Instruction of effector T cell programs by flexible dendritic cells

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Chapter 6

Commensal Gram-negative bacteria prime human dendritic cells for enhanced IL-23 and IL-27 expression and enhanced Th1 development


Abstract

Dendritic cells (DC) are the main orchestrators of specific immune responses. Depending on microbial information they encounter in peripheral tissues, they promote the development of Th1, Th2 or unpolarized Th cell responses. In this study we have investigated the immunomodulatory effect of non-pathogenic intestinal Gram-negative (Escherichia coli, Bacteroides vulgatus, Veillonella parvula, Pseudomonas aeruginosa) and Gram-positive (Bifidobacterium adolescentis, Enterococcus faecalis, Lactobacillus plantarum and Staphylococcus aureus) bacteria on human monocyte-derived DC (moDC). None of the Gram-positive bacteria (GpB) primed for preferential Th1 or Th2 development. In contrast, despite the low levels of IL-12 they induce, all Gram-negative bacteria (GnB) primed moDC for enhanced Th1 cell development, which was dependent on IL-12 and an additional unidentified co-factor. Strikingly, GnB-matured moDC expressed elevated levels of p19 and p28 mRNA, the critical subunits of IL-23 and IL-27 respectively, suggesting that the IL-12 family members are jointly responsible for their Th1 driving capacity. Maturation of moDC with single cell wall components from GnB (i.e. LPS) or GpB (i.e. PGN and LTA) resulted in Th cell profiles that did not fit the profiles obtained with whole bacteria, and could not explain the induction of the IL-12 family members nor Th1 priming by GnB. All together, our results suggest that not only IL-12, but also the novel cytokines IL-23 and IL-27, are instrumental in driving Th1 responses and are exclusively elevated in GnB-primed moDC, indicating that the expression of the different IL-12 family members, i.e. IL-23 and IL-27, is dictated by different priming conditions of immature DC.

Submitted for publication
Commensal Gram-negative bacteria prime human DC for enhanced Th1 development

**Introduction**

The gastrointestinal tract is colonized by a large variety of different commensal bacteria. The intestinal microflora is the chief stimulus for the mucosal and systemic immune system (1-3), that is under homeostatic conditions in symbiosis with the host and supplies a role in host nutrition, intestinal permeability and protection against invasive and resident pathogens (4,5).

Specific immune responses at mucosal sites are initiated by resident immature myeloid DC, that are specialized in antigen capture and processing. Upon exposure to microbial and/or inflammatory products, DC increase their expression of MHC-II and costimulatory molecules and migrate to the draining lymph nodes where they start adaptive immune responses by presenting processed antigen to naive Th cells (6). In addition, mature DC also determine the class of immune response by instructing naive Th cells to develop into either effector Th1-, Th2 cells or a mixed phenotype by the selective expression of polarizing molecules (7). DC-derived molecules that drive the development of Th1 cells include IL-12, IL-18, IFN-\(\gamma\) and ICAM-1 (6,7). Furthermore, the novel IL-12 family members IL-23, a heterodimer consisting of the p19 and p40 subunit, or IL-27, composed of the subunits p28 and EBI3, can be important players in this respect as well (8). IL-23 functions primarily on effector T cells, prolonging and sustaining their IFN-\(\gamma\) production, whereas IL-27 has a profound effect on especially naive Th cells and is of crucial importance for the initial and early IFN-\(\gamma\) production, either alone or in synergy with IL-12 (9,10).

The Th cell polarizing capacity of mature DC is strongly dictated by the type of pathogen or reactivity product in infected tissues, that prime DC at their immature sentinel stage. Ideally, DC with the ability to promote Th1 responses will develop after exposure of immature DC to (compounds of) intracellular pathogens, like mycobacteria or viruses (reviewed in (11);(12)). Likewise, tissue factors (13), innocuous fed antigens (14), enteric pathogens (15-17) or non-pathogenic microflora bacteria may influence the Th cell polarizing capacity of gut-resident DC and thereby contribute to the class of gut-associated and systemic effector Th cell responses. Previous studies investigating the immunomodulatory capacities of intestinal microflora bacteria in human PBMC (18), human monocytes (19), mouse bone marrow-derived DC (20) or in vivo mouse models (21,22) have demonstrated differences in either APC - or T cell cytokine secretion patterns, supporting the concept that microflora bacteria can affect DC by influencing their expression levels of Th cell polarizing signals. Therefore, the aim of the present study was to investigate the modulation of Th cell instructive signals of human DC by randomly selected commensal GnB and GpB.

