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THE INNATE AND ADAPTIVE IMMUNE SYSTEM OF THE INTESTINAL EPITHELIUM

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SUMMARY

It is increasingly being appreciated that innate immune mechanisms play a key role for the tuning of adaptive immunity in the gut and maintenance of mucosal homeostasis. Innate responses use preformed and rapidly synthesised effectors and sensors that lead to spontaneous activation or modulation of cellular functions, aiming at elimination of microbial challenges and return of the local tissue to a basal state with minimal pathology. Efficient adaptive immunity depends on postnatal 'education' of specific immune cells and induction of immunological memory in processes that are significantly modulated by innate immune responses. The intestinal immune system has through evolution developed two layers of adaptive non-inflammatory defence: Immune exclusion provided by secretory antibodies to limit epithelial penetration and host invasion of microorganisms as well as other potentially dangerous antigens; and immunosuppressive mechanisms to inhibit overreaction against innocuous luminal antigens – often referred to collectively as 'oral tolerance'. Both these strategies depend on co-operation of adaptive immune mechanisms with the innate defence system, including crosstalk between the intestinal epithelium and various lamina propria cells. This review focuses on immunological mechanisms taking place at the epithelial level.

INTRODUCTION

The body is under constant threat of attack by viruses, bacteria and parasites. Evolution has therefore provided mammalians with several complex and potent layers of defence. Microorganisms have inhabited Earth for at least 2.5 billion years, and the power of immunity is a result of co-evolution in which particularly the commensal bacteria have shaped the body's defence functions (*Hooper* and *Gordon*, 2001; *Bäckhed* et al., 2005). In humans, the critical role of the immune system becomes clinically apparent when it is defective. Thus, inherited and acquired immunodeficiency states, or more subtle immunoregulatory defects, are characterised by increased susceptibility to infectious diseases that are sometimes caused by the commensal microbiota which is normally considered to be non-pathogenic (*Haller* and *Jobin*, 2004; *Sansonetti*, 2004; *Yan* and *Polk*, 2004).

The immune system can be divided into two general arms: Innate (natural or non-specific) and adaptive (acquired or

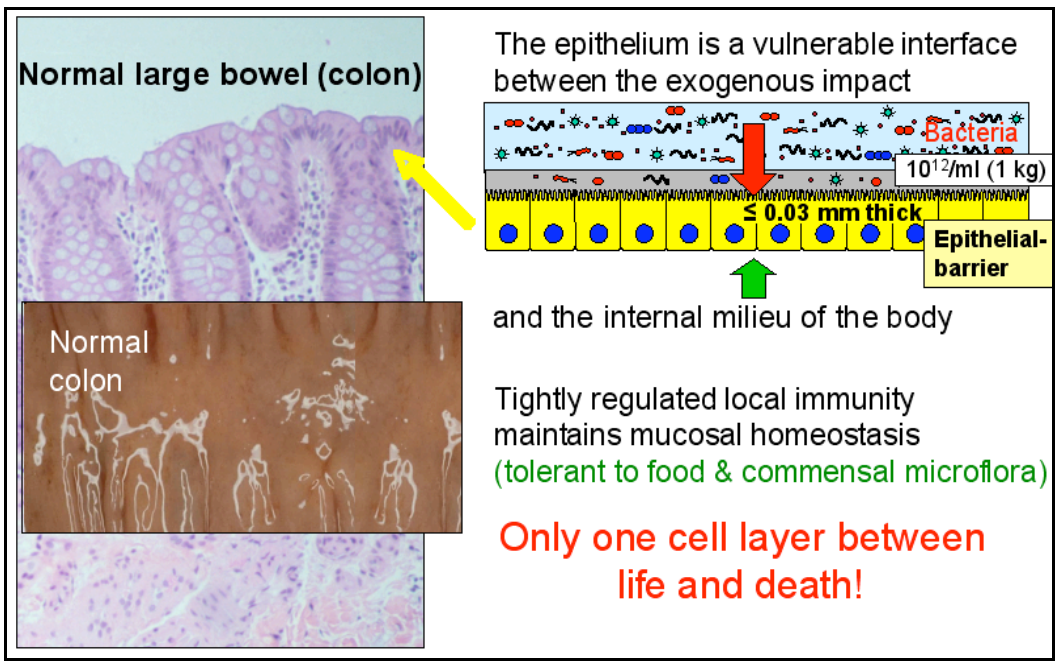

Figure 1: Mucosal homeostasis is maintained in the gut by tightly regulated immune mechanisms. Left panel: Histology and macroscopic picture of normal colon. Right panel: Schematic depiction of the monolayered epithelium covered by mucus and its enormous bacterial challenge, which can cause deadly disease if the epithelial barrier deteriorates.

specific) immunity, which work together synergistically (*Chaplin*, 2003; *Sompayrac*, 2003). Notably, the adaptive immune system developed rather late in the phylogeny, and most species survive without it. However, this is not true for mammalians, which have an extremely sophisticated adaptive immune system of both systemic and mucosal (local) type. There appears to be a great redundancy of mechanisms in both systems providing robustness to ensure that essential defence functions are preserved.

The success of such a complex overall strategy is evidenced by the fact that most humans have a normal gut. This is indeed remarkable because the enormous surface area (some 300 m^2 in an adult) is covered only by a monolayered, quite vulnerable epithelium exposed to an enormous load of commensal bacteria (Figure 1). Tightly regulated local immunity is clearly needed to maintain mucosal homeostasis (*MacDonald* and *Monteleone*, 2005).

INNATE IMMUNITY

The attempt of an infectious agent to enter the body will immediately be recognised and counteracted by the innate immune system (*Chaplin*, 2003), which comprises surface barriers, soluble factors, professional phagocytes, and dendritic cells (DCs). Together, these functions constitute a primary layer of natural defence against invading microorganisms, with the common goal of restricting their entry into the body by providing: (a) physical/structural hindrance and clearance mechanisms (epithelial linings of skin and mucosae,

mucus, ciliary function, peristalsis); (b) chemical factors (pH of body fluids, numerous antimicrobial proteins and peptides such as lysozyme, lactoferrin, peroxidase, and defensins); and (c) phagocytic cells (e.g. neutrophils, eosinophils, monocytes/macrophages, and immature DCs). Challenges of the innate system often lead to activation of adaptive immunity, which aids substantially the recovery from infection, as discussed below.

Triggering of innate immunity

The recognition molecules involved in innate immunity are encoded in the germline (*Chaplin*, 2003). Basically, this system is therefore quite similar among healthy individuals and shows no apparent memory effect; re-exposure to the same pathogen will normally elicit more or less the same type of response. The actual cellular receptors sense microbial molecular structures that are conserved and often essential for survival of the microorganisms. Such structures include for instance endotoxin or lipopolysaccharide (LPS), teichoic acid, peptidoglycan, and unmethylated CpG motifs of DNA (*Beutler* and *Rietschel*, 2003); together they are traditionally called pathogen-associated molecular patterns (PAMPs) but they also occur in commensal bacteria (*Medzhitov*, 2001) and are therefore preferably called microbe-associated molecular patterns (MAMPs).

It remains unclear whether the intestinal microbiota through its MAMPs induces a distinct molecular program in the innate immune system, which could explain that the indigenous microbiota is normally tolerated by the host (*Nagler-Anderson*, 2001; *Philpott* et al., 2001). The relative absence of microbial recognition structures from the apical surface of normal gut epithelium (see later), as well as potential attenuation of their signalling by certain commensal bacteria (e.g., NF-κB-mediated responses), also appear of crucial importance to this end (*Kobayashi* et al., 2002; *Haller* and *Jobin*, 2004; *Rakoff-Nahoum* et al., 2004; *Kelly* et al., 2004, 2005).

The cellular receptors of the innate immune system that recognise PAMPs or MAMPs as activation signals are called pattern recognition receptors (PRRs), many of which belong to the so-called Toll-like receptors (TLRs). PRRs are expressed mainly by macrophages and DCs, but also by a variety of other cell types including T and B cells as well as epithelial cells (see later).

Engagement of PRRs with their intracellular signalling pathways causes cellular activation; in the case of DCs, this leads to maturation accompanied by production of various cytokines and up-regulation or down-regulation of surface molecules according to strictly defined kinetics (*Ricciardi-Castagnoli* and *Granucci*, 2002). Such cellular modulation will critically influence further development of both innate and adaptive immunity.

In summary, there are both stereotypical and selective responses of innate host cells to different types of microorganisms. In this manner, they can imprint their 'signatures' on the immune system. Thus, the plasticity of the innate system prepares the ground for a targeted and powerful function of subsequent adaptive immune responses (*Liew*, 2002).

ADAPTIVE IMMUNITY

T and B cells

In peripheral blood, the lymphocytes comprise 20-25% of the leukocytes. All T lymphocytes (or T cells) express selectively cluster of differentiation (CD)3, and all B lymphocytes (or B cells) ex-

press selectively CD19 and CD20 molecules. A particular subset of T lymphocytes usually called T-helper (Th) cells expresses CD4, whereas most cytotoxic T cells express CD8. Adaptive immunity depends on the functional properties of T and B cells and is directed by their antigen-specific surface receptors, which show a random and highly diverse repertoire (*Chaplin*, 2003; *Sompayrac*, 2003).

Lymphocytes originate in the bone marrow from a common lymphoid stem cell. Further development and maturation of T and B cells occur in the thymus and bone marrow, respectively (so-called primary lymphoid organs). Mature but yet unprimed ('naïve') T and B cells enter the bloodstream and become disseminated to secondary lymphoid organs such as the spleen, lymph nodes and mucosa-associated lymphoid tissue (MALT).

Certain adhesion molecules and receptors for chemokines (chemo-attractant cytokines) enable adherence of immune cells to specialised vascular endothelium and their migration into the lymphoid organs, which are anatomically and functionally organised to facilitate interactions between professional antigen-presenting cells (APCs) such as mature DCs and lymphocytes (*Kunkel* and *Butcher*, 2002; *Brandtzaeg* and *Pabst*, 2004; *Brandtzaeg* and *Johansen*, 2005). Antigens are carried into these immune-inductive structures from epithelial surfaces via draining lymph – passively as soluble molecules or particles (e.g. infectious agents), and actively by migrating DCs – as well as directly from mucosal surfaces by 'membrane' or 'microfold' (M) cells in MALT (*Neutra* et al., 2001).

Each T and B cell bears antigen receptors with a certain specificity, which differs between individual clones of lymphocytes (*Chaplin*, 2003). A clone consists of daughter cells derived by proliferation from a single ancestor cell ; so-called clonal expansion. The total population of T and B cells in a human may be able to recognise some 1011 different antigens. This remarkably diverse antigen receptor repertoire is generated during lymphocyte development by random rearrangement of a limited number of receptor genes. Even without priming, the adaptive immune system would thus be able to respond against an enormous number of antigens, but the detection of any single antigen could be limited to relatively few lymphocytes, perhaps only 1 in 1,000,000.

It follows from the above that a primary immune response usually generates an insufficient number of specific lymphocytes to eliminate the invading pathogen. However, when an antigen receptor is engaged by its corresponding antigen, the lymphocyte generally becomes activated (primed), ceases temporarily to migrate, enlarges (blast transformation) and proliferates rapidly so that, within 3-5 days, there are numerous daughter cells – each specific for the antigen that initiated the primary immune response.

Such antigen-driven clonal expansion accounts for the characteristic delay of several days before adaptive immunity becomes effective in defending the body against an infection. In addition to the effector cells generated by clonal expansion and differentiation, also so-called memory cells are generated; these may be very long-lived and are the basis of immunological memory characteristic of adaptive immunity (*Chaplin*, 2003). Functionally, immunological memory enables a more rapid and effective secondary (or 'anamnestic') immune response upon re-exposure to the same antigen. In contrast to innate immunity, the antigen recognition profile of the adaptive immune system reflects the individual's lifetime exposure to stimuli from infectious agents and other antigens, and will consequently differ among individuals.

Immune response and immune reaction

Induction of immune responses

The purpose of adaptive immunity is primarily to combat infections by preventing colonisation of pathogens and keeping them out of the body (immune exclusion), and also to seek out specifically and destroy invading microorganisms (immune elimination). In addition, specific immune responses are, through regulatory mechanisms, involved in avoidance of overreaction (hypersensitivity or allergy) against harmless antigens as well as discrimination between components of 'self' and 'non-self' (*Chaplin*, 2003). Autoimmunity occurs when the latter control mechanism breaks down.

