Interactions between Epithelial Cells and Dendritic Cells in Bacterial Handling

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The role of the intestinal microflora in the development and correct functionality of the immune system is becoming increasingly evident. A perturbation of the gastrointestinal microflora or unwanted immune responses to this flora have been demonstrated to play a critical role in the pathogenesis of inflammatory bowel disease (IBD) in experimental animal models but recently also in tumorigenesis. It has been proposed to modify the intestinal microflora via the administration of probiotics in IBD patients. In order to better understand how probiotics could be beneficial to the host, it is important to understand how bacteria are handled at mucosal surfaces and how dendritic cells and epithelial cells communicate with each other to ‘tolerate’ the intestinal flora. This article is intended to summarize recent advances on the function of gut immune cells and on some of the features that have been documented on the immunophenotypic characteristics of some probiotic strains.

Key words: dendritic cells, bacteria, epithelial cells, intestinal inflammation

The gut microflora contributes to several intestinal functions, including the development of the mucosal immune system and homeostasis, the absorption of complex macromolecules, the synthesis of aminoacids and vitamins and the protection against pathogenic microorganisms. The exact composition of the intestinal microflora is not yet defined since as many as \(10^{13} - 10^{14}\) microorganisms inhabit our gut. Only recently, new developments in molecular microbiology have allowed start identifying different species through the sequencing of the microbiome (the genome associated to the commensal flora present within the intestinal mucosa or the feces) (15, 48). From the analysis of fecal specimens of two adult individuals (29), it appears that the human genome has significantly increased the metabolic potential by acquiring new functions from the microbiota. Thus human beings can be viewed as superorganisms where their genetic material is complemented with that of the microbiota to carry out complex metabolic functions. However, microbiota differ greatly between individuals and within the same individual between different segments of the gut, suggesting that the microflora is in continuous evolution. Whether this is due to specific requirements that can be provided by some microbes and not by others or whether the mucosal immune system plays a role in shaping the intestinal flora remains an open question. There are bacteria that are more persistent as they attach to mucosal surfaces, whereas others are rapidly transiting (79), but both of them seem to undergo profound changes in IBD patients versus healthy people (9, 78, 79, 91). It is becoming clear that overreaction to the intestinal flora is responsible for the development of inflammatory reactions, including IBD. Mice rendered genetically more susceptible to experimental colitis fail to develop the disease if raised under germ-free conditions, i.e. without commensal flora (16). The involvement of bacteria in human IBD is attested by at least four factors: 1. Antibiotics ameliorate the severity of the disease (17); 2. Genetic susceptibility to IBD has been associated with mutations in the CARD15/NOD2 (31, 34, 63), CARD4/NOD1 (55) or TLR4 (12, 23) genes that code for sensors of bacteria; 3. Proximal diversion of the fecal stream reduces inflammation in CD patients with distal ileum or colonic disease (11); IBD patients have lamina propria (LP) T cells reactive to their autologous flora, whereas normal individuals react only to heterologous flora (14). However, a correlation between IBD development and specific pathogens has not been clearly demonstrated, suggesting that similarly to mice, there is an overreaction to the patient’s own microflora. Thus it has been speculated that changes in the intestinal flora can contribute to the development of IBD leading to a recently proposed concept of dysbiosis, i.e. an unbalance between protective and harmful intestinal bacteria (93).

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But what is the effect of changes in the composition of microflora?

The close interaction between bacteria and mucosal surfaces can have several effects. The interaction of epithelial cells (ECs) with the commensal bacteria can play a non-inflammatory role. Bacteroides thetaiotamicron or non-virulent mutants of Salmonella typhimurium can interfere with the activation of NF-κB that is downstream of Toll-like receptor (TLR) signaling either by triggering binding of peroxisome-proliferator-activated receptor γ (PPAR-γ) with the NF-κB subunit Rel-A in the nucleus (42) or by blocking the degradation of IkBa, an intracellular inhibitor of NF-κB (61).

Therefore, the induction of an inflammatory response in ECs depends on the ability of invasive pathogens to activate pattern recognition receptor (PRR) signaling pathways and on that of commensals to perturb the same signaling pathways. In this regard, the continuous signaling by the commensal flora via TLRs is protective against chemical induced colitis (dextran sulphate sodium, DSS-colitis) (1, 63). Altogether these data suggest that the microflora is protective rather than harmful.

