

## Nature and function of gastrointestinal antigen-presenting cells

Antigen-presenting cells (APCs) of the human gut are heterogeneous, including both macrophages, a variety of dendritic cells and B cells. They are found both in gut-associated lymphoid tissue and in the mucosal lamina propria, especially beneath the surface epithelium. APCs have diverse phenotypes and therefore probably different functions in various locations; their expression levels of a variety of costimulatory molecules are most likely important for immunological decision making of stimulated T cells, either locally in the gut or in regional lymph nodes to which migrating APCs (dendritic cells) carry antigen. Thus, APCs are involved in active immunity as well as in induction of oral tolerance. However, their precise role in food allergy remains to be defined.

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Under given conditions, most cell types can present antigenic peptides to T cells. However, only macrophages, dendritic cells (DCs) and B cells constitutively express the antigen-presenting major histocompatibility complex, or MHC (HLA in humans), class II molecules as well as important costimulatory adhesion molecules and therefore are called professional antigen-presenting cells (APCs). These three cell types can take up exogenous antigens, process them in the endocytic pathway and transport immunogenic peptides in the polymorphic groove of their class II molecules to the cell surface, where the complex of “foreign plus self” is seen by the T-cell receptor (TCR) of CD4<sup>+</sup> T helper (Th) cells (Fig. 1). This is a prerequisite for induction of immunity, and only professional APCs can normally activate naive T cells to become memory/effector cells. Conversely, most cells of the body express MHC class I molecules, which can present endogenous peptides (e.g. derived from virus proteins in the cytosol of infected cells, or from autoantigens) to CD8<sup>+</sup> cytotoxic T cells. The genetic impact on immune responses is to a great extent exerted through the peptide-binding properties of the polymorphic grooves of class II and class I molecules, with additional influences from T-cell receptor variability and the multiple genes regulating expression of proteins involved in antigen processing, cell adhesion and cytokine/chemokine production (1).

It follows that the professional APCs are central in specific or adaptive immunity despite principally belonging to the innate or natural immune system (2, 3). Thus, APCs are able to sense “danger signals” by their pattern recognition receptors (e.g. Toll-like receptors), which can interact with molecular struc-

tures common to several infectious agents, including lipopolysaccharide (LPS) or endotoxin of Gram-negative bacteria (4), certain carbohydrates and bacterial DNA (CpG) motifs (5). Interactions via these receptors activate APCs, which thereby become efficient in presenting antigens, while their endocytic capacity may decrease (5). Because APCs also have migratory properties, especially the DCs, they largely determine the tissue site in which primary immune responses will take place (5, 6). Also, their expression level of various costimulatory molecules (e.g. CD80/CD86 or B7.1/B7.2, and CD40) will influence the differentiation process of naive T cells in terms of cytokine production – that is, a Th1 (IFN- $\gamma$ , IL-2 and TNF- $\alpha$ ) versus a Th2 (IL-4, IL-5, IL-10 and IL-13) profile. Interaction of the T cell CD28 receptor with B7.1 favors the former and, with B7.2, the latter cytokine profile (7). The Th1/Th2 paradigm is important in relation to food allergy, because IgE production as a basis for type I (atopic) hypersensitivity is highly dependent on IL-4 (Fig. 1). Also, homeostatic cross-regulation should ideally take place between the Th1 and Th2 responses (8).

Macrophages are derived from monocytes, which may also give rise to various types of DCs. Recent information suggests a remarkable DC heterogeneity, and certain types of DCs are derived from special bone marrow precursors (5). Immature DC subsets are found both in the circulation and in most peripheral tissues where, after endocytosis of exogenous antigen, they generally migrate via draining lymphatics to regional lymph nodes and perform antigen presentation there (5, 6).

### Mucosal antigen-presenting cells

In the normal gut, macrophages and DCs are preferentially localized to certain regions – that is, in the lamina propria below the surface epithelium (9) and in the T-cell zones and dome regions of Peyer's patches and other organized parts of the gut-associated lymphoid tissue (GALT). However, these cell types are remarkably rare in the membrane (M)-cell pockets within the follicle-associated epithelium (FAE) of GALT (10). In these unique immune-inductive sites, which are specialized extensions of germinal centers (10), naive and memory B cells probably represent the most important APCs (11).