Both the primary and secondary adaptive immune responses depend on professional APCs, which express major histocompatibility complex (MHC) class II determinants (in humans: HLA-DR, -DQ and -DP) as genetically determined restriction elements for $CD4^+$ Th cells (*Chaplin*, 2003). In this manner, the T-cell receptors can specifically recognise short immunogenic peptide sequences of the APC-processed antigen that each is presented in the polymorphic grove of an MHC molecule. The immune response may also involve polymorphic MHC class I molecules and $CD8^+$ T cells with cytotoxic and/or suppressive potential (*Chaplin*, 2003). All of these cell categories are present in secondary lymphoid organs and at immunological effector sites where the primed immune cells extravasate by means of homing molecules, which differ markedly between the systemic and the mucosal immune system (*Kunkel* and *Butcher*, 2002; *Brandtzaeg* and *Johansen*, 2005).

A long-lasting secondary immune response gives rise to abundant differentiation of effector cells and release of biologically active substances, aiming at neutralisation and elimination of antigens through a variety of targeted strategies. Such immunological effector mechanisms, and the non-specific biological amplification often triggered by them via hyperactivation of innate immunity, are collectively referred to as immune reactions. Adaptive immunity is thus based on specific immune responses but expressed by an array of cellular and humoral immune reactions.

The effector cells of the B-cell system are the terminally differentiated antibody-producing plasma cells that provide so-called humoral immunity. Antibodies are constituted by five immunoglobulin (Ig) classes (IgG, IgA, IgM, IgD, and IgE). The antigen-specific receptor on the surface of the B cells is a membrane-bound form of Ig produced by the same cell (*Chaplin*, 2003; *Sompayrac*, 2003). Engagement of surface Ig by corresponding antigen will – in cooperation with 'help' provided by cognate Th cells – initiate B-cell differentiation and clonal expansion. The resulting effector B cells can then differentiate into plasma cells that secrete large amounts of antibody with the same specificity as that of the antigen receptor expressed by the progenitor B lymphocyte. While IgM (primary response) and IgG (secondary response) dominate systemic humoral immunity, IgA is normally the predominant antibody class of mucosal immunity (see later).

Immune reactions

Immune reactions aim at immune elimination – a term that refers to mechanisms involved in removal of foreign material that has penetrated the epithelial barrier. Thus, when required, immune elimination can constitute a 'second line' of mucosal defence that depends partly on systemic immune effectors such as serum-derived antibodies in addition to locally produced antibodies, often operating in combination with cytotoxicity intraepithelial $CD8^+$ T cells. Immune elimination is generally enhanced by innate non-specific amplifi-

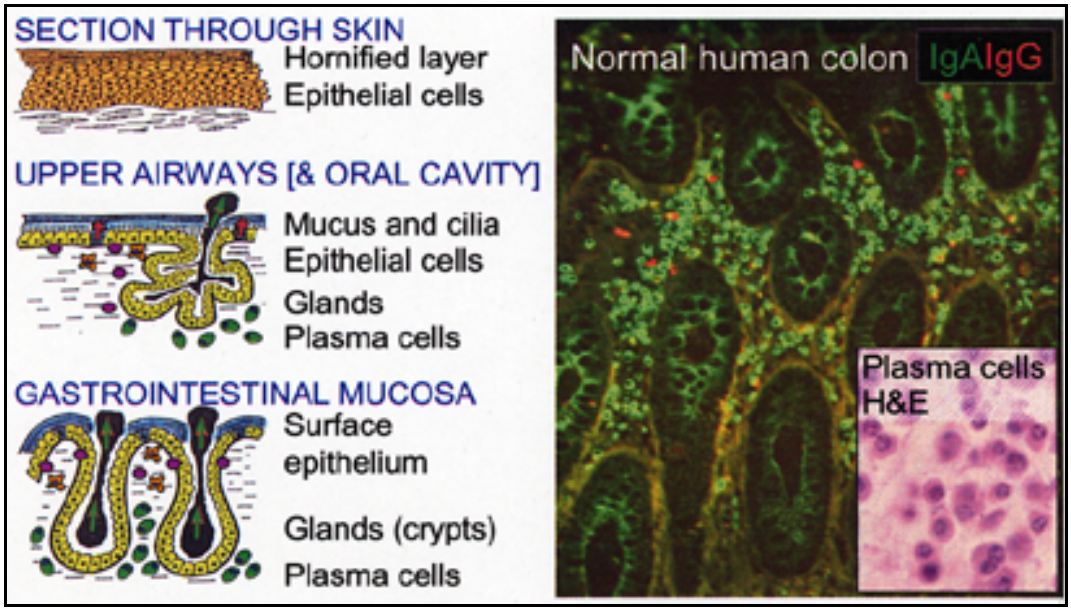

Figure 2: The cellular basis of secretory immunity. Left panel: Schematic depiction of cross-section through gastrointestinal and airway mucosae compared with skin. Because of their specialised function, mucosal epithelia are generally monolayered and protected by various innate defence mechanisms and a sophisticated B-cell system with IgA-producing plasma cells that co-operate with an export system for secretory antibodies (green arrows). Right panel: Paired immunofluoresence staining of IgA- and IgG-producing plasma cells in normal human colon, showing the remarkable dominance of the former class and its export through crypt epithelium outside of the goblet cells which are black. Immunofluoresence photograph from the author's laboratory.

cation mechanisms generating various themes of inflammation.

This scenario may evolve into overt immunopathology and inflammatory disease if satisfactory antigen removal and immunoregular control are not rapidly achieved (*Brandtzaeg* et al., 2005; *MacDonald* and *Montelone*, 2005). Such an adverse development is apparently part of the pathogenesis in various gut disorders including food allergy, coeliac disease, and inflammatory bowel disease (IBD).

MUCOSAL IMMUNITY

Homeostasis-promoting mechanisms

Mucosal immunity can be viewed as a first line of defence that aims at reducing the need for systemic immunity, which can engage forceful pro-inflammatory mechanisms to enable immune elimination when required to save life. This scenario can be reviewed as a 'two-edged sword' which may cause immunopathology and tissue damage as discussed above.

During evolutionary modulation, the mucosal immune system has generated two non-inflammatory layers of defence: (a) immune exclusion performed by secretory antibodies to restrict epithelial

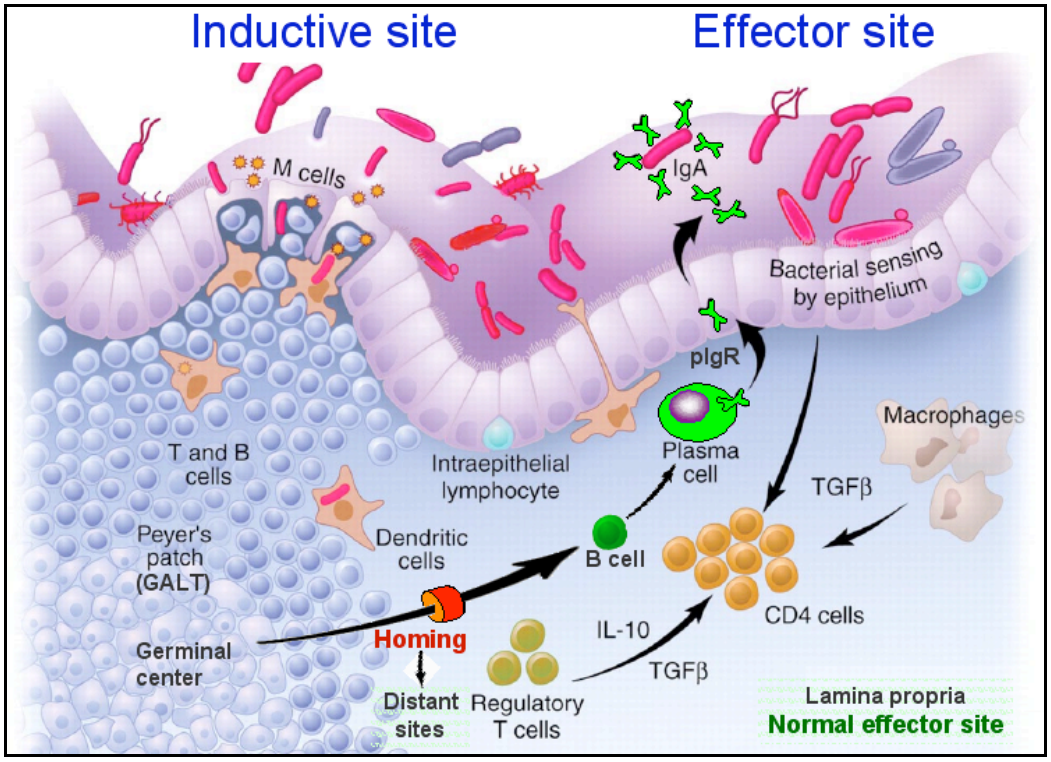

Figure 3: Illustration of inductive site for intestinal immunity (left) represented by Peyer's patch which in numbers up to 250 makes up a major part of the human gut-associated lymphoid tissue (GALT). The follicle-associated epithelium contains antigen-sampling M cells. The germinal centre of activated follicle generates memory/effector B cells which, together with primed mucosal T cells, home to mucosal effector sites, constituted largely by the intestinal lamina propria. Here the T cells perform immune regulation and provide various cytokines which, together with bacteria sampled from the lumen by dendritic cells as indicated, provide second signals for B cells. The latter differentiate into plasma cells mainly producing dimeric IgA which is exported to the lumen by the polymeric Ig receptor (pIgR) to become secretory IgA. Antibodies of this class then coat commensal bacteria. The antibody export may be enhanced by cytokines and epithelial sensing of bacteria (see Figure 7). Modified from *MacDonald* and *Monteleone* (2005).

penetration and host invasion of microorganisms as well as other potentially dangerous antigens; and (b) immunosuppressive mechanisms to avoid local and peripheral hypersensitivity to innocuous luminal antigens. The latter mechanisms are collectively referred to as 'oral tolerance' when induced via the gut (*Brandtzaeg*, 1996; *Mowat*, 2003) and probably explains why overt and persistent allergy to food proteins is relatively rare (*Bischoff* et al., 2000). A similar down-regulatory tone of the immune system normally develops against antigenic components of the commensal intestinal microbiota (*Duchmann* et al., 1997; *Helgeland* and *Brandtzaeg*, 2000; *Moreau* and *Gaboriau-Routhiau*, 2000).

Oral tolerance appears be a rather robust adaptive immune function in view of the fact that more than a ton of food may pass through the gut of an adult every year. This results in a substantial uptake of intact antigens, usually without causing harm. However, the neonatal period is particularly critical, both with

regard to infections and priming for allergic disease, because the mucosal barrier function and the immunoregulatory network are poorly developed for a variable period after birth (*Holt*, 1995; *Brandtzaeg*, 2002). Notably, the postnatal development of mucosal immune homeostasis depends on the establishment of a normal microbiota as well as on adequate timing and dose of dietary antigens when first introduced (*Brandtzaeg*, 1996, 1998; *Moreau* and *Gaboriau-Routhiau*, 2000).

The intestinal IgA system that provides secretory immunity is the best-studied part of mucosal immunity (Figure 2). In fact, the gut mucosa contains at least 80% of the body's activated B cells – terminally differentiated to Ig-producing blasts and plasma cells (*Brandtzaeg* and *Johansen*, 2005). Their generation depends on complex mechanisms of B-cell induction and homing as discussed below.

Immune stimulation in mucosa-associated lymphoid tissue

Inductive mucosal tissue structures

Gut-associated lymphoid tissue (GALT) constitutes a major part of MALT and comprises Peyer's patches, the appendix and numerous solitary or isolated lymphoid follicles (*Brandtzaeg* et al. 1987, 1999; *Brandtzaeg* and *Pabst*, 2004; *Brandtzaeg* and *Johansen*, 2005). All these structures are believed to represent inductive sites contributing to intestinal immune responses, while the lamina propria and epithelial compartment principally constitute effector sites (Figure 3).