The results of the present study suggest that non-pathogenic intestinal GnB induce the expression of Th1 polarizing signals in human moDC. In contrast, GpB did prime for
neither Th1 nor Th2 development. Th1 development induced by GnB-primed moDC is likely to be mediated by the joint action of different IL-12 family members.

Materials & Methods

Antibodies, cytokines and reagents

Human rIL-4 (sp. act. 1x10^8 U/mg) was obtained from PBH (Hanover, Germany). Human rGM-CSF (sp. act. 1.11x10^7 U/mg) was a gift of Schering-Plough (Uden, The Netherlands). Human rIFN-γ (sp. act. 8x10^7 U/mg) and a neutralizing rabbit IgG to human IL-12 were gifts from Dr. P.H. van der Meide (U-cytech, Utrecht, The Netherlands). Human IL-18 binding protein (BP) was a gift from Amgen (Thousand Oaks, CA). Neutralizing Abs to human IL-10 were obtained from BD Pharmingen (San Diego, CA). Neutralizing sheep antisera to human type I IFN (Jivari: 450.000 neutralizing U/ml anti-IFNα plus 3.000 U/ml anti-IFNβ and Kaaeli: 30.000 U/ml anti-IFNα plus 30.000 U/ml anti-IFNβ) were gifts from Dr. I. Julkunen (National Public Health Institute, Helsinki, Finland) (23). LPS (E.coli), peptidoglycan (PGN) and lipoteichoic acid (LTA; both from S.aureus) were purchased from Sigma-Aldrich (St. Louis, MO). Plastics were purchased from Greiner Bio-one (Alphen aan de Rijn, The Netherlands).

Intestinal bacteria

The following bacteria were obtained from the Culture Collection of the University of Göteborg (Göteborg, Sweden) and cultured as described before (19): *Escherichia coli* (strain 24), *Bacteroides vulgatus* (strain 4940), *Veillonella parvula* (strain 5123), *Pseudomonas aeruginosa* (strain 5123) (all Gram-negative) and *Bifidobacterium adolescentis* (strain 18363), *Enterococcus faecalis* (strain 19916), *Lactobacillus plantarum* (isolated from healthy human gastro-intestinal mucosa; (19)), *Staphylococcus aureus* (strain 1800) (all Gram-positive). The bacteria were washed in PBS and killed by a 15 minute exposure to UV-light and stored at -80°C. Killing of the bacteria was confirmed by replating of the UV-exposed bacteria.

In vitro generation and maturation of DC from monocytes

Immature DC were generated by culture of peripheral blood monocytes (0.5x10^6 cells/well) in 24-well culture plates (Costar, Cambridge, MA) in Iscove’s modified Dulbecco’s medium (IMDM; Life Technologies Ltd., Paisley, UK) containing gentamycin (86 μg/ml; Duchefa, Haarlem, The Netherlands) and 10% FCS (Hyclone, Logan, UT), supplemented with rGM-CSF (500 U/ml) and rIL-4 (250 U/ml), as previously described (24). On day 6, maturation of iDC was induced by the maturation factors (MF) IL-1β (25 ng/ml) and TNFα (50 ng/ml) (both purchased from Peprotech, Rocky Hill, NJ) in presence or absence of 1.10^7 UV-killed bacteria/ml. After 48h, full maturation into CD83⁺ mature effector DC (mDC) was confirmed by flowcytometric analysis.
Commensal Gram-negative bacteria prime human DC for enhanced Th1 development

Analysis of cell surface molecule expression by flow cytometry

Mouse anti-human mAbs against the following molecules were used: CD80, CD86, CD14 (all purchased by BD Pharmingen), CD54 (CLB), CD1b (Diacline Research, Besançon, France), CD83 (Immunotech, Marseilles, France). Bound mAb were detected by FITC-conjugated goat F(ab')2 anti-mouse IgG and IgM (Jackson Immunoresearch Laboratories Inc., West Grove, PA).