**INTESTINAL BARRIER**

It is important to note that the mucosal barrier is formed by several layers of protection. The first is provided by the intestinal epithelium (59, 74). It opposes a physical, electric and chemical barrier against luminal bacteria. The permeability of the barrier is regulated by the presence of both tight junctions (TJ) between epithelial cells (ECs) and a negatively charged mucous glycocalyx. TJ seal adjacent ECs to one another and regulate solute and ion flux between cells (77). The glycocalyx sets the size of macromolecules that can reach the apical membrane of ECs (24) and opposes an electric barrier to bacteria. Finally, ECs and Paneth cells, specialized cells located at the base of the crypt of intestinal villi, release antimicrobial peptides including defensins and cathelicidins that target broad classes of microorganisms (27). The intestinal epithelial barrier is further complicated by the presence of two important cell types that are interspersed between ECs and play a crucial role in sampling the luminal content: (microfold) M cells (45) and DCs (4, 6, 43). M cells are found primarily in the follicle-associated epithelium (FAE) of Payer’s patches (PP) but they have been recently described to be scattered also among the absorptive epithelium where they could potentially transport antigens to the lamina propria (LP) (38). M cells, differently from ECs, do not have an organized brush border and are more permissive to antigen uptake (24). DCs are phagocytic cells that are scattered throughout the intestinal epithelium (53). DCs play a major role in the activation or inhibition of T cell activity and as such play a major role in controlling the development of immunity (35). It has been described that DCs are able to send dendrites out like periscopes into the lumen for bacterial uptake (67, 68). The integrity of the epithelial barrier is preserved because DCs express TJ proteins and can establish new TJ-like structure with adjacent ECs (68). These ‘creeping’ DCs are typical myeloid DCs (62, 67). The number of EC extension is greatly reduced after antibiotic treatment and is controlled by the activation of ECs via bacterial products, indicating that bacteria play a major role in their formation (8). Interestingly, DCs in CX3CL1 (fractalkine) receptor-deficient mice are unable to spread their dendrites across the epithelial barrier, indicating the involvement of CX3CL1 in driving the extension of the dendrites (62). The GALT can be divided into inductive sites where the immune response is initiated and effector sites where immune cells carry out their function (59, 60). PP, mesenteric lymph nodes (MLN) and isolated lymphoid follicles are important inductive sites for mucosal immune responses whereas the epithelium and the lamina propria of the mucosa are considered effector sites for antibody production and T cell responses.

**IMMUNE CELLS AND HOMEOSTASIS**

DCs play an active role in bacterial uptake across mucosal surfaces and have unique functions that allow the generation of mucosal immune responses. Moreover, DCs can intercalate between ECs and can interact directly with the luminal bacteria and with all the TLR ligands that are carried by commensal or pathogenic bacteria. Therefore, important questions arise: how can DCs avoid the induction of exaggerated inflammatory responses towards commensal bacteria? Is there any relationship between the unique phenotype of mucosal DCs and the regulation of gut immune homeostasis? One possibility is that DCs sense differently commensal versus pathogenic bacteria. However, rather than a difference between dangerous and non-dangerous bacteria it seems that Gram negative versus Gram positive bacteria are differentially sensed by DCs. In fact, Gram negative bacteria (including commensals) activate DCs that induce a Th1 type of response, by contrast, Gram positive bacteria are more prone to induce Th2 type of responses (83). Certain probiotics belonging to the Lactobacillus species (L. reuteri and L. casei, but not L. plantarum) can drive tolerogenic DCs (82). Therefore,
it is not possible to generalize on the possibility of DCs to sense differently commensals, but some species could participate in downmodulating DC function. It is becoming clear that the relationship between DCs and the microenvironment are profoundly affecting the functional properties of tissue DCs. This has been demonstrated in the spleen (90, 102), but there are strong evidences that a similar situation is occurring also in the gut. In fact, the ability of intestinal DCs to induce gut-tropism during T cell priming (40, 57, 86) and reactivation (58) and to promote TH2 T cell responses (2, 3, 36, 37, 59, 99), as well as IgA antibody production (76), strongly favors this hypothesis. As intestinal ECs are in close contact with DCs, they could play an active role in driving mucosal DC differentiation. This is indeed the case because ECs release constitutively thymic stromal lymphopoietin (TSLP), a molecule involved in driving TH2 differentiation by DCs (84, 85). Interestingly, DCs exposed to EC-conditioning are unable to release IL-12 and to drive TH1 type of T cell responses even after activation with TH1-inducing pathogens (Fig. 1) (71). Therefore, it is likely that resident DCs even though they have the chance to contact directly the bacteria, they are unable to activate inflammatory cells and this can help maintaining the homeostasis of the gut. In fact, nearly 70% of individuals affected by a TH1-mediated chronic inflammatory disease like in Crohn’s disease (44) have undetectable levels of TSLP and this correlates with the inability of intestinal ECs to regulate DC function (71). Therefore, resident DCs that are actively involved in taking up bacteria at steady-state do not drive inflammatory responses and this can explain why the intestinal immune homeostasis is preserved even though DCs are continuously exposed to TLR ligands. Further, DCs isolated from the lamina propria or MLN have the ability to drive T regulatory cells in a retinoic acid and TGF-β dependent way (10, 89). Regulatory T cells can control vigorous T cell proliferation and are required to maintain immune homeostasis. Several types of regulatory T cells (T regs) have been described that seem to have at least two different origins (7, 73, 95). The naturally occurring ones are characterized by the expression of CD25 and are be generated within the thymus (72) or in the gut (10, 89). A unique transcription factor, Foxp3, is required for the generation and maintenance of natural T regs, and represents their more specific marker (22, 98). The inducible T reg cells have their origin in the periphery in response to specific stimuli and are further subdivided into Tr1 that release high amounts of IL-10 and Th3 that release TGF-β (56).