The domes of GALT are covered by a characteristic follicle-associated epithelium, which contains antigen-sampling M cells. These very thin and bell-shaped specialised epithelial cells transport effectively live and non-proliferating antigens (especially particles) from the gut lumen into the organised lymphoid tissue (*Neutra* et al. 2001). Many enteropathogenic infectious agents use the M cells as portals of entry, so they represent extremely vulnerable parts of the surface epithelium. However, such 'gaps' in the epithelial barrier are needed to facilitate efficient induction of mucosal immunity.

GALT structures resemble lymph nodes with B-cell follicles, intervening T-cell zones and a variety of APCs such as macrophages and DCs, but there are no afferent lymphatics (*Brandtzaeg* and *Pabst*, 2004). Exogenous stimuli therefore come directly from the epithelial surfaces mainly via the M cells, probably aided by DCs which may penetrate the epithelial layer with their processes (*Rescigno* et al. 2001). Induction and regulation of mucosal immunity hence takes place primarily in GALT but also to some lesser extent at the effector sites (Figure 3).

Priming and dispersion of intestinal B cells

Antigens are presented to naïve T cells in GALT by APCs after intracellular processing (degradation) to immunogenic peptides. In addition, luminal peptides may be taken up and presented by B-lymphocytes and epithelial cells to subsets of intra- and sub-epithelial T lymphocytes (*Brandtzaeg* et al. 1999). Both professional mucosal APCs, B cells, and the small-intestinal villous epithelium, as well as the follicle-associated epithelium of GALT surrounding the M cells, express MHC class II molecules – in humans particularly HLA-DR (see later) – in addition to classical and non-classical MHC class I molecules (*Brandtzaeg* et al. 1987; *Christ* and *Blumberg*, 1997). As discussed previously, such molecules are essential for an antigen-presenting function (*Chaplin*, 2003). Interestingly, MHC class II-expressing naïve and memory B-lymphocytes abound juxtaposed to the M cells. Such B cells may present antigens efficiently to T cells in

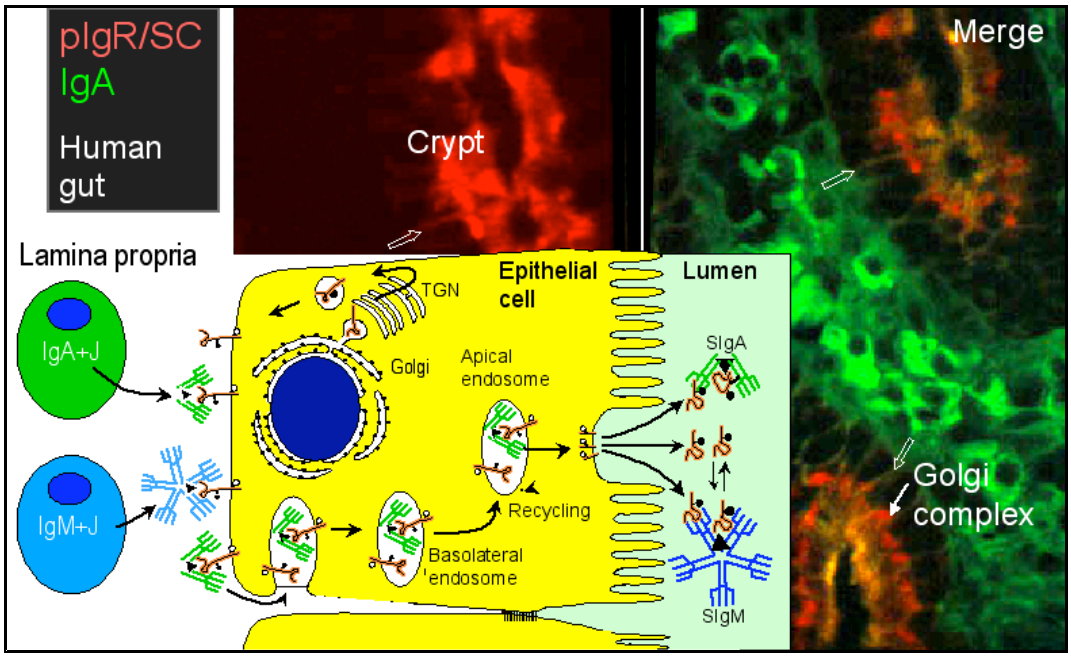

Figure 4A: Epithelial export of secretory antibodies. In the background is shown paired immunofluoresence staining for pIgR/SC (left panel) together with IgA (right panel, merge) from crypt region of human gut mucosa. Superimposed is a schematic representation of the various steps involved in the generation of SIgA and SIgM as explained in the text. Transmembrane SC is synthesized in the rough endoplasmic reticulum and sorted through the Golgi complex/trans-Golgi network (TGN) where it is revealed as pure red immunofluoresence (right panel, solid arrow). The mature form is terminally glycosylated (solid dots), them phosphorylated (open dots) and expressed basolaterally as functional pIgR (open arrows in both immunofluoresence panels). J chain-containing dimeric IgA (IgA+J) and pentameric IgM (IgM+J) are produced by lamina propria plasma cells, and both ligands are endocytosed and transcytosed by pIgR to reach recycling apical endosomes (yellow colour in right panel). Here pIgR is cleaved to release SIgA and SIgM to the lumen complexed with bound SC as well as free SC derived from unoccupied pIgR. Covalent stabilisation of SIgA occurs by disulfide exchange with bound SC (one S-S bridge indicated), whereas free SC in excess is needed to retain non-covalently bound SC in SIgM (dynamic equilibrium indicated).

cognate down-regulatory or immunostimulatory interactions (*Brandtzaeg* et al. 1999).

T cells primed in GALT release cytokines such as transforming growth factor (TGF)-ß and interleukin (IL)-10, which may drive the differentiation of mucosal B cells to predominantly IgA-committed plasma blasts, although their regulation still remains unclear (*Brandtzaeg* et al. 2001; *Fagarasan* and *Honjo*, 2003; *Brandtzaeg* and *Johansen*, 2005). Most B cells primed by 'first signals' in GALT structures migrate rapidly via draining lymphatics to mesenteric lymph nodes where they are further stimulated (*Brandtzaeg* and *Pabst*, 2004; *Brandtzaeg* and *Johansen*, 2005); they may then reach peripheral blood and become seeded by preferential homing mechanisms into distant mucosal effector sites, particularly the intestinal lamina propria where they finally develop to Ig-producing plasma cells (Figure 3).

This terminal differentiation is modulated by 'second signals' from lamina propria $CD4^+$ T cells, antigen-

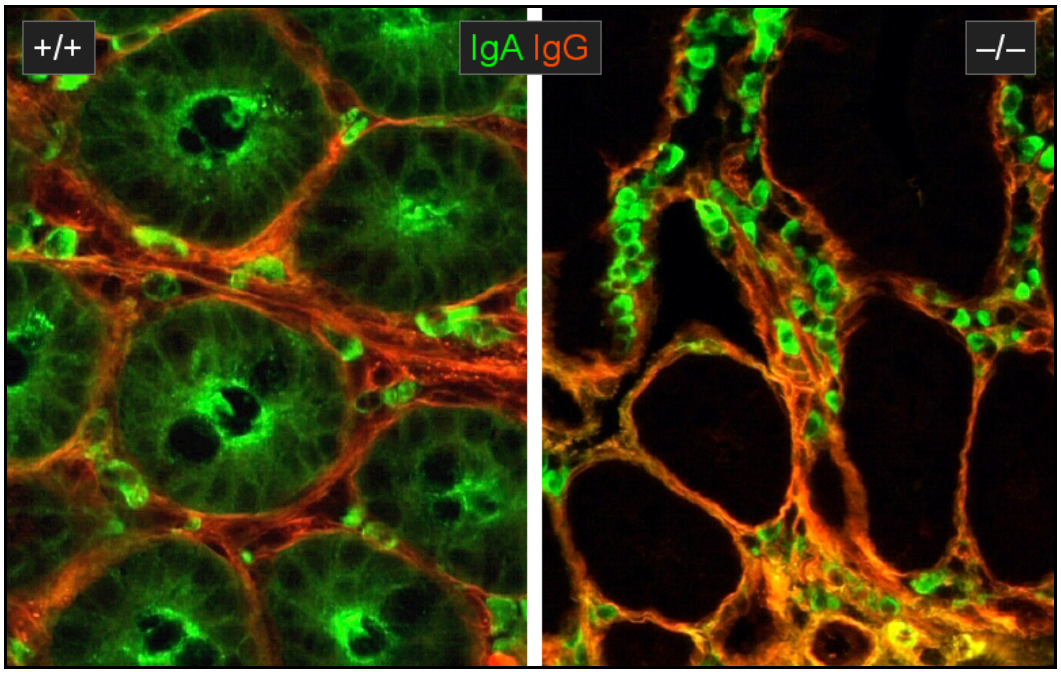

Figure 4B: Epithelial export of secretory antibodies. Paired immunofluoresence staining for IgA and IgG in gut of wild-type mouse (+/+) and pIgR knockout mouse (-/-), the latter showing no epithelial uptake of IgA in cross-section of colonic crypts despite a large number of IgA-producing plasma cells in the lamina propria. There is a high level of serum-derived IgG surrounding the crypts, but no visible epithelial uptake of this isotype. Immunofluoresence photographs from the author's laboratory.

sampling DCs, and available cytokines (Figure 3). Most B cells included in the homing to mucosal effector sites apparently belong to clones of an early maturation stage, as indicated by their high level of J chain (see later) regardless of concomitant isotype – although the IgA class normally predominates (*Brandtzaeg* et al., 1999). J chain-containing dimeric IgA and pentameric IgM are finally translocated to the lumen as secretory IgA (SIgA) and secretory IgM (SIgM) by the polymeric Ig receptor (pIgR) as discussed below. In the lumen, SIgA will coat commensal bacteria (Figure 3) as first demonstrated almost 40 years ago (*Brandtzaeg* et al., 1968). This coating reduces their access to the epithelial surface and protects against bacterial overgrowth and invasion (*Macpherson* et al., 2005).

Antibody-mediated mucosal defence

Secretory immunity and immune exclusion

As alluded to above, the unique and efficient export of SIgA and SIgM antibodies to the epithelial surfaces is mediated by pIgR, also known as membrane secretory component (SC), which is cleaved and sacrificed as bound SC to stabilise the secretory antibodies in the lumen (Figure 4A). The ligand site specific for this receptor depends on the small peptide called 'joining' or J chain that is incorporated selectively into dimeric IgA and pentameric IgM (*Brandtzaeg*, 1974a,b; *Brandtzaeg* and *Prydz*, 1984; *Brandtzaeg* and *Johansen*, 2005). It has been estimated that such pIgR-mediated transport of dimeric IgA on average results in the daily delivery of approximately 3 g of SIgA into the

Table 1: Antimicrobial effects of SIgA antibodies

- Perform non-inflammatory extracellular and intracellular immune exclusion by inhibiting epithelial adherence and invasion
- Polyreactive ('innate-like') activity provides cross-protection
- SIgA (particularly SIgA2) is quite stable (bound SC stabilizes both isotypes)
- Mucophilic and lectin-binding properties (via bound SC in both isotypes and mannose in IgA2)
- Dimer provides efficient agglutination and virus neutralisation

intestinal lumen of an adult human (*Conley* and *Delacroix*, 1987). SIgA acts as the first line of adaptive immune defence against microorganisms and other antigens, and regulates the host response to pathogens as well as commensal bacteria.

Notably, however, in face of the indigenous microbiota, the intestinal IgA system of mice behaves in a somewhat 'primitive' or 'innate-like' way by exhibiting high capacity, broad specificity, and low affinity (*Macpherson* et al., 2005; *Brandtzaeg* and *Johansen*, 2005). Even in humans, SIgA antibodies show a substantial level of cross-reactivity (*Bouvet* and *Fischetti*, 1999). The mucosal IgA system is therefore well designed to provide antibody-mediated immune exclusion of the extremely diverse intestinal microbiota with a multitude of redundant epitopes, while at the same time allowing a persistent host-commensal bacterial mutualism.

Defence functions of SC and secretory antibodies

Free SC is generated by apical cleavage and epithelial release of unoccupied pIgR (Figure 4A). Interestingly, free SC has been shown to possess several innate immune properties (*Phalipon* and *Corthésy*, 2003). This fact suggests that it phylogenetically has originated from the natural defence system like many other proteins involved in adaptive immunity. Importantly, SC has been exploited as the only identifiable epithelial receptor involved in secretory immunity as documented by the phenotype of pIgR knockout mice (*Johansen* et al., 1999), which have no active external transport of dimeric IgA and pentameric IgM (Figure 4B).

