

Uptake and presentation of orally administered antigens

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Abstract

The mucosae of the gastrointestinal tract are continuously exposed to a myriad of antigens and microorganisms that the immune system has to discriminate between dangerous and harmless. Entry of pathogenic microorganisms occurs mainly via M cells that are concentrated in the follicle-associated epithelium overlying the Peyer's Patches (PPs). M cells are very selective and do not allow entry of all microorganisms. We have recently described an additional mechanism by which dendritic cells (DCs) can monitor the contents of the intestinal lumen. DCs send dendrites outside the epithelium, like periscopes. It is not clear whether this mechanism is constitutively active or is induced in response to signals from epithelial cells that have been in contact with pathogens or high numbers of non pathogenic bacteria in the lumen. Therefore, deciphering the signals that are released by epithelial cells after the encounter with mucosal antigens is of paramount importance to understand the ability of the DCs to respond to the different antigens and to mount immune or tolerogenic responses.

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1. Introduction

Dendritic cells (DCs) are professional antigen presenting cells. They can be found in two distinct activation forms: immature and mature cells. DCs are distributed as immature cells in non-lymphoid organs and in the blood where they perform a sentinel function for incoming pathogens [1–6]. Immature DCs are characterized by the capacity to take up antigens and to phagocytose macroparticles [7,8]. During infection or inflammation, DCs are mobilized in and out of peripheral tissues [9,10] and activated DCs are targeted to secondary lymphoid organs [11,12]. Here, DCs have the unique function, among antigen presenting cells, to activate naive T cells. Moreover, it has been shown that immature DCs are able to constitutively migrate into peripheral lymphoid organs where they present endogenous antigens to T cells and induce tolerance rather than immunity [13]. Therefore, DCs play an important role both in the induction of immunity and of tolerance. The presence of DCs in intestinal mucosal tissues and especially in tissues underlying the mucosal epithelium both of intestinal villi and Peyer's Patches has been

extensively described. DCs do not play just a passive role in microbial entry; they are rather actively involved in taking up antigens. We have shown that during infection, lamina propria DCs are able to open the tight junctions between adjacent epithelial cells and to capture bacteria directly across the mucosal epithelium [14]. The epithelial barrier is preserved because DCs express tight junction proteins whose level is regulated by bacteria or bacterial products, and they establish tight junction-like structures with neighbouring epithelial cells [14]. Interestingly, lamina propria DCs can discriminate between pathogenic and non-pathogenic bacteria. In fact, when the intestine is infected with pathogenic bacteria, a large migration of DCs from the mucosa, presumably to the mesenteric node, is induced [14]. Thus, DCs play an active role in bacterial handling across mucosal surfaces, but how can DCs sense the presence of bacteria from the apical side of the mucosal epithelium and discriminate between pathogenic and commensal bacteria? Do DCs have an intrinsic ability to distinguish between dangerous and non-dangerous antigens, or is this a feature conferred by the local microenvironment? We know from previous studies that bacteria, regardless of their pathogenicity, easily activate DCs from the spleen or from the bone marrow [15]. This results in secretion of pro- and anti-inflammatory cytokines and in the upregulation of

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those molecules, which are necessary for their antigen presenting function and for cell migration [16,17]. This suggests that DCs by themselves are unable to discriminate between pathogenic and non-pathogenic bacteria. Thus, what is the role of epithelial cells that are the first cells encountering bacteria in the intestinal lumen? Can they perform a control action on DC function according to the type of encountered microorganism?

2. Contribution of epithelial cells to the activation of DCs

To address these issues, we have analyzed the role of epithelial cell-derived factors in mediating DC-epithelial cell cross-talk in terms of DC recruitment and activation. We used an in vitro co-culture model that we set up in our laboratory.

