right that IgA alone is present in immunocytes and throughout the stroma; SC alone is present in the Golgi zones (arrows) adjacent to nuclei of glandular acinar cells; both IgA and SC (mixed colour) are present basolaterally (open arrows) on acinar cells and apically in their cytoplasm. Original magnification: $\times 180$

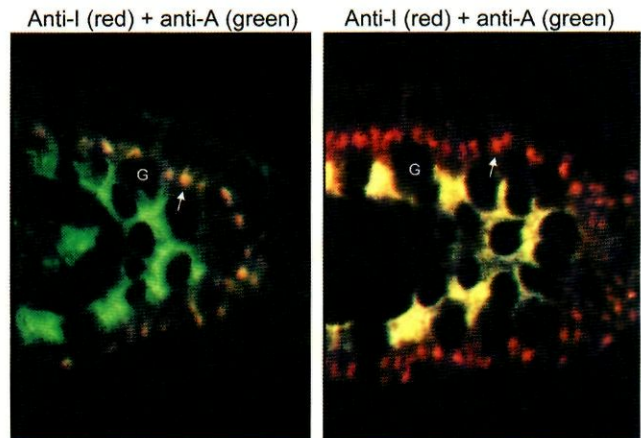


Fig. 24. Paired immunofluorescence for unoccupied pIgR/SC and bound SC, in tissue section of human colonic mucosa. Double exposures of same field showing crypt after incubation with red anti-I determinant (specific for unoccupied pIgR or free SC) + green anti-A determinant (accessible on unoccupied pIgR/SC as well as bound SC). The two antigenic determinants show distinct differential distribution with a relative dominance of I in the Golgi zones (arrows), and of A in the remaining cytoplasm and (weakly) also basolaterally. Right panel represents prolonged exposure time for red (anti-I) emission. Note that goblet cells (G) are devoid of both markers. Magnification: $\times 180$.

two ligands, or in their diffusion properties across the stromal matrix and basement membrane.

We have recently examined the impact of these variables on the epithelial transport of pIgA and pentameric IgM by employing polarized Madin-Darby canine kidney (MDCK) cells transfected to express the human pIgR (282). When the affinity of pIgA and pentameric IgM for human free SC in solution was determined *in vitro*, the latter ligand bound with considerably higher avidity (77, 94). Also, we found that the transfected pIgR bound polyclonal pIgA with somewhat lower affinity than monoclonal pentameric IgM, but the result depended on the individual characteristics of the latter (272, 282). Anyhow, both pIgA and pentameric IgM were found to be internalized quickly with similar rates at 37°C, and endocytosis was completed within 5 min (282). Moreover, both ligands were handled with similar efficiency during transcytosis in our test system (Fig. 25).

Possible ligand-enhanced transport

The regulation of human pIgR appears to differ from that of the rabbit and rat receptors, for which transcytosis is stimulated by bound ligand. Thus, rabbit pIgR complexed with human pIgA was transcytosed somewhat faster than unoccupied or anti-SC (Fab)-reacted receptor (283, 284). Such ligand-mediated

enhancement of the pIgR transport rate was also noted to operate for the rat receptor *in vivo* (285). Contrary to these observations, we found that the human pIgR transcytosis rate was not influenced by binding of either pIgA or pentameric IgM at concentrations reported to stimulate rabbit pIgR transcytosis (282). Thus, our results suggested that different levels of receptor saturation do not influence the rate of epithelial antibody transport mediated by the human pIgR (Fig. 26).

Altogether, species differences seem to exist with regard to regulation of pIgR transcytosis. It should be noted, however, that although human pIgA was found to stimulate this process when mediated by the rabbit pIgR, the reported effect was rather small and most pronounced with a mutant receptor that showed reduced efficiency of apical sorting (283, 284). Therefore, the biological significance of such ligand stimulation is questionable. Importantly, substantial amounts of human pIgR are continuously transcytosed across secretory epithelial cells *in vivo* even without any bound ligand (286). This observation has been further substantiated by comparing the level of SC in parotid saliva from IgA-deficient and hypogammaglobulinaemic subjects with normal controls; the amount of free SC (plus IgM-bound SC) in the former two categories of secretion was found to be of the same magnitude as that of free and bound (mainly SIgA-associated) SC in normal controls (278).

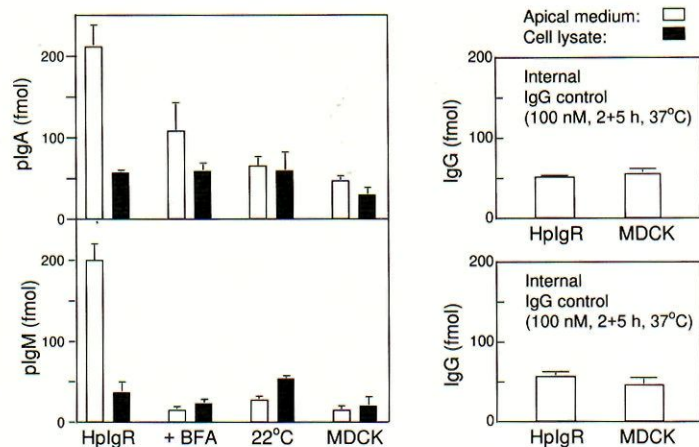


Fig. 25. Translocation of dimeric IgA (pIgA) and pentameric IgM (pIgM) by the human pIg receptor (HpIgR) in transfected MDCK cells.

Transport after basolateral addition of 100 nM pIgA or pIgM, with IgG as an internal control, was measured over a time period of 5 h after equilibration for 2 h. When indicated, brefeldin A (+BFA, 10 μ g/ml) was added to the basal medium, or incubation took place at 22°C. The apical medium was harvested and the cells were solubilized. Each test sample was analyzed in triplicates by enzyme-linked immunosorbent assay (ELISA) with reference to control samples from untransfected cells (MDCK). Data expressed as mean (+1 SD) of three filters. [Modified from (282)]

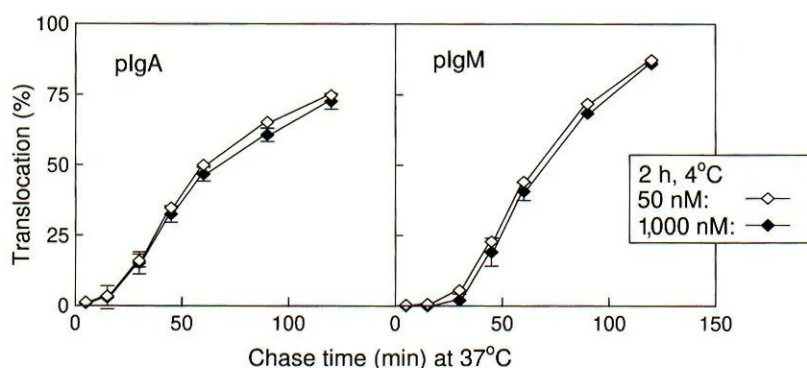


Fig. 26. No effect of ligand concentration on the rate of dimeric IgA (pIgA) and pentameric IgM (pIgM) translocation in MDCK cells expressing human pIgR. Cells were incubated with 125 I-labelled pIgA or pIgM as indicated for 2 h at 4°C, washed for 10 min at 4°C and chased at 37°C. Translocation is expressed as the cumulative appearance of 125 I-pIgA or 125 I-pIgM in the apical medium. Each point represents mean (\pm SD) of three filters. [Modified from (282)]

Thus, no evidence exists to support the notion that ligand-mediated stimulation of pIgR transcytosis takes place in humans.

Evolutionary significance of enhanced pIgA transport

IgM was the first Ig class to evolve and is found in external secretions of lower vertebrates (287, 288). All characterized mammalian species, and also chickens (289), possess IgA in addition to IgM. The evolution of IgA as the major secretory antibody class has introduced interesting changes conducive to mucosal homeostasis. Although our *in vitro* data showed that human pIgR-mediated transport of pentameric IgM is just as efficient as that of pIgA, we concluded that external translocation of the latter polymer is markedly favoured *in vivo* because of its better diffusion through the extracellular matrix and basement membranes (282). Therefore, the reduced size of pIgA may reflect a compromise between its advantageous external

translocation and the number of antigen binding sites carried to the secretions.

The pIgA molecule as such is well adapted to the proteolytic environment in external secretions (32, 290). In addition, its stabilization with covalently bound SC endows SIgA with increased resistance against digestive enzymes (277, 291) and bacterial proteases (32). Moreover, the lacking ability of human pIgA to activate the classical complement pathway (99, 292) is desirable to avoid altered mucosal homeostasis by inflammatory mediators generated at effector sites with persistent antigen exposure (see below). Altogether, pIgA appears to be a "smaller and smarter" antibody molecule than pentameric IgM in terms of mucosal defence.

Regulation of pIgR expression

Human pIgR is constitutively expressed even early in fetal life (143) and it can be upregulated postnatally by various cyto-

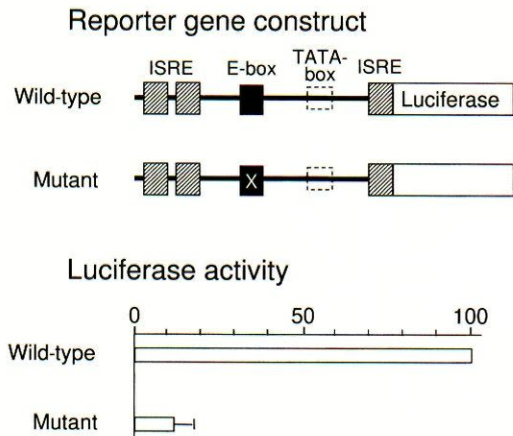


Fig. 27. Dependency on E-box in the promoter of the human pIgR/SC gene for its constitutive expression. Schematic diagram (top) of the proximal promoter region and reporter genes with a wild type and mutant E-box (X) as indicated. Result (bottom) of this mutation in SC gene promoter-driven reporter (luciferase) assay is given in percentage of wild-type activity; it is reduced to that of the TATA box alone (not shown).

[Data from (300)]

kines, particularly IFN- γ and IL-4 (182), which in some studies have been shown to act synergistically (293, 294). Also the proinflammatory cytokines TNF- α and IL-1 enhance SC expression in the HT-29 adenocarcinoma cell line (182, 295, 296), especially together with IFN- γ and butyrate (295), a bacterial fermentation product that abounds in the large bowel. Conversely, the stimulatory effect of IL-4 is selectively decreased by butyrate (295).

Transcriptional regulation of the human pIgR/SC gene

Our recent molecular experiments with HT-29 cells have demonstrated that IFN- γ , IL-4 and TNF- α enhance pIgR/SC expression by transcriptional activation (266, 297), and these cytokines can thus provide an immunoregulatory link between the level of local immune responses and the antibody transport function of pIgR (on the right in Fig. 16). We and others have cloned and characterized the pIgR/SC gene including its promoter region, and putative binding sites for various transcription factors, including glucocorticoid and androgen receptors, have been identified (298–302). A composite DNA element constituting an E-box consensus motif (Fig. 27), which binds proteins of the basic-helix-loop-helix/leucine-zipper (bHLH/LZ) family, was shown to be most important for constitutive pIgR/SC transcription in a reporter gene assay (300). A recent

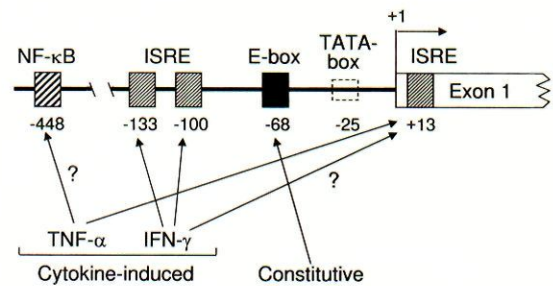


Fig. 28. Schematic depiction of regulatory elements in the human pIgR/SC gene. Diagram delineates DNA elements believed to be important for constitutive expression (see Fig. 27), IFN- γ -induced upregulation (all three ISREs included), and TNF- α -induced upregulation (an upstream NF- κ B binding site in the promoter and an ISRE in exon 1), with central base positions in relation to transcription start site indicated. Bent arrow marked +1 denotes the transcription start site and stippled open box a consensus TATA sequence. Question marks indicate putative cytokine-mediated induction not yet clearly shown by reporter gene assay.

study of the murine pIgR promoter has implicated upstream stimulatory factor (USF)-1 and possibly USF-2 as the bHLH/LZ members necessary for constitutive pIgR/SC expression (303).

Sequence analysis of the human gene has identified a number of putative DNA elements that contribute to its cytokine-enhanced expression (301). Three IFN-stimulated response elements (ISREs), two upstream of transcription start (centred around position -133 and -100) and one in exon 1 (centred around position +13), are potential sites for gene induction mediated by IFN- γ and TNF- α (Fig. 28). Piskurich et al. (301) investigated the role of these ISREs in IFN- γ -induced pIgR transcription. In a reporter gene construct spanning from position -563, deletion of the ISRE in exon 1 reduced IFN- γ -stimulated transcription. When the upstream region was deleted to position -280, deletion of the same ISRE had no effect. Further deletion to position -95, thereby removing the two upstream ISREs, completely abolished gene induction by IFN- γ , clearly implicating a role of these regulatory elements. Gel electrophoresis mobility shift assays (GEMSA) demonstrated protein binding to all three ISREs, but only binding to the ISRE in exon 1 was induced by IFN- γ . By supershift with corresponding antibody, this inducible factor was demonstrated to be IFN regulatory factor (IRF)-1. Furthermore, an IRF-1 promoter-based reporter gene was induced by IFN- γ in HT-29 cells, suggesting that IRF-

1 could be one of the *de novo* factor(s) required for IFN- γ -stimulated upregulation of pIgR expression in these cells. This possibility was strongly supported by results obtained in knockout mice lacking IRF-1 (304).

