

Chapter 2

Intestinal Permeability and Transport of Food Antigens

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Abbreviations

ALA	Alpha-lactalbumin
APC	Antigen-presenting cell
BLG	β -lactoglobulin
CCR	CC chemokine receptor
CXCR	CXC chemokine receptor
DC	Dendritic cell
Fc ϵ RII (CD23)	Low affinity Fc receptor II for IgE
FcRn	Neonatal Fc receptor
GALT	Gut-associated lymphoid tissue
GIT	Gastrointestinal tract
IC	Immune complex
IEC	Intestinal epithelial cell
IFN- γ	Interferon gamma
ILF	Isolated lymphoid follicle
Fcgbp	Fc-gamma-binding protein
JAM-A	Junctional adhesion molecule A
LT	Leukotriene
M cells	Microfold cell

MCP	Monocyte chemoattractant protein
MHC	Major histocompatibility complex
MIIC	Major histocompatibility complex class II-enriched compartment
MLCK	Myosin light chain kinase
MLN	Mesenteric lymph node
MUC	Mucin glycoproteins
OT	Oral tolerance
PG	Prostaglandin
pIgR	Polymeric immunoglobulin receptor
PI3K	Phospho-inositol 3 kinase
PP	Peyer's patch
PRP	Pattern recognition receptor
SIgA	Secretory immunoglobulin A
RELMbeta	Resistin-like molecule beta
TEER	Transepithelial electrical resistance
TFF	Trefoil factor peptide
TGF- β	Transforming growth factor beta
Th	T helper lymphocyte
Tj	Tight junction
TLRs	Toll-like receptors
TNF	Tumour necrosis factor
Treg	T regulatory lymphocyte
Zo	Zonula occludens

Summary The intestinal epithelium forms a selective barrier, which favours fluxes of nutrients, regulates ion and water movements, and limits host contact with the massive intraluminal load of dietary antigens and microbes. However, this barrier is not fully impermeable to macromolecules; in the steady state, the transepithelial passage of small amounts of food-derived antigens and microorganisms participates in the induction of a homeostatic immune response dominated by an immune tolerance to dietary antigens and the local production of secretory immunoglobulin A. Conversely, primary or secondary defects of the intestinal barrier can lead to excessive entrance of dietary or microbe-derived macromolecules, which are putative contributors to the pathogenesis of a wide range of human diseases, including food allergy and inflammatory bowel disease, and could even be related to autoimmune diseases and metabolic syndrome. Although a majority of dietary proteins are totally degraded by digestive enzymes and are absorbed in the form of nutrients (amino acids or dipeptides/tripeptides), some can resist both the low pH of the gastric fluid and proteolysis by enzymes, providing large immunogenic peptides or even intact proteins to reach the small intestinal lumen.

2.1 Intestinal Permeability of Antigens

The intestinal epithelium forms a selective barrier, which favours fluxes of nutrients, regulates ion and water movements, and limits host contact with the massive intraluminal load of dietary antigens and microbes. However, this barrier is not fully impermeable to macromolecules; in the steady state, the transepithelial passage of small amounts of food-derived antigens and microorganisms participates in the induction of a homeostatic immune response dominated by immune tolerance to dietary antigens and the local production of secretory immunoglobulin A (SIgA). Conversely, primary or secondary defects of the intestinal barrier can lead to excessive entrance of dietary or microbe-derived macromolecules, which are putative contributors to the pathogenesis of a wide range of human diseases, including food allergy and inflammatory bowel disease, and could even be related to autoimmune diseases and metabolic syndrome [1, 2]. The intestinal barrier and more particularly the paracellular pathway have recently been reinforced and suggested as a therapeutic target to treat or prevent diseases driven by luminal antigens [1].

Dietary antigens are available for intestinal transport: Although the majority of dietary proteins are totally degraded by digestive enzymes and are absorbed in the form of nutrients (amino acids or dipeptides/tripeptides), some can resist both the low pH of the gastric fluid and proteolysis by enzymes, providing large immunogenic peptides or even intact proteins to reach the small intestinal lumen (Fig. 2.1).