APCs in various mucosal regions may differ functionally from their counterparts elsewhere, and they may be ultimate decision-makers for mucosal T-cell responses (12, 13). Thus, mucosal APCs are not only involved in active immunity, but most likely contribute to downregulatory mechanisms, as the local immune system is able to discriminate between harmful antigens (pathogens) and those which are innocuous (dietary proteins and the indigenous microbiota). Local and peripheral hypo-responsiveness, or suppression of proinflammatory immune mechanisms elicited by the latter types of antigen in the gut, is often referred to as "oral tolerance". This mucosally induced phenomenon probably has its basis in a variety of biological strategies (14), and DCs appear to be of utmost importance both because of their migratory (antigen-transporting) properties (15) and because innocuous antigens normally are unable to activate them to a high level locally (9, 13).

### Antigen-presenting cells in normal intestinal lamina propria

Dense accumulations of large mononuclear cells with variable CD68 expression, strong pan-MHC class II (HLA-DR>-DP>-DQ) expression and weak CD4 expression occur in a band-like manner beneath the surface epithelium in the normal human gut (9, 16–18). Studies in rats have shown that some of these putative APCs send dendritic processes through the basement membrane up to the epithelial tight junctions (19, 20), but in the human gut this is a rare phenomenon (unpublished observations). Nevertheless, these mucosal resident APCs probably are in transition from macrophages to true DCs (21). As reviewed elsewhere (22), such local differentiation would fit with their phenotypic characteristics *in situ* and functional properties *in vitro*.

The intestinal resident APCs are negative or barely positive for the LPS receptor CD14, but in inflammatory bowel disease (IBD) putative APCs with a high level of CD14 become recruited to the lesion (9, 22). Concurrently, upregulation of both B7.1 and B7.2 occurs, particularly the former costimulatory molecule, which is hardly seen *in situ* in the normal state, in contrast

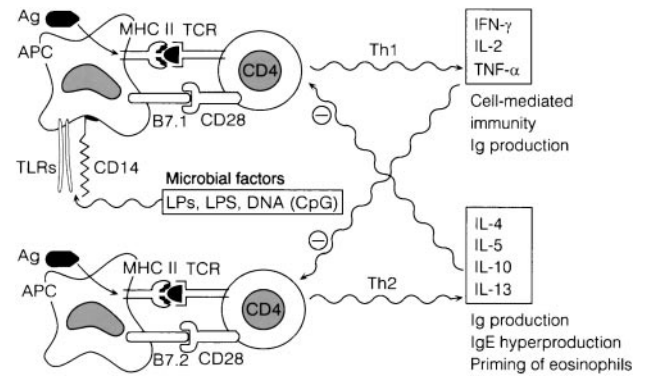


**Figure 1.** Schematic representation of polarized patterns of cytokines produced by activated T helper (Th) cells. When naive CD4<sup>+</sup> T cells are primed by antigen-presenting cell (APC), providing adequate costimulatory signals, they may differentiate to Th1 or Th2 cells. Such skewing of the immune response depends on the presence of microenvironmental factors, including cytokines and danger signals from microbial products interacting with APC receptors, thereby determining the expression level of various costimulatory signals. For simplicity, only the lipopolysaccharide (LPS) receptor CD14 and Toll-like receptors (TLRs) are indicated, together with the costimulatory molecules B7.1 and B7.2. Th1 cells produce predominantly IFN- $\gamma$ , IL-2, and TNF- $\alpha$ , while Th2 cells are mainly capable of IL-4, IL-5, IL-10 and IL-13 secretion. Distinct Th1 and Th2 patterns are further promoted by negative (-) cross-regulatory feedback loops as indicated. Ag, antigen. MHC II, major histocompatibility complex class II molecules. TCR, T-cell receptor.

to detectable levels of B7.2 (9). Experimental *in vivo* data have suggested that B7.2 drives induction of low-dose tolerance (23). However, it is not known how oral tolerance to innocuous antigens normally is maintained in the human gut, although an IL-10-producing T regulatory cell 1 (Tr1) subset has been implicated (24, 25), which would be different from the Th2 and Th3 (TGF- $\beta$ -producing) subsets believed to explain oral tolerance in mice (26). Altogether, it appears that the human intestinal immune system preferentially responds with a Th1 profile (Fig. 1) not only in disorders such as IBD (22) and celiac disease (27), but even in the seemingly normal state in response to food antigens (28). This also appears to be true for lamina propria T cells in the duodenal mucosa of children with cow's milk hypersensitivity (29), and might to some extent be explained by the high level of IL-12 expression observed for putative APCs in the domes of human Peyer's patches (28).