This system is particularly suitable to dissect out the contribution of epithelial cell-derived factors or bacterial products in the activation of DCs. It allows simplifying the mucosal barrier to just three players: DCs, epithelial cells and bacteria in a spatial distribution similar to that found in vivo. Thus, it can help to identify possible interactions and factors responsible for the ability or inability of DCs to 'sense' different bacterial strains or to deal with innocuous food antigens. With this system, it is possible to incubate DCs together with a monolayer of epithelial cells and to seed bacteria from the

apical surface of epithelial cells. Alternatively, DCs are incubated with supernatants of epithelial cells previously treated with bacteria. Therefore, it is possible to distinguish between soluble factors released by epithelial cells either constitutively or in response to bacteria, from the direct interaction of DCs with epithelial cells and/or bacteria.

We observed that incubation of invasive *Salmonellae*, but not of non-invasive bacteria, from the apical side of epithelial cell monolayers in the absence of DCs induced the release of pro-inflammatory cytokines, such as IL8 [18]. As we could not detect any bacteria from the lower chamber of the epithelial monolayer, this suggests that activation of epithelial cells is due either to bacteria staying intracellularly or to bacterial products translocated across the monolayer to basolateral membrane receptors. Interestingly, incubation of DCs with culture supernatant of epithelial cells treated with invasive bacteria, but not with non-invasive bacteria, induced DC activation as attested by an increased number of cells expressing high levels of activation markers, CD83 and DC-LAMP [18]. Activation of DCs could be due simply to bacterial products that have crossed the epithelial cell monolayer. However, when we incubated murine DCs that can respond to bacteria or bacterial products together with the same epithelial cells supernatants, only some cell activation was observed [18]. This correlated with the detection of traces of bacterial lipopolysaccharide (LPS). Indeed, when we incubated murine DCs derived from C3H/HeJ mice that are mutated

