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Review Article

Role of Natural Killer and Dendritic Cell Crosstalk in Immunomodulation by Commensal Bacteria Probiotics

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A cooperative dialogue between natural killer (NK) cells and dendritic cells (DCs) has been elucidated in the last years. They help each other to acquire their complete functions, both in the periphery and in the secondary lymphoid organs. Thus, NK cells' activation by dendritic cells allows the killing of transformed or infected cells in the periphery but may also be important for the generation of adaptive immunity. Indeed, it has been shown that NK cells may play a key role in polarizing a Th1 response upon interaction with DCs exposed to microbial products. This regulatory role of DC/NK cross-talk is of particular importance at mucosal surfaces such as the intestine, where the immune system exists in intimate association with commensal bacteria such as lactic acid bacteria (LAB). We here review NK/DC interactions in the presence of gut-derived commensal bacteria and their role in bacterial strain-dependent immunomodulatory effects. We particularly aim to highlight the ability of distinct species of commensal bacterial probiotics to differently affect the outcome of DC/NK cross-talk and consequently to differently influence the polarization of the adaptive immune response.

1. Introduction

Dendritic cells (DCs) and natural killer (NK) cells play a critical role in early defenses against cancer and infections, and evidence of interactions between these two cell types has accumulated in the last years [1–7]. This interaction might results in NK cell activation, DC maturation, or DC death, depending on the activation status of both cell types. Thus, the outcome of NK/DC crosstalk is likely to influence the innate as well as the subsequent adaptive immune responses [8]. This crosstalk can be promoted by pathogen-derived products that activate different innate immune cell types directly and simultaneously through their Toll-like receptors (TLRs) [9]. Indeed, DCs and NK cells have developed different, but partially overlapping, systems to identify pathogen-associated danger signals and they are, therefore, differently involved in the detection of various microorganisms.

DCs are critical for initiating immune responses against both pathogenic and nonpathogenic bacteria. In an immature stage, DCs reside in peripheral tissues, continuously sampling the microenvironment, sensing the presence of pathogens, and releasing chemokines and cytokines to amplify the immune response [10]. It has been clearly evidenced that, depending on the nature of the stimuli received, myeloid DCs can develop into different subsets that possess unique biological functions, determined by the combination of surface molecule expression and cytokine secretion [10]. In part, these different outcomes are influenced by exposure of the DCs to microbial products. Therefore, the regulatory role of DCs is of particular importance at mucosal surfaces such as the intestine, where the immune system exists in intimate association with the commensal bacteria such as lactic acid bacteria (LAB) [11]. Interestingly, recent studies have demonstrated that different strains of LAB posses the ability to finely regulate myeloid DCs maturation, polarizing

the subsequent T cell activity toward Th1, Th2, or even Treg responses [12–14].

Natural killer (NK) cells distinguish between normal healthy cells and abnormal cells by using a sophisticated repertoire of cell surface receptors [15, 16], playing a key role in the immune response to certain infections and malignancies by direct cytolysis of infected or transformed cells and by secretion of potent immune mediators [7]. Human gut-associated lymphoid tissues harbour various NK cell subsets, which are certainly involved in maintaining homeostasis between the intestinal microbiota and the mucosal immune system [17]. In addition, a human NK-like cell subset expressing NKp44 and IL-22 but lacking classic NK cells molecules such as perforin has been more recently identified [18–20]. Gut-associated NK cells might play an important role in mucosal homeostasis and protective immune responses, particularly under microbial challenge.

In addition, although evidence of a direct action of commensal bacteria, including LAB, on NK cells is still elusive, recent studies suggested that LAB-induced DC regulation might affect NK cell activity. It has been reported that DCs matured by LAB consistently induce activation and promote proliferation and cytotoxicity in autologous NK cells, and that strains of different LAB species differ importantly in their capacity to induce IFN-γ production in NK cells via DCs [14].

This review addresses NK/DC interactions in response to gut-derived LAB and the implications of LAB strain-dependent immunomodulatory effects. Finally, we discuss the potential in vivo impact of commensal bacteria on NK/DC interplay in mucosal tissues, with particular regard to the ability of distinct species of commensal bacterial probiotics to differently polarize the adaptive immune response.

