Lactobacilli activate human dendritic cells that skew T cells toward T helper 1 polarization

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Professional antigen-presenting dendritic cells (DCs) are critical in regulating T cell immune responses at both systemic and mucosal sites. Many *Lactobacillus* species are normal members of the human gut microflora and most are regarded as safe when administered as probiotics. Because DCs can naturally or therapeutically encounter lactobacilli, we investigated the effects of several well defined strains, representing three species of *Lactobacillus* on human myeloid DCs (MDCs) and found that they modulated the phenotype and functions of human MDCs. *Lactobacillus*-exposed MDCs up-regulated HLA-DR, CD83, CD40, CD80, and CD86 and secreted high levels of IL-12 and IL-18, but not IL-10. IL-12 was sustained in MDCs exposed to all three *Lactobacillus* species in the presence of LPS from *Escherichia coli*, whereas LPS-induced IL-10 was greatly inhibited. MDCs activated with lactobacilli clearly skewed $CD4^+$ and $CD8^+$ T cells to T helper 1 and Tc1 polarization, as evidenced by secretion of IFN-γ, but not IL-4 or IL-13. These results emphasize a potentially important role for lactobacilli in modulating immunological functions of DCs and suggest that certain strains could be particularly advantageous as vaccine adjuvants, by promoting DCs to regulate T cell responses toward T helper 1 and Tc1 pathways.

Lactobacilli are part of the commensal microbial flora of the intestinal tract of humans and mammals and are generally recognized as nonpathogenic. Dense cultures of viable organisms may be administered by a variety of mucosal routes (1–4). It is well documented that the intestinal microflora contributes to the health of the host with various bacterial species known to modulate immune responses (1–5). The mechanisms of such immune modulations are unknown. However, it has been demonstrated that the cell wall of these bacteria contain immunomodulatory components such as cell surface components and peptidoglycan that may play an important role in activating immune-competent cells in the intestine (3). Furthermore, functional involvement of the intestinal microflora in modulation of immune responses and maintenance of homeostasis highlights the critical role of the microbiota in our intestine (6, 7). Furthermore, intestinal microbiota, which include various species of *Lactobacillus*, interact regularly with cells of the colon, which include professional antigen-presenting cells and intestinal epithelial cells (8–10). Moreover, it has recently been reported that lactobacilli may facilitate the polarization of the naive immune system by skewing it away from T helper 2 (Th2) toward Th1 responses, and thus promoting humoral and cell mediated immunity (11).

Dendritic cells (DCs) play a pivotal role in immunological responses by priming adaptive immunity. Immature DCs migrate through the bloodstream and home to various tissues where they confront invading pathogens. Migratory DCs in the periphery, lymphatic, and nonlymphatic organs then undergo phenotypic and functional changes, including up-regulation of cell surface expression of costimulatory and adhesion molecules and production of inflammatory chemokines and cytokines (12–15). Along with antigen uptake and processing, these functional changes in the DCs augment and direct both humoral and adaptive immune responses (12, 13). Depending on the microbial stimulus encountered, DCs can promote the development of unprimed, naive T cells toward Th1, Th2, or unpolarized T cell responses (12, 13).

The gastrointestinal tract is colonized by an assortment of commensal bacteria, which are the primary stimulus for the intestinal immune system (16). Therefore, DCs residing in several compartments of the gut regularly encounter nonpathogenic organisms of the gut microflora, including *Lactobacillus* species (15, 16). It has been postulated that *Lactobacillus* cells may modulate DC properties, including their ability to activate specific immune responses at mucosal sites (14–16). A balance of DC stimulation and tolerance after an encounter with *Lactobacillus* cells in the gut may be important to maintain the homeostasis required for symbiotic bacteria to perform their critical functions in host nutrition, intestinal permeability, and protection against foreign, pathogenic microbes (16). In this study, we examined DC responses to three *Lactobacillus* species and investigated whether or not these bacteria could induce T cell immune responses in immature human DCs.

Materials and Methods

Bacterial Strains. *Lactobacillus gasseri* (ATCC no. 19992), *Lactobacillus johnsonii* (ATCC no. 33200), and *Lactobacillus reuteri* (ATCC no. 23272) were obtained from American Type Culture Collection. *Lactobacillus* species were inoculated at 1% and propagated in de Man, Rogosa, and Sharpe broth (MRS, Difco) at 37°C for 15 h. Subsequently, 10 ml of each culture was then transferred to 500 ml of fresh MRS and incubated at 37°C for 8 h until mid-log phase. Cells were then harvested by centrifugation, washed with PBS (50 ml), and added to immature myeloid DCs (MDCs). To kill *Lactobacillus* cells (10^{11} colony-forming units per ml), the bacteria were exposed to UV-light for 15 min and frozen at −80°C. Complete loss of cell viability was verified by plate counts on MRS medium. The dry cell weight of bacterial concentrations was determined by freeze-drying aliquots and correcting for buffer salt content. LPS from *Escherichia coli* was purchased from Sigma (SF3–82).

Abs, Cytokines, and Reagents. Murine mAbs were: HLA-DR, CD3, CD4, and CD8 (Becton Dickinson); CD62L (Caltag, South San Francisco, CA), CD83 (Pharmingen), CD40, HLA-ABC (R & D Systems), CD1a (DAKO), CD80, CD45RA, CD45RO, and CD69 (Beckman Coulter, Fullerton, CA). Recombinant human granulocyte/macrophage colony-stimulating factor was purchased from BioSource International (Camarillo, CA). Recombinant human IL-4 was purchased from R & D Systems. All ELISA reagents were purchased either from Pharmingen, R & D Systems, or BioSource International.