Cytokine production by DC

Mature DC (2x10^4 cells) were stimulated with mouse CD40L-expressing mouse plasmacytoma cells (J558 cells, 2x10^4 cells; a gift from Dr. P. Lane, University of Birmingham, Birmingham, UK) in 96-well flat-bottom culture plates (Costar) in IMDM containing 10% FCS, in a final volume of 200 µl. Supernatants were harvested after 24 h and stored at -20°C until the levels of IL-12 and IL-10 secretion were measured by ELISA, as described elsewhere (24).

Real-time quantitative RT-PCR analyses of p19, p28, p40 and EBI3 mRNA

Quantitative analysis of p19, p28, p40 and EBI3 mRNA expression was performed in mature DC (5x10^4 cells) stimulated with J558 cells (5x10^4 cells) in the presence or absence of IFN-γ (1000 U/ml; to analyze mRNA expression of p19 and p40), in 96-well flat-bottom culture plates (Costar), in IMDM plus 10% FCS, for 6 hours and lysed for total RNA extraction, using a NucleoSpin RNA Isolation Kit (Macherey-Nagel, Duren, Germany). First strand cDNA was synthesized, using a cDNA-synthesis kit (MBI Fermentas, St Leon-Rot, Germany). Quantification of p19, p28, p40, EBI3 and, as a control, β2-microglobulin (B2m) transcripts was performed by real-time quantitative PCR, using a Biorad iCycler (iCycler iQ Multi-Color Real Time PCR Detection System; Biorad, Hercules, CA) based on specific primers and general SYBR green (iQ SYBR Green supermix, 2x, Biorad, Hercules, CA) fluorescence detection. The primer sequences were the following: 5’ p19 primer, TCGGCACGAGAAACAACCTGAG; 3’ p19 primer, TGGGGAACATCATTTTGATGCT; 5’ p28 primer, GCGGAAATCTCACCCTGCAG; 3’ p28 primer, CCGGAGGGTTGAATCCTGGA; 5’ p40 primer, ATTAGGTCATGGTGGATGC; 3’ p40 primer, AATGCTGCGATTTTTGCGGC; 5’ EBI3 primer, CGTGCCTTTCTACAGAGCA; 3’ EBI3 primer, GACGTAGTACCTGGCTCGG; 5’ β2m primer, AAGATTCAGGTTTACTACGTC; 3’β2m primer, TGATGCTGCTATCATGC; resulting in the amplification of PCR-products of 353 bp (p19), 285 bp (p28), 297 bp (p40) or 294 bp (β2m). The reaction protocol was identical for all PCR-products: first a three min incubation at 94°C, followed by 45 cycles of sequential incubations at 94°C (30 seconds), 60°C (30 seconds), and finally 72°C (1 minute) for data collection. A bulk cDNA sample of CD40L-stimulated human moDC was used as a standard and normalization to B2m was performed for each sample.

Isolation of naive Th cells

PBL were isolated by density gradient centrifugation on Percoll (Pharmacia), and thereafter CD45RA^-CD45RO^CD4^ Th cells were isolated to high purity (>98% as assessed
by flow cytometry) through one-step high-affinity negative selection columns (R&D Systems), according to the manufacturer’s instructions.

**Stimulation and culture of naive Th cells**

Purified naive Th cells (2×10^4 cells) were cocultured with mature DC (5×10^3 cells) in 200 µl culture medium in the presence of superantigen *Staphylococcus aureus* enterotoxin B (SEB) (100 pg/ml; Sigma), in 96-well flat-bottom culture plates (Costar). At day 5, rIL-2 (10 U/ml, Cetus Corp.) was added and the cultures were expanded for the next 7 days.