2. NK-DC Interactions: Molecular Mechanisms

Several in vitro studies show a central role of DC-derived IL-12, IL-18, and type I IFN in the triggering of NK cell functions. IL-12 seems to be important to induce the secretion of IFN-γ by NK cells in several systems: LPS-activated monocyte-derived DCs, splenic DCs [21, 22], or poly(I:C)-stimulated myeloid DC [22]. IL-18 may act in synergy with IL-12 to induce the secretion of IFN-γ by NK cells but also to enhance cytotoxicity, at least when NK cells are stimulated with human CD34+ derived DCs [23]. Type I IFNs have also been shown to enhance cytotoxicity of NK cells [3, 24]. Although all types of DCs can secrete type I IFN, the main producer of these cytokines are plasmacytoid dendritic cells (pDCs), particularly when activated through TLR7 and TLR9 by virus components [25]. Nevertheless, NK cells may be activated in an IL-12-, IL-18- and type I IFN-independent manner. In fact, DCs from IL-12- and IL-18-deficient mice are able to induce IFN-γ secretion by NK cells. In mice, this capability might be under the control of IL-2 secreted by bone marrow-derived DCs activated by bacterial components [26].

IL-15 produced by mature monocyte-derived DCs appears to be particularly important to stimulate NK cell proliferation. Interestingly, this effect may require the membrane-bound form of IL-15, as the proliferation is abrogated by physical separation of DCs and NK cells [21].

Despite the large mass of data showing the role of soluble factors in NK cells activation, early studies in mice suggest the involvement of cell-to-cell contact [1]. Transwell separation of the two populations could abrogate DC-dependent NK cells' cytotoxicity induction [1]. Contact through an "immunological synapse" may be necessary for the polarized secretion of IL-12 or of other cytokines by DCs toward NK cells [27] as well as for ligand-receptor interaction [28].

Likewise, it is probably through such synaptic formations that NK cells may kill DCs. Several groups have observed that NK cells recognize and lyse monocyte-derived DCs in vitro [2, 29] in a cell-to-cell contact dependent manner. It has been described that NK/DC ratio is a critical factor to induce NK cells-mediated DC death. Whereas a low ratio (1 : 5) leads to DCs maturation, a higher NK/DC ratio (5 : 1) causes killing of immature DCs by the autologous NK cells [29]. Interestingly, DC subsets display different susceptibilities to lysis by NK cells; human pDCs were not lysed by IL-2 activated NK cells whereas mDCs isolated directly from blood underwent only a limited lysis [22].

Moreover, mature DCs are protected from NK cell lysis by acquiring a higher expression of HLA I molecules [30]. Beside the inhibitory receptors, NK cells activating receptors play a primary role in DC targeting. The activating receptor NKp30 appears to be an important candidate during this interaction, since the single blocking of this receptor inhibits NK cell-mediated lysis of immature DCs [2].

In peripheral tissues, the bidirectional crosstalk between NK cells and DCs has been proposed to play a relevant role in the mechanisms leading to the selection of DCs with maximal capability of T cell priming [4, 31]. In particular, distinct studies have demonstrated that human NK cells have the capability to induce DC maturation [22, 29, 32]. This might be important when pathogen-related molecules or inflammation are not present to drive DC maturation and, therefore, an effective antigen presentation.

The molecular mechanisms that regulate this specific part of the human NK/DC crosstalk have been also clarified. It has been found that, at low NK/DC ratio, NK-DC interactions induces cytokine production (especially, TNF-α and IL-12) by DCs as well as the upregulation of a series of molecules involved in antigen presentation. This stimulating effect may depend on cell-to-cell contact as well as TNF-α released by NK cells [29, 32].

3. Crosstalk between NK Cells and Plasmacytoid DCs

The crosstalk of NK cells with pDCs has not been investigated as much as with myeloid DCs. Remarkably, NK cells and pDCs share different specific receptors and are thus likely to respond to similar stimuli and possibly to be involved in the same phases of the innate immune response [33, 34].

Cytolytic activity of NK cells has long been known to be enhanced by IFN-α [35], and pDCs, also known as type I interferon-producing cells [36, 37], have been shown to be

required for NK cell-mediated lysis of virus-infected target cells [38].