A crucial protective role of secretory antibodies is supported by the fact that such mice have 'leaky' mucosal epithelia (*Johansen* et al., 1999). Also, similarly to J-chain knockout mice that likewise lack both SIgA and SIgM, they show decreased resistance against bacterial toxins and early colonisation of the gut epithelium by pathogens (*Lycke* et al., 1999; *Uren* et al., 2005).

Altogether, animal and cell culture experiments have suggested that SIgA antibodies promote intestinal homeostasis by neutralising viruses and bacterial products through non-inflammatory mechanisms at various levels (Table 1). In addition to traditional luminal neutralisation, it has been demonstrated that dimeric IgA antibodies – when exported by pIgR – can remove antigens from the lamina propria and neutralise viruses within the epithelium or block their transcytosis through polarised epithelial cells (*Mazanec* et al., 1993, 1995; *Burns* et al., 1996; *Bomsel* et al., 1998; *Alfsen* et al., 2001; *Robinson* et al., 2001; *Feng* et al., 2002; *Huang* et al., 2005). It has also been reported that dimeric IgA can neutralise bacterial LPS within intestinal epithelial cells (*Fernandez* et al., 2003), suggesting a novel intracellular, non-cytotoxic and anti-inflammatory role for this antibody class during its export.

Although SIgA is the chief effector of immune exclusion, SIgM also contributes – particularly in the new-born

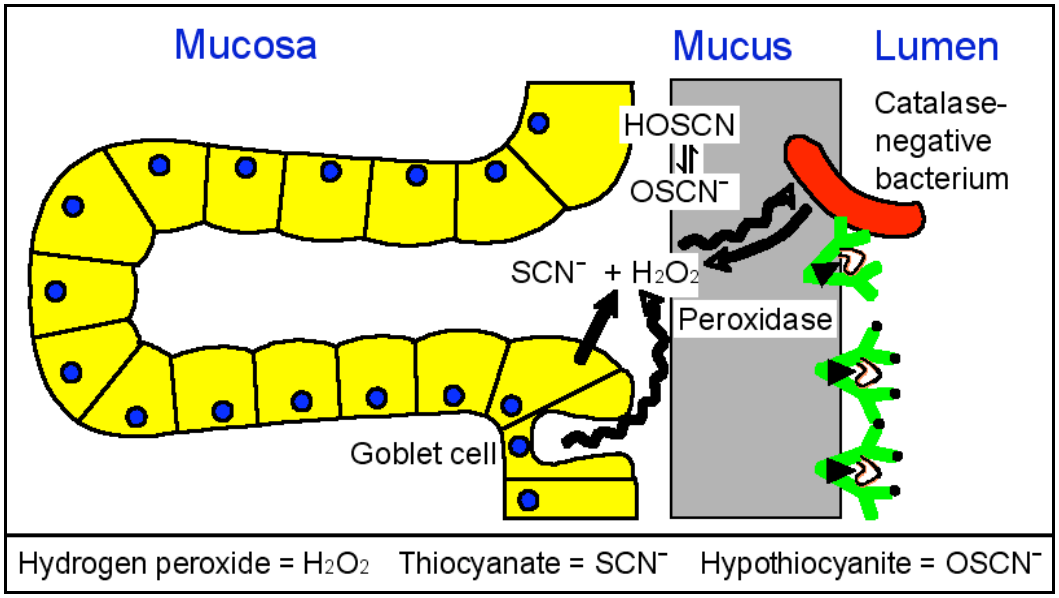

Figure 5: Schematic illustration of the co-operation between SIgA and the peroxidase defence system in mucus layer of colonic mucosa. Details are discussed in the text.

period and in IgA deficiency (*Brandtzaeg* and *Nilssen*, 1995). In addition, there may be some contribution to immune exclusion by serum-derived or locally produced IgG antibodies transferred passively to the lumen by paracellular leakage (*Persson* et al., 1998) or perhaps to some extent exported by the neonatal Fc receptor (FcRn) expressed by the gut epithelium (*Yoshida* et al., 2004). Notably, however, because IgG is complement-activating, its contribution to surface defence is potentially pro-inflammatory, which could jeopardise the epithelial barrier function (*Brandtzaeg* and *Tolo*, 1977).

Interactions between secretory immunity and innate defence factors

Several studies have shown that mucosal immunity may be enhanced by co-operation between SIgA and innate defence factors. Thus, SIgA antibodies can clearly promote the bacteriostatic effect of lactoferrin, apparently by inhibiting bacterial production of iron-chelating agents which may interfere with its function (*Rogers* and *Synge*, 1978).

SIgA can also promote the broad antimicrobial spectrum of the peroxidase defence system, independently of antibody specificity (*Tenovuo* et al., 1982) – probably because of the mucophilic properties of bound SC in SIgA (*Phalipon* and *Corthésy*, 2003) which, by its cross-reactivity, retains bacteria for prolonged and focused action of the enzyme with its biocidal product hypothiocyanate (Figure 5). Human milk is rich in both lactoperoxidase and leukocyte-derived myeloperoxidase (*Moldoveanu* et al., 1982) which survives enzymatically active in gastric juice (*Gothefors* and *Marklund*, 1975). Moreover, peroxidase is produced by colonic goblet cells and therefore appears to be part of the innate intestinal defence (*Venkatachalam* et al., 1970).

There is a possibility that catalase-positive bacteria may resist the oxidising effects derived from H_2O_2 (Figure 5). However, bacterial catalase is restricted to the cytoplasm or periplasm and would therefore not be expected to alter sub-

Table 2: Effect on bacterial overgrowth of co-operation between SIgA and other defence mechanisms as revealed in various clinical conditions with (+) or without (-) defective adaptive or innate immunity

Variables					
• SigA	+	+	-	-	-
• Peristalsis	+	-*	+	+	-*
• Gastric Acid	+	-**	+	-**	-**
Result					
• Jejunal colonisation	-	-	-	-	+

*Complete vagotomy
**Gastric drainage (gastroenterostomy) or pernicious anemia

Modified from *McLoughlin* et al. (1978)

stantially the availability of H_2O_2 as substrate for peroxidase, and some bacteria may even produce H_2O_2. Importantly, catalase-positive pathogens have been shown to be cleared efficiently from the airways *in vivo* by the peroxidase defence system (*Gerson* et al., 2000).

Immune reactions that take place at the epithelial surface may furthermore stimulate the release of mucus from goblet cells and thereby reinforce the mucosal barrier against penetration of soluble molecules and microorganims (*Walker* et al., 1982; *Walker* and *Bloch*, 1983). Through its affinity for bound SC, the mucus may further enhance its 'flypaper' effect – topically retaining SIgA antibodies with complexed antigens (*Lim* and *Rowley*, 1982; *Phalipon* and *Corthésy*, 2003).

Antigens present in immune complexes trapped in the mucus layer are more rapidly degraded by proteolytic enzymes than free antigens (*Walker* et al., 1975). It has been proposed that IgA1 is especially miscible with mucus because of certain structural homologies, whereas the function of IgA2 may take place mainly in the external fluid (*Clamp*, 1980). This may be particularly relevant in the distal gut where there is a predominance of local IgA2 production (*Kett* et al., 1986); and this subclass is also more resistant to proteolytic degradation than IgA1 (*Kilian* et al., 1996).

The co-operation between SIgA and the important innate defence function provided by the 'germicidal barrier' of gastric acid, as well as by intestinal peristalsis, was strikingly demonstrated in a study of bacterial overgrowth in the jejunum of vagotomised patients; mucosal protection deteriorated when IgA deficiency was combined with a suboptimal function of these two non-specific protective mechanisms (*McLoughlin* et al., 1978). As always when IgA is selectively lacking (*Brandtzaeg* and *Johansen*, 2005), the study subjects necessarily had compensatory SIgM in their gut; however, although several antimicrobial activities have been identified for this antibody class (*Brandtzaeg* et al., 1987), it can clearly not adequately replace SIgA (Table 2). This is also shown by the fact that IgA-deficient subjects have raised levels of serum IgG antibodies to dietary antigens and an increased frequency of allergic and autoimmune disorders, including coeliac disease (*Brandtzaeg* and *Nilssen*, 1995). The same is claimed to be true for Crohn's disease, with some 20-fold increased incidence (L. Hammarström, personal communication).

Neonatal mucosal immunity

In contrast to several animal species, the human foetus acquires circulating

Table 3: Colonisation of germ-free mice with *Bacteriodes thetaiotaomicron* increases intestinal expression of genes involved in epithelial barrier function and nutritient absorption

Gene product	Fold Δ over germ-free
Barrier function	
• Polymeric Ig receptor (pIgR/SC)	2.6 ± 0.7
• Small proline-rich protein 2a (sprr2a)	205 ± 64
• Decay-accelerating factor (DAF)	5.7 ± 1.5
Nutrient absorption	
• Na+/glucose co-transporter (SGLT-1)	2.6 ± 0.9
• Co-lipase	6.6 ± 1.9
• Liver fatty acid-binding protein (L-FABP)	4.4 ± 1.4

Modified from Hooper et al. (2001)

maternal IgG via the placenta and not as a result of breast-feeding. Also notably, intestinal uptake of SIgA antibodies from breast milk is of no importance for systemic immunity in humans, except perhaps in the preterm neonate (*Brandtzaeg*, 2002). Although so-called 'gut closure' normally occurs in humans mainly before birth, the mucosal barrier function may not be fully established until after 2 years of age; the different variables involved in this process remain poorly defined.

Only occasional traces of SIgA and SIgM occur in human intestinal fluid during the first postnatal period, whereas some IgG is often present – reflecting bulk transfer from the lamina propria, which after 34 weeks of gestation contains readily detectable maternal IgG (*Brandtzaeg* et al., 1991). IgA-producing plasma cells are normally undetectable in the intestinal mucosa before the infant is 10 days of age, but thereafter a rapid increase takes place – IgM often remaining predominant up to 1 month. Little increase of intestinal IgA production usually takes place after 1 year. A much faster establishment of secretory immunity may be seen in developing countries because of a more massive mucosal exposure to microorganisms (*Brandtzaeg* et al., 1991).

Critical role of breast-feeding in infancy

At least 90% of all pathogens use the mucosae as portals of entry. Thus, mucosal infections are a major killer below the age of 5 years – being responsible for more than 14 million deaths of children annually in developing countries. In those parts of the world, infants are highly dependent on SIgA antibodies from breast milk to protect their mucosae; epidemiological data suggest that the risk of dying from diarrhoea is reduced 14-24 times in sucklings (*Anonymous*, 1994).

Although the value of breast-feeding in westernised countries is clinically most apparent in preterm infants, population studies show that exclusively breast-fed infants are in general better protected against a variety of infections and probably also against allergy, asthma and coeliac disease (*Brandtzaeg*, 2002). This strongly suggests that the mucosal barrier function in newborns can be reinforced by breast-feeding. Experiments in neonatal rabbits have convincingly demonstrated that SIgA is a crucial anti-microbial component of breast milk (*Dickinson* et al., 1998), in addition to a variety of other factors that may enhance mucosal homeostasis (*Brandtzaeg*, 2002).