The intestinal transport of molecules from the intestinal lumen to *lamina propria* can occur through two distinct mechanisms: the paracellular diffusion through tight junctions (TJs) between adjacent intestinal epithelial cells (IECs) and the transcellular transport involving endocytosis/exocytosis (transcytosis) mediated or not by membrane receptors.

2.1.1 Paracellular Transport Pathways

There are five types of gut epithelial cells: absorptive columnar cells (enterocytes), goblet, endocrine, Paneth, and M (microfold) cells. Epithelial cohesion and polarity are maintained by the apical junctional complex, which is composed of tight and adherens junctions, and by the sub-adjacent desmosomes (Fig. 2.2) [1]. TJs are located apically and represent the barrier and the rate-limiting factor for the paracellular permeation of molecules. Paracellular transport across the epithelial barrier is highly regulated and is able to leave access not only to small molecules but also to larger molecules, including small peptides and bacterial lipopolysaccharides through the ‘leak pathway’ [4]. The width of the lateral space below the TJ is around 75 Å, being wider in comparison to 4–9 Å for the TJ pores in villi or 50–60 Å for the TJ pores in crypts [5]. The other structures shown in Fig. 2.2 maintain cell proximity, but are not rate limiting as TJs. The difference in the width of TJ pores in villi and crypts also suggests a decreasing gradient of paracellular permeability from crypt to villous IECs.

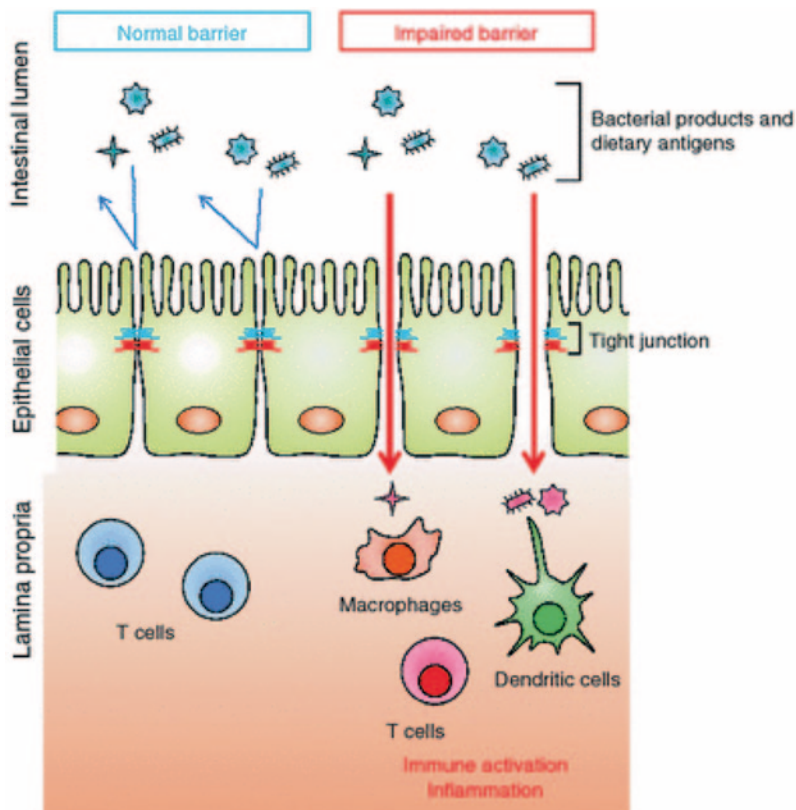


Fig. 2.1 Barrier function of intestinal tight junctions (TJs). The intestinal epithelium provides a physical barrier to luminal bacteria, toxins, and antigens. The barrier is organized by different barrier components, including the TJs. The TJs regulate the paracellular passage of ions, solutes, and water between adjacent cells. Luminal noxious macromolecules cannot penetrate the epithelium because of the TJ barrier; however, TJ barrier impairment allows the passage of noxious molecules, which can induce the excessive activation of mucosal immune cells and inflammation. Therefore, intestinal barrier defects are associated with the initiation and development of various intestinal and systemic diseases. (The figure is reprinted from Ref [3] with kind permission from Springer Science and Business Media)

TJs are multi-protein complexes mainly composed of proteins, such as membrane proteins occludin and claudins, junctional adhesion molecule A (JAM-A), and tricellulin, which control the permeability of TJs.