In humans, the pDC pattern of TLR expression is profoundly different from that of myeloid DCs. pDCs do not express TLR1, 2, 3, 4, 5, or 6 but express TLR7 that recognizes viral RNA and TLR9 that recognizes CpG-rich unmethylated DNA from bacteria and DNA viruses. Signaling by TLRs expressed on pDCs drives the production of type I IFN that can directly activate antiviral responses and augment both innate and adaptive immunity to viral as well as nonviral infections [25]. Similar to NK cells [39], pDCs express TLR9. Because both NK cells and pDCs express TLR9, under appropriate conditions, they can potentially be activated by the same invading pathogen simultaneously. The abundant release of type I IFN by pDCs [40], stimulated through TLR9 [41], suggests that the NK cells/pDCs interaction can result in enhanced antiviral innate protection. Through TLR9, the NK/pDC interaction results in upregulation of NK cell-mediated cytotoxicity against various tumor cell targets [3, 24, 42]. This effect is strongly reduced by antibodies against IFN-α, thus indicating a primary role for this cytokine in regulating NK cell functions [24]. Indeed, a sharp up-regulation of CD69 surface expression, confirmed that, under these conditions, NK cells become activated. However, only the small $CD56^{bright}$ NK cell subset can proliferate in the presence of pDCs and TLR9 ligands [24]. Nevertheless, pDC-induced NK cell proliferation appears to be IL-15-independent since, differently from monocyte-derived DCs [21], surface IL-15 is not detectable in TLR-stimulated pDCs [43]. In turn, NK cells are capable of promoting pDC maturation and of up-regulating their production of IFN-α in response to CpG [22]. It is of note that, although NK cells cannot exert an editing program on pDCs, owing to the poor susceptibility of these cells to NK cell-mediated lysis, when cocultured with TLR9-stimulated pDCs, NK cells acquire lytic activity against monocyte-derived immature DCs [24].

4. Lactic Acid Bacteria and Probiotics

The gastrointestinal tract constitutes an important interface between host and environment and, as such, has the dual role of excluding pathogens while facilitating the absorption of nutrients. It is colonized by an estimated 10^{14} microbes, with the density of colonization increasing from the stomach to the distal colon. Commensal bacteria participate in both tasks of the gastrointestinal tract: some help in the absorption of otherwise indigestible nutrients, especially complex carbohydrates, and some contribute to colonization resistance, that is, the ability to inhibit colonization or overgrowth of allegedly pathogenic microorganisms by (i) producing antimicrobial substances, (ii) competing for adhesion sites and nutrients, and (iii) by stimulating the immune system.

Lactic acid bacteria (LAB) are a group of commensal bacteria characterized by their main metabolite and comprise bacteria belonging to several genera (*Lactococcus, Streptococcus, Enterococcus, Leuconostoc,* and *Lactobacillus*). Moreover, the genus *Bifidobacterium* is often mentioned in LAB contexts, although the main fermentation product of bifidobacteria is acetic acid. LAB (hereafter, comprising bifidobacteria) are predominantly nonpathogenic, Gram positive, catalase negative, nonsporeforming, facultative anaerobe, and obligate fermentative. These bacteria have traditionally been applied in food fermentations due to their lactic acid production contributing acidity and thereby prolonged conservation to the foodstuff, and to the pleasant flavor of other metabolites [44]. Some strains of lactobacilli and bifidobacteria are acid and bile tolerant and can be isolated from the mammal gastrointestinal system. In the stomach and in the upper part of the small intestine, lactobacilli outnumber other bacteria whereas bifidobacteria are mainly present in the anaerobic colon, as the dominant genus in breast-fed infants and in adulthood coexisting with many other bacterial strains [45]. As a consequence, food containing viable LAB, such as yoghurt, has gained a reputation of benefiting intestinal well-being, and LAB strains with assumed health effects have been termed "probiotic" (prolife, as opposed to antibiotic). The definition of probiotics currently employed by the World Health Organization is "live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" [46].

"Probiotic" can also designate other microorganisms, for instance certain strains of *E. coli* and *Saccharomyces cerevisiae*, but the majority of commercial probiotics are LAB [47]. The effect of LAB on intestinal health can be assigned to their ability to competitively exclude pathogenic bacteria, to degrade indigestible food components (i.e., fibre or lactose in lactose-intolerant individuals) to valuable nutrients, and finally to support epithelial cell survival and intestinal wall integrity [48].