It was recently shown that overexpression of IRF-1 sufficed to activate the pIgR promoter, and that this induction depended on the ISRE in exon 1 (C. S. Kaetzel, personal communication). This ISRE has also been implicated in TNF- α -induced upregulation of pIgR (Fig. 28): in a reporter gene construct extending from -280, its deletion abolished the effect of TNF- α but not that of IFN- γ (302). However, induction mediated by TNF- α through this ISRE was modest compared to that obtained with IFN- γ , and we have strong evidence to suggest that the effect of TNF- α is additionally supported by regulatory elements found in intron 1 of the human pIgR/SC gene (H. Schjerven, P. Brandtzaeg, F.-E. Johansen, unpublished data). The IFN- γ -inducible binding of nuclear factor(s) to the ISRE in exon 1 was recently confirmed by us, and we demonstrated that the same factor(s) was also induced by TNF- α , albeit more weakly than by IFN- γ (297). We have furthermore identified a DNA element in intron 1 that is crucial for the IL-4-mediated activation of the human pIgR/SC gene (305).

Immunohistochemical observations in coeliac disease, chronic gastritis and Sjögren's syndrome harmonize with an immune response-associated enhancement of secretory immunity (Fig. 16); signs of upregulated pIgR/SC expression and increased receptor-mediated uptake of pIgA are seen in the secretory epithelium of all these immunologically active lesions (306). Nevertheless, the epithelial transport capacity may be insufficient in certain patients with an exceptionally expanded intestinal IgA immunocyte expansion, resulting in excessive amounts of pIgA in serum (307).

Non-inflammatory humoral defence of mucosae

Role of secretory antibodies and free SC in immune exclusion
The main purpose of the secretory antibody system is, in cooperation with innate mucosal defence mechanisms, to perform immune exclusion (Figs 2 & 29). Most importantly, SIgA inhibits colonization and invasion by pathogens (32). Both pIgA and pentameric IgM antibodies internalized by the pIgR may even inactivate viruses (e.g. rotavirus) inside epithelial cells and carry the pathogens and their products back to the lumen (44–47). The agglutinating and virus-neutralizing antibody effect of pIgA is in fact superior compared with monomeric antibodies (27, 32, 290), and SIgA antibodies can block microbial invasion quite efficiently (308). Thus, individuals negative for human immunodeficiency virus (HIV) who live together with HIV-

positive partners for several years, often appear to be protected by specific SIgA antibodies in their genital tract (309).

A potentially important additional defence function is the reported ability of SIgA antibodies to induce loss of bacterial plasmids that code for adherence-associated molecules and resistance to antibiotics (310). Also, SIgA may enhance the sticking of certain bacteria to mucus, interfere with growth factors (e.g. iron) and enzymes necessary for pathogenic bacteria and parasites (27, 32), and exert positive influences on the inductive phase of mucosal immunity by promoting relevant antigen uptake in GALT (28). The latter possibility adds to the importance of breast-feeding in providing a supply of SIgA antibodies to the infant's gut (311). Moreover, induction of SIgA responses has been shown to interfere significantly with mucosal uptake of macromolecules in experimental animals (27), and pIgR/SC knockout mice show evidence of increased mucosal leakiness and uptake of *Escherichia coli* antigens (29).

Collectively, therefore, the functions of locally produced pIgA would be to inhibit mucosal colonization of microorganisms as well as penetration of soluble antigens (Fig. 29). Notably, because of its stability (see above), SIgA can retain its antibody activity for remarkably prolonged periods in a hostile environment such as the oral cavity (291). This immune exclusion function is most likely reinforced by the relatively high levels of cross-reacting SIgA antibodies present in external secretions (312). These polyreactive "natural" antibodies apparently are designed for urgent protection before an adaptive immune response is elicited; they are therefore reminiscent of innate immunity (313), although their site and mechanism of induction remain unclear (see above). In addition, interaction of SIgA with the intestinal superantigen protein Fv (Fv fragment binding protein) may build an "immune fortress" by forming large complexes of intact or degraded antibodies with different specificities, thereby probably reinforcing immune exclusion in the gut (314).

Interestingly, free SC can inhibit epithelial adhesion of *E. coli* (315) and bind the potent toxin of *Clostridium difficile* (316). Moreover, a pneumococcal surface protein (SpsA) has recently been shown to interact directly with both free and bound SC (317). Altogether, these biological properties of SC suggest that it phylogenetically has originated from the innate defence system like many other proteins involved in specific immunity.

Role of IgA-mediated immune elimination

Magnitude of mucosal uptake of soluble antigens

Intact antigenic material from the gut lumen has been shown to cross the mucosal barrier and enter the blood stream even in normal adults, although the actual amount of uptake remains

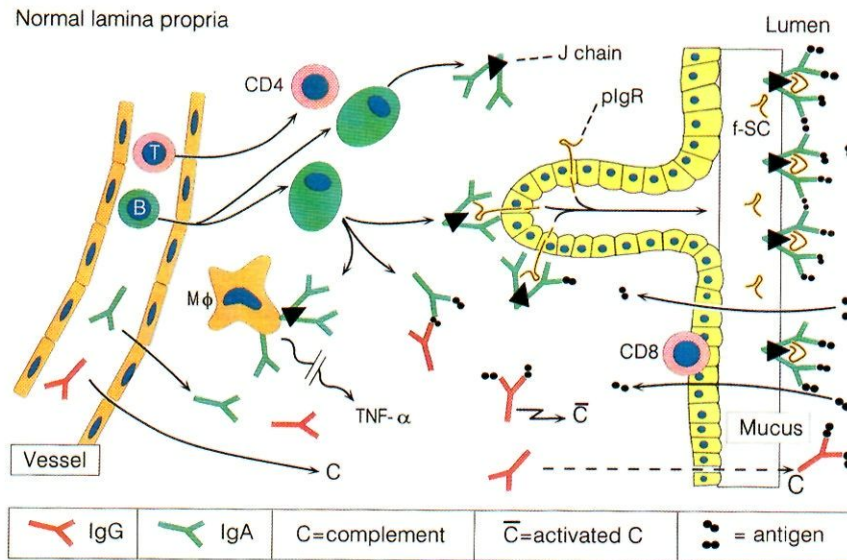


Fig. 29. Schematic depiction of normal mucosal homeostasis in the gut. Contributing biological variables are represented as a critical balance between available immunoglobulin classes (for simplicity, only IgG and IgA are indicated). Secretory IgA is generated from dimeric IgA with associated J chain produced by lamina propria immunocytes and transported by the transmembrane secretory component (SC or pIgR) to the lumen, together with variable amounts of unoccupied cleaved receptor called free SC (f-SC). Secretory IgA antibodies act in a first-line defence by performing antigen exclusion in mucus on the epithelial surface (to the right). Antigens circumventing this barrier meet corresponding serum-derived IgG antibodies in the lamina propria. The resulting immune complexes activate complement, and inflammatory

mediators are formed within the mucosa, thus causing a temporarily increased external transfer of IgG antibodies by leakage between epithelial cells to the lumen (broken arrow). A persistent and adverse inflammatory development is normally inhibited by blocking antibody activities (competition for antigen depicted) exerted in the lamina propria by serum-derived monomeric IgA as well as by locally produced monomeric and especially dimeric IgA. Both types of IgA molecules may, independent of their antibody specificities, also inhibit mediator release (TNF- α depicted) from activated phagocytic cells such as macrophages (M ϕ). Moreover, antigens may be returned in a non-inflammatory way to the lumen by the pIgR-mediated transport mechanism after being bound to J chain-containing dimeric IgA antibody as indicated.

uncertain. Work performed in experimental animals with ^{125}I -labelled albumin has been difficult to interpret due to marker instability (318). Both degradation of the protein molecule and release of the label can result in considerable overestimation of penetrability as determined by scintillation counting compared with data based on immunological quantification. However, several human studies have reported absorption of intact dietary antigens in healthy adults. Paganelli & Levinsky (319) found up to 3 ng/ml of β -lactoglobulin in peripheral blood after an intake of 1.2 l of bovine milk. Kilshaw & Cant (320) often detected bovine β -lactoglobulin and ovalbumin in both human serum and breast milk, the levels ranging from 110 pg/ml to 6.4 ng/ml in the latter fluid. Husby et al. (321) detected up to 10 ng/ml of ovalbumin, which corresponds to about 10^{-5} of the amount consumed. Most of the antigen appeared in the circulation after 2–5 h and was partly present in immune complexes (see below).

Routes of intestinal antigen uptake

Several routes may be visualized for the penetration of intact antigen through the normal intestinal epithelium: paracellular diffusion bypassing the tight junctions or via epithelial discontinuities such as the cell extrusion zones of the villus tips; translocation through enterocytes by endocytosis and subsequent exocytosis; or transport by M cells in GALT. The relative importance of these mechanisms for uptake of soluble antigens from the gut lumen in health and disease remains unknown; and the consequences in terms of sensitization or induction of oral tolerance probably depend on the route of uptake as well as on the nature of the antigen (soluble, lectin-like, or particulate). A detailed discussion of these possibilities is given elsewhere (27, 50, 322).

Putative mechanisms of immune elimination

It may be envisioned that IgA antibodies contribute to local homeostasis not only by performing immune exclusion on the mucosal surface, but also by trapping of antigens in the lamina propria (Fig. 29). Both these IgA-mediated blocking mechan-

isms would be non-inflammatory in competition with corresponding complement-activating IgG antibodies (27, 292). In addition, epithelial transport of pIgA (or pIgM)-containing immune complexes might also be an efficient non-inflammatory antigen clearance mechanism (Fig. 29) as suggested by *in vitro* experiments; thus, pIgR-expressing polarized epithelial monolayers were shown to translocate undegraded antigen bound to pIgA antibody from the basolateral side to the apical medium (44). Interestingly, monomeric IgA or IgG antibodies, when cross-linked via antigen to pIgA of the same specificity, could be included in the pIgR-mediated external transcytosis (44). Therefore, the secretory epithelium may participate in the clearance of immune complexes directly at the mucosal site where they are most likely to be formed.

Additional IgA-mediated putative homeostatic mechanisms

Experimental evidence moreover suggests that IgA in several ways might influence local homeostasis through interaction with Fc α RI (CD89) on leukocytes. By expressing its ligand-binding activity in the membrane-distal EC1 domain (323), this receptor appears to be well designed for complexing with all molecular forms of IgA. Thus, monomeric IgA – and particularly pIgA or IgA-containing immune complexes – are able to suppress the attraction of neutrophils, eosinophils and monocytes, thereby reducing the availability of the numerous potent inflammatory mediators that may be released from these cells (27). Also, IgA can downregulate the secretion of proinflammatory cytokines such as TNF- α from activated monocytes (324), and perhaps that from lamina propria macrophages as well (Fig. 29). Finally, activation of neutrophils and monocytes resulting in generation of reactive oxygen intermediates (respiratory burst) might likewise be inhibited by IgA (325). On the other hand, pIgA or aggregated monomeric IgA can trigger resting monocytes to show increased activity such as TNF- α secretion (326) and is also able to cause rather brisk degranulation of eosinophils (327). This proinflammatory potential of the local IgA system probably reflects a need for enhanced antigen elimination when immune exclusion fails (see below). Altogether, the divergent *in vitro* results emphasize the fact that the contribution of IgA to normal mucosal homeostasis *in vivo* must be remarkably fine-tuned.

Individual variations in the development of mucosal homeostasis

The postnatal development of the IgA-producing cell system shows large individual variations (143). On the basis of IgA measurements in serum, it has been suggested that infants and children at hereditary risk of atopy have a retarded post-natal development of their IgA system (328, 329). Perhaps their SIgA-mediated immune exclusion and other noninflammatory

mucosal antigen handling mechanisms are transiently deficient. This notion was later supported by quantitation of jejunal immunocytes; a significantly reduced IgA response to luminal antigens, without any IgM compensation, was indicated in the mucosa of atopic children (330). Another study showed an inverse relationship between the serum IgE concentration and the number of IgA-producing cells in jejunal mucosa of food-allergic children (331). More recently it was reported that infants born to atopic parents have a significantly higher prevalence of salivary IgA deficiency than age-matched control infants (332). Interestingly, Kilian et al. (333) found that the throats of 18-month-old infants with presumably IgE-mediated clinical problems, contained significantly higher proportions of IgA1 protease-producing bacteria than age-matched healthy controls. Therefore, a combination of reduced epithelial barrier function and hereditary elevated IgE responses might often underlie the pathogenesis of mucosal hypersensitivity, at least in many of the atopic children.