**Cytokine production by Th cells**

On day 14, the quiescent Th cells were restimulated with PMA (10 ng/ml) and ionomycin (1 µg/ml; Sigma) for 6 h, the last 5 hrs in the presence of Brefeldin A (10 µg/ml; Sigma), to determine single-cell IL-4 and IFN-γ production by intracellular flowcytometric analysis. Cells were fixed in 2% paraformaldehyde (PFA; Merck, Darmstadt, Germany), permeabilized with 0.5% saponin (ICN Biochemicals; Cleveland, OH) and stained with anti-human IFN-γ-FITC and anti-human IL-4-PE (both from BD Pharmingen).

**Results**

**Intestinal GnB but not GpB prime moDC for a high Th1 polarizing capacity**

To investigate whether intestinal commensal bacteria can modulate the Th cell polarizing capacity of human DC, immature moDC were cultured with a panel of intestinal GnB (*E. coli*, *B. vulgatus*, *V. parvula* and *P. aeruginosa*) or GpB (*B. adolescentis*, *E. faecalis*, *L. plantarum* and *S. aureus*) in the presence of the maturation factors (MF) IL-1β and TNFα to induce equal maturation in both groups, as GpB did not induce full maturation independently, in contrast to GnB (data not shown). The primed mature DC were used to stimulate naive Th cells with SEB (100 pg/ml), which were restimulated after 10 days to evaluate their acquired cytokine profile. Figure 1A demonstrates that all GnB primed moDC for an enhanced Th1 cell development, although not as strongly as priming with high level rIFN-γ. In contrast, GpB did not prime for an enhanced Th1 – or Th2 cell polarizing capacity in moDC. Figure 1B indicates that the dosage of 10^7 GnB (approximately 1:100 ratio) was optimal to prime for a strong Th1 polarising capacity in moDC, whereas 10^6 bacteria (ratio 1:10) gave a partial effect and 10^5 bacteria (ratio 1:1) was clearly insufficient. With respect to optimal cytokine induction a similar dose-dependent effect was found for both GnB and GpB in a previous study with peripheral monocytes (19).
Th1 polarization by GnB-primed moDC is blocked by neutralizing anti-IL-12, although IL-12p70 production is not increased

To explore the role of IL-12 in the Th1 driving effect of GnB-primed moDC, blocking Abs to IL-12 were added to the coculture of naive Th cells and mature moDC. The Th1 polarization driven by GnB-primed moDC was completely blocked by neutralizing anti-IL-12, whereas the Th cell cytokine profile obtained with moDC matured in the presence of GpB was hardly affected (Figure 2A). However, IL-12p70 production was not increased, in GnB-nor in GpB-matured moDC, in comparison to the control maturation condition with MF only (Figure 2B). Surprisingly, GnB-matured moDC expressed elevated levels of IL-10 (Figure 2C) instead. IL-10 neutralization experiments, however, showed that this IL-10 production did not affect their Th cell polarizing potential (Figure 2D).
Th1 polarization by Gnb-primed moDC is driven by IL-12 in synergy with an additional co-factor, which is not IL-18, type I IFNs or ICAM-1

Since Th1 polarization by Gnb-primed moDC was completely neutralized by polyclonal antibodies to IL-12 despite the fact that these cells produced only limited amounts of IL-12p70, it was hypothesized that apart from IL-12, an additional co-factor should play a key role in driving Th1 polarization. Likely candidates in this respect are the cytokines IL-18, IFN-α, IL-23, IL-27 or the membrane-bound molecule ICAM-1. To test this, blocking studies were performed by adding IL-18BP or anti-type I IFNs Abs, with or without anti-IL-12 Abs, during
the co-culture of bacteria-primed moDC and naive Th cells. In all conditions only neutralizing polyclonal IL-12 Abs were successful in blocking Th1 cell development, suggesting that the cytokines IL-18 (Figure 3A) or type I IFNs (Figure 3B) were not acting as Th1 driving co-factors with IL-12 under these conditions. This conclusion is further supported by the finding that neither IL-18 nor IFN-α was produced by bacteria-primed DC, as they could not be detected by ELISA (data not shown). A role for ICAM-1 or other costimulatory molecules, like CD80 or CD86, as co-factors for IL-12-induced Th1 polarization appeared not to be very likely, as no differences in their expression patterns were found on GnB or GpB-primed moDC (Figure 3C). Also CD83 expression was equal in all conditions, giving no indications for differences in Th cell response outcome (Figure 3C).