Data from studies in gnotobiotic, that is, noncolonized rodents and rodents with a controlled microbiota, however, suggest that LAB and other non-pathogenic, so-called commensal, gut bacteria enter in a complex relationship with their animal host, benefiting health also via interactions with both the intestinal and the systemic immune system. If the immune system is not stimulated by gut bacteria after birth it does not develop completely, comprises fewer T cells (including Tregs) [49, 50], dendritic cells [51], and generates weaker antibody responses [52]. Interestingly, specific recognition of commensal microorganisms occurs only in the gut and in the mesenteric lymph nodes [53], and this microbial stimulation might skew the immune system away from the neonatally dominating Th2 response towards a balanced Th1 immune profile [54].

5. LAB as Immunomodulators

Individual species of LAB bacteria possess varying immunomodulatory properties and may finely polarize the immune response. LAB mainly exert direct effects on antigen presenting cells such as dendritic cells, monocytes, macrophages, and, to a minor extent, B-cells, without necessarily being the source of antigen. Cytokines and maturation markers induced by LAB stimulation on APC are, together with the type of antigen encountered, determinants of an ensuing T-cell response [55]. Comparison of stimulation of monocytes/macrophages with LAB as opposed to Gram-negative

bacteria has shown that LAB preferentially induce IL-12 production whereas Gram-negative bacteria such as *E. coli* mainly induce IL-10 [56, 57].

Gastrointestinal-associated lymphoid tissue (GALT) harbours two subset of DCs: myeloid DCs (mDCs), expressing CD11c, and plasmacytoid DCs (pDCs), lacking CD11c and expressing BDCA-2 and ILT-7 [58]. Since myeloid and plasmacytoid DCs express different repertoires of TLRs, they are differently involved in the detection of various pathogens [59]. LAB stimulation of mDCs yields a complicated picture, as different strains of the same genus or even species of LAB can induce different amounts of IL-10 and IL-12. In addition to these Th1-Th2/Treg polarizing cytokines, LAB stimulation frequently leads to TNF-α and IL-6 production by DCs [60, 61]. Concomitantly with induction of cytokine production, distinct LAB induce different levels of maturation in DCs. Generally, LAB induce "semimature DCs", which express lower levels of CD40, CD80, CD86 than DCs matured with pathogenic Gram-positive bacteria and Gram-negative bacteria [62], and induction of maturation markers correlates with their capacity to induce IL-12 and TNF-α. Among gut-derived LAB, *Lactobacillus*-dependent induction of surface markers varies significantly with the strain whereas most bifidobacteria induce low levels of DC maturation [14]. The mucosal microbiota might also modulate IFN-α production by pDCs [63]. Although recent data have shown that LAB do not directly trigger activation of pDCs, *in vitro* studies have suggested a modulation of type I IFN production by the mucosal microenvironment on activated pDCs [63].

How probiotics could modulate activated pDCs remains an interesting field of investigation: it is hypothesized that commensal bacteria may act through specific intracytoplasmic TLRs with the help of humoral immunity or, alternatively, another intriguing scenario may envisage the cooperation of myeloid DCs in LAB-mediated pDC regulation [64].

6. Active Components of LAB

Consensus has not been reached regarding whether bacteria have to survive passage of the gastrointestinal tract to be termed probiotic. However, a picture is emerging where live bacteria are obviously required to reach the gut to metabolize fibres and to outgrow pathogens whereas similar in vitro immunomodulatory effects of LAB can be obtained with both live and dead bacteria [13]. For some immunomodulatory effects, cell integrity is required [65], but in other cases, bacterial components are stimulatory by themselves. Candidate active molecules of LAB are DNA [66, 67] and cell wall components [68, 69]. CpG DNA is a ligand of Toll-like receptor (TLR9) and cell wall peptidoglycan, lipoteichoic acid, and lipopeptides are recognised by TLR2 in conjunction with TLR1 or TLR6 [70]. LAB-ligation of TLRs results in translocation of NFκB to the nucleus with secretion of proinflammatory cytokines (IL-1β, TNFα, and IL-6) as a consequence. IL-12 production is also likely to be a product of TLR-stimulation, as expression of the subunit IL-12p40 in DC in the lamina propria of the small intestine requires binding of NFκB [71], but also intracellular Nucleotide-binding oligomerisation domain (NOD) receptors may recognize LAB peptidoglycan [72, 73]. Regarding the suppressive cytokine IL-10, it can be induced when microbes are recognised by TLR2 or via C-type lectin receptors such as DC-SIGN [55]. It is intriguing that, apparently, small differences in LAB surface structure result in distinct DCs maturation and cytokine induction patterns, which function as a kind of "strain-fingerprint" [74], although for most LAB these mechanisms still remain poorly understood.