Proinflammatory mucosal antigen handling

Interactions of SIgA and free SC

It has been claimed that SIgA can perform ADCC and promote phagocytosis via Fc α RI present on macrophages and granulocytes. However, this suggestion is poorly documented, and it is difficult to assess the possible outcome in terms of an inflammatory reaction, for instance caused by TNF- α release as discussed above. Interestingly, potentially tissue-damaging eosinophils possess not only Fc α RI but apparently also a receptor for free and bound SC (334). This probably explains that SIgA exhibits particularly strong eosinophil-degranulating properties (327, 335) and in this respect appears to be more potent than cross-linked IgE antibodies, which instead may be more involved in the recruitment and priming of these cells (336). Also, SIgA has been shown to induce degranulation of IL-3-primed basophils (337). Thus, by interacting with eosinophils and basophils (perhaps also mast cells?), SIgA provides a proinflammatory potential of the secretory immune system when immune exclusion fails, such as in parasitic mucosal infestations. However, it is at present difficult to know how the balance between SIgA and free SC may influence eosinophil activation, because free SC in solution (but presumably not on a surface) may be blocking in this respect (334, 335).

Putative function of IgG antibodies

Circulating IgG antibodies to food antigens are often present in normal individuals, although with a remarkably varying subclass contribution (338). Because approximately 50% of the

circulating pool of IgG is extravascularly distributed, these antibodies will be well represented locally in the intestinal mucosa. Parenteral immunization eliciting high levels of IgG antibodies may reduce mucosal antigen penetration (27), although some studies have reported increased uptake (339). Our *in vitro* results showed that IgG antibodies could retard mucosal penetration of the corresponding antigen; conversely, the penetration of an unrelated bystander macromolecule was significantly enhanced (318). A similar detrimental side effect of IgG antibody has been observed *in vivo* (340). This is probably explained by local formation of complement-fixing IgG-containing immune complexes (27) and activation of mast cells (339). The pro-inflammatory potential of maternal IgG present in the intestinal mucosa of the newborns, or of locally produced IgG antibodies, may be less important in infants who are breast-fed because milk SIgA antibodies, which have little or no complement-activating capacity (292), will exert a non-inflammatory blocking effect (311).

As long as the mucosal homeostasis remains in control, IgG-mediated immune reactions occurring in the mucosa will not cause clinical symptoms and may be viewed as a "second line" of defence (Fig. 29). However, in subjects with a pronounced regional IgE response to environmental or dietary antigens, immune-mediated degranulation of mucosal mast cells and activation of eosinophils are likely to result in a massive release of potent inflammatory mediators overruling the IgA- and tolerance-mediated local homeostatic mechanisms. Such "parasite-directed" pathotopic potentiation of the second line of mucosal defence is probably the basis of overt atopic mucosal allergy (341), and may be considered as a breakdown of mucosally induced tolerance. In IBD (ulcerative colitis and Crohn's disease), mucosal homeostasis is severely altered with signs of immunological hyperactivation, downregulated J chain expression and substantial local overproduction of IgG, including antibodies to gut bacteria (57). Together, these features are believed to signify break of oral tolerance to antigens from the indigenous bacterial flora accompanied by less restricted extravasation of leukocytes.

Concluding considerations

This review has focused on the induction of mucosal B cells and their effector functions after terminal differentiation to immunocytes. However, it is becoming increasingly evident that B cells also can exert important immunoregulatory activities both as efficient stimulatory APCs (342), tolerizing APCs (343), and by secreting a variety of cytokines (344). In the mucosal immune system such B-cell characteristics remain elu-

sive (30). Nevertheless, B cells located at the presumed sites of antigen entrance in GALT express the necessary costimulatory molecules to function as APCs, perhaps leading to enhanced diversification of mucosal immune responses, thereby explaining the observation that SIgA antibodies show broader specificity than comparable serum antibodies (30). Such putative diversification of secretory immunity is reflected by the deletions and insertions observed in the CDR regions of IgV genes analyzed from human intestinal immunocytes (226).

Are local IgA production and mucosally induced tolerance interrelated?

The relationship between induction of intestinal IgA responses and oral tolerance remains rather enigmatic (50). Experiments in CD8 knockout mice have suggested that this phenotype of T lymphocytes (the predominant IEL subset) is crucial for down-regulation of the mucosal B-cell system (345). The tone of hyporesponsiveness in the intestinal immune system appears to be quite robust, because even a strong immunogen such as cholera toxin (CT) is unable to abrogate it although oral tolerance cannot be induced in the presence of CT (345). On the other hand, TGF- β has been shown to be important to promote IgA switching (Fig. 16) also in mice immunized with CT (346), and this cytokine is in addition believed to be one of the major mediators of oral tolerance in murine test systems (51). It is as yet not possible to extrapolate such apparently contradictory information to the human mucosal immune system.

What is known about human mucosal B cells and their functions?

It is well established in the human gut that the mucosal immune system responds to infection with local IgA and IgM production (347), and it appears that the level of this response may determine whether clinical symptoms will occur or not (348). In experimental animals, antibody-dependent immune exclusion has been shown to operate even for small molecules such as chemical carcinogens (349, 350). However, a rational basis for manipulation of local immunity by vaccines is still not satisfactorily established. Altogether, the following facts and open questions can be summarized about the human mucosal B-cell system:

- 1) Secretory immunity depends on an intimate cooperation between mucosal B cells and the pIgR/SC-expressing epithelia. The obvious biological significance of the striking J chain expression shown by disseminated MALT-derived immunocytes is that IgA and IgM polymers with high affinity for pIgR/SC can be produced at secretory effector sites and become readily available for active external transport. This important

functional goal in terms of clonal differentiation, appears to be sufficient justification for the J chain also to be expressed by B cells terminating at such sites with IgG or IgD production; these immunocytes may be considered as a "spin-off" from early effector clones that through isotype switching are on their way to pIgA expression.

2) There is considerable evidence to support the notion that intestinal immunocytes are largely derived from B cells initially induced in GALT. However, insufficient knowledge exists concerning the relative importance of M cells, MHC class II-expressing epithelial cells, B cells and other professional APCs in the transport, processing and presentation of luminal antigens that take place in GALT to accomplish the extensive and continuous priming and expansion of mucosal B cells. Also, it is not clear how the germinal-centre reaction in GALT, compared with other parts of MALT such as the tonsils, so strikingly promotes preferential isotype switching to IgA and a high level of J chain expression.

3) Although the B-cell migration from GALT to the intestinal lamina propria is guided by rather well-characterized adhesion molecules, the chemotactic stimuli involved in extravasation and microcompartmental distribution of various B-cell subsets remain elusive. Also, the homing mechanisms of mucosal B cells appear to be remarkably regionalized, but they remain to be defined in the upper aerodigestive tract.

4) Retention and accumulation of B cells extravasated at secretory effector sites are influenced by antigen-driven local proliferation and differentiation. However, the relative roles of

stromal T cells and IELs, MHC class II-positive APCs and epithelial cells in providing the necessary stimulatory signals for proliferation and terminal differentiation of the local B cells are unknown.

5) The mucosal barrier normally allows some penetration of intact soluble antigens so there is probably always a need for immune elimination in the lamina propria. If immune exclusion is impaired (e.g. in IgA deficiency), or if there is too large an antigen load on the epithelial barrier (e.g. in chronic infection), activated nonspecific amplification mechanisms involved in immune elimination may cause hypersensitivity which is observed clinically as mucosal disease. This immunopathological development may be mediated by proinflammatory IgG and IgE antibodies as well as by hyperactivated APCs and T cells. Although these immunopathogenic mechanisms are rather well understood in several types of intestinal disorders such as atopic food allergy and coeliac disease, the cause of their initiation, possibly involving abrogation of oral tolerance, generally remains unexplained.

6) Clinical observations in immunodeficient patients have shown that SIgA, SIgM and IgG are not the only important components of the intestinal mucosal defence system. It is becoming increasingly evident that innate immunity is crucial and much more complex than previously believed; the co-operation between innate and adaptive mucosal immunity needs exploration to better understand how homeostasis of mucous membranes normally is maintained.

References

1. Hanson LA, Brandtzaeg P. The discovery of secretory IgA and the mucosal immune system. *Immunol Today* 1993;**14**:416–417.
2. Tomasi TB, Tan EM, Solomon A, Prendergast RA. Characteristics of an immune system common to certain external secretions. *J Exp Med* 1965;**121**:101–124.
3. South MA, Cooper MD, Wollheim FA, Hong R, Good RA. The IgA system. I. Studies of the transport and immunochemistry of IgA in the saliva. *J Exp Med* 1966;**123**:615–627.
4. Crabbé PA, Carbonara AO, Heremans JF. The normal human intestinal mucosa as a major source of plasma cells containing A-immunoglobulin. *Lab Invest* 1965;**14**:235–248.
5. Brandtzaeg P, Baklien K. Immunohistochemical studies of the formation and epithelial transport of immunoglobulins in normal and diseased human intestinal mucosa. *Scand J Gastroenterol* 1976;**11** (Suppl 36):1–45.
6. Brandtzaeg P. Two types of IgA immunocytes in man. *Nature* 1973;**243**:142–143.
7. Brandtzaeg P, Fjellanger I, Gjeruldsen ST. Immunoglobulin M: local synthesis and selective secretion in patients with immunoglobulin A deficiency. *Science* 1968;**160**:789–791.
8. Brandtzaeg P. Human secretory immunoglobulin M. An immunochemical and immunohistochemical study. *Immunology* 1975;**29**:559–570.
9. Brown WR, Isobe Y, Nakane PK. Studies on translocation of immunoglobulins across intestinal epithelium. II. Immunoelectron-microscopic localization of immunoglobulins and secretory component in human intestinal mucosa. *Gastroenterology* 1976;**71**:985–995.
10. Brandtzaeg P. Mucosal and glandular distribution of immunoglobulin components. Differential localization of free and bound SC in secretory epithelial cells. *J Immunol* 1974;**112**:1553–1559.
11. Brandtzaeg P. Mucosal and glandular distribution of immunoglobulin components. Immunohistochemistry with a cold ethanol-fixation technique. *Immunology* 1974;**26**:1101–1114.
12. Halpern MS, Koshland ME. Novel subunit in secretory IgA. *Nature* 1970;**228**:1276–1278.