Occludin interacts directly with membrane-associated *zonula occludens* (ZO-1, ZO-2, and ZO-3) proteins that regulate the perijunctional actinomyosin ring. Claudins determine the charge selectivity of the paracellular pathway [6]. Claudin-2 forms cation-selective channels in the intestinal TJs. A tightening function has been described for claudin-1, -3, -4, -5, -8, -11, -14, and -19 using knockout mouse models or a range of assays. Other claudins, such as claudin-2, -7, -10, -15, and -16, are pore-forming molecules likely to increase paracellular permeability [6]. The junctional protein JAM-A is also involved in the control of mucosal homeostasis by regulating the integrity and

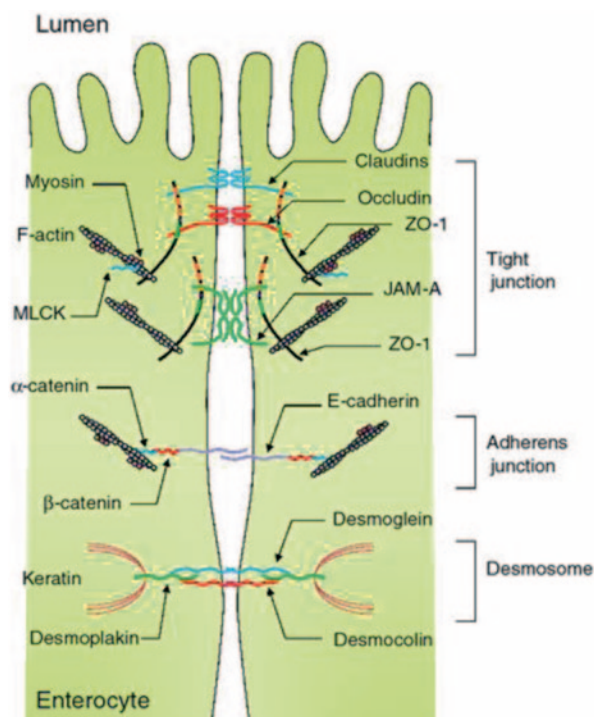


Fig. 2.2 Molecular structure of the intercellular junction of intestinal epithelial cells. The intercellular junctions of intestinal epithelial cells are sealed by different protein complexes, including tight junctions (*TJs*), adherens junctions, and desmosomes. The *TJs*, multiple protein complexes, are located at the apical ends of the lateral membranes of intestinal epithelial cells. The *TJ* complex consists of transmembrane and intracellular scaffold proteins. The extracellular loops of the transmembrane proteins (occludin, claudins, junctional adhesion molecules (*JAMs*), and tricellulin) create a permselective barrier in the paracellular pathways by haemophilic and heterophilic interactions with adjacent cells. The intracellular domains of the transmembrane proteins interact with the intracellular scaffold proteins such as zonula occludens (*ZO*) proteins and cingulin, which in turn anchor the transmembrane proteins to the actin cytoskeleton. Myosin light chain kinase (*MLCK*) is associated with the perijunctional actinomyosin rings and regulates paracellular permeability through myosin contractility. The adherens junctions along with desmosomes provide strong adhesive bonds between the epithelial cells and also intercellular communication, but do not determine paracellular permeability. (The figure is reproduced from Ref [3] with kind permission from Springer Science and Business Media)

permeability of epithelial barrier function and leukocyte migration [7]. A decreased expression of JAM-A and an increased inflammatory cytokine production have been reported in inflammatory bowel disease [8, 9]. In JAM-A-deficient mice, increased intestinal permeability is related to the upregulation of claudin-10 and claudin-15 [7].

Tricellulin has a critical role in the formation of the epithelial barrier [10]. It is an integral membrane protein contributing to the structure and function of tricellular contacts between neighbouring cells. Sodium caprate's enhancing effect on intestinal drug uptake is based on increased permeability in tricellular cell contacts, mediated by reversible removal of tricellulin from the tricellular TJ [11].