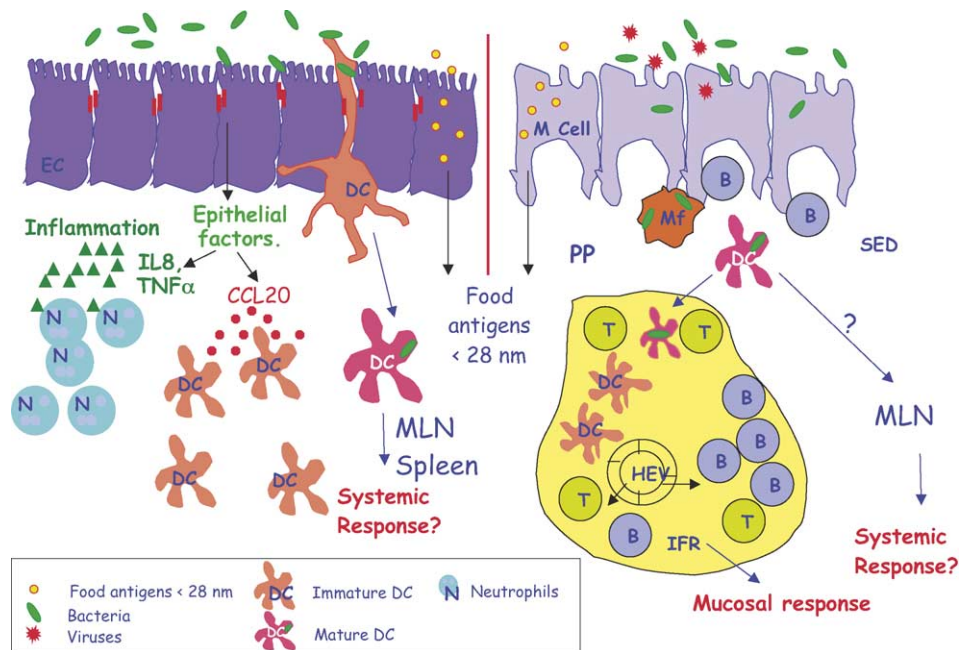


Fig. 1. Two non-mutually exclusive mechanisms of bacterial uptake have been described. The classical one (right part) is mediated by M cells. The alternative one (left part) is mediated by dendritic cells and is independent on the pathogenicity of the bacteria. The encounter of flagellated pathogens with epithelial cells induces the release of chemoattractants for CCR6-expressing immature DCs (CCL20) or for neutrophils (IL8). Bacteria entering through the M-cell dependent mechanism are eventually delivered to underlying DCs or Macrophages. It is likely that bacteria-loaded DCs residing in the subepithelial dome migrate into the interfollicular region of PP, whereas, bacteria-loaded lamina propria DCs migrate directly to the MLN or the spleen. Whether DCs from PP have access to peripheral lymph nodes is unclear. Entry of digested food antigens most likely takes place across both M cells and epithelial cells that allow entrance of particles smaller than 28 nm. It is still unclear where mucosal and systemic responses are initiated.

in the toll-like receptor 4 gene and cannot respond to LPS, DC activation was abrogated. This indicates that human DC activation that occurs only after incubation with supernatants of epithelial cells infected with invasive bacteria is primarily due to epithelial cell-derived molecules. Interestingly, both invasive and non-invasive *Salmonellae*, as long as they express flagellin, induce the release of CCL20, a chemokine responsible for the recruitment of immature DCs [18]. This is consistent with a recent report showing that flagellated *Salmonellae* can induce the release of CCL20 by epithelial cells [19]. This suggests that the presence of flagellated bacteria induces the recruitment of immature DCs regardless of their invasiveness or pathogenicity. In fact, *Bacillus subtilis*, a flagellated soil bacterium, is also able to induce CCL20 release by epithelial cells [18]. By contrast, only invasive bacteria induce DC activation. If, however, we switch to a situation in which DCs are coincubated with epithelial cells and can sense the bacteria directly across the monolayer, they are activated both by invasive and non-invasive bacteria (not shown), even by commensal bacteria as *Lactobacillus plantarum*, due to their capacity to cross the monolayer (not shown). Therefore, besides the typical M cell-dependent portal for bacterial entry (right part of Fig. 1), there is a second portal for bacterial penetration that is mediated by DCs (left part of Fig. 1).

It is still unclear what is the functional role of each mechanism, i.e. whether they are used to initiate different types of immune reactions such as mucosal or systemic. What is clear is that DCs do not act as ‘solo players’ in the induction of immune responses to ingested oral antigens. Epithelial cells, in fact, through ‘sensing’ the presence of pathogens from the intestinal lumen, can release pro-inflammatory cytokines or chemokines that control DC function. Indeed, we and others have shown that invasive *Salmonella* transduce activation signals to epithelial cells that release inflammatory chemokines as IL-8 and activating factors for DCs (Fig. 1). Moreover, both invasive and non-invasive *Salmonella*, because they express flagellin, induce epithelial cells to release CCL20 that will recruit immature DCs. The signals that promote DC migration across the monolayer after infection with non-invasive *Salmonellae* remain to be elucidated.

3. Conclusion

According to our data, DCs respond differently if they receive signals from invasive or non-invasive bacteria. In particular, invasive bacteria induce a broad DC activation primarily due to epithelial cell-derived factors, whereas non-invasive bacteria induce activation only of those DCs that they have contacted directly. It has been suggested recently that non-invasive and commensal bacteria have an immunosuppressive effect on epithelial cells [20]. It has yet to be established whether they also modulate DC functions. Our system could also be applied to determine the role of DCs in the induction of oral tolerance to food antigens. It is likely that DCs that

take up antigens in the absence of a danger signal (released by the antigen itself or by epithelial cells) can induce tolerance. It is also worth investigating the ability of different mucosal adjuvants to recruit DCs in the epithelium and to generate a pro-inflammatory environment similar to that induced by invasive bacteria in order to achieve full immuno-stimulatory capacity.

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