13. Mestecky J, Zikan J, Butler W. Immunoglobulin M and secretory immunoglobulin A: evidence for a common polypeptide chain different from light chains. *Science* 1971;**171**:1163–1165.
14. Bastian A, Kratzin H, Eckart K, Hilschmann N. Intra- and interchain disulfide bridges of the human J chain in secretory immunoglobulin A. *Biol Chem Hoppe Seyler* 1992;**373**:1255–1263.
15. Brandtzaeg P, Prydz H. Direct evidence for an integrated function of J chain and secretory component in epithelial transport of immunoglobulins. *Nature* 1984;**311**:71–73.
16. Vaerman J-P et al. Antibody against the human J chain inhibits polymeric Ig receptor-mediated biliary and epithelial transport of human polymeric IgA. *Eur J Immunol* 1998;**28**:171–182.
17. Vaerman JP, Langendries A, Giffroy D, Brandtzaeg P, Kobayashi K. Lack of SC/pIgR-mediated epithelial transport of a human polymeric IgA devoid of J chain: in vitro and in vivo studies. *Immunology* 1998;**95**:90–96.
18. Johansen F-E, Norderhaug IN, Roe M, Sandlie I, Brandtzaeg P. Recombinant expression of polymeric IgA: incorporation of J chain and secretory component of human origin. *Eur J Immunol* 1999;**29**:1701–1708.
19. Brandtzaeg P. Presence of J chain in human immunocytes containing various immunoglobulin classes. *Nature* 1974;**252**:418–420.
20. Brandtzaeg P. Immunohistochemical characterization of intracellular J-chain and binding site for secretory component (SC) in human immunoglobulin (Ig) producing cells. *Mol Immunol* 1983;**20**:941–966.
21. Castrignano SB, Carlsson B, Carneiro-Sampaio MS, Soderstrom T, Hanson LA. IgA and IgG subclass deficiency in a poor population in a developing country. *Scand J Immunol* 1993;**37**:509–514.
22. Brandtzaeg P, Nilssen DE. Mucosal aspects of primary B-cell deficiency and gastrointestinal infections. *Curr Opin Gastroenterol* 1995;**11**:532–540.
23. Cunningham-Rundles C. Disorders of the IgA system. In: Stiehm ER, eds. *Immunologic disorders in infants and children*. 4th ed. London: Saunders; 1996. p. 423–442.
24. McLoughlin GA, Hede JE, Temple JG, Bradley J, Chapman DM, McFarland J. The role of IgA in the prevention of bacterial colonization of the jejunum in the vagotomized subject. *Br J Surg* 1978;**65**:435–437.
25. Brandtzaeg P, Gjeruldsen ST, Korsrud F, Baklien K, Berdal P, Ek J. The human secretory immune system shows striking heterogeneity with regard to involvement of J chain positive IgD immunocytes. *J Immunol* 1979;**122**:503–510.
26. Cataldo F, Marino V, Ventura A, Bottaro G, Corazza GR. Prevalence and clinical features of selective immunoglobulin A deficiency in coeliac disease: an Italian multicentre study. Italian Society of Paediatric Gastroenterology and Hepatology (SIGEP) and "Club del Tenue" Working Groups on Coeliac Disease. *Gut* 1998;**42**:362–365.
27. Brandtzaeg P, Baklien K, Bjerke K, Rognum TO, Scott H, Valnes K. Nature and properties of the human gastrointestinal immune system. In: Miller K, Nicklin S, eds. *Immunology of the gastrointestinal tract*. Vol I. Boca Raton, Florida: CRC Press; 1987. p. 1–85.
28. Brandtzaeg P. Immune responses to gut virus infections. In: Farthing MJG, ed. *Viruses and the gut*. London: Smith Kline, French Laboratories Ltd; 1989. p. 45–54.
29. Johansen F-E, et al. Absence of epithelial IgA transport, with increased mucosal leakiness, in pIgR/SC-deficient mice. *J Exp Med* (In press).
30. Brandtzaeg P, et al. Regional specialization in the mucosal immune system: what happens in the microcompartments? *Immunol Today* 1999;**20**:141–151.
31. Langman JM, Rowland R. The number and distribution of lymphoid follicles in the human large intestine. *J Anat* 1986;**149**:189–194.
32. Goldblum RM, Hanson LA, Brandtzaeg P. The mucosal defense system. In: Stiehm ER, ed. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: WB Saunders; 1996. p. 159–199.
33. Brandtzaeg P, Farstad IN, Haraldsen G. Regional specialization in the mucosal immune system: primed cells do not always home along the same track. *Immunol Today* 1999;**20**:267–277.
34. Hanson LA, et al. Child health and the population increase. *Peace, Health and Development* 1993;**4**:31–38.
35. Anonymous. A warm chain for breastfeeding. *Lancet* 1994;**344**:1239–1241.
36. Wright AL, Bauer M, Naylor A, Sutcliffe E, Clark L. Increasing breastfeeding rates to reduce infant illness at the community level. *Pediatrics* 1998;**101**:837–844.
37. Saarinen UM, Kajosaari M. Breastfeeding as prophylaxis against atopic disease: prospective follow-up study until 17 years old. *Lancet* 1995;**346**:1065–1069.
38. Brandtzaeg P. Development of the intestinal immune system and its relation to coeliac disease. In: Mäki M, Collin P, Visakorpi JK, eds. *Coeliac disease. Proceedings of the Seventh International Symposium on Coeliac Disease*. Tampere: Coeliac Disease Study Group; 1997. p. 221–244.
39. Dickinson EC, et al. Immunoglobulin A supplementation abrogates bacterial translocation and preserves the architecture of the intestinal epithelium. *Surgery* 1998;**124**:284–290.
40. Berneman A, Belec L, Fischetti VA, Bouvet JP. The specificity patterns of human immunoglobulin G antibodies in serum differ from those in autologous secretions. *Infect Immun* 1998;**66**:4163–4168.
41. Persson CG, et al. Contribution of plasma-derived molecules to mucosal immune defence, disease and repair in the airways. *Scand J Immunol* 1998;**47**:302–313.
42. Mbawuike IN, Pacheco S, Acuna CL, Switzer KC, Zhang Y, Harriman GR. Mucosal immunity to influenza without IgA: An IgA knockout mouse model. *J Immunol* 1999;**162**:2530–2537.
43. Brandtzaeg P. Mucosal immunity in the female genital tract. *J Reprod Immunol* 1997;**36**:23–50.
44. Mazanec MB, Nedrud JG, Kaetzel CS, Lamm ME. A three-tiered view of the role of IgA in mucosal defense. *Immunol Today* 1993;**14**:430–435.
45. Burns JW, Siadat-Pajouh M, Krishnaney AA, Greenberg HB. Protective effect of rotavirus VP6-specific IgA monoclonal antibodies that lack neutralizing activity. *Science* 1996;**272**:104–107.
46. Bomsel M, et al. Intracellular neutralization of HIV transcytosis across tight epithelial barriers by anti-HIV envelope protein dIgA or IgM. *Immunity* 1998;**9**:277–287.
47. Fujioka H, et al. Immunocytochemical colocalization of specific immunoglobulin A with sendai virus protein in infected polarized epithelium. *J Exp Med* 1998;**188**:1223–1229.
48. Mowat AM, Viney JL. The anatomical basis of intestinal immunity. *Immunol Rev* 1997;**156**:145–166.
49. Christ AD, Blumberg RS. The intestinal epithelial cell: immunological aspects. *Springer Semin Immunopathol* 1997;**18**:449–461.
50. Brandtzaeg P. History of oral tolerance and mucosal immunity. *Ann N Y Acad Sci* 1996;**778**:1–27.
51. Strobel S, Mowat AM. Immune responses to dietary antigens: oral tolerance. *Immunol Today* 1998;**19**:173–181.

52. Rugtveit J, Bakka A, Brandtzaeg P. Differential distribution of B7.1 (CD80) and B7.2 (CD86) co-stimulatory molecules on mucosal macrophage subsets in human inflammatory bowel disease (IBD). *Clin Exp Immunol* 1997;**110**:104–113.
53. Viney JL, Mowat AM, O'Malley JM, Williamson E, Fanger NA. Expanding dendritic cells *in vivo* enhances the induction of oral tolerance. *J Immunol* 1998;**160**:5815–525.
54. Gütgemann I, Fahrer AM, Altman JD, Davis MM, Chien YH. Induction of rapid T cell activation and tolerance by systemic presentation of an orally administered antigen. *Immunity* 1998;**8**:667–673.
55. Sallusto F, Lanzavecchia A. Mobilizing dendritic cells for tolerance, priming, and chronic inflammation. *J Exp Med* 1999;**189**:611–614.
56. Scott H, et al. Immunopathology of gluten-sensitive enteropathy. *Springer Semin Immunopathol* 1997;**18**:535–553.
57. Brandtzaeg P, Haraldsen G, Rugtveit J. Immunopathology of human inflammatory bowel disease. *Springer Semin Immunopathol* 1997;**18**:555–589.
58. Rubin W, Fauci AS, Sleisenger MH, Jeffries GH. Immunofluorescent studies in adult celiac disease. *J Clin Invest* 1965;**44**:475–485.
59. Van der Heijden PJ, Stok W, Bianchi AT. Contribution of immunoglobulin-secreting cells in the murine small intestine to the total 'background' immunoglobulin production. *Immunology* 1987;**62**:551–555.
60. Brandtzaeg P, et al. Immunobiology and immunopathology of human gut mucosa: humoral immunity and intraepithelial lymphocytes. *Gastroenterology* 1989;**97**:1562–1584.
61. Turesson I. Distribution of immunoglobulin-containing cells in human bone marrow and lymphoid tissues. *Acta Med Scand* 1976;**199**:293–304.
62. Tseng J. Expression of immunoglobulin isotypes by lymphoid cells of mouse intestinal lamina propria. *Cell Immunol* 1982;**73**:324–336.
63. Liu Y-J, Banchereau J. The paths and molecular controls of peripheral B-cell development. *Immunologist* 1996;**4**:55–66.
64. Fossum S, Rolstad B, Tjernshaugen H. Selective loss of S-phase cells when making cell suspensions from lymphoid tissue. *Cell Immunol* 1979;**48**:149–154.
65. Mayrhofer G. Physiology of the intestinal immune system. In: Newby TJ, Stokes CR, eds. *Local immune responses of the gut*. Boca Raton, Florida: CRC Press; 1984. p. 1–96.
66. Farstad IN, Halstensen TS, Lazarovits AI, Norstein J, Fausa O, Brandtzaeg P. Human intestinal B-cell blasts and plasma cells express the mucosal homing receptor integrin $\alpha 4\beta 7$. *Scand J Immunol* 1995;**42**:662–672.
67. Bienenstock J, Befus D, McDermott M, Mirski S, Rosenthal K. Regulation of lymphoblast traffic and localization in mucosal tissues, with emphasis on IgA. *Fed Proc* 1983;**42**:3213–3217.
68. Husband AJ. Kinetics of extravasation and redistribution of IgA-specific antibody-containing cells in the intestine. *J Immunol* 1982;**128**:1355–1359.
69. Baklien K, Brandtzaeg P, Fausa O. Immunoglobulins in jejunal mucosa and serum from patients with adult coeliac disease. *Scand J Gastroenterol* 1977;**12**:149–159.
70. André C, André F, Fargier MC. Distribution of IgA1 and IgA2 plasma cells in various normal human tissues and in the jejunum of plasma IgA-deficient patients. *Clin Exp Immunol* 1978;**33**:327–231.
71. Crago SS, et al. Distribution of IgA1-, IgA2-, and J chain-containing cells in human tissues. *J Immunol* 1984;**132**:16–18.
72. Jonard PP, Rambaud JC, Dive C, Vaerman JP, Galian A, Delacroix DL. Secretion of immunoglobulins and plasma proteins from the jejunal mucosa. Transport rate and origin of polymeric immunoglobulin A. *J Clin Invest* 1984;**74**:525–535.
73. Kett K, Brandtzaeg P, Radl J, Haaijman JJ. Different subclass distribution of IgA-producing cells in human lymphoid organs and various secretory tissues. *J Immunol* 1986;**136**:3631–3635.
74. Burnett D, Crocker J, Stockley RA. Cells containing IgA subclasses in bronchi of subjects with and without chronic obstructive lung disease. *Clin Pathol* 1987;**40**:1217–1220.
75. Müller F, Frøland SS, Hvatum M, Radl J, Brandtzaeg P. Both IgA subclasses are reduced in parotid saliva from patients with AIDS. *Clin Exp Immunol* 1991;**83**:203–209.
76. Feltelius N, Hvatum M, Brandtzaeg P, Knutson L, Hällgren R. Increased jejunal secretory IgA and IgM in ankylosing spondylitis: normalization after treatment with sulfasalazine. *J Rheumatol* 1994;**21**:2076–2081.
77. Brandtzaeg P. Human secretory component. VI. Immunoglobulin-binding properties. *Immunochemistry* 1977;**14**:179–188.
78. Kilian M, Reinholdt J, Lomholt H, Poulsen K, Frandsen EV. Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. *APMIS* 1996;**104**:321–338.
79. Mestecky J, Russell MW. IgA subclasses. *Monogr Allergy* 1986;**19**:277–301.
80. Tarkowski A, Lue C, Moldoveanu Z, Kiyono H, McGhee JR, Mestecky J. Immunization of humans with polysaccharide vaccines induces systemic, predominantly polymeric IgA2-subclass antibody responses. *J Immunol* 1990;**144**:3770–3778.
81. Kett K, Baklien K, Bakken A, Kral JG, Fausa O, Brandtzaeg P. Intestinal B-cell isotype response in relation to local bacterial load: evidence for immunoglobulin A subclass adaptation. *Gastroenterology* 1995;**109**:819–825.
82. Brandtzaeg P, Karlsson G, Hansson G, Petruson B, Björkander J, Hanson LÅ. The clinical condition of IgA-deficient patients is related to the proportion of IgD- and IgM-producing cells in their nasal mucosa. *Clin Exp Immunol* 1987;**67**:626–636.
83. Valnes K, Brandtzaeg P, Elgjo K, Stave R. Quantitative distribution of immunoglobulin-producing cells in gastric mucosa: relation to chronic gastritis and glandular atrophy. *Gut* 1986;**27**:505–514.
84. Korsrud FR, Brandtzaeg P. Quantitative immuno histochemistry of immunoglobulin- and J-chain-producing cells in human parotid and submandibular glands. *Immunology* 1980;**39**:129–140.
85. Brandtzaeg P. Mechanisms of gastrointestinal reactions to food. *Environ Toxicol Pharmacol* 1997;**4**:9–24.
86. Bjerke K, Brandtzaeg P. Terminally differentiated human intestinal B cells. IgA and IgG subclass-producing immunocytes in the distal ileum, including Peyer's patches, compared with lymph nodes and palatine tonsils. *Scand J Immunol* 1990;**32**:61–67.
87. Nilssen DE, et al. Isotype distribution of mucosal IgG-producing cells in patients with various IgG subclass deficiencies. *Clin Exp Immunol* 1991;**83**:17–24.
88. Helgeland L, et al. The IgG subclass distribution in serum and rectal mucosa of monozygotic twins with or without inflammatory bowel disease. *Gut* 1992;**33**:1358–1364.
89. Flanagan JG, Rabbitts TH. Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing γ , ϵ and α genes. *Nature* 1982;**300**:709–713.
90. Papadea C, Check JJ. Human immunoglobulin G and immunoglobulin G subclasses: biochemical, genetic, and clinical aspects. *Crit Rev Clin Lab Sci* 1989;**27**:27–58.