Abbreviations: DCs, dendritic cells; Th, T helper; TLR, Toll-like receptor; MDC, myeloid DC.

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14. ABSTRACT

Professional antigen-presenting dendritic cells (DCs) are critical in regulating T cell immune responses at both systemic and mucosal sites. Many Lactobacillus species are normal members of the human gut microflora and most are regarded as safe when administered as probiotics. Because DCs can naturally or therapeutically encounter lactobacilli, we investigated the effects of several well defined strains, representing three species of Lactobacillus on human myeloid DCs (MDCs) and found that they modulated the phenotype and functions of human MDCs. Lactobacillus-exposed MDCs up-regulated HLA-DR, CD83, CD40, CD80, and CD86 and secreted high levels of IL-12 and IL-18, but not IL-10. IL-12 was sustained in MDCs exposed to all three Lactobacillus species in the presence of LPS from Escherichia coli, whereas LPS-induced IL-10 was greatly inhibited. MDCs activated with lactobacilli clearly skewed CD4(+) and CD8(+) T cells to T helper 1 and Tc1 polarization, as evidenced by secretion of IFN-gamma, but not IL-4 or IL-13. These results emphasize a potentially important role for lactobacilli in modulating immunological functions of DCs and suggest that certain strains could be particularly advantageous as vaccine adjuvants, by promoting DCs to regulate T cell responses toward T helper 1 and Tc1 pathways.

15. SUBJECT TERMS
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DCs. Peripheral blood mononuclear cells were isolated from the blood of healthy individuals by Ficoll gradient centrifugation. Peripheral blood mononuclear cells including monocytes (10^7 cells per well) were seeded in six-well plates for 2 h at 37°C. Nonadherent cells were removed by several washes by using PBS plus 2% heat inactivated FCS and frozen for autologous or allogeneic mixed lymphocyte reaction experiments. Adherent monocytes were cultured with human granulocyte/macrophage colony-stimulating factor (100 ng/ml) and human IL-4 (10 ng/ml) in complete medium consisted of RPMI 1640, 10% FCS, 1% L-glutamine, 1% penicillin/streptomycin, 50 μM 2-mercaptoethanol, 1% sodium pyruvate, and 1% nonessential amino acids (all from GIBCO) for 6 d (17, 18). MDCs on day 5 of culture were defined as immature MDCs. Immature MDCs were treated with *Lactobacillus* cells at various doses: 10:1, 100:1, or 1,000:1 colony-forming units per MDC for various times of 16, 48, or 72 h at 37°C. Treated MDCs were then harvested, centrifuged at 300 $\times$ *g* for 10 min, washed three times with PBS, and then used in several experiments. To detect intracellular IL-10 and IL-12 expression, MDCs were stimulated with lactobacilli or *E. coli* LPS (100 ng/ml) at 37°C for 2–3 d and treated with golgi-inhibitor for 4 h. MDCs were stained for CD1a or HLA-DR, fixed with 0.1% paraformaldehyde, permeabilized, and stained with IL-10 antigen-presenting cells and IL-12 FITC for 1 h at 4°C. Subsequently, cells were washed and analyzed by flow cytometry. In some experiments, the supernatants of MDCs activated with *Lactobacillus* species *or E. coli* LPS (100 ng/ml) were collected and assayed for IL-1β, TNF-α, IL-6, IL-10, IL-18, or IL-12 p70 by ELISA, according to standard protocols.

T Cell Proliferation. Isolated $CD4^+$ T cells were negatively purified by the depletion of $CD8^+$, $CD19^+$, $CD56^+$, $CD1a^+$, and $CD14^+$ cells by using specific Ab conjugated beads (Miltenyi Biotec, Auburn, CA). $CD8^+$ T cells were purified by depleting $CD4^+$ cells in analogous fashion (29). Monocyte-derived DCs were generated as described above and incubated with live or killed *Lactobacillus* cells, *E. coli* LPS (300 ng/ml), or with no supplement for 72 h at 37°C. MDCs were harvested and washed with PBS. Subsequently, MDCs were cocultured at graded doses with autologous or allogeneic $CD4^+$ or $CD8^+$ T cells (10^5 cells per well in a 96-well plate) for 4 d in complete RPMI medium 1640 where heat-inactivated 10% human AB^+ serum (Gemini Bio-Products, Woodland, CA) replaced FCS. Cells were pulsed with 0.5 μCi of [^{3}H]thymidine per well (New England Nuclear). [^{3}H]Thymidine incorporation was measured 16 h later by using a β-counter (Wallac TriLux, Perkin–Elmer). In some experiments naive $CD62L^+CD45RA^+CD45RO^-CD4^+$ or $CD62L^+CD45RA^+CD45RO^-CD4^+CD8^+$ T cells were sorted by a cell sorter from human adulate peripheral blood mononuclear cells. Subsequently, autologous or allogeneic CD4 (50×10^3 cells per well in a 96-well plate) or CD8 T cells were cocultured with MDCs, which were treated with live or killed *Lactobacillus* species as described above. Supernatants of the cocultures were harvested on day 5 and subsequently assayed by ELISA to measure cytokines.

