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Interactions of bacterial pathogens with dendritic cells during invasion of mucosal surfaces

Francesca Granucci and Paola Ricciardi-Castagnoli*

Recent studies of mucosal immunity suggest a key role for dendritic cells in the regulation of gut immune responses, in both physiological and pathological conditions. Dendritic cells are widely distributed in the lamina propria of the gut and are involved in direct bacterial uptake across mucosal surfaces, which questions the role of dendritic cells in innate mucosal responses. Approximately 400 commensal microbial species are present in the gut lumen. So how do dendritic cells distinguish pathogens from luminal microflora? Are the cytokines and chemokines induced in dendritic cells tailored to the class of microbes being recognized? Several very important questions still need to be addressed.

Addresses
Department of Biotechnology and Bioscience, University of Milano-Bicocca, Piazza della Scienza, 2–20126 – Milano, Italy
*e-mail: paola.castagnoli@unimib.it

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Abbreviations

DC	dendritic cell
IL	interleukin
MAMP	microbial-associated molecular pattern
MR	mannose receptor
PAMP	pathogen-associated molecular pattern
TGF	transforming growth factor
TLR	Toll-like receptor

Introduction

Mucosal surfaces represent a very large proportion of the surface area of the body and so are exposed to large numbers of commensal bacteria (about 10^{14} microbial cells reside in 10^{13} human cells). This commensal microflora is particularly abundant and it colonizes the large intestine, the oropharynx and the female genital tract without causing any harm; in most cases it is beneficial for the host [1]. So, what is the evolutionary advantage and specific benefit for mammals in hosting such an extensive commensal microflora? This key question still needs to be answered, although the development of the systemic immune system, in terms of circulating natural and specific antimicrobial antibodies, largely depends on the colonization of the gut [2].

The continuous interface between the mucosal surface and the luminal milieu is a central feature of the gastrointestinal tract and a key determinant in many common inflammatory disorders. The gut seems to be a privileged environment for immune regulation, mainly because of the mucosal network that involves many cells of the innate response including dendritic cells (DCs).

In recent years, using new cell markers, it has become increasingly apparent that DCs are particularly abundant at mucosal sites and are recruited during infections to the site of mucosal inflammation. DCs are bone-marrow-derived phagocytic cells [3], which are present in tissues that interface with the external environment, such as the mucosa, where they perform a sentinel function for the recognition of invading pathogens, as well as a regulatory function to control mucosal immunity.

Understanding how DCs cross-talk with pathogenic and nonpathogenic bacteria might shed some light on the control of the immune response at mucosal sites. In this review, we discuss recent observations that might have significant implications in our understanding of the role of lamina propria dendritic cells in mucosal immunity.

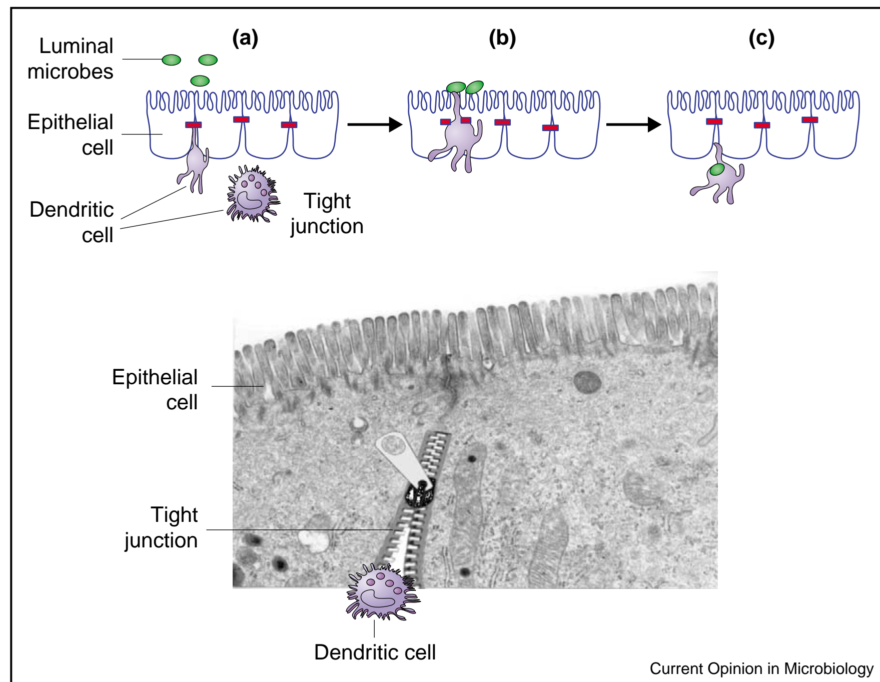
Dendritic cells in the lamina propria

In the gut, resident DCs have been described in Peyer's patches, forming a dense layer of cells in the subepithelial dome [4], beneath the follicle epithelium [5,6] and in the lamina propria [7], where they are distributed along the entire intestinal epithelium. In Peyer's patches, DCs are in close contact with M cells and co-localize with *Salmonella typhimurium* a few hours after bacterial injection into a ligated loop of the duodenum [8], indicating their key role in bacterial uptake.