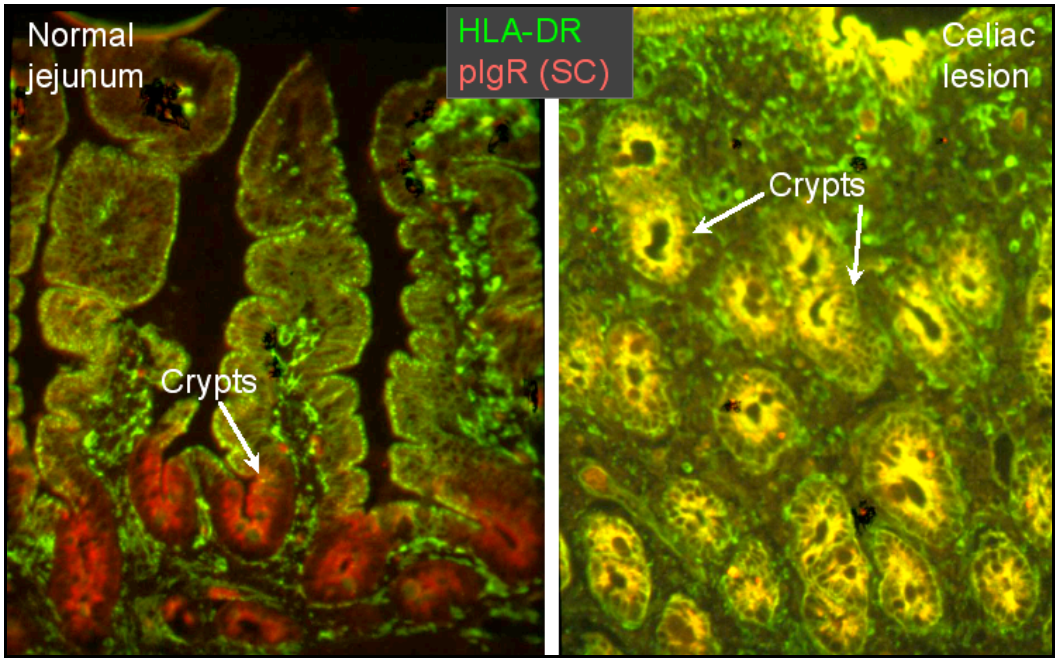

Figure 6: Paired immunofluoresence staining for HLA-DR and pIgR/SC in section of normal human jejunum (left) and jejunal lesion of untreated coeliac disease (right). In the normal state, DR is expressed apically by the villous epithelium as well as by numerous macrophages and dendritic cells in the lamina propria, while pIgR/SC expression is typically restricted to the crypt epithelium. In the coeliac lesion the hyperplastic crypts as well as the surface epithelium show co-expression of DR and pIgR/SC (yellow colour). Both components are presumably up-regulated by cytokines, particularly IFN-γ, derived from activated T cells and macrophages in the lesion (see Figure 7). Immunofluoresence photographs from the author's laboratory.

Regulation of pIgR expression

The postnatal colonisation of commensal bacteria is important both to establish and regulate an appropriate intestinal epithelial barrier function in the gut, including enhanced expression of pIgR (*Neish* et al., 2000; *Hooper* et al., 2001). This has been clearly demonstrated in mouse experiments (Table 3). Epithelial synthesis of pIgR/SC starts in human foetal life as early as around 20 weeks of gestation, but there is a peak of expression during the first couple of postnatal months – best revealed in salivary glands (*Brandtzaeg* et al., 1991) – most likely reflecting microbial colonisation of the mucosae which starts already during the vaginal delivery process.

Collectively, these observations show that the expression of pIgR is both constitutive and subjected to inductive transcriptional up-regulation, as first documented at the molecular level by our laboratory (*Johansen* and *Brandtzaeg*, 2004). Because one molecule of pIgR is consumed for every ligand of dimeric IgA or pentameric IgM transported, regulation of pIgR expression must be crucial for maintenance of intestinal homeostasis.

Coeliac disease is a good example of how the pIgR expression and IgA export can be indirectly enhanced via cytokines produced by activated mucosal T cells and macrophages (Figures 6 and 7, left panel). There is a remarkable level of interferon (IFN)-γ in the untreated

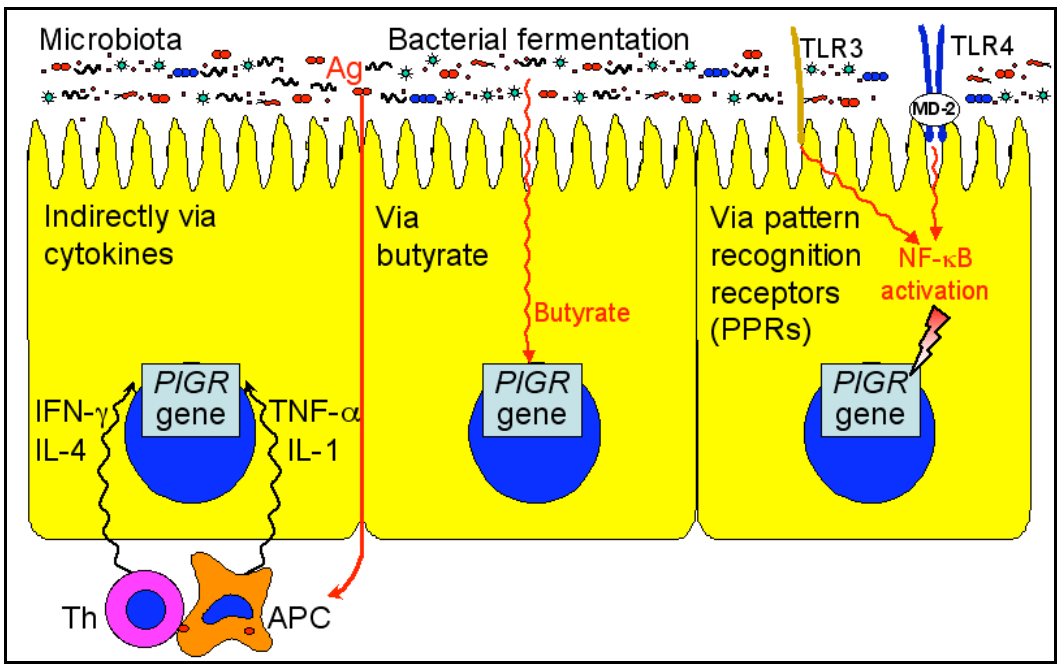

Figure 7: Schematic illustrations of three possible manners in which pIgR expression can be up-regulated by activation of its gene locus *PIGR*. Details are discussed in the text.

coeliac lesion, derived from gluten-specific $CD4^+$ lamina propria T cells (*Nilsen* et al., 1995, 1998) and activated intraepithelial $CD8^+$ T cells (*Olaussen* et al., 2002). Similar up-regulation of pIgR and epithelial IgA export is seen in Sjögren's syndrome and chronic gastritis – supporting the notion that cytokines provide an immunoregulatory link between increased local IgA production and enhanced output of SIgA during low-grade inflammation and infection (*Brandtzaeg* et al., 1992).

Table 4 summarises characteristics of the regulation of human pIgR/SC, and the various putative regulatory DNA elements identified in the PIGR locus are schematically depicted in Figure 8A (*Johansen* and *Brandtzaeg*, 2004). Our laboratory has recently characterised an enhancer region ('enhanceosome') in intron 1 of the human pIgR gene that is sufficient for induction by IL-4 and also contributes to tumour necrosis factor (TNF)-α responsiveness (Figure 8B). The minimal enhancer for IL-4-induced

Table 4: Characteristics of transcriptional regulation of the human *pIgR* gene (locus *PIGR*)

- Constitutively expressed in mucosal and glandular epithelia
- Expression upregulated by immunoregulatory (IFN-γ, IL-4, IL-13) and pro-inflammatory (TNF-α, IL-1) cytokines
- Upregulation of pIgR by cytokines depends on *de novo* protein synthesis
- Both promoter elements and intronic enhancer ('enhanceosome') contribute
- Increased expression correlates with enhanced ability to transport polymeric Igs which contain J chain (dimeric/trimeric IgA and pentameric IgM)

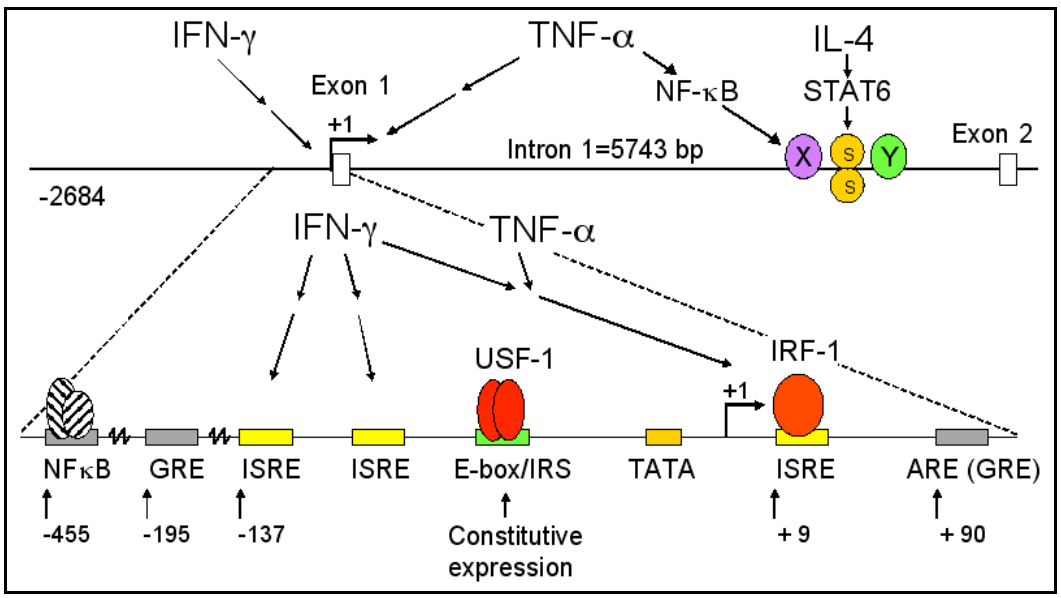

Figure 8A: Regulatory DNA elements in the human *pIgR* gene (locus *PIGR*) and their constitutive and cytokine-induced transcriptional activation. At the top is shown the overall orientation of the promoter, exon 1, and the extensive intron 1 with its pIgR enhancer element that binds signal transducer and activator of transcription (STAT)6 (S/S) and other potential transcriptional regulators (X and Y). DNA elements with some of the identified transcriptional factors in the upstream proximal promoter region and exon 1 are detailed below (GRE = glucocorticoid response element; ISRE = interferon-stimulated response element; USF = upstream stimulatory factor; IRF = IFN regulatory factor; ARE = androgen response element). Based on data from *Schjerven* et al. (2001).

transcription is a 250 bp region located more than 4 kb downstream of the transcription start site and containing at least seven target elements for different DNA-binding factors (*Schjerven* et al., 2000, 2001, 2003; *Johansen* and *Brandtzaeg*, 2004). A binding site for STAT6 (signal transducer and activator of transcription 6) is a crucial element in this enhancer (Figure 8A).

MICROBIAL IMPACT ON MUCOSAL IMMUNE REGULATION

The extended hygiene hypothesis

It is possible that suboptimal development of the SIgA-dependent mucosal barrier function and inadequate tolerance mechanisms together may explain the increasing frequency of certain diseases in industrialised countries – particularly allergies and autoimmune inflammatory disorders (*Brandtzaeg*, 2002; *Yazdanbakhsh* et al. 2002). Interestingly, we have recently demonstrated that milk-allergic children who do not 'grow out' of their allergy after a milk-free diet, have a relatively low level of $CD4^+CD25^+$ regulatory T (Treg) cells (*Karlsson* et al., 2004). This could explain their persistently deficient oral tolerance to cow's milk proteins.

Homeostatic effects of probiotics

On the basis of the so-called extended hygiene hypothesis (*Rautava* et al., 2004), several studies have evaluated the beneficial clinical effect of probiotic

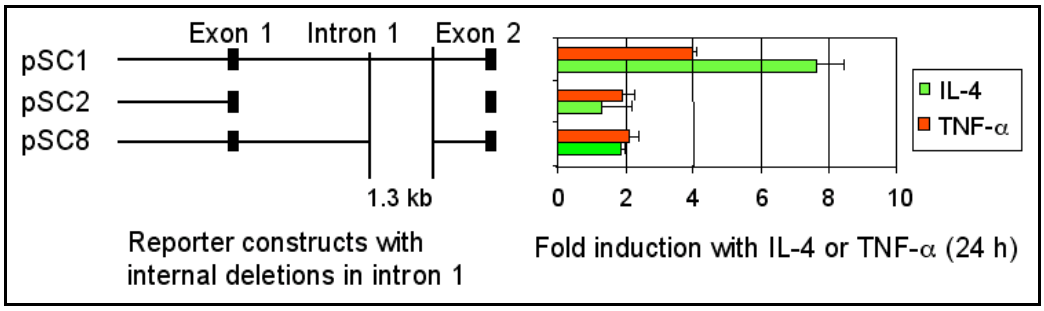

Figure 8B: Regulatory DNA elements in the human *pIgR* gene (locus *PIGR*) and their constitutive and cytokine-induced transcriptional activation. Luciferase reporter assay performed with HT-29 cells transiently transfected with the indicated constructs from the *PIGR* locus (left) and treated with IL-4 or TNF-α to observe induction of pIgR mRNA (right). Deletion of the 1.3-bp intronic enhancer abolishes responsiveness to both cytokines. Based on data from *Schjerven* et al. (2001).

bacterial preparations (*Collins* and *Gibson*, 1999; *Kirjavainen* and *Gibson*, 1999; *Isolauri* et al., 2001). Especially certain strains of commensal intestinal lactic acid bacteria (LAB), particularly lactobacilli and bifidobacteria, have been reported to enhance IgA responses (both in humans and experimental animals – apparently in a T cell-dependent manner (*Yasui* et al. 1995; *Malin* et al. 1996; *Prokesová* et al. 1998, 1999; *Moreau* and *Gaboriau-Routhiau*, 2000). A recent double-blind study of children with a family history of atopy, reported the prevalence of atopic eczema to be reduced by 50% at the age of 2 years after receiving the probiotic Lactobacillus GG strain daily for 6 months (*Kalliomäki* et al. 2001). It remains unknown whether this beneficial effect was mediated via SIgA enhancement or promotion of oral tolerance.