Flow Cytometry. MDCs (5×10^5) were incubated with mAbs or isotype control for 1 h at 4°C, washed extensively with PBS plus 0.2% FCS, fixed with 0.1% paraformaldehyde, and analyzed by a FACSCalibur four-laser cytometry by using standard CELLQUEST acquisition/analysis software (Becton Dickinson). At least 1×10^4 gated events per condition were acquired. FACS analysis for intracellular IL-10 and IL-12 was performed as described (19).

Confocal Microscopy. Untreated or treated MDCs with *Lactobacillus* species or with *E. coli* LPS were stained by using anti-HLA-DR Ab and an Alexa Fluor 488 conjugated anti-mouse secondary Ab, Texas red phalloidin, and Hoechst dye (Molecular Probes). Cells were visualized with a Bio-Rad 2000MP confocal multiphoton microscope. Image acquisition and subsequent contrast enhancement were done identically for each condition.

RT-PCR. RT-PCR amplifications were performed as described (20). Cycling conditions were 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The primers used to amplify *Toll-like receptor 2* (*TLR-2*) and *β-actin* genes were *TLR-2*, (forward) 5′-TTGAAGTTCTCCAGCTCCTG-3′ and (reverse) 5′-GCCAAAGTCTTGATTGATTG-3′ and *β-actin*, (forward) 5′-CAAGGCCAACCGCGAGAAGA-3′ and (reverse) 5′-GTCCCGGCCAGCCAGGTCCCA -3′.

Results

***Lactobacillus* Species Modulate the Phenotype and Function of MDCs.** Previous studies have shown that *Lactobacillus plantarum* and *Lactobacillus rhamnosus* can induce activation, as measured by

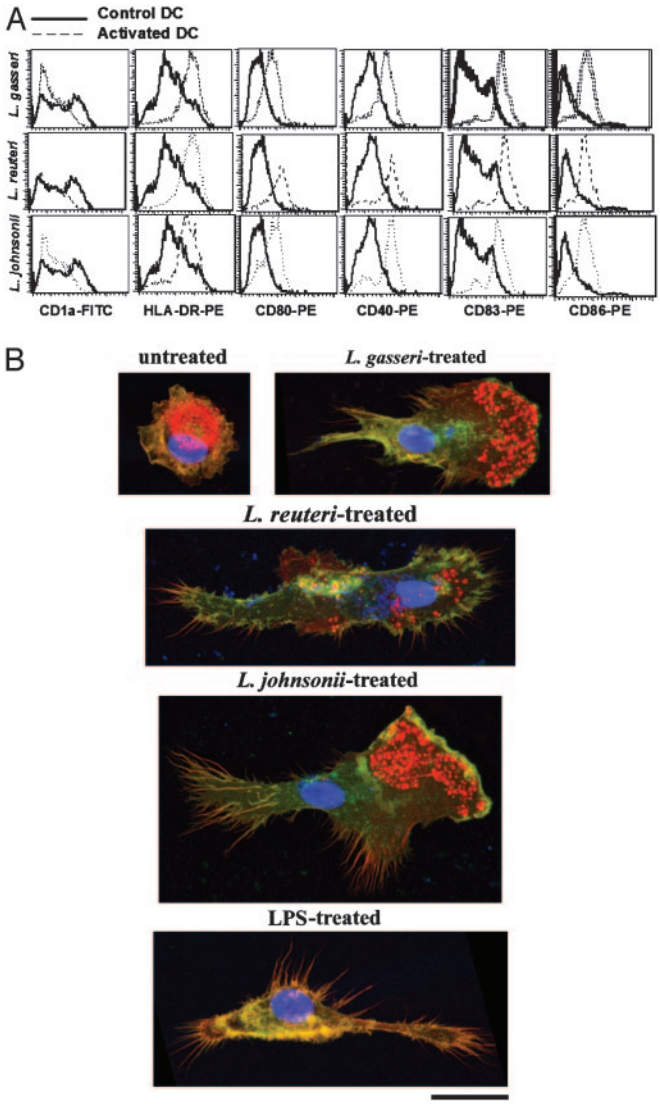

Fig. 1. FACS and confocal analysis of MDCs treated with different *Lactobacillus* species. (*A*) MDCs were treated with *Lactobacillus* cells (1,000 colony-forming units per DC) for 3 d. Treated (dotted line) and untreated (solid line) MDCs were harvested, washed extensively with PBS, stained with specific Abs for 1 h at 4°C, and analyzed by FACS. Experiments were repeated at least three times with similar results. (*B*) Treated or untreated MDCs with *Lactobacillus* species or *E. coli* LPS were stained with Ab to HLA-DR (green), a fluorescent marker of actin polymer (red), and Hoechst DNA stain (blue). (Scale bar, 20 μm.)