Paracellular permeability is related to the pores in the epithelial TJ determining a high-capacity, size-restricted pathway and a low-capacity, size-independent (or less restrictive) pathway that might be due to fixed (e.g. tricellular junctions, larger pores) or transient breaks (e.g. apoptosis) in the epithelial monolayer. The paracellular pathway accepts molecules with molecular mass < 600 Da (Fig. 2.1); permeation activity through this pathway can be measured from the diffusion of small inert probes (mannitol = $6.7 \text{ \AA}$, lactulose = $9.5 \text{ \AA}$). A large number of small pores together with a small number of large pores could explain the higher permeation of small-sized markers. Recent findings suggest that both small and large pores are defined by different TJ proteins, such as claudins and tricellulin, respectively. The paracellular diffusion of small molecules through TJ pores is driven by water movement due to transepithelial electrochemical or osmotic gradients.

2.1.2 Transcellular Transport Pathways

A transcellular transport pathway allows large antigenic molecules to gain access to the subepithelial compartment and to interact with local immune cells. Macromolecules can be sampled from the intestinal lumen into enterocytes by a vesicular transport (fluid phase or receptor-mediated endocytosis) and released basolaterally. M cells located in the follicle-associated epithelium of Peyer's patches (PPs) are involved in the transcytosis of bacteria. M cells express receptors on apical surfaces (Toll-like receptors, lectin-like microbial adhesins, $\alpha 5 \beta 1$ integrin, platelet-activating factor receptor, or glycoprotein-2) thus being specialized in transcytosis of bacteria. Columnar enterocytes, the major cell type in the small intestinal epithelium, can sample, transport, and/or process soluble antigens all along the intestine.

It has been shown that antigen transport through afferent lymphatics into the draining mesenteric lymph nodes (MLNs) is obligatory for oral tolerance (OT) induction. PPs are not mandatory for the development of OT. A rapid antigen uptake by dendritic cells (DCs) in the small intestinal *lamina propria* occurs after feeding mice with ovalbumin [2].

PPs seem to be dispensable for the induction of tolerance against soluble antigens: Oral administration of a soluble antigen in PP-deficient mice results in the same frequency of DCs and macrophages in MLNs, peripheral lymph nodes, and spleen [12]; knockout mice devoid of PPs, but with fully developed MLNs, are competent in establishing systemic tolerance against an antigen given by the oral route [13]. The data show that in the presence of MLNs, PPs are not required for OT induction and that the presence of MLNs is sufficient for OT induction [13]. Taken collectively, this suggests that antigen-presenting cells (APCs) loaded with antigens emanating from isolated lymphoid follicles (ILF; or from the mucosa) are sufficient to induce OT.

The transcellular transport of large particles (Fig. 2.3) has been traditionally ascribed to M cells overlying PPs and ILF in the distal part of the intestine [1]. Intestinal DCs have the capacity to sample bacteria directly in the intestinal lumen by