Interaction of bacteria with dendritic cells

DCs take up bacteria across the mucosal epithelium in the lamina propria [9••]. This finding was initially quite surprising, as the intestinal mucosa was thought to be almost inaccessible to microbes and very small molecules (<2000 Da). This is because of both a brush border on the luminal cell surface that opposes steric hindrance and a belt of tight junctions between cells that impedes the entry of bacteria and their metabolites [10]. Thus, the entry of pathogens was believed to occur mainly through the specialized M cells, which lack an organized brush border. The penetration of M cells by bacteria requires the expression of invasin proteins [11–13]; however, *S. typhimurium* deficient in invasion genes encoded by the *Salmonella* pathogenicity island 1 (SPI1) are still able

Figure 1



Lamina propria DCs gain access to luminal microbes and maintain the integrity of the epithelial barrier. DCs open the tight junctions between epithelial cells **(a)** and uptake bacteria directly into the gut lumen **(b)**. During this process, the epithelial barrier integrity is preserved as DCs can express tight junction-like structures with the epithelium that are subsequently shut off **(c)**.

to reach the spleen following oral administration [14], suggesting an M-cell independent pathway. This pathway has recently been elucidated [9••] as an alternative mechanism for bacterial uptake in the mucosal tissues, which is mediated by DCs. In this mechanism, DCs open the tight junctions between epithelial cells, extend dendrites from the epithelium and sample bacteria directly in the gut lumen (Figure 1). The molecular mechanism that preserves the integrity of the epithelial barrier during this process is the expression and modulation of tight junction proteins, such as occludin, claudin 1, Zonula occludens 1, and junctional adhesion molecule (JAM) by DCs [9••]. Occludin is constitutively expressed on immature DCs, but becomes downregulated upon microbial stimuli. This might be sufficient to loosen the epithelial tight junction, a destabilization process that is followed by the rapid formation of new junctions between the epithelium and the infiltrating DCs, thereby preserving the integrity of the epithelial barrier.

Recognition of pathogenic and nonpathogenic bacteria by dendritic cells

How DCs distinguish pathogens from luminal microflora is still unknown. One possibility is that resident DCs, in the absence of inflammation, express an innate receptor repertoire, which interacts with still unidentified microbial-associated molecular patterns (MAMPs) to elicit an anti-inflammatory cytokine response. Conversely, pathogens expressing pathogen-associated molecular patterns (PAMPs) engage pattern-recognition receptors (PRRs). These receptors, which include the family of Toll-like receptors (TLRs), are expressed on blood-recruited DCs.

TLRs belong to a large innate receptor repertoire family that has evolved to recognize conserved molecular patterns of microbial origin not expressed in the host (Table 1). Several TLRs [15••] are involved in the recognition of various PAMPs, but the mechanism of recognition has not yet been precisely determined. The stimulation of DC TLRs by PAMPs leads to the activation of signaling pathways that result in the transcription of inflammatory cytokines and antimicrobial

Table 1

TLR innate receptor repertoire.

PAMP (ligand)	Pathogen	PRR (receptor)
LPS	Gram −ve bacteria	TLR4, TLR2/6, CD14, LBP
LTA	Gram +ve bacteria	TLR4
Lipoprotein	Eubacteria	TLR2/6
CpG	Most bacteria	TLR9
peptidoglycan	Gram +ve bacteria	TLR2/6
flagelin	Many bacteria	TLR5
ds RNA	Viruses	TLR3

