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The Role of Toll-like Receptors (TLRs) in Bacteria-induced Maturation of Murine Dendritic Cells (DCs)

PEPTIDOGLYCAN AND LIPOTEICHOIC ACID ARE INDUCERS OF DC MATURATION AND REQUIRE TLR2*

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Toll-like receptors (TLRs) have been found to be key elements in pathogen recognition by the host immune system. Dendritic cells (DCs) are crucial for both innate immune responses and initiation of acquired immunity. Here we focus on the potential involvement of TLR ligand interaction in DC maturation. TLR2 knockout mice and mice carrying a TLR4 mutation (C3H/HeJ) were investigated for DC maturation induced by peptidoglycan (PGN), lipopolysaccharide (LPS), or lipoteichoic acids (LTAs). All stimuli induced maturation of murine bone marrow-derived DCs in control mice. TLR2$^{-/-}$ mice lacked maturation upon stimulation with PGN, as assessed by expression of major histocompatibility complex class II, CD86, cytokine, and chemokine production, fluorescein isothiocyanate-dextran uptake, and mixed lymphocyte reactions, while being completely responsive to LPS. A similar lack of maturation was observed in C3H/HeJ mice upon stimulation with LPS. DC maturation induced by LTAs from two different types of bacteria was severely impaired in TLR2$^{-/-}$, whereas C3H/HeJ mice responded to LTAs in a manner similar to wild-type mice. We demonstrate that DC maturation is induced by stimuli from Gram-positive microorganisms, such as PGN and LTA, with similar efficiency as by LPS. Finally, we provide evidence that TLR2 and TLR4 interaction with the appropriate ligand is essential for bacteria-induced maturation of DCs.

Dendritic cells (DCs)[1] are potent antigen-presenting cells and play a key role during the induction of a specific immune response toward microbial pathogens (1, 2). DCs that encounter invading microbes capture bacterial antigens and migrate to lymphoid organs, where they home to the T cell areas. While migrating to the lymph nodes, they shift from an endocytic/phagocytic immature stage to a mature stage of efficient T cell stimulation (1). This activation stage is named maturation and is accompanied by drastic morphological and functional changes. Only mature DCs have high levels of surface expression of MHC and costimulatory molecules that are essential for efficient T cell stimulation (2). Besides inducing adaptive immune responses, maturing DCs, as part of the innate immune system, secrete cytokines and chemokines to recruit immune cells to the site of infection (2). Maturation of DCs can be induced by microbial antigens, proinflammatory cytokines (*e.g.* TNF-α), and non-microbial stimuli such as CD40 ligand (3, 4).

Lipopolysaccharide (LPS), the major immunostimulatory cell wall component of Gram-negative bacteria, lipoteichoic acid (LTA) of Gram-positive bacteria, and bacterial peptidoglycan (PGN) stimulate immune competent host cells to release a multitude of mediators (5). Circulating LPS and other bacterial products are recognized by LPS-binding protein and transported to mCD14. Soluble CD14 enables CD14-negative cells to respond to LPS challenge (6–8).

Evidence from studies with C3H/HeJ LPS-hyporesponsive mice and *in vitro* experiments have led to the discovery that Toll-like receptors (TLRs) act as the signal-transducing coreceptors for recognition of pathogenic microorganisms (9–12). Several human homologues of the *Drosophila* Toll have been identified and termed "Toll-like receptors" (13). The characterization of TLR2- and TLR4-deficient mice demonstrated that TLR4 is involved in the recognition of LPS and LTA (14). TLR2 is involved in the response to PGN of Gram-positive bacteria and to lipopeptides from several microorganisms including spirochetes, mycobacteria, mycoplasma, whole bacteria, and yeast (reviewed in Ref. 15).

Recently, it was shown that human DCs express TLR1–5 (16). The expression pattern of TLRs on murine DCs has not been described yet; however, it is likely that murine DCs exhibit the same expression pattern as human DCs. We focused on the role of TLR2 and -4 for maturation of DCs. We examined DCs derived from TLR2-deficient and TLR4-defective C3H/HeJ mice for their ability to mature after stimulation with Gram-negative and Gram-positive cell wall components. Our results

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[1] The abbreviations used are: DC, dendritic cell; MHC, major histocompatibility complex; TNF-α, tumor necrosis factor α; LPS, lipopolysaccharide; LTA, lipoteichoic acid; PGN, peptidoglycan; TLR, Toll-like receptor; m, murine; GM-CSF, granulocyte/macrophage colony-stimulating factor; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; IL, interleukin; MIP, macrophage-inflammatory protein; MLR, mixed lymphocyte reaction.