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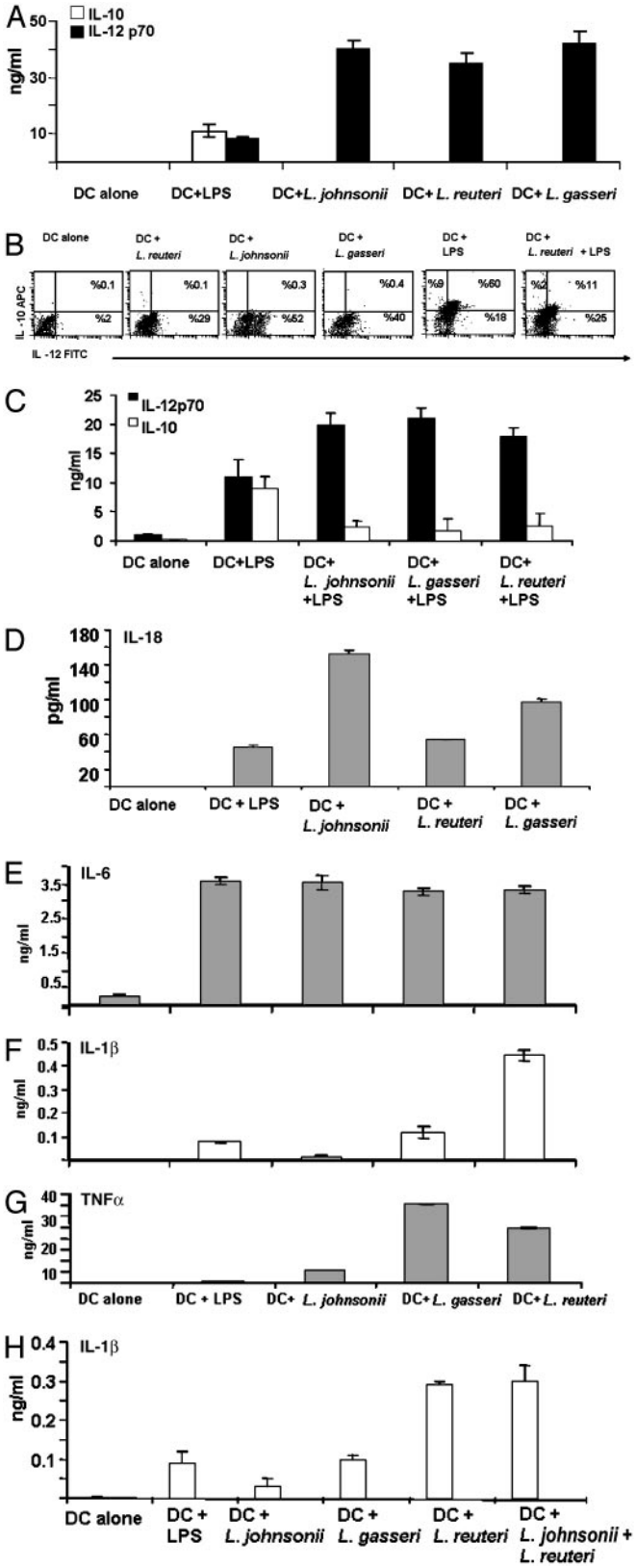

Fig. 2. Induction of bioactive IL-12, IL-18, and proinflammatory cytokines in MDCs treated with *Lactobacillus* cells. MDCs were treated with *Lactobacillus* species, *E. coli* LPS, or no supplement for 3 d at 37°C. (*A*) Supernatants of MDCs treated with *Lactobacillus* species, *E. coli* LPS, or no supplement were harvested after 72 h and analyzed for IL-10 and IL-12 by ELISA. Experiments were performed at least three times with similar results. (*B*) MDCs were treated with *Lactobacillus* species, *E. coli* LPS, *L. reuteri* and *E. coli* LPS, or no supplement for 48 h. MDCs were harvested and treated with Golgi inhibitor for 4 h. Cells were stained with anti-IL-10 allophycocyanin or anti-IL-12 FITC for 1 h on ice. Cells were washed and analyzed by flow cytometry. (*C*) DCs were treated with LPS alone or in combination with *Lactobacillus* species for 72 h. Cytokines were then analyzed by ELISA. (*D*) Supernatants of MDCs treated with *Lactobacillus* species, *E. coli* LPS, or no supplement were harvested after 72 h and analyzed for IL-18 by ELISA. Experiments were performed at least three times with similar results. (*E–H*) MDCs supernatants were harvested and analyzed for proinflammatory cytokines by ELISA. Results are representative of three independent experiments.

the expression of cell surface markers, of human MDCs, albeit at lower levels than Gram-negative bacteria such as *E. coli* or other pathogenic Gram-positive bacteria, such as *Klebsiella pneumoniae* (21–23). To determine whether this difference might vary among species, live *L. gasseri*, *L. johnsonii*, and *L. reuteri* cells at varying concentrations were incubated with MDCs at 37°C for up to 72 h. The data showed that DCs were activated by *Lactobacillus* at 16, 48, and 72 h, but the level of activation (based on cell surface markers) was not significantly different between any of the time points (data not shown). Therefore, the conditions selected for all experiments were 1,000 colony-forming units of lactobacilli per one MDC for 72 h at 37°C. All three *Lactobacillus* species modulated the MDC phenotype by up-regulating HLA-DR, activation of costimulatory molecules CD40, CD80, CD83, and CD86, and down-regulation of CD1a (Fig. 1*A*). As expected, DCs treated with *E. coli* LPS up-regulated HLA-DR and the costimulatory molecules (data not shown). As seen in Fig. 1*B*, MDCs captured *Lactobacillus* cells, which were visualized by staining their DNA with Hoechst dye (Blue) and using confocal microscopy.