91. Takahashi T, et al. The joining (J) chain is present in invertebrates that do not express immunoglobulins. *Proc Natl Acad Sci USA* 1996;**93**:1886–1891.
92. Eskeland T, Brandtzaeg P. Does J chain mediate the combination of 19S IgM and dimeric IgA with the secretory component rather than being necessary for their polymerization? *Immunochemistry* 1974;**11**:161–163.
93. Brandtzaeg P. Complex formation between secretory component and human immunoglobulins related to their content of J chain. *Scand J Immunol* 1976;**5**:411–419.
94. Brandtzaeg P. Role of J chain and secretory component in receptor-mediated glandular and hepatic transport of immunoglobulins in man. *Scand J Immunol* 1985;**22**:111–146.
95. Hendrickson BA, et al. Lack of association of secretory component with IgA in J chain-deficient mice. *J Immunol* 1996;**157**:750–754.
96. Ma JK, et al. Generation and assembly of secretory antibodies in plants. *Science* 1995;**268**:716–719.
97. Brewer JW, Randall TD, Parkhouse RM, Corley RB. IgM hexamers? *Immunol Today* 1994;**15**:165–168.
98. Wiersma EJ, Collins C, Fazel S, Shulman MJ. Structural and functional analysis of J chain-deficient IgM. *J Immunol* 1998;**160**:5979–5989.
99. Sorensen V, Sundvold V, Michaelsen TE, Sandlie I. Polymerization of IgA and IgM: roles of Cys³⁰⁹/Cys⁴¹⁴ and the secretory tailpiece. *J Immunol* 1999;**162**:3448–3455.
100. Brandtzaeg P, Korsrud FR. Significance of different J chain profiles in human tissues: generation of IgA and IgM with binding site for secretory component is related to the J chain expressing capacity of the total local immunocyte population, including IgG and IgD producing cells, and depends on the clinical state of the tissue. *Clin Exp Immunol* 1984;**58**:709–718.
101. Stott SI. Biosynthesis and assembly of IgM. Addition of J-chain to intracellular pools of 8S and 19S IgM. *Immunochemistry* 1976;**13**:157–163.
102. Brewer JW, Corley RB. Late events in assembly determine the polymeric structure and biological activity of secretory IgM. *Mol Immunol* 1997;**34**:323–331.
103. Parkhouse RMF, Della Corte E. Control of IgA biosynthesis. In: Beers RF, Bassett EG, eds. *The role of immunological factors in infections, allergic, and autoimmune processes*. New York: Raven Press; 1976. p. 389–401.
104. Roth R, Mather EL, Koshland ME. Intracellular events in the differentiation of B lymphocytes to pentamer IgM synthesis. In: Pernis B, Vogel HJ, eds. *Cells of immunoglobulin synthesis*. London: Academic Press; 1979. p. 141–151.
105. Kett K, Brandtzaeg P, Fausa O. J-chain expression is more prominent in immunoglobulin A2 than in immunoglobulin A1 colonic immunocytes and is decreased in both subclasses associated with inflammatory bowel disease. *Gastroenterology* 1988;**94**:1419–1425.
106. Bull DM, Bienenstock J, Tomasi TB. Studies on human intestinal immunoglobulin A. *Gastroenterology* 1971;**60**:370–380.
107. MacDermott RP, et al. Altered patterns of secretion of monomeric IgA and IgA subclass 1 by intestinal mononuclear cells in inflammatory bowel disease. *Gastroenterology* 1986;**91**:379–385.
108. Kutteh WH, Koopman WJ, Conley ME, Egan ML, Mestecky J. Production of predominantly polymeric IgA by human peripheral blood lymphocytes stimulated in vitro with mitogens. *J Exp Med* 1980;**152**:1424–1429.
109. Moldoveanu Z, Egan ML, Mestecky J. Cellular origins of human polymeric and monomeric IgA: intracellular and secreted forms of IgA. *J Immunol* 1984;**133**:3156–3162.
110. Nagura H, Brandtzaeg P, Nakane PK, Brown WR. Ultrastructural localization of J chain in human intestinal mucosa. *J Immunol* 1979;**123**:1044–1050.
111. Moro I, Iwase T, Komiyama K, Moldoveanu Z, Mestecky J. Immunoglobulin A (IgA) polymerization sites in human immunocytes: immunoelectron microscopic study. *Cell Struct Funct* 1990;**15**:85–91.
112. Della Corte E, Parkhouse RME. Biosynthesis of immunoglobulin A (IgA) and immunoglobulin M (IgM). Requirement for J-chain and a disulphide exchange enzyme for polymerization. *Biochem J* 1973;**136**:597–606.
113. Mestecky J, Winchester RJ, Hoffman T, Kunkel HG. Parallel synthesis of immunoglobulins and J chain in pokeweed mitogen-stimulated normal cells and in lymphoblastoid cell lines. *J Exp Med* 1977;**145**:760–765.
114. Roth RA, Koshland ME. Identification of a lymphocyte enzyme that catalyzes pentamer immunoglobulin M assembly. *J Biol Chem* 1981;**256**:4633–4639.
115. Delamette F, Marty MC, Panijel J. In vitro study of IgM polymerization. *Cell Immunol* 1975;**19**:262–275.
116. Meng YG, Criss AB, Georgiadis KE. J chain deficiency in human IgM monoclonal antibodies produced by Epstein-Barr virus-transformed B lymphocytes. *Eur J Immunol* 1990;**20**:2505–2508.
117. Bjerke K, Brandtzaeg P. Terminally differentiated human intestinal B cells. J chain expression of IgA and IgG subclass-producing immunocytes in the distal ileum compared with mesenteric and peripheral lymph nodes. *Clin Exp Immunol* 1990;**82**:411–415.
118. Nilssen DE, Brandtzaeg P, Frøland SS, Fausa O. Subclass composition and J-chain expression of the “compensatory” gastrointestinal IgG cell population in selective IgA deficiency. *Clin Exp Immunol* 1992;**87**:237–245.
119. Mosmann TR, Gravel Y, Williamson AR, Bauml R. Modification and fate of J chain in myeloma cells in the presence and absence of immunoglobulin secretion. *Eur J Immunol* 1978;**8**:94–101.
120. Ahmed R, Gray D. Immunological memory and protective immunity: understanding their relation. *Science* 1996;**272**:54–60.
121. Bjerke K, Brandtzaeg P. Immunoglobulin- and J-chain-producing cells associated with the lymphoid follicles of human appendix, colon and ileum, including the Peyer’s patches. *Clin Exp Immunol* 1986;**64**:432–441.
122. Bjerke K, Brandtzaeg P, Rognum TO. Distribution of immunoglobulin producing cells is different in normal human appendix and colon mucosa. *Gut* 1986;**27**:667–664.
123. Korsrud FR, Brandtzaeg P. Immunohistochemical evaluation of J-chain expression by intra- and extra follicular immunoglobulin-producing human tonsillar cells. *Scand J Immunol* 1981;**13**:271–280.
124. Brandtzaeg P. Immune functions and immunopathology of palatine and nasopharyngeal tonsils. In: Bernstein JM, Ogra PL, eds. *Immunology of the ear*. New York: Raven Press; 1987. p. 63–106.
125. Koshland ME. The coming of age of the immunoglobulin J chain. *Annu Rev Immunol* 1985;**3**:425–453.
126. Rinkenberger JL, Wallin JJ, Johnson KW, Koshland ME. An interleukin-2 signal relieves BSAP (Pax5)-mediated repression of the immunoglobulin J chain gene. *Immunity* 1996;**5**:377–386.
127. Shin MK, Koshland ME. Ets-related protein PU.1 regulates expression of the immunoglobulin J-chain gene through a novel Ets-binding element. *Genes Dev* 1993;**7**:2006–2015.
128. Rao S, Karray S, Gackstetter ER, Koshland ME. Myocyte enhancer factor-related B-MEF2 is developmentally expressed in B cells and regulates the immunoglobulin J chain promoter. *J Biol Chem* 1998;**273**:26123–26129.

129. Turner CA, Mack DH, Davis MM. Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. *Cell* 1994;**77**:297–306.
130. Takayasu H, Brooks KH. IL-2 and IL-5 both induce μ , and J chain mRNA in a clonal B cell line, but differ in their cell-cycle dependency for optimal signaling. *Cell Immunol* 1991;**136**:472–485.
131. Randall TD, Parkhouse RM, Corley RB. J chain synthesis and secretion of hexameric IgM is differentially regulated by lipopolysaccharide and interleukin 5. *Proc Natl Acad Sci USA* 1992;**89**:962–966.
132. Tigges MA, Casey LS, Koshland ME. Mechanism of interleukin-2 signaling: mediation of different outcomes by a single receptor and transduction pathway. *Science* 1989;**243**:781–786.
133. Kang CJ, Sheridan C, Koshland ME. A stage-specific enhancer of immunoglobulin J chain gene is induced by interleukin-2 in a presecretor B cell stage. *Immunity* 1998;**8**:285–295.
134. McCune JM, Fu SM, Kunkel HG. J chain biosynthesis in pre-B cells and other possible precursor B cells. *J Exp Med* 1981;**154**:138–145.
135. Hadju I, Moldoveanu Z, Cooper M, Mestecky J. Ultrastructural studies of human lymphoid cells. p and J chain expression as a function of B cell differentiation. *J Exp Med* 1983;**158**:1993–2006.
136. Max EE, Korsmeyer SJ. Human J chain gene. Structure and expression in B lymphoid cells. *J Exp Med* 1985;**161**:832–849.
137. Kubagawa H, Burrows PD, Grossi CE, Mestecky J, Cooper MD. Precursor B cells transformed by Epstein-Barr virus undergo sterile plasma-cell differentiation: J-chain expression without immunoglobulin. *Proc Natl Acad Sci USA* 1988;**85**:875–879.
138. Iwase T, et al. Early expression of human J chain and μ chain gene in the fetal liver. *Cell Struct Funct* 1993;**18**:297–302.
139. Bertrand FE, Billips LG, Gartland GL, Kubagawa H, Schroeder HW. The J chain gene is transcribed during B and T lymphopoiesis in humans. *J Immunol* 1996;**156**:4240–4244.
140. Savidge TC. The life and times of an intestinal M cell. *Trends Microbiol* 1996;**4**:301–306.
141. Brandtzaeg P. Immunological functions of adenoids. In: van Cauwenberge P, Wang D-Y, Ingels K, Bachert C, eds. The nose. The Hague: Kugler Publications; 1998. p. 233–246.
142. Brandtzaeg P. Regionalized immune function of tonsils and adenoids. *Immunol Today* 1999;**20**:383–384.
143. Brandtzaeg P, Nilssen DE, Rognum TO, Thrane PS. Ontogeny of the mucosal immune system and IgA deficiency. *Gastroenterol Clin North Am* 1991;**20**:397–439.
144. Kapasi ZF, et al. Follicular dendritic cell (FDC) precursors in primary lymphoid tissues. *J Immunol* 1998;**160**:1078–1084.
145. Tarlinton D. Germinal centers: form and function. *Curr Opin Immunol* 1998;**10**:245–251.
146. Chaplin DD, Fu Y. Cytokine regulation of secondary lymphoid organ development. *Curr Opin Immunol* 1998;**10**:289–297.
147. Fu YX, Huang G, Wang Y, Chaplin DD. B lymphocytes induce the formation of follicular dendritic cell clusters in a lymphotoxin α dependent fashion. *J Exp Med* 1998;**187**:1009–1018.
148. Liu Y-J, de Bouteiller O, Fugier-Vivier I. Mechanisms of selection and differentiation in germinal centers. *Curr Opin Immunol* 1997;**9**:256–262.
149. MacLennan ICM. Germinal centers. *Annu Rev Immunol* 1994;**12**:117–139.
150. Liu Y-J, Arpin C. Germinal center development. *Immunol Rev* 1997;**156**:111–126.
151. MacLennan IC, et al. The changing preference of T and B cells for partners as T-dependent antibody responses develop. *Immunol Rev* 1997;**156**:53–66.
152. Garside P, Ingulli E, Merica RR, Johnson JG, Noelle RJ, Jenkins MK. Visualization of specific B and T lymphocyte interactions in the lymph node. *Science* 1998;**281**:96–99.
153. Lindhout E, Koopman G, Pals ST, de Groot C. Triple check for antigen specificity of B cells during germinal centre reactions. *Immunol Today* 1997;**18**:573–577.
154. Ehrenstein MR, O'Keefe TL, Davies SL, Neuberger MS. Targeted gene disruption reveals a role for natural secretory IgM in the maturation of the primary immune response. *Proc Natl Acad Sci USA* 1998;**95**:10089–10093.
155. Förster R, Mattis AE, Kremmer E, Wolf E, Brem G, Lipp M. A putative chemokine receptor, BLR1, directs B cell migration to defined lymphoid organs and specific anatomic compartments of the spleen. *Cell* 1996;**87**:1037–1047.
156. Goodnow CC, Cyster JG. Lymphocyte homing: the scent of a follicle. *Curr Biol* 1997;**7**:R219–R222.
157. Gunn MD, Ngo VN, Ansel KM, Ekland EH, Cyster JG, Williams LT. A B-cell-homing chemokine made in lymphoid follicles activates Burkitt's lymphoma receptor-1. *Nature* 1998;**391**:799–803.
158. Legler DF, Loetscher M, Roos RS, Clark-Lewis I, Baggiolini M, Moser B. B cell-attracting chemokine 1, a human CXC chemokine expressed in lymphoid tissues, selectively attracts B lymphocytes via BLR1/CXCR5. *J Exp Med* 1998;**187**:655–660.
159. Bleul CC, Schultze JL, Springer TA. B lymphocyte chemotaxis regulated in association with microanatomic localization, differentiation state, and B cell receptor engagement. *J Exp Med* 1998;**187**:753–762.
160. Szabo MC, Butcher EC, McEvoy LM. Specialization of mucosal follicular dendritic cells revealed by mucosal addressin-cell adhesion molecule-1 display. *J Immunol* 1997;**158**:5584–5588.
161. Brandtzaeg P, Halstensen TS. Immunology and immunopathology of tonsils. *Adv Otorhinolaryngol* 1992;**47**:64–75.
162. Lampert IA, Schofield JB, Amlot P, Van Noorden S. Protection of germinal centres from complement attack: decay-accelerating factor (DAF) is a constitutive protein on follicular dendritic cells. A study in reactive and neoplastic follicles. *J Pathol* 1993;**170**:115–120.
163. Pulendran B, van Driel R, Nossal GJ. Immunological tolerance in germinal centres. *Immunol Today* 1997;**18**:27–32.
164. Cleary AM, Fortune SM, Yellin MJ, Chess L, Lederman S. Opposing roles of CD95 (Fas/APO-1) and CD40 in the death and rescue of human low density tonsillar B cells. *J Immunol* 1995;**155**:3329–3337.
165. Koopman G, Keehnen RM, Lindhout E, Zhou DF, de Groot C, Pals ST. Germinal center B cells rescued from apoptosis by CD40 ligation or attachment to follicular dendritic cells, but not by engagement of surface immunoglobulin or adhesion receptors, become resistant to CD95-induced apoptosis. *Eur J Immunol* 1997;**27**:1–7.
166. Grouard G, Durand I, Filgueira L, Banchereau J, Liu YJ. Dendritic cells capable of stimulating T cells in germinal centres. *Nature* 1996;**384**:364–367.
167. Randall TD, Heath AW, Santos-Argumedo L, Howard MC, Weissman IL, Lund FE. Arrest of B lymphocyte terminal differentiation by CD40 signaling: mechanism for lack of antibody-secreting cells in germinal centers. *Immunity* 1998;**8**:733–742.
168. Arpin C, Banchereau J, Liu YJ. Memory B cells are biased towards terminal differentiation: a strategy that may prevent repertoire freezing. *J Exp Med* 1997;**186**:931–940.