Similarly, there is some hope that immunisation with mycobacterial antigens or bacterial CpG oligonucleotides might skew the cytokine profile towards Th1 and thereby – through cross-regulation – dampen Th2-dependent allergic (atopic) symptoms (*von Reyn* et al., 1997; *Hopkin* et al., 1998; *Wohlleben* and *Erb*, 2001). New-borns are in fact able to mount a Th1-type immune response when appropriately stimulated (*Marchant* et al., 1999). It is also possible that DNA from probiotic bacteria may induce Treg cells that enhance the immunoregulatory homeostasis because certain strains of LAB appear to act as well by subcutaneous or peritoneal injection as by the oral route (*Sheil* et al., 2004; *Foligné* et al., 2005a,b).

Microbial enhancement of pIgR expression

Up-regulation of pIgR expression by microbes and their metabolic products is another intriguing possibility to enhance homeostatic immune functions in the gut as alluded to above. A role for commensal bacteria to this end was suggested by the observation that intestinal pIgR mRNA levels were increased almost three-fold when germ-free mice were colonised with *Bacteroides thetaiotaomicron*, a prominent bacterium of the intestinal flora (Table 3). Also, it was recently observed that infection with reovirus up-regulates expression of pIgR in the human colonic epithelial cell-line HT-29 (*Pal* et al., 2005); this might reflect interaction with epithelial PRRs as discussed later. Changes in composition of the intestinal microflora may thus explain the reported roles for passive and adaptive immunity in the ontogeny of pIgR expression in mice (*Jenkins* et al., 2003).

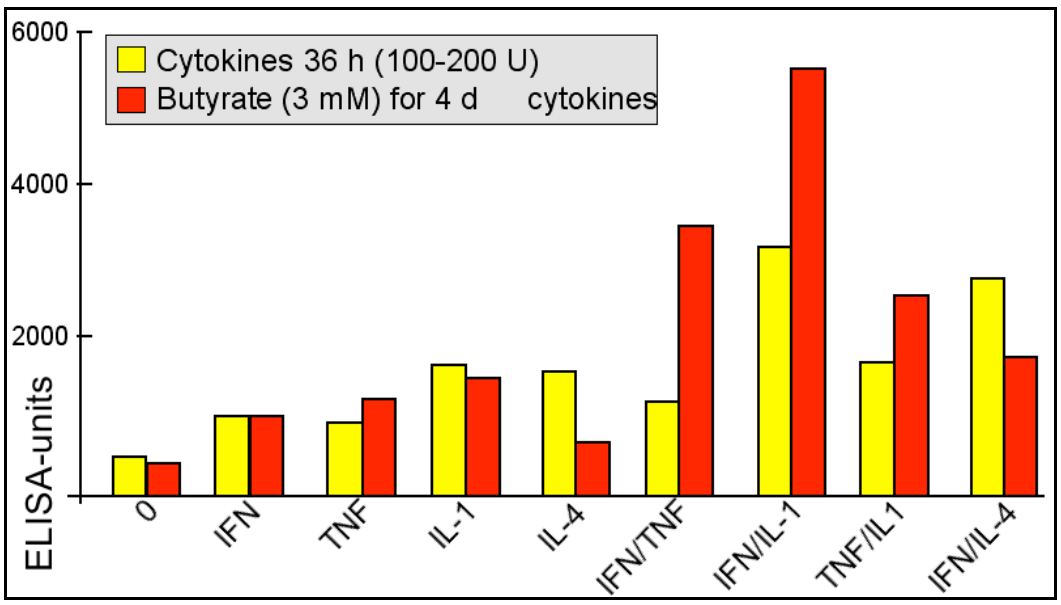

Figure 9A: Effects of butyrate and prebiotics on pIgR expression in gut epithelial cells. Pre-incubation of HT-29 cells for 4 days with butyrate as indicated enhances the pIgR-inducing effects (shown as ELISA-units) of TNF-α and IL-1 or these cytokines in combination with IFN-γ, whereas the effect of IL-4 is reduced (based on data from *Kvale* and *Brandtzaeg,* 1995).

Altogether, commensal bacteria may be important for maintaining the 'tone' of intestinal pIgR expression above its constitutive level. This notion is in keeping with a role for certain strains of commensal gut bacteria to enhance homeostatic immunoregulatory mechanisms – observed directly by administration of probiotics such as LAB (see earlier) or indirectly by adding prebiotics to the diet. Oligosaccharides, for instance, can both promote the growth of LAB and act as a substrate for intestinal formation of butyrate (C-C-COONa) – a fermentation product of many anaerobic bacteria in the normal colonic microbiota. Butyrate is an important energy source for colonic epithelial cells and, notably, it can increase gene transcription levels through specific DNA sequences (*Glauber* et al., 1991).

In this context (Figure 7, middle panel), it is of considerable interest that when we pre-treated HT-29 cells with butyrate, the effect on pIgR/SC expression induced by some cytokines, and particularly various combinations of cytokines, was remarkably enhanced (Figure 9A) – with the exception of the effect of IL-4 which was reduced (*Kvale* and *Brandtzaeg*, 1995). In line with our observations, suggesting an overall positive effect in the distal gut, *Nakamura* et al. (2004) reported that mouse pups receiving dietary fructo-oligosaccharides showed significantly enhanced pIgR expression in ileal and colonic epithelium (Figure 9B), as well as increased export of SIgA into ileal loops and faeces.

Intestinal pattern recognition receptors

The Drosophila gene called Toll gave name to TLRs, which are trans-membrane proteins functioning as PRRs to recognise a variety of microbial constituents or products and trigger innate

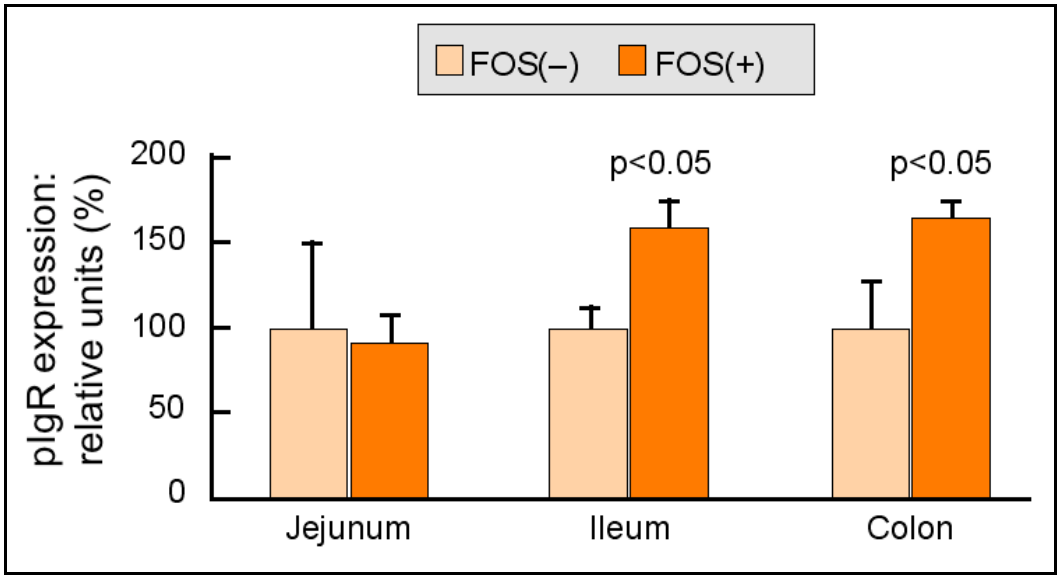

Figure 9: Effects of butyrate and prebiotics on pIgR expression in gut epithelial cells. Effect of dietary fructo-oligosaccharides (FOS) on the expression of pIgR (shown as relative units) in the distal gut of mouse pups (based on data from *Nakamura* et al., 2004).

cellular responses (*Medzhitov*, 2001; *Akira*, 2003). To date, at least 11 TLRs acting singly or in combination have been identified (*Beutler*, 2004; *Cario*, 2005). TLR4 and TLR2 act as the sole conduits for signalling from LPS – an integral component of the outer membranes of Gram-negative bacteria. The classical LPS receptor CD14 is anchored in the cell membrane by glycosylphosphatidyl-inositol; in complex with its ligand and TLR4 or TLR2, it represents an important link between innate and adaptive immunity. The same is true for other TLRs which recognise additional PAMPs or MAMPs (Figure 10).

This immunoregulatory link operates partly via 'shared' and partly via 'specific' signalling pathways generally leading to NF-κB activation, DC maturation, release of pro-inflammatory cytokines (*Modlin*, 2000; *Kaisho* and *Akira*, 2001; *Medzhitov*, 2001), including the Th1-inducing IL-12 and IL-18, and expression of chemokines and co-stimulatory molecules (*Manigold* et al., 2000; *McInnes* et al., 2000; *Cario*, 2005). Moreover, certain unmethylated CpG motifs of bacterial DNA have been shown to promote Th1-cell activity through interaction with TLR9 (*Klinman* et al., 1996; *Kadowaki* et al., 2001; *Peng* et al., 2001).

Although not yet extensively studied in the human gut, sub-epithelial APCs reportedly express certain TLRs. Thus, mRNA for TLR2 and TLR4 has been detected in isolated lamina propria macrophages (*Smith* et al., 2001), but the proteins were undetectable by immunohistochemistry in the normal state although a substantial fraction of the sub-epithelial macrophage-like putative APCs showed positive staining in IBD lesions (*Hausmann* et al., 2002). Also, only extremely low levels of CD14 are normally present on these cells, and their cytokine response is usually poor after LPS stimulation (*Rugtveit* et al., 1997a; *Smith* et al., 2001).