***Lactobacillus* Species Induce Bioactive IL-12 and IL-18 and Proinflammatory Cytokines in MDCs.** The production of chemokines and cytokines is another critical function of DCs in response to microbial stimulation (12). Therefore, the production of the proinflammatory cytokines IL-12 (Fig. 2 *A–C*) and IL-18 (Fig. 2*D*), as well as the antiinflammatory cytokine IL-10 (Fig. 2 *A–C*) were examined. Killed (data not shown) or live *Lactobacillus*-treated MDCs produced bioactive IL-12 p70, but not IL-10, as determined by ELISA (Fig. 2*A*). This observation was confirmed by intracellular staining (Fig. 2*B*). In contrast, MDCs stimulated with *E. coli* LPS secreted high levels of IL-12 p70, IL-10, and IL-18, as expected (Fig. 2 *A–D*). To investigate whether *Lactobacillus* activation inhibited LPS-mediated IL-10 induction, MDCs were activated with live or killed (data not shown) *L. gasseri*, *L. johnsonii* (data not shown), or *L. reuteri* plus *E. coli* LPS for 2 d. Under these conditions, LPS promoted the production of IL-10 only in 11% of MDCs, compared with the high (60%) induction of IL-10 with *E. coli* LPS in the absence of *L. reuteri* (Fig. 2 *B* and *C*). Lethally-irradiated *Lactobacillus* species (10 μg/ml) also activated MDCs to secrete IL-12 p70 and IL-18, but not IL-10, respectively (data not shown). Moreover, IL-6 was induced in MDCs at similar levels when treated with each *Lactobacillus* species or *E. coli* LPS (Fig. 2*E*). IL-1β was minimally induced in MDCs treated with *L. johnsonii*, *L. gasseri*, or *E. coli* LPS but was induced in MDCs treated with *L. reuteri* (Fig. 2*F*). TNF-α production was observed in MDCs treated with *Lactobacillus* species or *E. coli* LPS (Fig. 2*G*). To test whether *L. johnsonii* can inhibit the elevated production of IL-1β induced by *L. reuteri*, DCs were treated with live or killed (data not shown) *L. johnsonii* and *L. reuteri*. Data showed that *L. johnsonii* did not inhibit the high secretion of IL-1β induced by *L. reuteri* in DCs (Fig. 2*H*).

Induction of T Cell Proliferation and Activation by MDCs Treated with *Lactobacillus* Species. DCs affect the adaptive immune response by priming T cells to proliferate, become activated, and

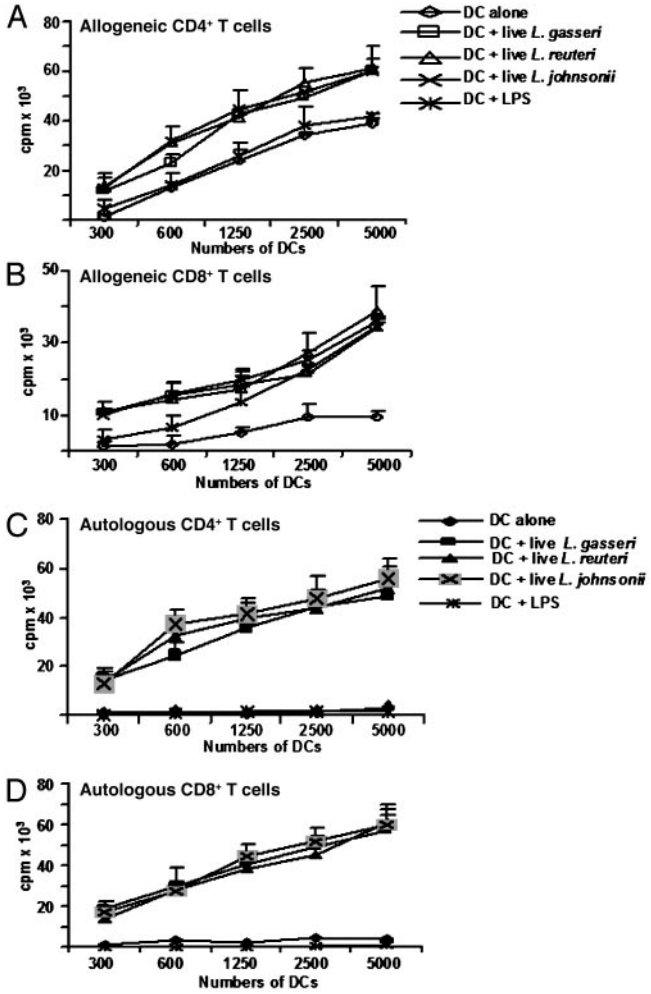

Fig. 3. Induction of T cell proliferation by MDCs treated with live *Lactobacillus* species. MDCs were treated with live *Lactobacillus* species, *E. coli* LPS (100 ng/ml), or no supplement for 72 h at 37°C. Treated and untreated MDCs were harvested, washed extensively with PBS, and at graded doses cocultured with purified allogeneic or autologous $CD4^+$ or $CD8^+$ T cells (10^5 cells per well in a 96-well plate) for 4 d at 37°C. [^{3}H]Thymidine incorporation was measured in triplicate by using a β-counter. Results are representative of three independent experiments.