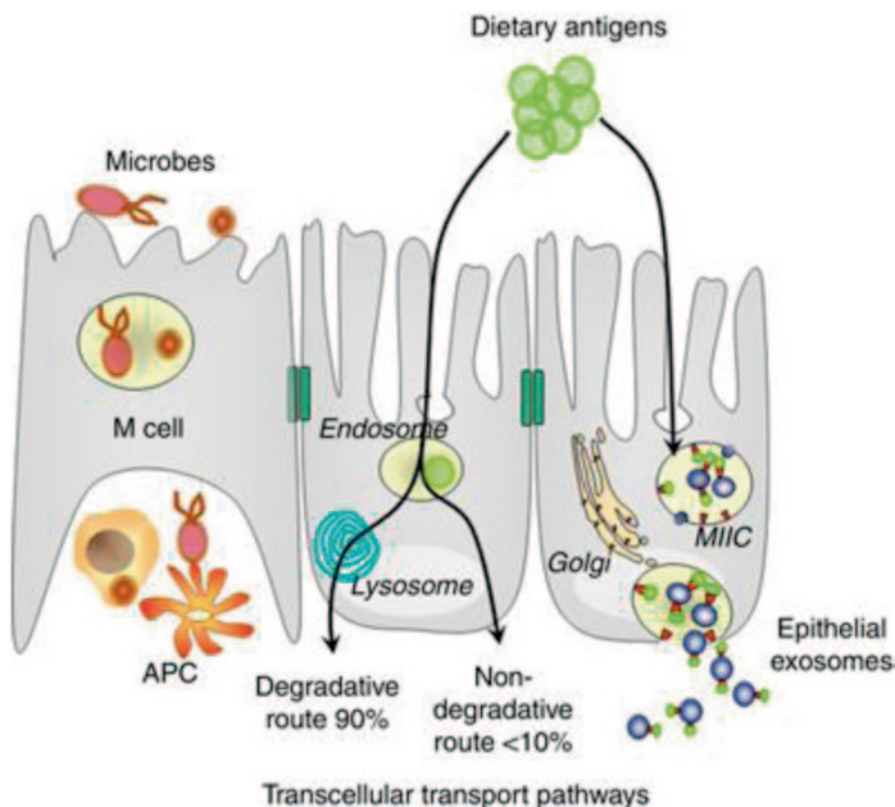


Fig. 2.3 Transcellular transport pathways. Under steady-state condition, molecules of molecular weight (MW) >600 Da (such as food antigens, peptides) are sampled by the epithelial cells by endocytosis at the apical membrane and transcytosis toward the lamina propria. During transcytosis, full-length peptides or proteins are partly degraded in acidic and lysosomal compartments and released in the form of amino acids (total degradation) or breakdown products (partial degradation) at the basolateral pole of enterocytes. Early *endosomes* containing partially degraded food antigens meet the major histocompatibility complex (MHC) class II-enriched compartment (*MIIC*) where exogenous peptides are loaded on MHC class II molecules. Inward invagination of *MIIC* compartment lead to the formation of exosomes, which are small membrane vesicles (40–90 nm) bearing MHC class II/peptide complexes at their surface. Exosomes can diffuse in the basement membrane and interact with local immune cells. Exosome-bound peptides are much more potent than free peptides to interact with dendritic cells and stimulate peptide presentation to T cells. (Figure reprinted by permission from Macmillan Publishers Ltd from Ref [1])

extending dendrites between epithelial cells. The role of M cells in the sampling of soluble antigens is not exclusive, and food antigens present in the proximal intestine can be transported through columnar enterocytes. In *ex vivo* studies of animal and human intestinal mucosa, the uptake of food antigens by IECs was associated with a powerful epithelial degradation.

Using chambers with tritiated food proteins (^3H -lysine) added to the apical compartment, transport and degradation during transcytosis could be quantified by

analysis of ^3H -labeled fragments released in the basolateral compartment. These studies indicated that only small amounts of intact protein are transcytosed, around 0.1% of luminal concentration. Epithelial cells process proteins into peptides of $\sim 1,500$ -Da molecular weight, which is a size compatible with the peptide-binding pocket of antigen-presenting major histocompatibility complex (MHC) class II molecules. Large proteins taken up by IECs were released on their basolateral side either as immunogenic peptides ($\sim 40\%$) or fully degraded into amino acids ($\sim 50\%$), with only a minor fraction crossing the epithelium in their intact form ($< 10\%$). Large peptides or intact proteins released into the *lamina propria* might then be taken up by local APCs. It is also possible that protection from the total degradation might occur during the transcytosis process.

Antigenic material may also reach the *lamina propria* within exosomes, small membrane vesicles (~ 80 nm in diameter), derived from MHC class II-expressing enterocytes. Exosomes are formed when MHC class II-loading compartments fuse with endosomes containing only partially degraded proteins (Fig. 2.3). It has been shown that exosomes can be taken up efficiently by APCs. It is uncertain whether this takes place *in vivo*, and the relative importance of this mechanism in the induction or the maintenance of OT compared with the other APCs remains difficult to evaluate.

In professional APCs, exogenous antigens are endocytosed at the cell surface and are processed in early endosomes before reaching MHC class II-enriched compartments (MIICs) where antigen-derived peptides are loaded on MHC class II molecules. In this compartment, exosomes are formed by inward invagination of the MIIC-limiting membrane and they carry MHC class II/peptide complexes at their surface [14]. MIICs can either be directed to the lysosomal compartment or fuse with the plasma membrane and release exosomes into the extracellular medium.