7. LAB Shaping NK/DC Crosstalk

Because LAB have a profound modulatory effect on DCs and because DCs in turn are potent activators of NK cells, it is reasonable to expect that DCs that have encountered LAB and undergo maturation can stimulate NK cells. This hypothesis has been recently addressed, and it is now clear that human monocyte-derived DCs [14], blood DCs [75], mouse splenic [76] and lymph node DCs [77] matured by IL-12-inducing LAB activate NK cells to produce IFN-γ, which is in accordance with the belief that IL-12 is essential for IFN-γ production in NK cells. Similarly, LAB-stimulated monocytes produce IL-12 and induce IFN-γ production in NK cells [73–75]. Interestingly, intestine-near mesenteric lymph node NK cells respond to LAB-matured DC with a stronger IFN-γ production than their spleen counterparts [77]. LAB, which do not induce IL-12 in DCs, are equally interesting, because DCs matured by these LAB do not induce IFN-γ production in NK cells, but, added together with IL-12-inducing LAB, they reduce IL-12 production by DCs and thereby IFN-γ production by NK cells. Although the ratio of IL-10/IL-12 induced by non-IL-12-inducing LAB is high, IL-10 is not responsible for this suppression [14]. Rather, LAB with different properties may compete for receptor sites including TLR2 [73]. In addition, when coculturing NK cells with DCs matured by LAB, irrespective of their IL-12-inducing capacities, increased NK cell proliferation and cytotoxicity can be observed [14]. Thus, some LAB might actively contribute to Th1 skewing via the intermediate of NK cells producing IFN-γ whereas a broader panel of LAB strains may increase NK cell number and cytotoxic potential owing to their mutual interaction with dendritic cells (Figure 1).

8. Gut-Derived LAB Induce Species-Dependent IFN-γ Release by NK Cells via DC Activation

In a study comparing three LAB (*L. acidophilus, L. reuteri, and B. bifidum*), only DCs matured by *L. acidophilus* induced high amounts of IFN-γ release by NK cells, suggesting that not all LAB have this capability [14]. It is generally accepted that IL-12 induces IFN-γ production in human NK cells [4]. IFN-γ production by NK cells is required to induce Th1 responses in lymph nodes [78], emphasizing the importance of bacterial regulation of IL-12 production in DCs. Remarkably, it is still not completely elucidated how *L. acidophilus* induces IL-12 production in DCs, although the mechanism involves recognition by TLR2 [73] and phagocytosis [79].

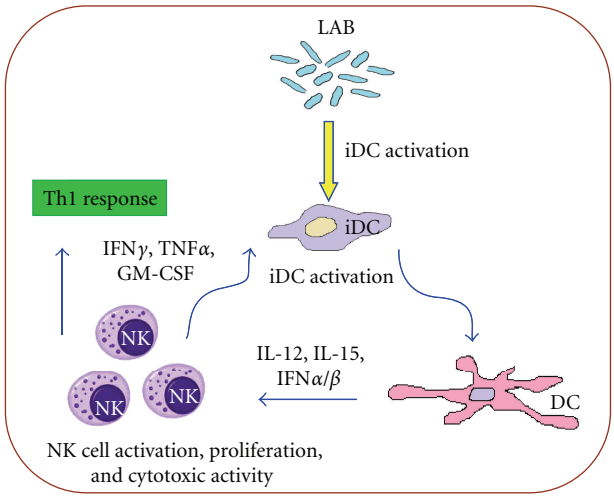

Figure 1: Bi-directional activation between dendritic cell and natural killer (NK) cells in the presence of commensal bacteria. Immature dendritic cells (iDC) are activated and matured by commensal bacteria, for example, lactic acid bacteria (LAB). These LAB-activated mature dendritic cells (DC) produce cytokines able to activate NK cell cytotoxicity and induce their proliferation. Activated NK cells can in turn, via the release of relevant cytokines, recruit (GM-CSF) and activate iDC (TNF-α and IFN-γ). Alternatively, activated NK cells can exert an *editing* of DC by killing some of the iDC. At the same time, the early release of IFN-γ by NK cells interacting with LAB-activated DC, most likely in secondary lymphoid organs such as the mesenteric lymph nodes, is critical for shaping the following adaptive immune response toward a type 1 T cell response. Remarkably, some LAB display opposite outcomes and could hamper T cell type 1 polarization.