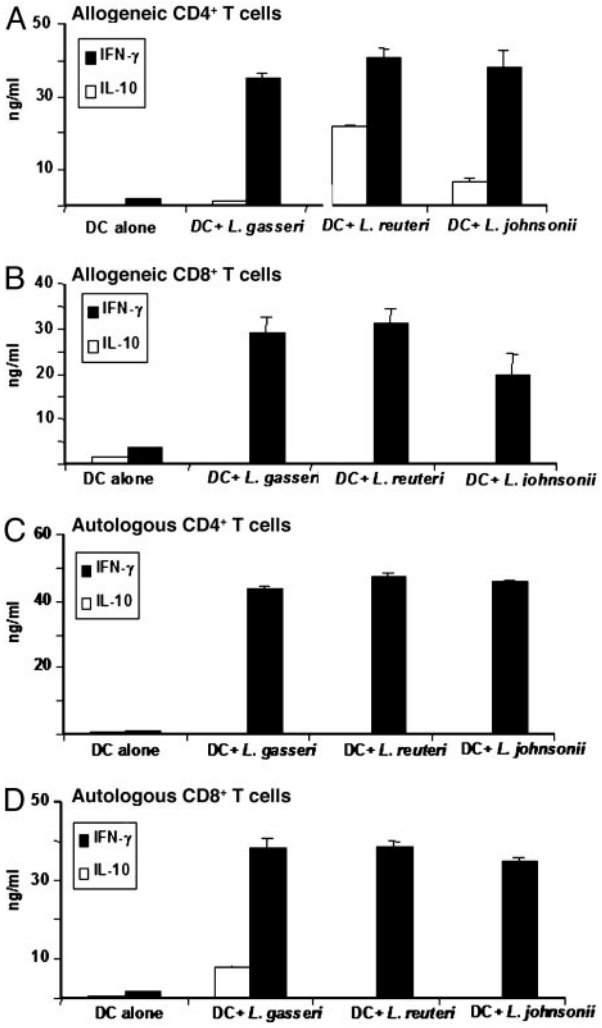

Fig. 4. MDCs treated with *Lactobacillus* species skew T cells toward Th1 immune responses. MDCs were treated with live *Lactobacillus* species, *E. coli* LPS (100 ng/ml), or no supplement for 3 d. MDCs were harvested and cocultured with allogeneic or autologous $CD4^+$ or $CD8^+$ T cells for 4 d at 37°C. Supernatants of T cells cocultured with MDCs treated with *Lactobacillus* species, *E. coli* LPS, or no supplement were collected and assayed for cytokines by ELISA. All experiments were repeated at least three times.

produce cytokines (12). When MDCs were activated with live (Fig. 3 *A* and *B*) or killed (data not shown) lactobacilli, they enhanced the proliferation of allogeneic $CD4^+$ and $CD8^+$ T cells, confirming their capacity to enhance priming of T cells. As expected, MDCs activated with *E. coli* LPS increased $CD4^+$ T cell proliferation and enhanced the proliferation of allogeneic $CD8^+$ T cells (Fig. 3 *A* and *B*). Interestingly, MDCs activated with live (Fig. 3 *C* and *D*) or killed (data not shown) lactobacilli strongly induced the proliferation of autologous $CD4^+$ and $CD8^+$ T cells. By contrast, no T cell proliferation was observed in autologous $CD4^+$ or $CD8^+$ T cells when cocultured with MDCs activated with *E. coli* LPS (Fig. 3 *C* and *D*). MDCs activated with live (Fig. 4*A*) or killed (data not shown) lactobacilli induced high levels of IFN-γ in both allogeneic T cells; however, IL-10 production was observed in allogeneic $CD4^+$ with only minimal induction of IL-10 in $CD8^+$ T cells (Fig. 4*B*). When MDCs were treated with various *Lactobacillus* species, the MDCs induced high levels of IFN-γ but minimal or no IL-4, IL-10, or IL-13 in autologous $CD4^+$ or $CD8^+$ T cells (Fig. 4 *C* and *D* and data not shown). Furthermore, in an attempt to show whether activated MDCs with lactobacilli can enhance the proliferation and activation of naive $CD4^+$ or $CD8^+$ T cells, MDCs were treated with live or killed (data not shown) cells and then were cocultured with naïve allogeneic or autologous $CD4^+$ and $CD8^+$ T cells. Activated MDCs with lactobacilli enhanced allogeneic T cell proliferation (Fig. 5 *A* and *B*) and induced the secretion of IFN-γ and IL-2, but not IL-4 or IL-13 by naive $CD4^+CD62L^+CD45RA^+CD45RO^-$ or $CD8^+CD62L^+CD45RA^+CD45RO^-$ T cells (Fig. 5 *C* and *D*). Mild enhancement of IL-10 was observed, similar to that shown previously in Fig. 4 *A* and *B*. In contrast, no proliferation or activation of naive T cells was induced by autologous MDCs treated with live or killed (data not shown) lactobacilli. These results may indicate that *Lactobacillus*-activated MDCs induce the activation of effector memory T cells specific for bacterial components.