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- **Figure 3**: Th1 polarization by GnB-primed moDC is driven by IL-12 in synergy with an unidentified co-factor, which is not IL-18, type I IFNs or ICAM-1.

Generation of immature moDC and maturation conditions are described in the legend to figure 1. (A) and (B) Naïve Th cells (2x10⁴ cells/well) were stimulated with differentially matured DC (5x10⁵ cells/well) and superantigen SEB (100 pg/ml) in the absence or presence of IL-18BP (A) or anti type I IFNs Abs (B), with or without anti-IL-12 Abs. After 12 days, IFN-γ and IL-4 productions per cell were determined by intracellular FACS-analysis as described in the legend to figure 1. (C) CD14, CD80, CD83, CD86 and ICAM-1 (CD54) expression on mature bacteria-treated DC was analyzed by flowcytometry.
GnB-primed moDC express elevated levels of IL-23 and IL-27 mRNA

The novel IL-12 family member IL-23, composed of p19 and p40 subunits, can elevate IFN-γ in Th cells and is also blocked by polyclonal antibodies to IL-12 (because of sharing the p40 subunit with IL-12). The Th1 polarizing capacity of GnB-primed moDC, therefore, may be explained by the additional production of IL-23 as well. In addition, the other novel IL-12 family member IL-27, composed of p28 and EBI3 subunits, may synergize with IL-12 in this Th1 bias. The p19 and p28 subunits are the limiting factors in the p19-p40 heterodimer of IL23 and the p28-EBI3 heterodimer of IL-27 respectively, like for the p35 subunit in the IL-12 molecule. The expression of p19, p28, p40 and EBI3 subunits was analyzed by real-time RT-PCR in either CD40L-stimulated moDC (p28 and EBI3) or in CD40L plus IFN-γ-stimulated moDC (p19 and p40), since p19 mRNA expression was only marginal in CD40L-stimulated moDC. Strikingly, p19 and, in particular, p28 were significantly elevated in GnB-treated moDC (Figure 4A). Furthermore, in contrast to p28 mRNA, which was expressed at levels similar to the levels of control MF-treated moDC, the levels p19 mRNA were slightly elevated in the GpB-primed moDC. The levels of p19 and p28 in the GnB-primed moDC were as high as in IFN-γ-primed moDC. EBI3 and p40 expression was the same in all conditions (Figure 4B). Although additional blocking studies are necessary to draw firm conclusions on the contribution of either IL-23 and/or IL-27 to the Th1 polarization by GnB-primed moDC (unfortunately there are no specific neutralizing Abs available yet), these data indicate that IL-23 and/or IL-27 may act as a cofactor, together with IL-12, in the induction of Th1 responses by GnB-primed moDC.
Modulation of human DC function by purified cell wall components of GnB or GpB