TABLE I
PGN induces the up-regulation of CD86 and MHC class II in a dose-dependent manner during DC maturation

DCs were cultured in the presence of the indicated concentration of PGN for 24 h. MHC class II (I-A^b) and CD86 expression was analyzed by FACScan. Shown is the mean fluorescence intensity. Data represent one experiment out of three with similar results.

PGN	Mean fluorescence intensity	
	CD86	MHC II
μg/ml		
Control	6.4	25.4
0.1	9.0	54.1
1.0	10.4	57.2
10	22.5	63.0

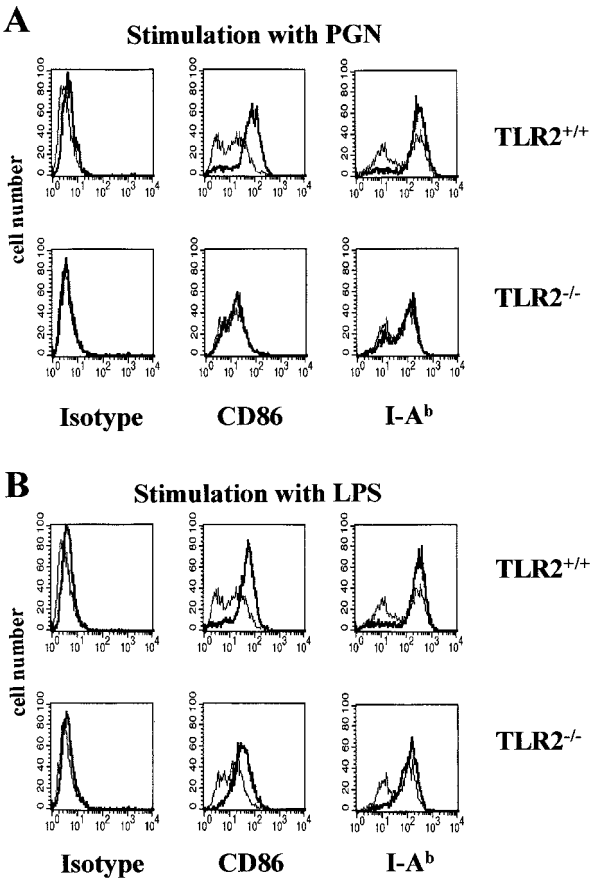

FIG. 1. **Expression of costimulatory molecules and of MHC class II molecules in DCs derived from TLR2$^{-/-}$ mice upon stimulation with PGN.** DCs from TLR2$^{-/-}$ and wild-type littermates were cultured in the presence of 10 μg/ml PGN (*A*) or 10 ng/ml *S. minnesota* Re 595 LPS (*B*) for 24 h. MHC class II (I-A^b) and CD86 expression were analyzed by FACScan. Unstimulated DCs (*thin lines*) were compared with PGN and LPS-stimulated DCs (*bold lines*). Data shown are representative of three separate experiments with identical results.

indicate that interaction with the corresponding ligands of TLRs during bacteria-induced maturation is required for both the up-regulation of cell surface MHC class II and costimulatory molecules on mature DCs. Cytokine secretion of DCs also depends on TLR integrity, because it is severely impaired in TLR2-deficient mice upon stimulation with PGN and highly purified LTA and in C3H/HeJ mice upon stimulation with LPS and lipid A.