Despite this propensity for the induction of TH2 and Tregs by mucosal DCs, TH1 and CTL responses are
effectively generated to mucosal pathogens and are required to fight intracellular microorganisms (28, 32, 49, 54, 96). Whether this involves the same or different DC subsets as those responsible for mucosal responses and tolerance induction, remains to be established. However, it is conceivable that resident mucosal DCs are ‘educated’ by ECs to initiate non-inflammatory responses, whereas DCs recruited after bacterial invasion might retain their ability to respond in an inflammatory mode. In fact, infection by flagellated bacteria like Salmonella spp. induces the recruitment of DCs in the intestinal epithelium (62, 68) via the release of CCL-20 by ECs (81). These non-conditioned newly recruited DCs might be responsible for the induction of TH1 responses to invasive bacteria. This hypothesis is supported by in vitro three-partite studies in which DCs were seeded from the basolateral membrane of EC monolayers shortly before apical bacterial application (71). Interestingly, due to their ability to creep between ECs and to contact bacteria directly, DCs were ‘qualitatively’ similarly activated regardless of the invasiveness or pathogenicity of the apical bacteria. Bacteria-activated DCs produced both IL-12 and IL-10 and skewed towards a TH1 phenotype (71). This suggests that non-conditioned DCs can drive the induction of inflammatory responses provided that they are not subject to EC conditioning before their encounter with bacteria. Bystander DCs that do not contact directly the bacteria are activated by EC-derived factors to non-inflammatory DCs producing IL-10 and TARC (CCL-17) and inducing or recruiting TH2 T cells, probably as a feedback mechanism to turn off the inflammatory response (70). We have now evidence that these DCs also drive T regulatory cells (Iliev et al. unpublished).

**IMMUNE CELL-BACTERIA INTERPLAY**

Given the role that plays the microflora in the development of the immune system, it is likely that also the immune system plays a role in shaping the intestinal microflora. What characterizes the mucosal immune system is the precarious balance between the induction of tolerance towards commensal bacteria and the ability to promptly respond to infectious insult by initiating a protective immune response. As described above, the mucosal immune system has the very difficult task to avoid inflammation that can generate from the continuous exposure to luminal bacteria. In the absence of concomitant activation stimuli, LP-DCs are probably involved in the induction of oral tolerance. In fact expansion of DCs in vivo enhanced tolerance induction after antigen feeding (94). It is possible that antigen-loaded DCs migrate to MLN which is the preferential site for naive T cell activation and expansion after oral feeding of soluble antigen (47). Conversely, particulate antigen, including bacteria, is most likely taken up in PP as mice lacking PP are perfectly competent to induce antibody response towards soluble but not towards particulate (microsphere) antigen (46).