169. Ngo VN, Tang HL, Cyster JG. Epstein-Barr virus-induced molecule 1 ligand chemokine is expressed by dendritic cells in lymphoid tissues and strongly attracts naive T cells and activated B cells.
J Exp Med 1998;**188**:181–191.
170. Liu YJ, Barthélemy C, de Bouteiller O, Arpin C, Durand I, Banchereau J. Memory B cells from human tonsils colonize mucosal epithelium and directly present antigen to T cells by rapid up-regulation of B7-1 and B7-2.
Immunity 1995;**2**:239–248.
171. Slifka MK, Ahmed R. Long-lived plasma cells: a mechanism for maintaining persistent antibody production.
Curr Opin Immunol 1998;**10**:252–258.
172. Strober W, Sneller MC. Cellular and molecular events accompanying IgA B cell differentiation.
Monogr Allergy 1988;**24**:181–190.
173. Conley ME, Bartelt MS. In vitro regulation of IgA subclass synthesis. II. The source of IgA2 plasma cells.
J Immunol 1984;**133**:2312–2316.
174. Zan H, Cerutti A, Dramitinos P, Schaffer A, Casali P. CD40 engagement triggers switching to IgA1 and IgA2 in human B cells through induction of endogenous TGF- β : evidence for TGF- β but not IL-10-dependent direct $S\mu \rightarrow S\alpha$ and sequential $S\mu \rightarrow S\gamma$, $S\gamma \rightarrow S\alpha$ DNA recombination.
J Immunol 1998;**161**:5217–5225.
175. Arpin C, et al. The normal counterpart of IgD myeloma cells in germinal center displays extensively mutated IgVH gene, C μ C δ switch, and λ light chain expression.
J Exp Med 1998;**187**:1169–1178.
176. Brandtzaeg P, Johansen F-E, Baekkevold ES, Farstad IN. Compartmentalization of the human mucosal immune system. A tonsillar B-cell subset (sIgD⁺IgM⁺CD38⁺) homes to regional effector sites but not to the gut [Abstract].
Scand J Immunol 1998;**47**:620.
177. Ruan MR, Akkoyunlu M, Grubb A, Forsgren A. Protein D of *Haemophilus influenzae*. A novel bacterial surface protein with affinity for human IgD.
J Immunol 1990;**145**:3379–3384.
178. Janson H, Heden LO, Grubb A, Ruan MR, Forsgren A. Protein D, an immunoglobulin D-binding protein of *Haemophilus influenzae*: cloning, nucleotide sequence, and expression in *Escherichia coli*.
Infect Immun 1991;**59**:119–125.
179. Parkhouse RME, Cooper MD. A model for the differentiation of B lymphocytes with implications for the biological role of IgD.
Immunol Rev 1977;**37**:105–126.
180. Schrader CE, George A, Kerlin RL, Cebra JJ. Dendritic cells support production of IgA and other non-IgM isotypes in clonal microculture.
Int Immunol 1990;**2**:563–570.
181. Fayette J, et al. Human dendritic cells skew isotype switching of CD40-activated naive B cells towards IgA1 and IgA2.
J Exp Med 1997;**185**:1909–1918.
182. Brandtzaeg P. Molecular and cellular aspects of the secretory immunoglobulin system.
APMIS 1995;**103**:1–19.
183. Nilsen EM, Lundin KEA, Krajci P, Scott H, Sollid LM, Brandtzaeg P. Gluten-specific, HLA-DQ restricted T cells from coeliac mucosa produce cytokines with Th1 or Th0 profile dominated by interferon γ .
Gut 1995;**37**:766–776.
184. Fujihashi K, et al. Human appendix B cells naturally express receptors for and respond to interleukin 6 with selective IgA1 and IgA2 synthesis.
J Clin Invest 1991;**88**:248–252.
185. Brière F, et al. Interleukin 10 induces B lymphocytes from IgA-deficient patients to secrete IgA.
J Clin Invest 1994;**94**:97–104.
186. Defrance T, Vanbervliet B, Brière F, Durand I, Rousset F, Banchereau J. Interleukin 10 and transforming growth factor β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A.
J Exp Med 1992;**175**:671–682.
187. Kimata H, Fujimoto M. Induction of IgA1 and IgA2 production in immature human fetal B cells and pre-B cells by vasoactive intestinal peptide.
Blood 1995;**85**:2098–2104.
188. Boirivant M, Fais S, Annibale B, Agostini D, Delle Fave G, Pallone F. Vasoactive intestinal polypeptide modulates the in vitro immunoglobulin A production by intestinal lamina propria lymphocytes.
Gastroenterology 1994;**106**:576–582.
189. Pascual DW, Xu-Amano JC, Kiyono H, McGhee JR, Bost KL. Substance P acts directly upon cloned B lymphoma cells to enhance IgA and IgM production.
J Immunol 1991;**146**:2130–2136.
190. Fujihashi, et al. γ/δ T cell-deficient mice have impaired mucosal immunoglobulin A responses.
J Exp Med 1996;**183**:1929–1935.
191. Jahnsen FL, Farstad IN, Aanesen JP, Brandtzaeg P. Phenotypic distribution of T cells in human nasal mucosa differs from that in the gut.
Am J Respir Cell Molec Biol 1998;**18**:392–401.
192. Kett K, Scott H, Fausa O, Brandtzaeg P. Secretory immunity in celiac disease: cellular expression of immunoglobulin A subclass and joining chain.
Gastroenterology 1990;**99**:386–392.
193. Watanabe M, et al. Interleukin 7 is produced by human intestinal epithelial cells and regulates the proliferation of intestinal mucosal lymphocytes.
J Clin Invest 1995;**95**:2945–2953.
194. Butcher EC, Picker LJ. Lymphocyte homing and homeostasis.
Science 1996;**272**:60–66.
195. Briskin M, et al. Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue.
Am J Pathol 1997;**151**:97–110.
196. Streeter PR, Berg EL, Rouse BT, Bargatzke RF, Butcher EC. A tissue-specific endothelial cell molecule involved in lymphocyte homing.
Nature 1988;**331**:41–46.
197. Shyjan AM, Bertagnolli M, Kenney CJ, Briskin MJ. Human mucosal addressin cell adhesion molecule-1 (MAdCAM-1) demonstrates structural and functional similarities to the $\alpha 4\beta 7$ -integrin binding domains of murine MAdCAM-1, but extreme divergence of mucin-like sequences.
J Immunol 1996;**156**:2851–2857.
198. Denis V, et al. Selective induction of peripheral and mucosal endothelial cell addressins with peripheral lymph nodes and Peyer's patch cell-conditioned media.
J Leukoc Biol 1996;**60**:744–752.
199. Berg EL, McEvoy LM, Berlin C, Bargatzke RF, Butcher EC. L-selectin-mediated lymphocyte rolling on MAdCAM-1.
Nature 1993;**366**:695–698.
200. Berlin C, et al. $\alpha 4$ integrins mediate lymphocyte attachment and rolling under physiologic flow.
Cell 1995;**80**:413–422.
201. Pachynski RK, Wu SW, Gunn MD, Erle DJ. Secondary lymphoid-tissue chemokine (SLC) stimulates integrin $\alpha 4\beta 7$ -mediated adhesion of lymphocytes to mucosal addressin cell adhesion molecule-1 (MAdCAM-1) under flow.
J Immunol 1998;**161**:952–956.
202. Farstad IN, Halstensen TS, Lien B, Kilshaw PJ, Lazarovits AI, Brandtzaeg P. Distribution of $\beta 7$ integrins in human intestinal mucosa and organized gut-associated lymphoid tissue.
Immunology 1996;**89**:227–237.
203. Farstad IN, Halstensen TS, Kvale D, Fausa O, Brandtzaeg P. Topographic distribution of homing receptors on B and T cells in human gut-associated lymphoid tissue. Relation of L-selectin and integrin $\alpha 4\beta 7$ to naive and memory phenotypes.
Am J Pathol 1997;**150**:187–199.
204. Baggiolini M. Chemokines and leukocyte traffic.
Nature 1998;**392**:565–568.

205. Del Pozo MA, Sánchez-Mateos P, Sánchez-Madrid F. Cellular polarization induced by chemokines: a mechanism for leucocyte recruitment? *Immunol Today* 1996;**17**:127–131.
206. Nakano H, et al. Genetic defect in T lymphocyte-specific homing into peripheral lymph nodes. *Eur J Immunol* 1997;**27**:215–221.
207. Gunn MD, et al. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J Exp Med* 1999;**189**:451–460.
208. Campbell JJ, Hedrick J, Zlotnik A, Siani MA, Thompson DA, Butcher EC. Chemokines and the arrest of lymphocytes rolling under flow conditions. *Science* 1998;**279**:381–384.
209. Middleton J, et al. Transcytosis and surface presentation of IL-8 by venular endothelial cells. *Cell* 1997;**91**:385–395.
210. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD, Williams LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc Natl Acad Sci USA* 1998;**95**:258–263.
211. Campbell JJ, et al. 6-C-kine (SLC), a lymphocyte adhesion-triggering chemokine expressed by high endothelium, is an agonist for the MIP-3 β receptor CCR7. *J Cell Biol* 1998;**141**:1053–1059.
212. Willmann K, et al. The chemokine SLC is expressed in T cell areas of lymph nodes and mucosal lymphoid tissues and attracts activated T cells via CCR7. *Eur J Immunol* 1998;**28**:2025–2034.
213. Postigo AA, Sanchez-Mateos P, Lazarovits AI, Sanchez-Madrid F, de Landazuri MO. $\alpha 4\beta 7$ integrin mediates B cell binding to fibronectin and vascular cell adhesion molecule-1: expression and function of $\alpha 4$ integrins on human B lymphocytes. *J Immunol* 1993;**151**:2471–2483.
214. Ohtsuka A, Piazza AJ, Ernak TH, Owen RL. Correlation of extracellular matrix components with the cytoarchitecture of mouse Peyer's patches. *Cell Tissue Res* 1992;**269**:403–410.
215. Gretz JE, Anderson AO, Shaw S. Cords, channels, corridors and conduits: critical architectural elements facilitating cell interactions in the lymph node cortex. *Immunol Rev* 1997;**156**:11–24.
216. Bianchi E, Bender JR, Blasi F, Pardi R. Through and beyond the wall: late steps in leukocyte transendothelial migration. *Immunol Today* 1997;**18**:586–591.
217. Farstad IN, Norstein J, Brandtzaeg P. Phenotypes of B and T cells in human intestinal and mesenteric lymph. *Gastroenterology* 1997;**112**:163–173.
218. Fujisaka M, Ohtani O, Watanabe Y. Distribution of lymphatics in human palatine tonsils: a study by enzyme-histochemistry and scanning electron microscopy of lymphatic corrosion casts. *Arch Histol Cytol* 1996;**59**:273–280.
219. Hub E, Rot A. Binding of RANTES, MCP-1, MCP-3, and MIP-1 α to cells in human skin. *Am J Pathol* 1998;**152**:749–757.
220. Bradley LM, Watson SR. Lymphocyte migration into tissue: the paradigm derived from CD4 subsets. *Curr Opin Immunol* 1996;**8**:312–320.
221. Rott LS, Briskin MJ, Andrew DP, Berg EL, Butcher EC. A fundamental subdivision of circulating lymphocytes defined by adhesion to mucosal addressin cell adhesion molecule-1. Comparison with vascular cell adhesion molecule-1 and correlation with $\beta 7$ integrins and memory differentiation. *J Immunol* 1996;**156**:3727–3736.
222. Guy-Grand D, Griscelli C, Vassalli P. The gut-associated lymphoid system: nature and properties of the large dividing cells. *Eur J Immunol* 1974;**4**:435–443.
223. Parrott DM. The gut as a lymphoid organ. *Clin Gastroenterol* 1976;**5**:211–228.
224. Bos NA, et al. Monoclonal immunoglobulin A derived from peritoneal B cells is encoded by both germ line and somatically mutated VH genes and is reactive with commensal bacteria. *Infect Immun* 1996;**64**:616–623.
225. Dunn-Walters DK, Boursier L, Spencer J. Hypermutation, diversity and dissemination of human intestinal lamina propria plasma cells. *Eur J Immunol* 1997;**27**:2959–2964.
226. Fischer M, Kuppers R. Human IgA- and IgM-secreting intestinal plasma cells carry heavily mutated V_H region genes. *Eur J Immunol* 1998;**28**:2971–2977.
227. Dunn-Walters DK, Isaacson PG, Spencer J. Sequence analysis of human IgVH genes indicates that ileal lamina propria plasma cells are derived from Peyer's patches. *Eur J Immunol* 1997;**27**:463–467.
228. Lue C, et al. Intraperitoneal immunization of human subjects with tetanus toxoid induces specific antibody-secreting cells in the peritoneal cavity and in the circulation, but fails to elicit a secretory IgA response. *Clin Exp Immunol* 1994;**96**:356–363.
229. Quiding-Järbrink M, et al. Human circulating specific antibody-forming cells after systemic and mucosal immunizations: differential homing commitments and cell surface differentiation markers. *Eur J Immunol* 1995;**25**:322–327.
230. Quiding-Järbrink M, et al. Differential expression of tissue-specific adhesion molecules on human circulating antibody-forming cells after systemic, enteric, and nasal immunizations. A molecular basis for the compartmentalization of effector B cell responses. *J Clin Invest* 1997;**99**:1281–1286.
231. Kantele A, et al. Homing potentials of circulating lymphocytes in humans depend on the site of activation: oral, but not parenteral, typhoid vaccination induces circulating antibody-secreting cells that all bear homing receptors directing them to the gut. *J Immunol* 1997;**158**:574–579.
232. Halstead TE, Hall JG. The homing of lymph-borne immunoblasts to the small gut of neonatal rats. *Transplantation* 1972;**14**:339–346.
233. Parrott DM, Ferguson A. Selective migration of lymphocytes within the mouse small intestine. *Immunology* 1974;**26**:571–588.
234. Bode U, Wonigeit K, Pabst R, Westermann J. The fate of activated T cells migrating through the body: rescue from apoptosis in the tissue of origin. *Eur J Immunol* 1997;**27**:2087–2093.
235. Janeway CA, et al. Quantitative variation in Ia antigen expression plays a central role in immune regulation. *Immunol Today* 1984;**5**:99–105.
236. Thrane PS, Sollid LM, Haanes HR, Brandtzaeg P. Clustering of IgA-producing immunocytes related to HLA-DR-positive ducts in normal and inflamed salivary glands. *Scand J Immunol* 1992;**35**:43–51.
237. Newman RA, Ormerod MG, Greaves MF. The presence of HLA-DR antigens on lactating human breast epithelium and milk fat globule membranes. *Clin Exp Immunol* 1980;**41**:478–486.
238. Brandtzaeg P. The secretory immune system of lactating human mammary glands compared with other exocrine organs. *Ann N Y Acad Sci* 1983;**409**:353–381.
239. Eerola E, Jalkanen S, Granfors K, Toivanen A. Immune capacity of the chicken bursectomized at 60 h of incubation. Effect of bursal epithelial cells and bursal epithelium-conditioned medium on the production of immunoglobulins and specific antibodies in vitro. *Scand J Immunol* 1984;**19**:493–500.
240. Kremmidiotis G, Zola H. Changes in CD44 expression during B cell differentiation in the human tonsil. *Cell Immunol* 1995;**161**:147–157.
241. Merville P, et al. Bcl-2⁺ tonsillar plasma cells are rescued from apoptosis by bone marrow fibroblasts. *J Exp Med* 1996;**183**:227–236.