This corresponds to Michelsen et al. [80], who showed that maturation and IL-12 production can be induced in murine DCs via TLR2 recognizing purified peptidoglycan and lipoteichoic acid. Of note, IL-12 induction in mDC by strong IL-12-inducing LAB such as *L. acidophilus* has recently been shown to be preceded and partially depend on IFN-β production by the same cells [79]. How this early IFN-β production may affect the DC's interaction with NK cells remains to be investigated.

Being non-IL-12 inducing LAB strains, *B. bifidum* and *L. reuteri* inhibit *L. acidophilus*-induced IL-12 production in DCs and, accordingly, abrogate IFN-γ production by NK cells [14]. This dominant IL-12-inhibitory property of a mixture of LAB may be of importance in the intestine where a large variety of distinct species coexists. The inhibitory components of these bacteria are seemingly soluble and may highlight that such compounds reach gut DC compartments that intact bacteria do not usually access [14, 73].

9. LAB Increasing In Vivo NK Cell Cytotoxic Potential

A number of probiotic LAB, both IL-12-inducing and non-IL-12-inducing strains, have been tested for their ability to increase in vivo NK cell activity as a measure of innate immune activity. Intervention studies have been conducted in healthy volunteers, in which the cytolytic potential of NK cells against standard tumor target cell lines has been measured at several stages of the intervention. Strong evidence of an increase in NK cell activity after probiotic supplementation has been found in elderly individuals [81–83] and in habitual smokers [84]. Often, the increment in NK cell cytolytic activity is lost when probiotic supplementation is terminated [82, 83], reflecting that probiotic bacteria hardly permanently colonize the host. DCs, and not other accessory cells, are likely to be the cell type responsible for the increase in NK cell activity, as DCs present in Peyer's patches and the lamina propria of the intestine have access to the commensal microflora and continuously migrate to mesenteric lymph nodes [84]. In a human study comparing intake of fibre and *L. casei* to reduce recurrence of colon tumors, *L. casei* administration was the preferred intervention [85]. In mice, increased cytolytic potential of NK cells after ingesting probiotic bacteria has been correlated to reduction in tumour incidence [86]. In these studies, however, in addition to a direct effect of NK cells on tumours, NK cells activation may have promoted Th1 polarization and thereby a cytotoxic T cell antitumour response. Investigators have attempted to assign the increase in NK cytolytic activity to an increase in NK cell number or in per-cell cytotoxicity, since both phenomena indeed occur [82, 87].

10. Direct Stimulation of NK Cells by LAB

Direct interaction between LAB and NK cells may occur in the epithelium, where NK cells reside among the intraepithelial lymphocytes [78]. Even if NK cells have previously been shown to be the lymphocyte population most sensitive to activation by LAB, it happens only in the presence of innate accessory cells [88]. These observations, however, do not rule out that NK cells in the gut may be able to directly detect LAB, possibly by interaction between bacterial CpG DNA and TLR9, which is present in NK cells [39]. Studying the direct effect of LAB stimulation on both polyclonally activated and nonactivated NK cells, IFN-γ production was not observed (L.N.F, unpublished data), suggesting that LAB do not interact with TLR9 on NK cells, but rather with TLR2 on accessory cells. TLR2 has been shown to be absent or expressed in low amounts in NK cells [89, 90], and its ligation only activates NK cells in the presence of exogenous IL-12 [91]. On the contrary, induction of cytotoxic activity in NK cells by LAB has been observed both in the presence and absence of accessory cells [92].

To our knowledge, only Yun and colleagues [93] have observed a direct stimulatory effect of gastro-intestinal bacteria on cytokine production by highly pure NK cells using *Helicobacter pylori*. This bacterium may interact with one of the TLRs shown to be functional in NK cells recognizing bacterial products: TLR5 that binds flagellin [94] and TLR9 that detects unmethylated CpG motifs in bacterial DNA [39], and not TLR2, which is expected to be the main receptor involved in the recognition of LAB [95].