In an attempt to identify the bacterial components that prime moDC for the Th1 polarizing capacity, the role of some obvious candidate compounds was tested by adding them to cultures of maturing moDC, and evaluating the impact on DC cytokine production and Th cell polarization. These include lipopolysaccharide (LPS), a component of the outer cell wall of Gnb, peptidoglycan (PGN), a component of the cell wall of all bacteria, but in particular of Gpb, and lipoteichoic acid (LTA), present only in Gpb. Figure 5A demonstrates that neither LPS nor LTA influenced the Th cell polarizing capacity of moDC when present during maturation, while PGN primed for Th1 cell development. This Th1 driving effect was IL-12-dependent, as shown by the parallel IL-12 blocking studies. However, PGN did not prime for high IL-12 production in mature moDC (Figure 5B), but instead, primed for high IL-10 production, in a similar fashion as priming with whole Gnb (Figure 5C). Interestingly, p19 mRNA, but not p28 mRNA, was strongly elevated in PGN-primed DC, which is in line with previous reports (25) (Figure 5D). Again, EBI3 and p40 mRNA was expressed to a similar extent in all groups (Figure 5E). Furthermore, when moDC were matured in the presence of PGN plus LTA, like in whole Gpb, the Th1 polarizing effect of PGN was dominant (Figure 5A). Together, these data show that the priming action of whole bacteria cannot easily be attributed to a single cell wall component since the effect of the purified compounds tested here was not in line with the priming effects of whole bacteria and cannot explain either the induction of IL-27 nor the priming for Th1 development.
Figure 5: Modulation of human DC function by purified cell wall components of GnB or GpB. Immature DC were generated as described elsewhere. Maturation was induced by addition of LPS (10 μg/ml), PGN (10 μg/ml), LTA (10 μg/ml) or PGN plus LTA. (A) After 48 h mature moDC were harvested, washed and cocultured (5x10^5 cells/well) with naive Th cells (2x10^4 cells/well) in the absence or presence of neutralizing anti-IL-12. After 12 days, IFN-γ and IL-4 production per cell were determined by intracellular FACS-analysis as described in the legend to figure 1. Mature DC (2.10^4 cells/well) were stimulated with mouse CD40L-expressing mouse plasmaclloyd cells (J558 cells, 2x10^5 cells/well) in the presence of absence of rIFN-γ (1000 U/ml) to induce the production of IL-12p70 (B) and IL-10 (C). After 24h, supernatants were collected and IL-12p70 and IL-10 production were measured by ELISA. Mature DC were stimulated as described in the legend to figure 4. After 6 hours, the cells were lysed, and cDNA was synthesized. The relative number of p19, p28 (D), p40 or EBI3 (E) transcripts was determined by real-time quantitative RT-PCR as described in the legend to figure 4. The data presented here are the mean ± SEM of 4 independent experiments. Statistical analysis was performed by a Student's t-test. * P<0,05 and ** P< 0,01.
**Discussion**

In the present study we demonstrate that GnB, but not GpB, prime human moDC for enhanced capacity to drive Th1 responses, which is in part dependent on IL-12, but also involves an additional co-factor for which IL-27 and/or IL-23 are likely candidates as mRNA of the p19 subunit of IL-23 and the p28 subunit of IL-27 is enhanced in GnB-primed moDC. The type 1 priming capacity of GnB is not easily attributed to a single component of their cell wall, as purified major cell wall compounds of either GnB or GpB did not yield Th cell profiles identical to those obtained with whole bacteria. These data suggest that commensal Gram-negative microflora bacteria can have immunomodulatory functions, in which the novel IL-12 family members IL-23 and/or IL-27 may play a crucial role.

The induction of IFN-γ in response to GnB (26) has recently also been described for murine splenocyte cultures and bone marrow-derived DC (BM-DC). However, with respect to the GnB, this was accomplished via an IL-12-independent pathway and mainly via the cytokines IL-18 and type 1 interferons, whereas it remained unclear which cell types did produce these factors. This finding is in contrast with the results from the present study with human cells, showing that the induction of IFN-γ was dependent on IL-12 and another factor, probably IL-27 and/or IL-23, but clearly not IFN-α or IL-18. However, we cannot exclude the possibility that these cytokines, perhaps produced by other cell types, may contribute to elevate IFN-γ production by Th cells during inflammatory responses evoked by GnB in vivo.

Our data demonstrate that the upregulation of p19 and p28 mRNA expression in the GnB-primed DC was comparable to those in IFN-γ-primed moDC. This is in line with previous reports demonstrating that IFN-γ is a major enhancing signal of all IL-12 family members, which includes IL-12 (27), IL-23 (9,28) and IL-27 (10). The role of IL-12 in the protection against intracellular protozoan, fungal, bacterial and viral infections may not be as crucial as originally thought. Interestingly, patients with mutations in the *IL-12p40* or the *IL-12Rβ1* gene have a relatively mild phenotype and only some may develop chronic courses of salmonellosis or mycobacteriosis, suggesting that other Th1-polarizing cytokines are effective as well in clearing of infections, in particular other than salmonellosis and mycobacteriosis (29,30). Indeed, mice lacking IL-12 still develop polarized Th1 responses to some viral or mycobacterial infections (31,32), provided p40 subunits are present (33,34), which thus suggests a role for other p40-related and p40-dependent proteins, such as IL-23. an additional role for IL-27 in mycobacteriosis follows form experiments with mice deficient in WSX-1, a receptor chains of IL-27. These mice show impaired early IFN-γ production and poorly differentiated granulomas when treated with BCG (35). These studies all suggest that the separate IL-12 family members have overlapping in the clearing of particular infections, albeit that IL-12 and IL-27 act at early and IL-23 may act at later stages of T cell
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differentiation. However, it cannot be ruled out that they have unique functions as well, in particular in innate immunity.