Epithelium-derived exosomes may be potent vehicles involved in the intestinal antigen presentation. *In vitro* studies have shown that peptides released when bound to exosomes interacted very efficiently with DCs and could potentially stimulate antigen-specific T cell clones at much lower concentration than free peptides [15]. *In vivo* administration of epithelial exosomes can prime an immunogenic or a tolerogenic immunogenic response [14]. Recent studies demonstrated that the serum or isolated serum exosomes obtained from ovalbumin-fed mice and administered intraperitoneally to naive recipient mice abrogated allergic sensitization in the recipients [16]. It also seems that *in vivo* outcome of immune stimulation by epithelium-derived exosomes depends on the nature of the APCs, and on their conditioning by epithelium-derived factors [17]. In the intestine, both the mucosal microenvironment and local effector cells are probably key players in determining the outcome of the immune response to exosome-derived epitopes.

The transcytosis of food antigens occurs primarily by a fluid-phase endocytosis of proteins at the apical membrane of enterocytes; however, under different circumstances, pathogenic antigens can access the mucosa through the expression of immunoglobulin (Ig) receptors at the apical surface of enterocytes, thereby allowing their entry in the form of immune complexes (ICs).

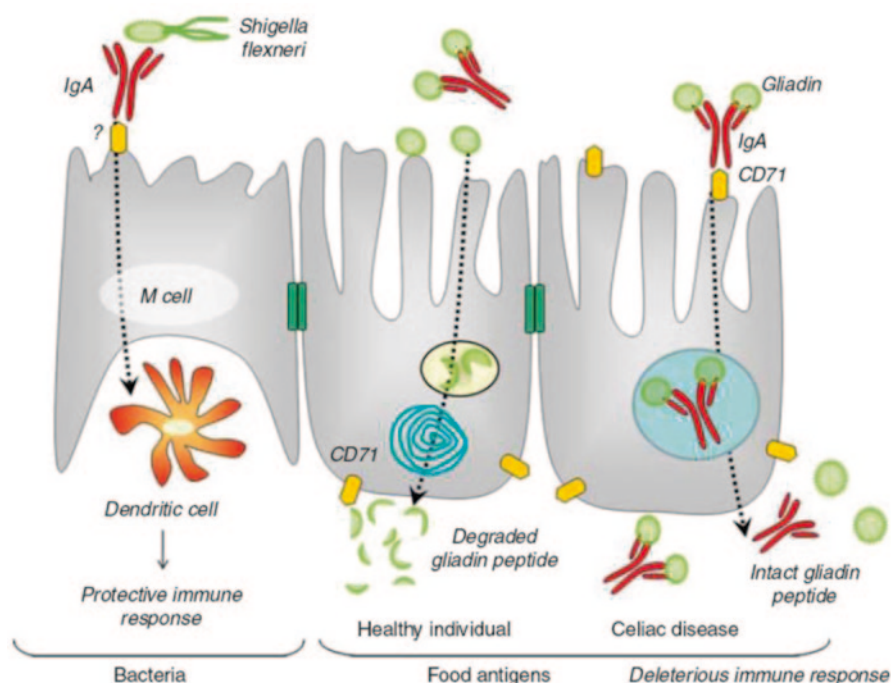


Fig. 2.4 Immunoglobulin (Ig)A-mediated retrotransport of luminal antigens. IgA is a protective mucosal immunoglobulin secreted in the intestinal lumen through polymeric immunoglobulin receptor (pIgR) in the form of secretory IgA (SIgA). Whereas the major role of SIgA is to contain microbial and food antigens in the intestinal lumen, in some pathological situations, an abnormal retrotransport of SIgA immune complexes can allow bacterial or food antigens entry in the intestinal mucosa, with various outcomes. Indeed, SIgA can mediate the intestinal entry of SIgA/*Shigella flexneri* immune complexes through M cells and interact with dendritic cells, inducing an inflammatory response aimed at improving bacterial clearance and the restoration of intestinal homeostasis. In celiac disease, however, SIgA allows the protected transcytosis of gliadin peptides, a mechanism more likely to trigger exacerbated adaptive and innate immune responses in view of the constant flow of gluten in the gut and to precipitate mucosal lesions. Indeed, whereas in healthy individuals, undigested gliadin peptides are taken up by non-specific endocytosis in enterocytes and entirely degraded/detoxified during transepithelial transport, in contrast, in active celiac disease, the ectopic expression of CD71 (the transferrin receptor also known as IgA receptor) at the apical membrane of epithelial cells favours the retrotransport of IgA immune complexes and inappropriate immune responses. (Figure reprinted by permission from Macmillan Publishers Ltd from Ref [1])