The mechanisms of bacterial entrance depend on their pathogenicity. Most of the pathogens have developed strategies to penetrate ECs or to facilitate M cell invasion (for a review see (74)), whereas non-invasive bacteria can enter mucosal surfaces either through M cells or DCs. M cells can release their ‘cargo’ to underlying phagocytic cells, including DCs, that can migrate to the interfollicular region of PP for T and B cell interactions, whereas DCs that take up bacteria directly across mucosal surfaces are likely to migrate to MLN. Interestingly, MLN set the border for mucosal compartment avoiding systemic spread of commensal-loaded DCs (51). Both mechanisms do not discriminate between invasive pathogenic and non-invasive commensal bacteria. An alternative mechanism for antigen entry across a mucosal surface that also targets DCs and could be used for bacterial internalization, has been recently described (101). It is mediated by neonatal Fc receptors (FcRn) expressed by adult human (but not mouse) intestinal epithelial cells that transport IgG across the intestinal epithelial barrier, and after binding with cognate antigen in the intestinal lumen, recycles the immune complexes back to the LP (101). Antigens bound by IgG are less susceptible to degradation within the epithelial cells because endosomes formed after uptake by FcRn do not readily fuse with lysosomes. FcRn transport directs and delivers the antigens in the form of immune complexes directly to DCs lying in the LP. As DCs can be activated by immune complexes, it would be interesting to know whether DCs internalize the immune complexes via the FcγRs or via FcRn (both of which are expressed by DCs) and whether these receptors differentially affect DC function. The role of IgA and their secretory form (sIgA) in facilitating the internalization of opsonized bacteria still needs to be investigated but it is known that IgA-coated antigens although being excluded from epithelial cell binding, are facilitated in their access across M cells (97) and it has been recently shown that they are targeted directly to DCs present in the dome region of PPs (69). Finally, DCs can process antigens from apoptotic intestinal epithelial cells, both in the steady state (33) and following infection (21), which constitutes another mechanism of DC antigen uptake that directly involves interactions with the
mature DCs that are found in interfollicular T cell areas. Localized in the sub epithelial dome, below the FAE and find in the PP both immature DCs that are mainly differently from other peripheral tissues, it is possible to the FAE and T and B cell activation. Therefore, differently from other peripheral tissues, it is possible to find in the PP both immature DCs that are mainly localized in the sub epithelial dome, below the FAE and mature DCs that are found in interfollicular T cell areas.

PROBIOTICS IN DISEASE PREVENTION AND CURE

The demonstration that protective bacteria can exist and can prevent the development of colitis in genetically susceptible mice, has prompted the study of probiotics. The current definition of probiotics by FAO/WHO is: ‘Live microorganisms which when administered in adequate amounts confer a health benefit on the host’. The use of probiotics in postoperative prophylaxis as well as in patients under remission has been proposed (18, 41, 75). The beneficial effect of probiotics can be summarized as a reinforcement of the mucosal barrier against deleterious agents (20). Probiotics can increase the resistance of the epithelial barrier, compete for intestine colonization, reduce mucous degradation and modulate the immune response. These characteristics are probiotic- and strain-specific indicating that every probiotic could have a different therapeutic usage.

Studies in animal models of Inflammatory Bowel Disease (IBD) are very encouraging and suggest that probiotics can have a regulatory role on the establishment of inflammation and of colitis (41). A recent report showed that oral administration of VSL#3, a mixture of 8 different lactic acid bacteria (LAB), to mice can ameliorate chemical induced colitis (Trinitrobenzenesulfonic acid, TNBS-colitis), if administered during a remission phase, through the development of TGF-β regulatory cells (13). The clinical application of probiotics in IBD derives from the encouraging results of clinical trials using VSL#3 in prevention and treatment of pouchitis (30) or ulcerative colitis (UC) (5) or using a mixture of probiotics and prebiotics in UC (26). The efficacy of probiotics in the treatment or maintenance of remission in CD patients remains unsubstantiated (19). Very few placebo-controlled trials have been performed and most of them do not meet the requirements for statistical significance. Most of the trials have been conducted with different probiotic strains and on different study populations limiting a direct comparison. One well-controlled study using Lactobacillus casei rhamnosus (LGG) on preventing postoperative endoscopic recurrence of CD following intestinal resection of inflamed tissue has shown negative results (64). In this study, 47 well-defined patients were randomized to receive LGG or placebo and no other medications. The endoscopic recurrence rate was very similar in the two groups or even slightly higher in the probiotic group as compared to the placebo one. In 1997 Malchow compared the non-pathogenic E. coli Nissle 1917 with placebo for one year in 32 patients with active colonic CD who were also treated with standardized prednisolone schedule (52). Remission rates were similar in the two groups but a lower relapse rate was observed in the probiotic group (33% versus 64%). Whether the positive clinical outcome of this trial as compared to the one conducted by Pantera et al. is due to the different probiotic strain that has been used (E. coli Nissle 1917 versus LGG), to the enrolled population (active disease versus post-surgery) or to the different therapeutic regimen (immunosuppressants versus none) is hard to say. Also in other studies beneficial effects of different probiotics or mixtures of pro- and prebiotics could be detected in trials with a limited number of patients and without placebo control (25, 41, 75, 88, 92).