In IBD, on the other hand, expression of CD14 on recently recruited monocyte-like macrophages is strongly elevated, while the co-stimulatory mole-

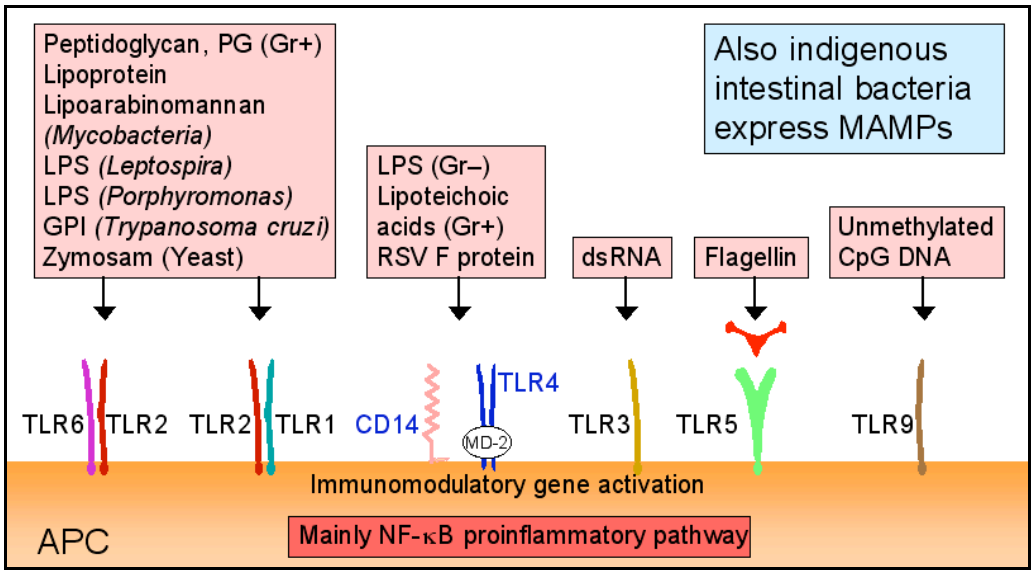

Figure 10: Ligand specificities of Toll-like receptors (TLRs). A variety of pathogen-associated molecular patterns (PAMPs), preferably called microbe-associated molecular patterns (MAMPs), are recognised by highly conserved TLRs expressed on the surface of antigen-presenting cells (APCs) and a variety of other cell types. Recognition of endotoxin or lipopolysaccharide (LPS) from Gram-negative bacteria by TLR4 is aided by two accessory proteins, CD14 and MD-2. TLR2 recognises a broad range of structurally unrelated ligands and functions in combination with several (but not all) other TLRs, including TLR1 and TLR6. TLR3 is involved in recognition of double-stranded RNA (dsRNA). TLR5 is specific for bacterial flagellin, whereas TLR9 is a receptor for unmethylated CpG motifs which are abundant in bacterial DNA. Gr+: Gram-positive; Gr-: Gram-negative; GPI: glycophosphoinositol; RSV: respiratory syncytial virus. Adapted from *Medzhitov* (2001).

cules B7.1 (CD86) and B7.2 (CD80) are up-regulated on putative APCs below the surface epithelium (*Rugtveit* et al., 1997b). The LPS-induced pro-inflammatory cytokine response of macrophage-like cells isolated from IBD lesions is concomittantly increased (*Rugtveit* et al., 1997a). Presumably, also the antigen-presenting potential in the lesions is increased because monocyte-derived macrophages can be skewed towards DC differentiation under the influence of TNF-α (*Chomarat* et al., 2003).

Epithelial sensing of microorganisms

The intestinal epithelium appears to have inherent mechanisms to protect itself against activation from the luminal side unless production of pro-inflammatory cytokines and chemokines is needed in defence against invading microorganisms (*Philpott* et al., 2001; *Sansonetti*, 2004; *Yan* and *Polk*, 2004). Thus, epithelial cells apparently possess sensing systems that allow discrimination between pathogenic and non-pathogenic bacteria in order to initiate an inflammatory reaction only when elimination of invading pathogens is needed (Figure 11). Interestingly, non-pathogenic *Salmonella* strains are able to block the NF-κB transcription pathway in human gut epithelial cells *in vitro* and thereby reduce basolateral IL-8 secretion in response to pro-inflammatory stimuli, including apical infection with wild-type *S. typhimurium* (*Neish* et al., 2000).

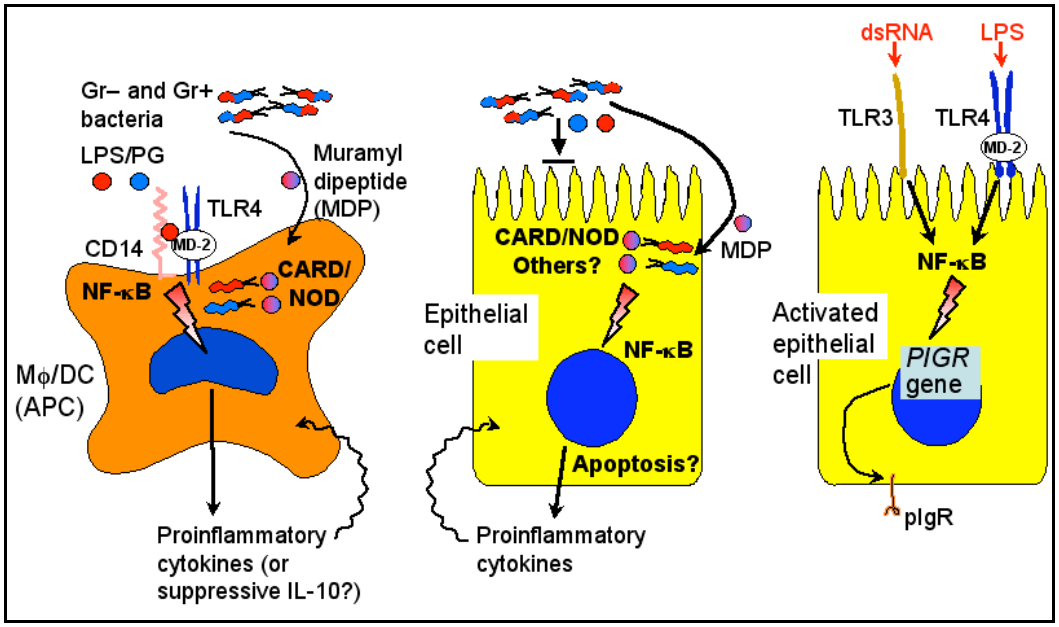

Figure 11: Comparison of the sensing of bacteria and bacterial products by professional antigen-presenting cell (APC) and epithelial cells. Left: TLRs and CD14 expressed by APCs sense Gram-negative (Gr-) and Gram-positive (G+) bacteria or their bacterial products such as lipopolysaccharide (LPS, red dots), peptidoglycan (PG, blue dots), and muramyl dipeptide (MDP, red/blue dots) by APCs; subsequent NF-κB signals may lead to inflammation, apoptosis or immunosuppression. Middle: The mechanisms by which epithelial cells recognise and respond to bacteria remain rather unclear, but they are normally quite refractory to signals from the luminal site; this is probably important for tolerance to commensal bacteria. However, inflammation in response to invasive pathogens promotes bacterial clearance and host survival. One relevant intracellular recognition system is represented by CARD/NOD molecules which sense Gr- or Gr+ bacteria or their MDP motifs. This event leads to secretion of proinflammatory cytokines and cellular activation. Right: Activated epithelial cells show enhanced expression of TLRs. Recognition of double-stranded RNA (dsRNA) and LPS by TLR3 and TLR4, respectively, induces enhanced pIgR expression via activation of the *PIGR* gene (see Figure 12). MØ = macrophage, DC = dendritic cell.

Studies on the expression of CD14 and TLRs on human intestinal epithelial cells have provided inconsistent results, but the HT-29 adenocarcinoma cell line expresses low levels of TLR3 and TLR 4 (*Schneeman* et al., 2005). Double-stranded RNA (dsRNA), a by-product of viral replication, has been identified as a ligand for TLR3 (Figure 10), and epithelial cells can apparently be activated by LPS via TLR4 in a CD14-independent manner (*Böcker* et al., 2003).

The expression of both TLR3 and TLR4 has been shown to be up-regulated on epithelial cells in IBD lesions, particularly so for TLR4 (*Cario* and *Podolsky*, 2000), although others have failed to confirm this by immunohistochemistry (*Hausmann* et al., 2002). While the signalling cascades for TLR3 and TLR4 are similar, recent studies have shown that differential usage of adaptor molecules may result in different biological responses (*Yamamoto* et al., 2002, 2003; *Hoebe* et al., 2003; *Oshiumi* et al., 2003).

In collaboration with our laboratory, Dr. C.S. Kaetzel's group recently showed that pIgR mRNA and protein expression was strongly up-regulated in

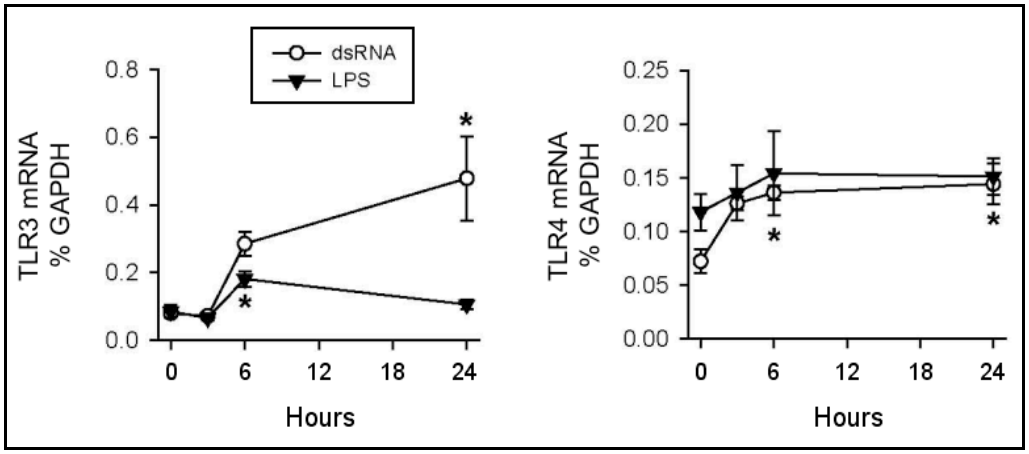

Figure 12A: Differential expression of mRNA for TLR3 and TLR4 and their ligand-induced effect on pIgR expression in HT-29 cells via the *PIGR* intronic-1 enhancer. Levels of TLR3 (left) and TLR4 (right) mRNA in HT-29 cells stimulated with double-standard RNA (dsRNA, 100 μg/ml) or lipopolysaccharide (LPS, 1 μg/ml) for 24 hours, normalised to the mRNA level of 'house-keeping' gene (GAPDH).

response to both dsRNA/TLR3 and LPS/TLR4 signalling in HT-29 cells (*Schneeman* et al., 2005). By contrast, dsRNA but not LPS increased the mRNA level for TLR3 and TLR4 (Figure 12A), although the protein expression of both receptors was enhanced by the respective ligands; this suggested that TLR4 could have been transported to the cell surface from intracellular stores.

A binding site for NF-κB in the previously discussed intron 1 enhancer of the pIgR gene (Figure 8) was shown by reporter assays with differently mutated cDNA constructs from the PIGR locus to be critical for transcriptional activation in response to TLR3 and TLR4 signalling (Figure 12B). Analysis of several cytokine/chemokine gene products, including IL-8, demonstrated that TLR3 signalling resulted in a more pronounced pro-inflammatory response than did TLR4. These data suggested that signalling through TLR4 up-regulates pIgR expression while minimising initiation of inflammation.

Altogether, epithelial TLR engagement by microbes and their products might serve to augment pIgR expression (Figure 11, right panel) and thereby enhance export of SIgA, thus linking the innate and adaptive immune responses to viruses and bacteria. The differential epithelial activation induced via TLR3 and TLR4 implies that particularly the latter receptor may promote IgA-mediated homeostasis in the presence of commensal Gram-negative bacteria.

Experiments in mice have suggested that also other epithelial TLRs are involved in maintenance of intestinal homeostasis (*Rakoff-Nahoum* et al., 2004; *Bambou* et al., 2004). In addition, evidence is accumulating to suggest that such homeostasis is significantly influenced by cross-talk between epithelial cells and underlying lamina propria cells, particularly macrophages and DCs (*Haller* et al., 2000; *Rimoldi* et al., 2005).