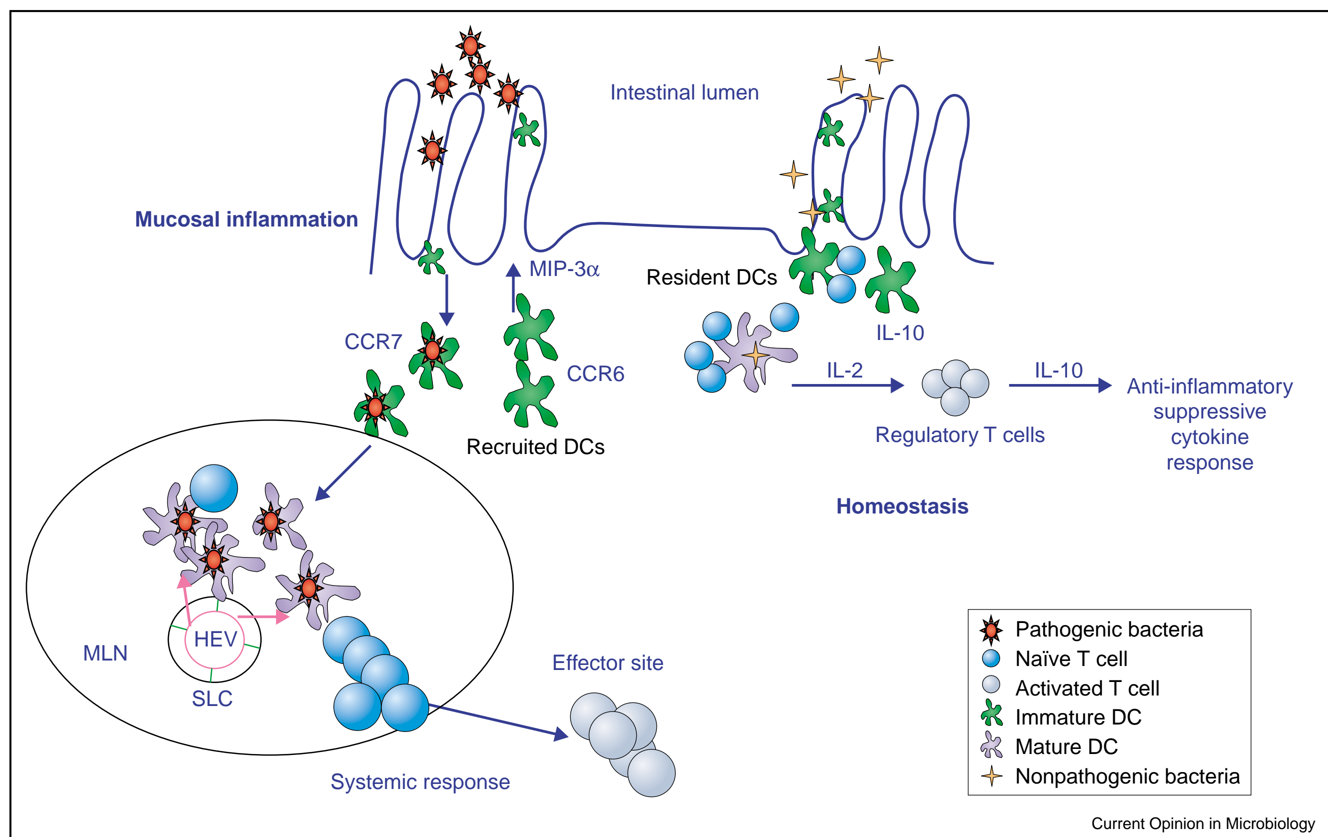
*LPS, lipopolysaccharide; LTA, lipoteichoic acid; PRR, pattern-recognition receptor.

genes. In addition, DCs express mannose receptors (MRs) that belong to the C-type lectin family and scavenger receptors, an extended receptor family [16] that has a broad specificity and interesting features in signaling. It has recently been suggested that MRs as well as DC-SIGN, another C-type lectin, could counteract TLR-mediated DC activation. How MRs or DC-SIGN signals affect DCs is not yet clear. However, it has been recently shown that glycolipid lipoarabinomannan induces an intracellular signal via DC-SIGN leading to the production of the immunosuppressive IL-10. Thus, the diverse expression and regulation of receptors of the innate repertoire by resident DCs versus blood-recruited DCs, might explain their plasticity in responding to different environmental stimuli.