Up-Regulation of TLR-2 in MDCs by Lactobacilli. Because MDCs were efficiently activated, we investigated whether TLR-2 was induced after DC engagement of *Lactobacillus* cells. Expression of

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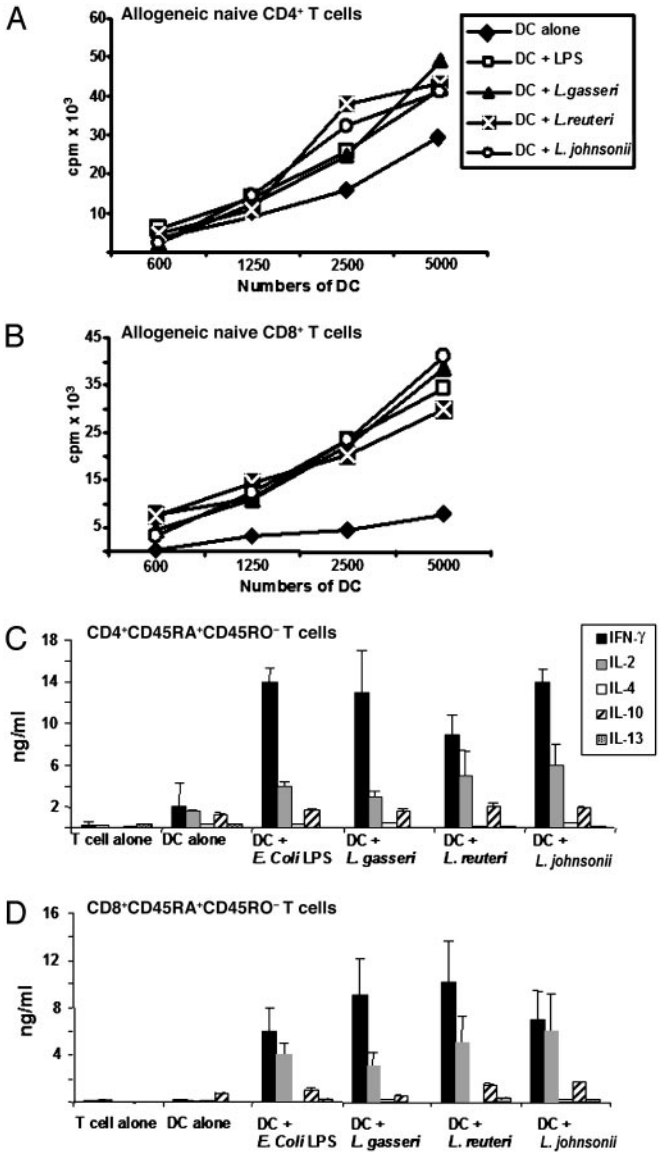

Fig. 5. Activation of naive T cells by *Lactobacillus*-activated MDCs. Sorted naive $CD4^+$ and $CD8^+$ T cells (50×10^3 cells per well in a 96-well plate) were cocultured for 4 d with allogeneic or autologous MDCs, which were treated with live *Lactobacillus* species or with *E. coli* LPS. Cells were pulsed for the last 16 h with 0.5 μCi of [^{3}H]thymidine per well. [^{3}H]Thymidine incorporation was measured by using a β-counter. Cytokines released in the supernatants of the cocultures were assayed by ELISA.

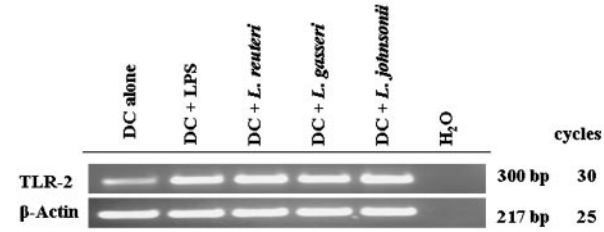

Fig. 6. Total RNA was isolated from MDC activated with *Lactobacillus* species, *E. coli* LPS, or untreated MDC. RT-PCR for TLR-2 and β-actin was performed. Data shown are representative of three experiments.

TLR-2 was undetectable on MDC surface by FACS analysis (data not shown). Therefore, RT-PCR analysis was performed on activated MDCs by using primers that were specific for RNA transcripts encoding either TLR-2 or β-actin. MDCs activated with lactobacilli or *E. coli* LPS showed a 3-fold induction of TLR-2 transcripts whereas β-actin expression remained constant, as expected (Fig. 6).

Discussion

The commensal microbial flora of the intestinal tract harbors Gram-positive and Gram-negative bacteria that may be involved in homeostasis of gut-associated immunity (3–5, 16). Recent studies have highlighted the effects of probiotic bacteria on immune competent cells (3, 4, 24–26). In the present study, we investigated the adjuvanticity of three *Lactobacillus* species on immature human MDCs and showed that *Lactobacillus* cells induced activation and maturation of MDCs. Furthermore, we found that *Lactobacillus*-exposed MDCs secreted bioactive IL-12, a critical factor in switching naive or memory T cells to Th1 responses, which are proinflammatory and lead to robust immunity against infections and other diseases (27).