242. Husband AJ, Monié HJ, Gowans JL. The natural history of cells producing IgA in the gut. *Ciba Found Symp* 1977;**46**:29–42.
243. Lange S, Nygren H, Svennerholm A-M, Holmgren J. Antitoxic cholera immunity in mice: influence of antigen deposition on antitoxin-containing cells and protective immunity in different parts of the intestine. *Infect Immun* 1980;**28**:17–23.
244. Pierce NF, Gowans JL. Cellular kinetics of the intestinal immune response to cholera toxoid in rats. *J Exp Med* 1975;**142**:1550–1563.
245. Ogra PL, Karzon DT. The role of immunoglobulins in the mechanism of mucosal immunity to virus infection. *Pediatr Clin North Am* 1970;**17**:385–400.
246. Hopkins S, et al. A recombinant *Salmonella typhimurium* vaccine induces local immunity by four different routes of immunization. *Infect Immun* 1995;**63**:3279–3286.
247. Kantele A, et al. Differences in immune responses induced by oral and rectal immunizations with *Salmonella typhi* Ty21a: evidence for compartmentalization within the common mucosal immune system in humans. *Infect Immun* 1998;**66**:5630–5635.
248. Eriksson K. Specific-antibody-secreting cells in the rectums and genital tracts of nonhuman primates following vaccination. *Infect Immun* 1998;**66**:5889–5896.
249. Mizoguchi A, Mizoguchi E, Chiba C, Bhan AK. Role of appendix in the development of inflammatory bowel disease in TCR- α mutant mice. *J Exp Med* 1996;**184**:707–715.
250. Dunkley ML, Husband AJ. The role of non-B cells in localizing an IgA plasma cell response in the intestine. *Reg Immunol* 1990-91;**3**:336–340.
251. McDermott MR, Bienenstock J. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. *J Immunol* 1979;**122**:1892–1898.
252. Sminia T, van der Brugge-Gamelkoorn GJ, Jeurissen SH. Structure and function of bronchus-associated lymphoid tissue (BALT). *Crit Rev Immunol* 1989;**9**:119–150.
253. VanCott JL, Brim TA, Lunney JK, Saif LJ. Contribution of antibody-secreting cells induced in mucosal lymphoid tissues of pigs inoculated with respiratory or enteric strains of coronavirus to immunity against enteric coronavirus challenge. *J Immunol* 1994;**152**:3980–3990.
254. Nadal D, Albini B, Chen CY, Schl  pfer E, Bernstein JM, Ogra PL. Distribution and engraftment patterns of human tonsillar mononuclear cells and immunoglobulin-secreting cells in mice with severe combined immunodeficiency: role of the Epstein-Barr virus. *Int Arch Allergy Appl Immunol* 1991;**95**:341–351.
255. Perry LL, Feilzer K, Portis JL, Caldwell HD. Distinct homing pathways direct T lymphocytes to the genital and intestinal mucosae in *Chlamydia*-infected mice. *J Immunol* 1998;**160**:2905–2914.
256. Picker LJ, et al. Differential expression of lymphocyte homing receptors by human memory/effector T cells in pulmonary versus cutaneous immune effector sites. *Eur J Immunol* 1994;**24**:1269–1277.
257. Wagner N, et al. Critical role for $\beta 7$ integrins in formation of the gut-associated lymphoid tissue. *Nature* 1996;**382**:366–370.
258. Abitorabi MA, Mackay CR, Jerome EH, Osorio O, Butcher EC, Erle DJ. Differential expression of homing molecules on recirculating lymphocytes from sheep gut, peripheral, and lung lymph. *J Immunol* 1996;**156**:3111–3117.
259. Quiding-J  rbrink M, Granstr  m G, Nordstr  m I, Holmgren J, Czerkinsky C. Induction of compartmentalized B-cell responses in human tonsils. *Infect Immun* 1995;**63**:853–857.
260. Balmelli C, Roden R, Potts A, Schiller J, De Grandi P, Nardelli-Haeffliger D. Nasal immunization of mice with human papillomavirus type 16 virus-like particles elicits neutralizing antibodies in mucosal secretions. *J Virol* 1998;**72**:8220–8229.
261. Hordnes K, Tynning T, Kvam AI, Jonsson R, Haneberg B. Colonization in the rectum and uterine cervix with group B streptococci may induce specific antibody responses in cervical secretions of pregnant women. *Infect Immun* 1996;**64**:1643–1652.
262. Crowley-Nowick PA, et al. Rectal immunization for induction of specific antibody in the genital tract of women. *J Clin Immunol* 1997;**17**:370–379.
263. Kozlowski PA, Cu-Uvin S, Neutra MR, Flanagan TP. Comparison of the oral, rectal, and vaginal immunization routes for induction of antibodies in rectal and genital tract secretions of women. *Infect Immun* 1997;**65**:1387–1394.
264. Bentley AM, et al. Expression of endothelial and leukocyte adhesion molecules intercellular adhesion molecule-1, E-selectin, and vascular cell adhesion molecule-1 in the bronchial mucosa in steady-state and allergen-induced asthma. *J Allergy Clin Immunol* 1993;**92**:857–868.
265. Jahnsen FL, Haraldsen G, Aanesen JP, Haye R, Brandtzaeg P. Eosinophil infiltration is related to increased expression of vascular cell adhesion molecule-1 in nasal polyps. *Am J Respir Cell Molec Biol* 1995;**12**:624–632.
266. Norderhaug IN, Johansen F-E, Schjerv  n H, Brandtzaeg P. Regulation of the formation and epithelial transport of secretory immunoglobulins. *Crit Rev Immunol* (In press).
267. Brandtzaeg P. Polymeric IgA is complexed with secretory component (SC) on the surface of human intestinal epithelial cells. *Scand J Immunol* 1978;**8**:39–52.
268. Mostov KE, Blobel G. A transmembrane precursor of secretory component. The receptor for transcellular transport of polymeric immunoglobulins. *J Biol Chem* 1982;**157**:11816–11821.
269. Brandtzaeg P. Immunohistochemical studies on various aspects of glandular immunoglobulin transport in man. *Histochem J* 1977;**9**:553–572.
270. Brandtzaeg P, Baklien K. Intestinal secretion of IgA and IgM: a hypothetical model. *Ciba Found Symp* 1977;**46**:77–108.
271. Valnes K, Brandtzaeg P, Elgjo K, Stave R. Specific and nonspecific humoral defense factors in the epithelium of normal and inflamed gastric mucosa. Immunohistochemical localization of immunoglobulins, secretory component, lysozyme, and lactoferrin. *Gastroenterology* 1984;**86**:402–412.
272. R  e M, Norderhaug IN, Brandtzaeg P, Johansen F-E. Fine-specificity of ligand-binding domain 1 in the polymeric immunoglobulin receptor: importance of the CDR2-containing region for IgM interaction. *J Immunol* 1999;**162**:6046–6052.
273. Norderhaug IN, Johansen F-E, Kraj  i P, Brandtzaeg P. Domain deletions in the human polymeric Ig receptor disclose differences between its dimeric IgA and pentameric IgM interaction. *Eur J Immunol* (In press).
274. Conley ME, Delacroix DL. Intravascular and mucosal immunoglobulin A: two separate but related systems of immune defense? *Ann Intern Med* 1987;**106**:892–899.
275. Lindh E. Increased resistance of immunoglobulin A dimers to proteolytic degradation after binding of secretory component. *J Immunol* 1975;**114**:284–286.
276. Crottet P, Corthesy B. Secretory component delays the conversion of secretory IgA into antigen-binding competent F(ab')₂: a possible implication for mucosal defense. *J Immunol* 1998;**161**:5445–5453.