2.1.3 Antigen Transcytosis Through Immune Complexes

IgA-Mediated Transport IgA is the most representative Ig isotype at the mucosal surface. A common receptor-mediated IgA transport mechanism in the intestine is the basal-to-apical secretion of dimeric IgA in the form of secretory SIgA through the polymeric Ig receptor [18]. The major role of SIgA is to bind and keep microbial and food antigens in the intestinal lumen (Fig. 2.4). Although SIgAs are mainly

devoted to restricting the entry of exogenous antigens in the intestinal mucosa, some cases of apical-to-basal retrotransport have been reported. SIgA also triggers migration of DCs to the T cell-rich regions of PPs, and regulates expression of CD80 and CD86 on DCs in PPs, MLNs, and spleen. These results provide evidence that mucosal SIgA re-entering the body exerts a function of Ag delivery that contributes to effector and/or regulatory pathways characteristic of the intestinal mucosal compartment [19]. In some pathological situations, an abnormal retrotransport of SIgA ICs can allow bacterial or food antigens entry into the intestinal mucosa. This retrotranscytosis of SIgA–gliadin complexes may promote the entry of harmful gliadin peptides into the intestinal mucosa, thereby triggering an immune response and perpetuating intestinal inflammation [20]. This mechanism is likely to trigger an exacerbated immune response due to a constant flow of gluten in the gut. In healthy individuals, undigested gliadin peptides are taken up by non-specific endocytosis in enterocytes and degraded during transepithelial transport. However, in active celiac disease, the expression of CD71 (the transferrin receptor, or IgA receptor) at the apical membrane of epithelial cells favours the retrotransport of IgA ICs and inappropriate immune responses. The presence of large aggregates of gliadin-specific IgA in duodenal secretions, *lamina propria*, and serum of celiac patients could provide a danger signal promoting the rupture of OT and/or triggering tissue damage [20, 21].

IgE-Mediated Allergen Transport In food allergy, the low-affinity receptor for IgE, (FcεRII, CD23), is abnormally overexpressed in IECs [22]. Allergens complexed with IgE can bypass epithelial lysosomal degradation, resulting in the entry of a large amount of intact allergens into the mucosa (Fig. 2.5) [23]. High levels of interleukin (IL)-4, a Th2-type cytokine elevated in allergic diseases, upregulates the expression of CD23 at the apical side of IECs. An overexpression of CD23 at the apical side of enterocytes can drive the transport of intact IgE/allergen ICs from the intestinal lumen to the *lamina propria*. Evidence shows that IgE/CD23-mediated transport of allergens protect them from degradation in sensitized animals [24]. Large amounts of transported allergens into *lamina propria* may trigger mast cell degranulation and induce an allergic inflammatory cascade. This mechanism could participate in the rapid onset of intestinal symptoms in IgE-dependent food allergy [25].

IgG-Mediated Transport of Antigens IgGs are not classical secretory antibodies. Gastrointestinal secretions contain significant amounts of IgG suggesting their protective role [26]. Now, it is widely accepted that presence of IgG in intestinal lumen is not related to the non-specific diffusion of IgG through TJ as often suggested, but to a specific mechanism.

IgGs have initially been shown to bind the neonatal Fc receptor on IECs (FcRn) [27] in the acidic environment close to the apical membrane or in early endosomes of enterocytes (Fig. 2.5) [1]. This receptor-mediated transcytosis allows a protected transport of IgG and their release on the basal side of enterocytes where neutral pH induces their dissociation from the receptor [27]. In vitro studies have indicated that IgG ICs can also be shuttled from the apical to the basal pole of enterocytes through FcRn and vice versa [1].

Food Allergens

Biochemistry and Molecular Nutrition

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2014, XIII, 208 p. 19 illus., 9 illus. in color., Hardcover

ISBN: 978-1-4939-0840-0