Our data do not favor a critical role for LPS, the major cell wall component of GnB, in the DC-mediated Th1 development primed by these bacteria. In the tested conditions, only very high levels of LPS (up to 100 µg/ml; data not shown) could prime DC for a slight upregulation in the percentage of IFN-γ-producing Th cells, however to no extent in comparison to whole GnB. In vivo secreted LPS can induce IFN-γ production in pathological conditions, but it remains to be established whether LPS can induce Th1 cell development (via modulation of DC) under physiological conditions of GnB infection. Although LPS is a component of major biologic importance for GnB, various other molecular components of GnB may activate and polarize the moDC, including PGN (also part of the cell wall of GnB), porins, lipoproteins and outer membrane proteins (36). A role for an alternative component is highly likely according to a recent study by Resigno et al. (37) with Toll–like receptor (TLR)-deficient mice strain showing that GnB induce DC maturation via activation of TLR2, and not via TLR4, the major binding site of LPS. Similarly, our preliminary experiments (Smits, H.H. and van der Kleij, D.) using Toll-like receptor (TLR)-transfected cell lines indicated that GnB, such as E. coli, activate both TLR2 and TLR4 with high affinity and TLR1, 6, and 9 to a lesser extent, whereas purified LPS activates only TLR4. However, as TLR2-neutralization experiments did not block Th1 polarization induced by GnB-primed DC (data not shown), this suggest that the TLR2-ligating component in GnB is not likely to be responsible for Th1 priming. Nevertheless, a differential functional role of TLR2, TLR4, other TLR or other pattern recognition receptors in this respect, is highlighted by recent studies demonstrating that whole GnB induce other and more expanded gene programs in human DC than do individual cell wall compounds, like LPS (38).

In this study moDC were used as a model for resident immature DC present in peripheral tissues, such as the mucosal lining of the intestine. This model has the limitation that we cannot mimic the influence of local micro-environmental tissue factors on the ultimate Th cell polarizing capacity of tissue-specific DC. For example, TGF-β is abundantly present in the intestine and has been demonstrated to downregulate IL-12p70 production by DC (39,40). Indeed, DC isolated from gut-associated lymphoid tissue have been shown to be IL-12 deficient (13), in contrast to, e.g. DC isolated from the spleen. Therefore the question remains whether the results of this study can easily be extrapolated to acquired antimicrobial immune responses mounted in the intestine in vivo. Nevertheless, several studies have demonstrated that human intraepithelial (IEL) and lamina propria (LML) lymphocytes isolated from intestinal biopsies in non-pathological conditions can produce high levels of IFN-γ (41-43) in addition to high levels of IL-10 (44). (reviewed in (45)). Remarkably, this is also the phenotype of the effector Th cells obtained with GnB-primed moDC in the present study. Blocking studies with anti-IL-10 indicated that the high IL-10 production in the Th cells was
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strongly dependent on IL-10 secreted by GnB-primed moDC (data not shown & Figure 2C). This IL-10 production may also account for the generation of IgA antibodies in vivo, instrumental in (oral) tolerance induction and frequently found in the intestines against harmless food proteins or commensal bacteria (46).

This study demonstrates that GnB have a clear immunomodulatory effect on DC by the imprinting of a strong Th1 polarizing capacity. This capacity is only partly dependent on the activity of the classical Th1 polarising cytokine IL-12, and it is shown that this Th1 polarisation may as well be driven by the action of the novel IL-12 family members IL-27 and/or IL-23.

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References