11. Role of NK Cells in Th1 Polarization by LAB

Consumption of certain LAB has been shown to alleviate allergy [96, 97]. Matsuzaki and colleagues [98] observed that oral administration of *Lactobacillus casei*, strain *Shirota* (LcS), enhance innate immunity by stimulating the activity of splenic NK cells. Oral feeding with killed LcS was able to stimulate the production of Th1 cytokines, resulting in repressed production of IgE antibodies against ovalbumin in experimental mice. It is believed that LAB skew the allergic Th2 response to a Th1 response via the induction of IL-12 in antigen-presenting cells. Interestingly, NK cells are considered to play a key role in the induction of Th1 responses. It is not known whether NK cells secreting IFN-γ after interaction with DCs participate in Th1 polarization by LAB in vivo. Although the link between in vitro and in vivo activation of NK cells by LAB remains to be established, LAB may become a valuable tool to promote NK activation and thereby Th1 polarization, both as oral adjuvants and as stimulators of DCs exploited in immunotherapy. In addition, the presence of LAB early in life, deflecting the immune system towards a Th1 response, possibly through the intermediate NK cells, may aid in the prevention of Th2-mediated allergy. Finally, the evidence that weak IFN-γ-inducing LAB species are able to suppress the action of IFN-γ-inducing species while preserving NK cell-stimulatory activity could represent a pivotal mechanism in maintaining immunological homeostasis in the intestine or for contrasting Th1-mediated autoimmune diseases, such as Crohn's disease or even celiac disease. Moreover, LAB may represent a useful tool for modulating the cytokine balance during autoimmune diseases driven by Th17 cells in the absence of IFN-γ [99].

12. Concluding Remarks

Commensal bacteria are known to be involved in the maintenance of gut immune homeostasis, and now also emerge as potential NK cell modulators [14]. The crosstalk between NK cells and DCs suggests a critical role for NK cells in the initiation and regulation of immune responses. The considerable knowledge on the molecular basis of these cellular interactions offers opportunities for clinical intervention exploiting DC/NK cell cooperation. For instance, LAB mediate, via DC maturation, proliferation of NK cells and increase of their cytotoxicity. This indicates that LAB, similar to pathogenic microorganisms and inflammatory stimuli, allow DCs to signal to NK cells. Stimulation of NK cell proliferation and cytotoxicity can, therefore, be considered a general ability of LAB. An enlarged and more cytolytic pool of NK cells would be beneficial prophylactically in healthy individuals but also therapeutically in many disease conditions.

Purified NK cells rarely respond to commensal bacteria, and their activation apparently requires the presence of either pDCs or myeloid DCs, depending on the stimulus. A concept emerging from data regarding the crosstalk NK/pDCs is that type I IFN released by pDCs is a potent inducer of NK cell cytotoxicity, suggesting that interaction of NK cells with pDCs can result in enhanced antiviral innate protection. Furthermore, the mucosal microflora might be able to regulate IFN-α production by activated pDCs, although further research is currently highly required in this field of investigation [63, 64].

More generally, the regulatory role of DCs by LAB is of particular importance at mucosal surfaces such as the intestine, where the immune system exists in intimate association with the commensal bacteria [11]. It has been shown that different species of LAB posses the ability to finely regulate myeloid DC maturation and their interactions with NK cells, polarizing the subsequent T cell activity toward Th1, Th2, or even Treg responses [12–14]. This network can, therefore, control not only the strength and the quality of innate responses but also the subsequent adaptive responses, via both cell-to-cell interactions and cytokine release.

In conclusion, LAB potently initiate NK/DC interactions via DC maturation and, as a consequence of that, NK cells increase their cytolytic potential. However, different commensal bacteria have different effects on IFN-γ production by NK cells [14]. Since Th1-promoting LAB or their inhibitory counterpart (non-IL-12-inducing LAB) can easily be identified, these LAB strains may represent a useful tool for modulating the cytokine balance and to promote potent type-1 immune responses, as required in infection and cancer, or to contrast immune dysregulation associated to specific T cell polarization.

All these considerations provide a strong rationale for a combined targeting of NK cells and LAB-stimulated DCs in novel immunotherapeutic strategies, exploiting this cellular crosstalk not only in the treatment of intestinal inflammatory diseases but also in many other conditions for which appropriate immune interventions are required.

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