277. Berdoz J, Blanc CT, Reinhardt M, Kraehenbuhl JP, Cortes B. In vitro comparison of the antigen-binding and stability properties of the various molecular forms of IgA antibodies assembled and produced in CHO cells. *Proc Natl Acad Sci USA* 1999;**96**:3029–3034.
278. Brandtzaeg P. Structure, synthesis and external transfer of mucosal immunoglobulins. *Ann Inst Pasteur Immunol* 1973;**124C**: 417–438.
279. Prigent-Delecourt L, Coffin B, Colombel JF, Dehennin JP, Vaerman JP, Rambaud JC. Secretion of immunoglobulins and plasma proteins from the colonic mucosa: an in vivo study in man. *Clin Exp Immunol* 1995;**99**:221–225.
280. Gitlin D, Sasaki T. Immunoglobulins G, A, and M determined in single cells from human tonsil. *Science* 1969;**164**:1532–1534.
281. Alberini CM, Bet P, Milstein C, Sitia R. Secretion of immunoglobulin M assembly intermediates in the presence of reducing agents. *Nature* 1990;**347**:485–487.
282. Natvig IB, Johansen F-E, Nordeng TW, Haraldsen G, Brandtzaeg P. Mechanism for enhanced external transfer of dimeric IgA over pentameric IgM. Studies of diffusion, binding to the human polymeric Ig receptor, and epithelial transcytosis. *J Immunol* 1997;**159**:4330–4340.
283. Song W, Bomsel M, Casanova J, Vaerman JP, Mostov K. Stimulation of transcytosis of the polymeric immunoglobulin receptor by dimeric IgA. *Proc Natl Acad Sci USA* 1994;**91**:163–166.
284. Song W, Vaerman JP, Mostov KE. Dimeric and tetrameric IgA are transcytosed equally by the polymeric Ig receptor. *J Immunol* 1995;**155**:715–721.
285. Giffroy D, et al. In vivo stimulation of polymeric Ig receptor transcytosis by circulating polymeric IgA in rat liver. *Int Immunol* 1998;**10**:347–354.
286. Nagura H, Nakane PK, Brown WR. Secretory component in immunoglobulin deficiency and immunoelectron microscopic study of intestinal epithelium. *Scand J Immunol* 1980;**12**:359–363.
287. Portis JL, Coe JE. IgM the secretory immunoglobulin of reptiles and amphibians. *Nature* 1975;**258**:547–548.
288. St. Louis-Cormier EA, Osterland CK, Anderson PD. Evidence for a cutaneous secretory immune system in rainbow trout (*Salmo gairdneri*). *Dev Comp Immunol* 1984;**8**:71–80.
289. Mansikka A. Chicken IgA H chains. Implications concerning the evolution of H chain genes. *J Immunol* 1992;**149**:855–861.
290. Renegar KB, Jackson GD, Mestecky J. In vitro comparison of the biologic activities of monoclonal monomeric IgA, polymeric IgA, and secretory IgA. *J Immunol* 1998;**160**:1219–1223.
291. Ma JK, et al. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. *Nat Med* 1998;**4**:601–606.
292. Russell MW, Reinholdt J, Kilian M. Anti-inflammatory activity of human IgA antibodies and their Fab α fragments: inhibition of IgG-mediated complement activation. *Eur J Immunol* 1989;**19**:2243–2249.
293. Phillips JO, Everson MP, Moldoveanu Z, Lue C, Mestecky J. Synergistic effect of IL-4 and IFN- γ on the expression of polymeric Ig receptor (secretory component) and IgA binding by human epithelial cells. *J Immunol* 1990;**145**:1740–1744.
294. Denning GM. IL-4 and IFN- γ synergistically increase total polymeric IgA receptor levels in human intestinal epithelial cells. Role of protein tyrosine kinases. *J Immunol* 1996;**156**:4807–4814.
295. Kvale D, Brandtzaeg P. Constitutive and cytokine induced expression of HLA molecules, secretory component, and intercellular adhesion molecule-1 is modulated by butyrate in the colonic epithelial cell line HT-29. *Gut* 1995;**36**:737–742.
296. Hayashi M, et al. The polymeric immunoglobulin receptor (secretory component) in a human intestinal epithelial cell line is up-regulated by interleukin-1. *Immunology* 1997;**92**:220–225.
297. Nilsen EM, Johansen F-E, Kvale D, Krajci P, Brandtzaeg P. Different regulatory pathways employed in cytokine-enhanced expression of secretory component and epithelial HLA class I genes. *Eur J Immunol* 1999;**29**:168–179.
298. Krajci P, Kvale D, Taskén K, Brandtzaeg P. Molecular cloning and exon-intron mapping of the gene encoding human transmembrane secretory component (the poly-Ig receptor). *Eur J Immunol* 1992;**22**:2309–2315.
299. Verrijdt G, Swinnen J, Peeters B, Verhoeven G, Rombauts W, Claessens F. Characterization of the human secretory component gene promoter. *Biochim Biophys Acta* 1997;**1350**:147–154.
300. Johansen F-E, Boslöven B, Krajci P, Brandtzaeg P. A composite DNA element in the promoter of the polymeric immunoglobulin receptor promoter regulates its constitutive expression. *Eur J Immunol* 1998;**28**:1161–1171.
301. Piskurich JF, et al. Transcriptional regulation of the human polymeric immunoglobulin receptor gene by interferon- γ . *Mol Immunol* 1997;**34**:75–91.
302. Kaetzel CS, Blanch VJ, Hempen PM, Phillips KM, Piskurich JF, Youngman KR. The polymeric immunoglobulin receptor: structure and synthesis. *Biochem Soc Trans* 1997;**25**:475–480.
303. Martin MG. Characterization of the 5'-flanking region of the murine polymeric IgA receptor gene. *Am J Physiol* 1998;**275**: G778–G788.
304. Blanch VJ, Piskurich JF, Kaetzel CS. Cutting edge: coordinate regulation of IFN regulatory factor-1 and the polymeric Ig receptor by proinflammatory cytokines. *J Immunol* 1999;**162**:1232–1235.
305. Schjerven H, Nilsen EM, Brandtzaeg P, Johansen F-E. IL-4 and TNF- α enhance the expression of human plg-receptor through DNA elements in the first intron. 29th Ann. Meeting of SSI, Abstract 36. *Scand J Immunol* 1998;**47**:623.
306. Brandtzaeg P, Halstensen TS, Huitfeldt HS, Krajci K, Kvale D, Scott H, Thrane PS. Epithelial expression of HLA, secretory component (poly-Ig receptor), and adhesion molecules in the human alimentary tract. *Ann N Y Acad Sci* 1992;**664**:157–179.
307. Brandtzaeg P, Baklien K. Characterization of the IgA-immunocyte population and its product in a patient with excessive intestinal formation of IgA. *Clin Exp Immunol* 1977;**30**:77–88.
308. Hocini H, et al. High-level ability of secretory IgA to block HIV type 1 transcytosis: contrasting secretory IgA and IgG responses to glycoprotein 160. *AIDS Res Hum Retroviruses* 1997;**13**:1179–1185.
309. Mazzoli S, et al. HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals. *Nat Med* 1997;**3**:1250–1257.
310. Porter P, Linggood MA. Novel mucosal anti-microbial functions interfering with the plasmid-mediated virulence determinants of adherence and drug resistance. *Am N Y Acad Sci* 1983;**409**:564–578.
311. Brandtzaeg P. Development of the mucosal immune system in humans. In: Bindels JG, Goedhart AC, Visser HKA, eds. Recent developments in infant nutrition. London: Kluwer Academic Publishers; 1996. p. 349–376.
312. Quan CP, Berneman A, Pires R, Avrameas S, Bouvet JP. Natural polyreactive secretory immunoglobulin A autoantibodies as a possible barrier to infection in humans. *Infect Immun* 1997;**65**:3997–4004.

313. Bouvet JP, Dighiero G. From natural polyreactive autoantibodies to *à la carte* monoreactive antibodies to infectious agents: is it a small world after all? *Infect Immun* 1998;**66**:1–4.
314. Bouvet J-P, Pirès R, Quan CP. Protein Fv (Fv fragment binding protein): a mucosal human superantigen reacting with normal immunoglobulins. In: Zouali M, ed. *Human B-cell superantigens*. Austin: RG Landes; 1996. p. 179–187.
315. Giugliano LG, Ribeiro STG, Vainstein MH, Ulhoa CJ. Free secretory component and lactoferrin of human milk inhibit the adhesion of enterotoxigenic *Escherichia coli*. *J Med Microbiol* 1995;**42**:3–9.
316. Dallas SD, Rolfe RD. Binding of *Clostridium difficile* toxin A to human milk secretory component. *J Med Microbiol* 1998;**47**:879–888.
317. Hammerschmidt S, Talay SR, Brandtzaeg P, Chhatwal GS. SpsA, a novel pneumococcal surface protein with specific binding to secretory immunoglobulin A and secretory component. *Mol Microbiol* 1997;**25**:1113–1124.
318. Brandtzaeg P, Tolo K. Mucosal penetrability enhanced by serum-derived antibodies. *Nature* 1977;**266**:262–263.
319. Paganelli R, Levinsky RJ. Solid phase radioimmunoassay for detection of circulating food protein antigens in human serum. *J Immunol Methods* 1980;**37**:333–341.
320. Kilshaw PJ, Cant AJ. The passage of maternal dietary proteins into human breast milk. *Int Arch Allergy Appl Immunol* 1984;**75**:8–15.
321. Husby S, Jensenius JC, Svehag S-E. Passage of undegraded dietary antigen into the blood of healthy adults. Quantification, estimation of size distribution, and relation of uptake to levels of specific antibodies. *Scand J Immunol* 1985;**22**:83–92.
322. Sanderson IR, Walker WA. Uptake and transport of macromolecules by the intestine: possible role in clinical disorders (an update). *Gastroenterology* 1993;**104**:622–639.
323. Morton HC, van Zandbergen G, van Kooten C, Howard CJ, van de Winkel JGJ, Brandtzaeg P. Immunoglobulin-binding sites of human FcRI (CD89) and bovine Fc2R are located in their membrane-distal extracellular domains. *J Exp Med* 1999;**189**:1715–1722.
324. Wolf HM, Fischer MB, Pühringer H, Samstag A, Vogel E, Eibl MM. Human serum IgA downregulates the release of inflammatory cytokines (tumor necrosis factor- α , interleukin-6) in human monocytes. *Blood* 1994;**83**:1278–1288.
325. Wolf HM, Vogel E, Fischer MB, Rengs H, Schwarz H-P, Eibl MM. Inhibition of receptor-dependent and receptor-independent generation of the respiratory burst in human neutrophils and monocytes by human serum IgA. *Pediatr Res* 1994;**36**:235–243.
326. Devière J, et al. IgA triggers tumor necrosis factor α secretion by monocytes: a study in normal subjects and patients with alcoholic cirrhosis. *Hepatology* 1991;**13**:670–675.
327. Abu-Ghazaleh RI, Fujisawa T, Mestecky J, Kyle RA, Gleich GJ. IgA-induced eosinophil degranulation. *J Immunol* 1989;**142**:2393–2400.
328. Taylor B, Norman AP, Orgel HA, Stokes CR, Turner MW, Soothill JF. Transient IgA deficiency and pathogenesis of infantile atopy. *Lancet* 1973;**2**:111–113.
329. Soothill JF. Some intrinsic and extrinsic factors predisposing to allergy. *Proc R Soc Med* 1976;**69**:439–442.
330. Sloper KS, Brook CG, Kingston D, Pearson JR, Shiner M. Eczema and atopy in early childhood: low IgA plasma cell counts in the jejunal mucosa. *Arch Dis Child* 1981;**56**:939–942.
331. Perkiö M. Immunohistochemical study of intestinal biopsies from children with atopic eczema due to food allergy. *Allergy* 1980;**35**:573–580.
332. Van Asperen PP, et al. The relationship between atopy and salivary IgA deficiency in infancy. *Clin Exp Immunol* 1985;**62**:753–757.
333. Kilian M, Husby S, Host A, Halken S. Increased proportions of bacteria capable of cleaving IgA1 in the pharynx of infants with atopic disease. *Pediatr Res* 1995;**38**:182–186.
334. Lamkhioued B, Gounni AS, Gruart V, Pierce A, Capron A, Capron M. Human eosinophils express a receptor for secretory component. Role in secretory IgA-dependent activation. *Eur J Immunol* 1995;**25**:117–125.
335. Moteji Y, Kita H. Interaction with secretory component stimulates effector functions of human eosinophils but not of neutrophils. *J Immunol* 1998;**161**:4340–4346.
336. Peebles RS Jr, Liu MC, Adkinson NF Jr, Lichtenstein LM, Hamilton RG. Ragweed-specific antibodies in bronchoalveolar lavage fluids and serum before and after segmental lung challenge: IgE and IgA associated with eosinophil degranulation. *J Allergy Clin Immunol* 1998;**101**:265–73.
337. Iikura M, et al. Secretory IgA induces degranulation of IL-3-primed basophils. *J Immunol* 1998;**161**:1510–1515.
338. Brandtzaeg P, Halstensen TS, Hvatum M, Kvale D, Scott H. The serologic and mucosal immunologic basis of celiac disease. In: Walker WA, Harmatz PR, Wershil BK, eds. *Immunophysiology of the gut*. Bristol-Myers Squibb/Mead Johnson Nutrition Symposia. Vol 11. London: Academic Press; 1993. p. 295–333.
339. Berin MC, Kilian AJ, Yang PC, Groot JA, Taminiou JA, Perdue MH. Rapid transepithelial antigen transport in rat jejunum: impact of sensitization and the hypersensitivity reaction. *Gastroenterology* 1997;**113**:856–864.
340. Lim PL, Rowley D. The effect of antibody on the intestinal absorption of macromolecules and on intestinal permeability in adult mice. *Int Arch Allergy Appl Immunol* 1982;**68**:41–46.
341. Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993;**366**:421–428.
342. Mamula MJ, Janeway CA. Do B cells drive the diversification of immune responses? *Immunol Today* 1993;**14**:151–152.
343. Eynon EE, Parker DC. Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. *J Exp Med* 1992;**175**:131–138.
344. Pistoia V. Production of cytokines by human B cells in health and disease. *Immunol Today* 1997;**18**:343–350.
345. Grdic D, Hörnquist E, Kjerrulf M, Lycke NY. Lack of local suppression in orally tolerant CD8-deficient mice reveals a critical regulatory role of CD8⁺ T cells in the normal gut mucosa. *J Immunol* 1998;**160**:754–762.
346. Kim P-H, Eckmann L, Lee WJ, Han W, Kagnoff MF. Cholera toxin and cholera toxin B subunit induce IgA switching through the action of TGF- β 1. *J Immunol* 1998;**160**:1198–1203.
347. Sölköft J, Söberg B. Immunoglobulin-containing cells in the small intestine during acute enteritis. *Gut* 1972;**13**:535–538.
348. Agus SG, Falchuk ZM, Sessoms CS, Wyatt RG, Dolin R. Increased jejunal IgA synthesis *in vitro* during acute infectious non-bacterial gastroenteritis. *Am J Dig Dis* 1974;**19**:127–131.
349. Silbart LK, Keren DF. Reduction of intestinal carcinogen absorption by carcinogen-specific secretory immunity. *Science* 1989;**243**:1462–1464.
350. Rasmussen MV, Silbart LK. Preoral administration of specific antibody enhances carcinogen excretion. *Immunotherapy* 1998;**21**:418–426.

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