Defects in innate immune mechanisms may jeoparise mucosal homeostasis

It follows from the available information that defects in innate immune mechanisms should predispose to abro-

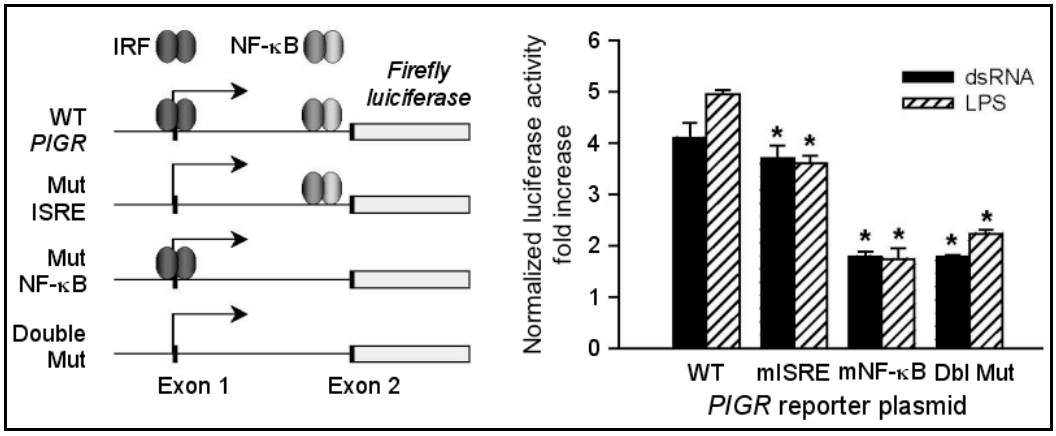

Figure 12B: Differential expression of mRNA for TLR3 and TLR4 and their ligand-induced effect on pIgR expression in HT-29 cells via the *PIGR* intronic-1 enhancer. Lusiferase reporter assay performed in HT-29 cells transiently transfected with the indicated constructs from the *PIGR* locus (left), wild-type (WT) or mutated (Mut/m), and treated for 24 hours with dsRNA (100 μg) or LPS (1 μg/ml) to observe induction of pIgR mRNA (right). Although reduced pIgR expression was observed after mutating the interferon-stimulated response element (ISRE) that binds IFN regulatory factor (IRF) in exon 1, a more striking effect was obtained by mutating the NF-KB element in the intronic enhancer, with no further effect seen after double (Dbl) mutation of the two elements. Asterisks indicate significant differences from untreated cells ($p<0.05$). Based on data from *Schneeman* et al. (2005).

gated mucosal homeostasis. The best proof of principle in this respect has been provided by the NOD2 family of intracellular sensor molecules carrying a C-terminal leucine-rich repeat domain; its PRR activity recognises unique muramyl dipeptide (MDP) motifs of peptidoglycans from both Gram-negative and Gram-positive bacteria (*Cario*, 2005).

The NOD2 (CARD15) gene is encoded by the IBD susceptibility locus (IBD1) on chromosome 16, and certain mutations of this gene is associated with clinical subsets of Crohn's disease patients, showing a striking dose effect of mutations that affect the PPR function of the molecules (*Hugot* et al., 2001; *Ogura* et al., 2001; *Abreu* et al., 2002; *Ahmad* et al., 2002; *Cuthbert* et al., 2002; *Hampe* et al., 2002; *Mathew* and *Lewis*, 2004). These seminal observations provide strong support for the possibility that aberrant 'sensing' of the intestinal microbiota is an early pathogenic event – perhaps involving deficient induction of NF-κB-mediated activation of epithelial Paneth cells leading to insufficient defensin and cryptidin production (*Lala* et al., 2003; *Kobayashi* et al., 2005) and impaired intestinal barrier function (Figure 13A).

Imbalanced triggering of innate immunity may in fact be a common theme for disease initiation on a polygenic susceptibility background in the pathogenesis of a spectrum of clinical IBD entities. In this scenario, it has been suggested that NOD2/CARD15-gene mutations may cause deficient signalling for down-regulatory mechanisms in the gut (*Judge* and *Lichtenstein*, 2002; *Bouma* and *Strober*, 2003), which in the normal state maintain lamina propria APCs/DCs in a quiescent state (Figure 13B). One possibility is that the function of NOD2 is to limit the pro-inflammatory effects mediated by TLR2 stimulation by peptidoglycan at the APC

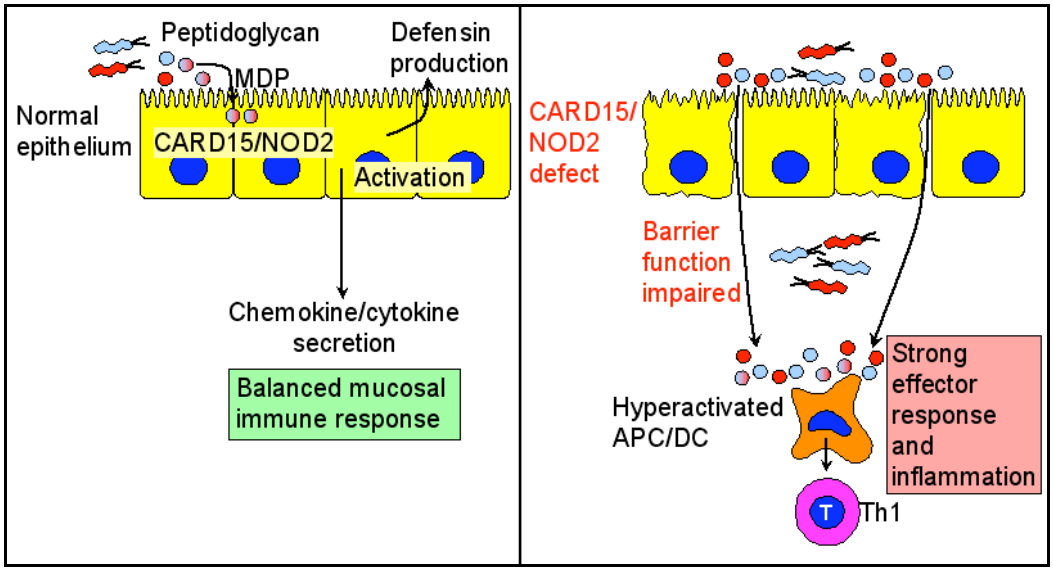

Figure 13A: Intestinal homeostasis depends on the epithelial barrier function and the balance between regulatory T (Treg) cells and Th1/Th2 effector cells as modulated by antigen-presenting cells (APCs), particularly conditioned dendritic cells (DCs). Normal epithelium senses muramyl dipeptide (MDP) motifs from Gram-positive and Gram-negative bacteria via intracellular CARD15/NOD2 molecules, which leads to activation with secretion of protective defensins and cytokines/chemokines (left). Mutations of CARD15/NOD2 may result in defective barrier function and APC hyperactivation which causes strong effector responses with mucosal inflammation (right). Modified from *Bouma* and *Strober* (2003).

surface (*Watanabe* et al., 2004); mutant NOD2 is unable to sense MDP, which leads to 'gain-of-function' for the TLR2 pathway with enhanced IL-12 and inflammation (Figure 13B).

However, this theory needs substantiation (*Cario*, 2005). It has alternatively been suggested that mutated NOD2 itself achieves gain-of-function whereby its N-terminal CARD domains become capable of activating Caspase-1 (*Maeda* et al., 2005). This enzyme may then cleave off the pro-domain of IL-1, leading to secretion of mature IL-1 which promotes the inflammatory process (Figure 13B). Admittedly, further studies are needed to discern the precise role of NOD2 in MDP recognition and subsequent intracellular signalling and cytokine/chemokine production. However, the near future will undoubtedly see a body of evidence also for other PRR mutations predisposing to intestinal inflammation.

Secondary alterations in the pIgR gene with defect transcription can furthermore result in deterioration of the intestinal barrier function, which may appear as patchy lack of pIgR/SC expression and absence of SIgA export (Figure 14). In ulcerative colitis we have observed a relationship between down-regulated pIgR/SC expression and the degree of hyperplastic epithelial lesions – overt dysplasia showing the most reduced immunohistochemical staining (*Rognum* et al., 1982). Expression of pIgR/SC protein and mRNA correlated and both variables were negatively related to the histological grade of dysplasia (*Krajci* et al., 1996), suggesting that this defect is a rather late event in the IBD process.

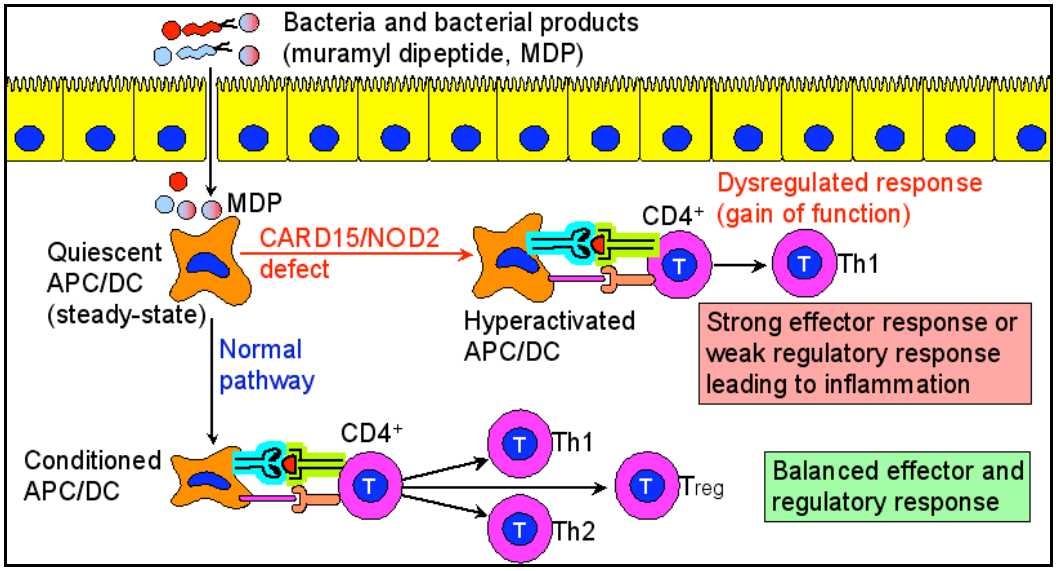

Figure 13B: Intestinal homeostasis depends on the epithelial barrier function and the balance between regulatory T (Treg) cells and Th1/Th2 effector cells as modulated by antigen-presenting cells (APCs), particularly conditioned dendritic cells (DCs). Mucosal APCs are normally quiescent and migrate in a steady state to mesenteric lymph nodes to become conditioned for induction of a balanced immune response. Mutations of CARD15/NOD2 may by different molecular mechanisms as discussed in the text, provide APCs with 'gain-of-function' that leads to disruption of the homeostatic balance between effector and regulatory cells. Modified from *Bouma* and *Strober* (2003).

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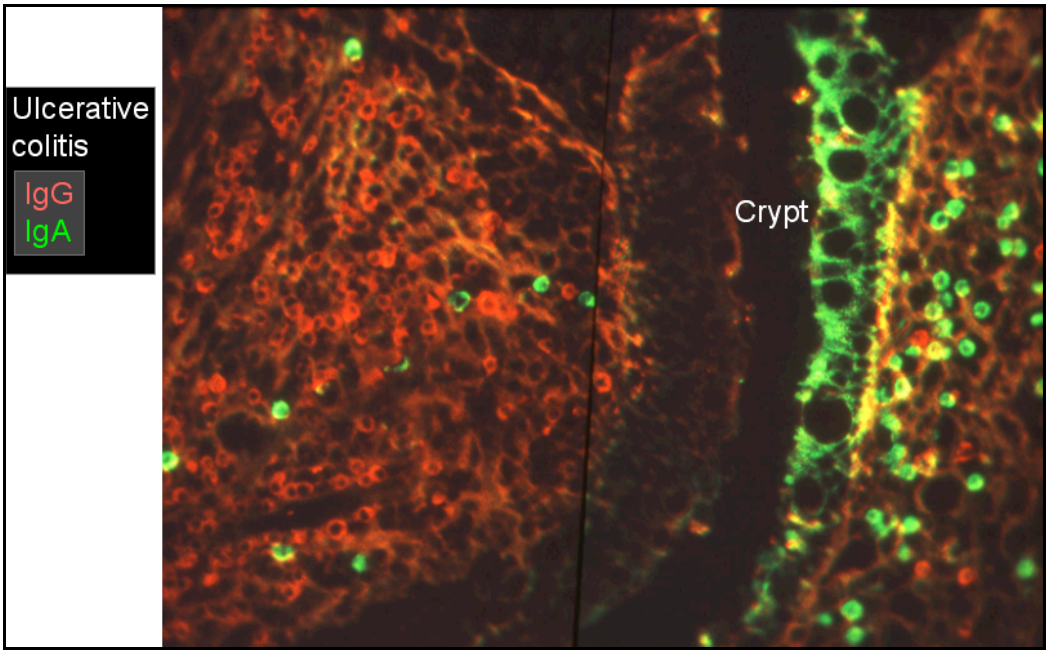

Figure 14: Patchy inflammation-induced defect in pIgR expression causes lack of epithelial IgA transport. Paired immunofluoresence staining for IgG and IgA in ulcerative colitis shows localised absence of IgA in the epithelium on one side of colonic crypt facing inflammatory infiltrate dominated by IgG-producing blasts and plasma cells. On the other side of the crypt, there is less inflammatory activity with more remaining IgA-producing cells, and the epithelium exports large amounts of IgA outside of the goblet cells which are black. Immunofluoresence photograph from the author's laboratory.

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