Interestingly, IL-12 production by MDCs induced by lactobacilli was not reversible when MDCs were simultaneously treated with *E. coli* LPS. This finding suggests that some strains may possess a property that establishes a continuous Th1 immune response by inducing bioactive IL-12 production but not IL-10 production. In agreement with these results, it has been shown that macrophages treated with lactobacilli activated NFκB and STAT signaling, resulting in secretion of IL-12 and IL-18 (28). In addition, some *Lactobacillus* species can differentially effect antigen-specific IgG_1 and IgG_2 Ab responses (29, 30), and inhibit Th2 cytokines (IL-4 and IL-5) derived from patients allergic to house dust mites (31). Our findings show both similarities and differences to previous studies with *Lactobacillus* and human MDCs (21–23). Similar to our current work, the previous studies found that human MDCs exposed to lactobacilli increased MHC, costimulatory, adhesion, and activation molecules (21–23). However, coculture of MDCs for 24 h with *L. rhamnosus* and *L. plantarum* did not elicit IL-2, IL-6, IL-8, or IL-12p70 (21–23, 32). Furthermore, work with murine monocyte-derived DCs by Drakes *et al*. (33) showed that probiotics containing lactobacilli could induce cell surface markers of maturation and activation, but the DCs produced IL-10 and not IL-12p70 (33). Another study by using mouse bone marrow-derived DCs found an induction of Th2 immune responses when DCs were treated with *L. reuteri* (34). In contrast, human MDCs treated with *L. reuteri* in the present study induced Th1 polarization. MDCs treated with *L. reuteri* or *L. johnsonii* induced the production of both IFN-γ and IL-10 in allogeneic $CD4^+$ T cells, but IL-10 was not induced in $CD8^+$ T cells. In contrast, *L. gasseri* induced a clear Th1 polarization pattern in both allogeneic $CD4^+$ and $CD8^+$ T cells. Together, these data suggest that lactobacilli can exert different effects on human immune cells, when compared with mouse immune cells. These differences with human MDCs may be due to several reasons, including differences in the *Lactobacillus* species, specific strains used, and the timing of sampling to assay cytokine production. IL-10 is thought to be a key for maintaining gut homeostasis and the antiinflammatory effects of probiotics (16). However, we found that the three *Lactobacillus* species induced IL-12p70, but not IL-10, in MDCs. These observations are further supported by the fact that MDCs activated with lactobacilli primed allogeneic $CD4^+$ and $CD8^+$ T cells and skewed them toward a Th1 response by secretion of IFN-γ. Lactobacilli were far more potent in priming $CD8^+$ T cells compared to *E. coli* LPS, which is likely due to the production of IL-12p70 and not IL-10 by *Lactobacillus*-exposed MDCs (35). All three species of lactobacilli clearly stimulated Th1 polarization in allogeneic $CD8^+$ T cells. *L. gasseri* induced

high levels of IFN-γ, but a low level of IL-10. In contrast, MDCs treated with *L. reuteri* or *L. johnsonii* induced IL-10 in allogeneic $CD4^+$ T cells. These species-specific effects are in accordance with previous observations that lactobacilli control the secretion of critical immune modulators, including IFN type I and II, IL-12, or IL-18 in a strain-dependent manner (28). The mechanisms underlying the different MDC responses induced by varying *Lactobacillus* species and strains are unknown.

Interestingly, *Lactobacillus*-activated MDCs also induced proliferation of autologous $CD4^+$ and $CD8^+$ T cells and induced their secretion of IFN-γ. The mechanism for this unusual property remains to be defined. However, for autologous naive $CD4^+$ or $CD8^+$ T cells, *Lactobacillus*-activated MDCs did not induce their proliferation or activation. These results may represent a recall response to endogenous lactobacilli or polyclonal T cell activation, as demonstrated previously for *Toxoplasma gondii* and in *Chlamydia trachomatis* infection (35–37).

The findings presented herein add to the complexity of current evidence indicating that intestinal bacteria and probiotics, including lactobacilli, help maintain gut homeostasis by balancing proinflammatory and antiinflammatory mucosal responses (13). We have shown that MDCs respond to certain lactobacilli with inflammatory cytokines that lead to the development of Th1 immune responses. Likely, this represents one piece of a larger, complex environment in which the responses of multiple immune cells, in combination with the responses of other critical intestinal cell types, such as epithelial cells, are responsible for the overall response to nonpathogenic commensal bacteria (16). The responses of the epithelium and other intestinal cells may either combine with or direct the DC response to the microbiota of the gut. In this way, the gut may maintain a balance between inflammatory responses to pathogens and natural intestinal homeostasis and function.

It has been shown that the two major bacterial cell wall components, peptidoglycan in the case of Gram-positive bacteria, and LPS in Gram-negative bacteria, are important molecular markers recognized by the immune system (38). Cell surface molecules such as TLRs and CD14 interact with peptidoglycan or LPS to control expression of several specific, inducible immune responses (38). Accordingly, TLR-2 has been shown to be a signal transducer for cells activated by peptidoglycan, lipoteichoic acid, bacterial lipoprotein, and LPS (39). The *Lactobacillus* species used in our studies, like LPS, up-regulated expression of TLR-2 transcripts. These data suggest that lactobacilli may deliver signals in MDCs through TLR-2, thereby promoting the activation of these cells.

In summary, three *Lactobacillus* species were able to activate MDCs to induce strong T cell immune responses. Both allogeneic T cell priming, as well as autologous T cell activation, was detected. This study indicates that various lactobacilli can be efficient immune modulators, but the signals for directing Th1 or Th2 responses are unknown, and appear to vary among strains and species. Because lactobacilli can activate MDCs, prime T cells, and induce Th1 cytokines, certain strains and species could be particularly useful for delivery of biotherapeutics and vaccines. This field is rapidly expanding as the potential for use of recombinant lactic acid bacteria in human health is being recognized and exploited (40).

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