The local immune system in the airways also responds to antigen stimulation in the presence of danger signals (infection/inflammation) with a Th1 profile (5, 30), but in subjects with atopic predisposition a Th2 profile may develop as the basis for type I (atopic) IgE-mediated allergy (31). In addition to genetic predisposition (1), the mucosal allergic pathogenesis could be determined by the "lymphoid" DC type, or DC2 – recently named plasmacytoid DCs (P-DCs), which can be identified by their high level of IL-

3-receptor (CD123) in nasal mucosa (32). *In vitro*, P-DCs have been shown to drive naive T cells towards a Th2 response with IL-4 and IL-5 production (33). Interestingly, we have been unable to detect P-DCs in intestinal lamina propria, even in IBD and celiac disease (unpublished observations). The apparent inability of this DC subset to extravasate at intestinal effector sites may in part explain the Th1 dominance of immune responses in the human gut as a result of insufficient cross-regulation from Th2 responses. The paucity of human intestinal Th2 responsiveness is emphasized by the fact that there is usually no detectable IgE production at this mucosal effector site, even in food-allergic individuals with atopy (34). Thus, there may be several mechanisms other than a mucosal Th2 response which could explain allergy to dietary antigens in the human gut (35), for example recruitment of mast cells armed with IgE from mesenteric lymph nodes, type III (immune complex)-mediated reactions and type IV (delayed type) hypersensitivity (36).

Interestingly, an accumulation of CD1d<sup>+</sup> cells was recently reported to take place in the duodenal lamina propria of nonatopic adult patients with verified intolerance to cow's milk both during symptomatic and asymptomatic periods (37). Although the actual cell type was not directly identified, the authors implied that B cells were mainly responsible for the CD1d expression (37). CD1 cell surface glycoproteins are non-MHC-encoded and nonpolymorphic antigen-presenting molecules (38). CD1d can apparently act as restriction element in the presentation of glycolipid antigens to natural killer T cells (39). However, it remains unknown whether CD1d molecules contribute in the pathogenesis of hypersensitivity to food proteins by presenting such antigens, or perhaps bacterial products, to T cells.

### Antigen-presenting cells in GALT

Live and dead luminal particles are preferentially taken up by GALT via M cells scattered throughout the FAE. These unique bell-shaped cells sample antigens unspecifically or by receptor-mediated uptake and can supposedly exert some processing of antigen. However, HLA-DR expression is at best faint on intestinal M cells (40), and there is no evidence for an antigen-presenting function of this epithelial cell type. HLA-DR-expressing professional APCs constituted by CD68<sup>+</sup> macrophages and CD11c<sup>+</sup> DCs, intermingled with B and T cells, abound in human GALT immediately below the FAE (41, 42). Conversely, the M-cell pockets that represent putative sites of initial antigen encounter, contain only occasional CD68<sup>+</sup> or CD11c<sup>+</sup> APCs but are packed with B and T cells (Fig. 2), on average in similar numbers (10, 42). The dominating CD4<sup>+</sup> T cells generally are of the

CD45R0<sup>+</sup> memory phenotype and express CD69 as a sign of early activation. Interestingly, both B and T cells show some proliferative activity (42), and the predominant (>60%) memory B cells are positive for the apoptosis resistance marker Bcl-2 and are often positive for the post-germinal center marker CD27, and they express high levels of B7 (CD80/CD86). Memory T cells juxtaposed to B cells often express CD154, or CD40L (10), suggesting cognate interactions in an antigen-presenting process (Fig. 2).

Internalization of cognate antigen by B cells via their surface Ig molecules is some 10 000-fold more efficient than the nonspecific uptake performed by other APCs (43). Therefore, the M-cell pockets might provide an efficient opportunity for memory B cells to present luminal antigens to cognate T cells. This interaction might lead to IL-2 secretion, and promote T-cell survival and proliferation. Enhanced diversification of mucosal immune responses, particularly to recall antigens, can thus be visualized (Fig. 2). Evidence does suggest that human secretory IgA antibodies have broader specificity than comparable serum antibodies (44, 45), which would be important to cope with the continuous antigenic drift and the changing strains of the indigenous microbial flora.