Dendritic cells produce the regulatory cytokine IL-2

The interaction of *Escherichia coli* with DCs has recently been studied using a global transcriptional approach with microarray technologies [17•]. In one of these studies [18••] a kinetic analysis of approximately 11 000 genes and expressed sequence tags (ESTs) from the transcriptome, following activation with Gram-negative non-invasive bacteria (*Escherichia coli* DH5α), was performed using microarrays. Three thousand transcripts that are differentially expressed following bacterial uptake were identified. These sequences mostly encoded enzymes, transcription factors, signal transduction molecules, proteins involved in cytoskeletal rearrangements, cytokines and chemokines. Among the more unexpected genes, the IL-2 transcript was transiently upregulated at early timepoints (4–6 hours) following a bacterial encounter. This observation was validated at the protein level and confirmed using other microbial stimuli *in vitro* and *in vivo* (F Granucci, S Feau, P Ricciardi-Castagnoli *et al.*, unpublished data). IL-2 is a growth factor for T cells, B cells and natural killer (NK) cells; thus its induction and upregulation in DCs by commensal bacteria might also have a key role in innate mucosal immunity [19].

Figure 2



A possible dual role for lamina propria DCs. (**a**) During an infection with pathogenic bacteria, DCs are recruited from the blood to the inflammatory site, where they phagocytose bacteria and exert a sentinel function by initiating an immune response. The antigen is transported from the lamina propria to the mesenteric lymph nodes (MLN) through the high endothelial venules (HEVs). In the MLN, unprimed antigen-specific T cells encounter activated DCs presenting pathogens and are primed to become effector cells, which then migrate to the effector site. (**b**) In non-pathogenic conditions, tissue-resident DCs exposed to commensal bacteria produce IL-10 and IL-2, thus inducing the differentiation of regulatory T cells and the activation of the their effector functions.

To test the role of DC-derived IL-2 in activating T-cell responses, we compared the ability of wild-type and IL-$2^{-/-}$ DCs to stimulate T cells in primary mixed lymphocyte reactions. IL-$2^{-/-}$ DCs were severely impaired in their ability to induce both CD4^{+} and CD8^{+} T-cell proliferation, indicating that IL-2 is a key molecule in conferring T-cell stimulatory capacity to DCs [18••]. In addition, IL-$2^{-/-}$ mice develop an autoimmune syndrome. In these IL-$2^{-/-}$ mice, exogenous CD4^{+} regulatory T cells prevent the autoimmune disease and downregulate antibody responses against foreign antigens [20]. Moreover, IL-2 signaling is essential for regulatory T-cell function. We propose that, in addition to T cells, mucosal DCs activated by MAMPs might produce IL-2. Thus, gut DCs, which are the only cells to activate T cells under physiological conditions, might be central to the generation of regulatory T cells, which are indeed very abundant among the intraepithelial lymphocytes. These regulatory T cells are characterized by their ability to produce large amounts of IL-10, which is considered to be a typical anti-danger signal (Figure 2).

Consistent with these results, IL-$2^{-/-}$ mice lack regulatory T cells and have severe mucosal inflammation resembling colitis [21]. The disease is present in mice housed in conventional animal facilities, whereas mice housed in specific pathogen-free (SPF) conditions have a very attenuated response. Following the bacterial colonization of IL-$2^{-/-}$ mice, colitis rapidly develops, indicating a mechanism for effector T-cell activation driven by luminal bacteria.

B cells also depend on IL-2 for proliferation. It is commonly known that there are two sources of IgA in the gut: Peyer's patches and the lamina propria, where many plasma cells have been observed. An alternative pathway for the induction of IgA that is independent of IgM expression has recently been described [22,23], suggesting a requirement for other molecular signals such as TGFβ. As the response to commensal bacteria does not require either T-cell help or organized follicular lymphoid tissue, it is believed that DCs could play a role in the activation of B cell response to microbial commensal antigens as DC–B cell interactions have been described in lymphoid tissues.

A dual role for lamina propria DCs in mucosal immunity is now emerging: the resident DC population, which has regulatory functions, maintains homeostasis and suppresses immune responses against commensal bacteria; second, during inflammation, DCs expressing the chemokine receptor CCR6 are recruited from the blood in response to the macrophage inflammatory protein MIP-3α chemotactic gradient formed during inflammatory responses (Figure 2). Transport of DCs from the lamina propria to the mesenteric lymph nodes in the absence of any antigenic or inflammatory stimuli has been proposed [24], although it is difficult to prove that chemokine gradients are not formed *in vivo*.

Conclusions

Over the past few years, progress has been made in understanding the mechanisms involved in the disruption of mucosal integrity leading to gut inflammation. The unique responsiveness of mucosal DC populations to inflammatory stimuli (their rapid kinetics of recruitment is equivalent to that of neutrophils) highlights their relevance as antigen sentinels at mucosal sites. In contrast, the nature of the interaction of DCs with the intact mucosa and the luminal microflora is still poorly understood. The resident mucosal DC population might have a constitutive transcriptional signature that differs from the recruited DC population, but this still needs to be determined.

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