PROBIOTICS IN THE CONTROL OF EPITHELIAL CELLS/DC INTERACTION

In order to better understand how probiotics work in vivo, we decided to undertake in vitro studies on a cell culture model system that allowed us to dissect the activity of probiotics on epithelial cells, on dendritic cells or on both. The rational being that since epithelial cells are the first line of defense towards luminal microorganisms, their function could be modulated by the presence of probiotics. Further, we have recently shown that bacteria can gain access across mucosal surfaces through dendritic cells (DCs). DCs can extend dendrites across epithelial cells and sample bacteria directly from the intestinal lumen (68). However, even though mucosal DCs are exposed to activating Toll-like receptor ligands, still the inflammatory response is kept at bay. How the mucosal immune system can limit the initiation of inflammatory reactions is unknown. We show that, at steady state, ECs condition anti-inflammatory DCs through the constitutive release of Thymic stromal lymphopoietin (TSLP) (71). EC-
conditioned DCs even though phenotypically activated by bacteria lose the ability to produce interleukin-12. EC-conditioned DCs release interleukin-6, interleukin-10 but not IL-12 and polarize T cells towards a mucosal non-inflammatory T helper-2 phenotype or T regulatory cells even after a strong Th1 inducer as Salmonella Typhimurium. This control is lost in Crohn’s disease (CD) patients.

From our first analysis, we compared *S. typhimurium* (SL1344), *L. plantarum* (NCIMB882) and *L. paracasei* (B 21060) for their ability to activate human monocyte-derived dendritic cells (hMo-DCs). We found that whereas all of the bacteria were equally capable to induce the phenotypic activation of hMo-DCs in terms of CD83 upregulation, they induced three different functional activation states. *S. typhimurium* induced the release of both IL-12 and IL-10, whereas both *Lactobacillus* strains induced primarily IL-10 and much less IL-12 both at 6 and 24 hr after incubation. This had great consequences in the ability of bacteria-activated DCs to polarized naive T cells. hMo-DCs were incubated with the different bacterial strains for 1 hr in medium without antibiotics. The cells were then extensively washed to eliminate extracellular bacteria and gentamyacin was added (100 μg/ml) to kill any remaining extracellular and intracellular bacteria. The cells were then incubated with peripheral blood allogeneic purified CD45RA+CD4+ naïve T cells. 5 Days later intracellular staining for IL-4 and IFN-γ was performed on DC-activated T cells to distinguish between Th2 and Th1 T cell polarization, respectively.

The percentage of IFN-γ producing T cells was reduced after activation with DCs incubated with both *Lactobacillus* strains suggesting that the latter have reduced ability to induce Th1-polarizing DCs. In addition, *L. paracasei* (B 21060) favored the development of non-inflammatory Th2 T cells (compare 8.4% versus 4.5% in *L. paracasei* versus L. *plantarum* activated cells). This suggests that whereas *Lactobacillus* species have reduced ability to drive Th1 T cells (most likely due to lower levels of IL-12 induction by DCs), *L. Paracasei* (B 21060) is even less inflammatory as it drives the preferential development of non-inflammatory Th2 T cells.

Altogether these results suggest that among different probiotics it is possible to select strains that have non-inflammatory properties and that could be more rationally employed to treat inflammatory bowel disease. Future studies on the activity of probiotics on intestinal epithelial cells and dendritic cells in co-culture will be instrumental to further select non-inflammatory probiotics.

**CONCLUSIONS**

Probiotics can be safely used in several inflammatory diseases but further studies are needed in order to understand what is their mechanism of action. Probiotics can differ between immunostimulatory and immunomodulatory, thus a thorough analysis of the properties of each strain should be investigated in in vitro models and preclinical models before their use in humans. Clinical trials that are placebo controlled would better address the role of probiotics in the prevention or cure of gut immune related disorders.

**REFERENCES**


EPITHELIAL CELL-DENDRITIC CELL CROSSTALK


23 and IL-27 expression and enhanced Th1 development.