A different result might be obtained if naive B cells,

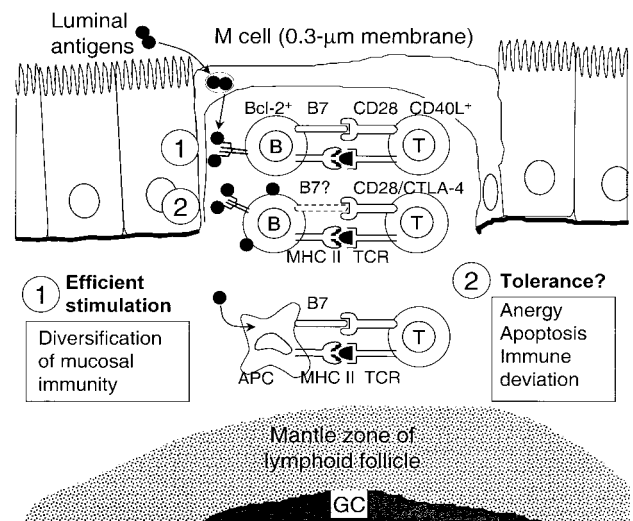


**Figure 2.** Hypothetical scheme for positive and negative immune regulation taking place in M-cell area of gut-associated lymphoid tissue. Memory B cell (Bcl-2<sup>+</sup> B7<sup>+</sup>) internalizes specific antigen efficiently and provides cognate stimulation of adjacent CD4<sup>+</sup>CD40L<sup>+</sup> T cell by antigen presentation and B7-CD28 ligation; this interaction probably leads to a diversified T-cell response that can further diversify the B-cell response (1). In competition with ordinary antigen-presenting cells (APCs), naive B cell with low or absent level of costimulatory molecules (B7?) internalizes antigen either specifically (depending on the dose?) and presents it to T cell, which might become tolerized. Interaction of B (naive or memory) and T cells via B7-CTLA-4 might also result in tolerance (2). MHC II, major histocompatibility complex class II molecules. TCR, T-cell receptor. GC, germinal center.

which lack the necessary costimulatory molecules, capture antigens in competition with a much smaller number of traditional APCs such as macrophages and DCs in the M-cell areas (Fig. 2). One possible outcome could be tolerance induction caused by T-cell anergy, depending on the differentiation of interacting T cells as well as on the nature and amount of available antigen (46). Moreover, engagement of the alternative high-affinity CD28-homologue B7 receptor, CTLA-4, which is expressed quite transiently on activated T cells, might provide inhibitory signals when the B7 levels are low (Fig. 2), possibly leading to apoptosis, anergy and downregulation of Th1- or Th2-type cytokines (11, 47). Altogether, it is tempting to speculate that the dominance of B cells over ordinary APCs in human M-cell pockets must have implications for the balance between adaptive immunity and mucosal tolerance, whereas the germinal center reaction in GALT follicles is mainly responsible for induction of memory B-cell clones, including their affinity maturation and Ig heavy chain switching (11, 48).

### Conclusions

The process of antigen presentation is central in induction and maintenance of adaptive immunity and therefore forms the basis of both specific immune defense and homeostatic tolerance mechanisms. The nature of APCs and the microenvironment in which these cells operate, will to a large extent determine the outcome of their interaction with T cells. The level of functional surface molecules expressed by APCs is crucial to this end, and reflects microenvironmental danger signals from microorganisms and other inflammatory stimuli. Intestinal epithelial cells also show antigen-presenting properties but lack essential costimulatory molecules, which may contribute to the downregulatory or suppressed immunological tone of T cells in the normal gut (49).

Although many genes necessarily are involved in hypersensitivity reactions against food components (1), the genetically determined antigen-binding groove of MHC molecules has a central position in the interaction between APCs and CD4<sup>+</sup> T cells (Fig. 1). This is highlighted in celiac disease patients who show a primary HLA class II association mainly expressed by DQ2 and to a minor extent by DQ8 (50). The major restriction element for presentation of gluten peptides to specific T cells has been identified; it is so critical in terms of peptide-binding affinity that deamidation of one particular glutamine to glutamic acid is crucial for induction of a strong T-cell response (50). The underlying deamidation of gluten may be caused by the process of baking or in the acid stomach environment, and even more efficiently by the endogenous enzyme tissue transglutaminase (50). Thus, celiac disease demonstrates how a dietary antigen can be modified both by food preparation and the digestive process, and even by an endogenous enzyme, to fit better in the polymorphic peptide-binding groove of MHC class II molecules on APCs. This illustrates an interesting relationship between exogenous antigens, endogenous factors, genes and the immune system, which under certain conditions may result in hypersensitivity rather than in oral tolerance.

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