EXPERIMENTAL PROCEDURES

Reagents—LPS from *Salmonella minnesota* Re 595 was purchased from Sigma. Synthetic lipid A was kindly provided by L. Hamann, Borstel Research Center, Borstel, Germany. PGN from *Staphylococcus*

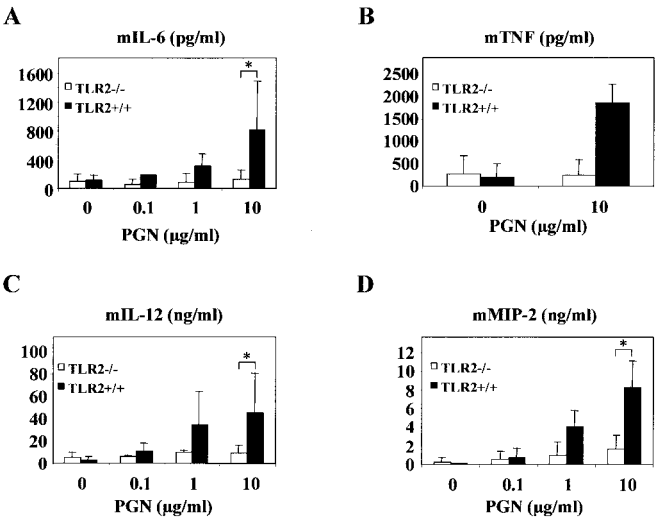

FIG. 2. **Expression of cytokines and chemokines in TLR2$^{-/-}$ DCs upon stimulation with PGN.** Bone marrow-derived immature DCs from TLR2$^{-/-}$ and wild-type littermates were cultured with the indicated concentrations of PGN for 4 (TNF-α) and 24 h (all others), respectively. Concentrations of IL-6 (*A*), TNF-α (*B*), IL-12p40 (*C*), and MIP-2 (*D*) in the culture supernatant were measured by ELISA. Mean values ± S.D. of four independent experiments (*A*, *C*, and *D*) or of two experiments (*B*) are shown. Statistical significance was determined by the Mann-Whitney-U test. $p < 0.05$ is indicated by *asterisks*.

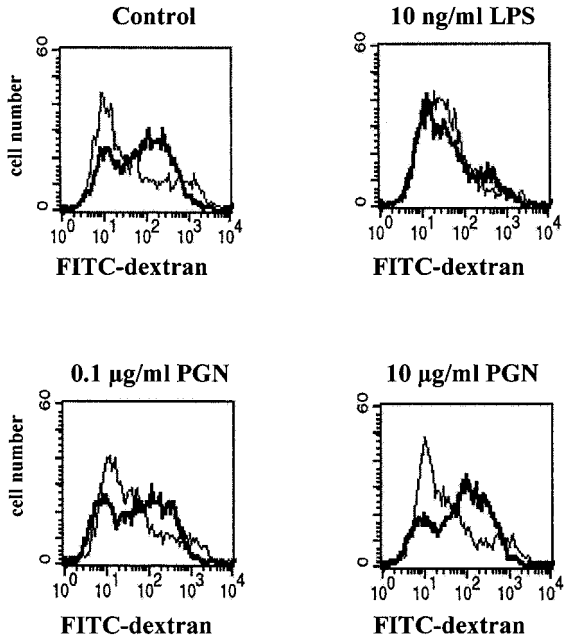

FIG. 3. **FITC-dextran uptake of DCs derived from TLR2$^{-/-}$ mice.** DCs from TLR2$^{-/-}$ mice were cultured with the indicated concentrations of PGN and LPS for 24 h. FITC-dextran uptake at 37 °C (*bold lines*) was assayed as described under "Experimental Procedures" and analyzed by flow cytometry. FITC-dextran uptake at 4 °C served as negative control (*thin lines*). Data shown are representative of two separate experiments with identical results.

aureus was provided by Simon J. Foster, Department of Molecular Biology and Biotechnology, University of Sheffield, Sheffield, UK. LTA from *Bacillus subtilis* (DSMZ 1087) and *S. aureus* (DSMZ 20233) was extracted using 1-butanol and purified by hydrophobic interaction chromatography on octyl-Sepharose as described elsewhere (17, 18). LTA preparation contained less than 30 pg of LPS per mg of LTA, as assessed by a chromogenic limulus amebocyte lysate (LAL) assay (Bio Whittaker, Walkersville, MD). Murine GM-CSF was obtained from supernatants of NIH3T3 cells stably transfected with mGM-CSF. All FITC-conjugated antibodies were obtained from PharMingen (Heidel-

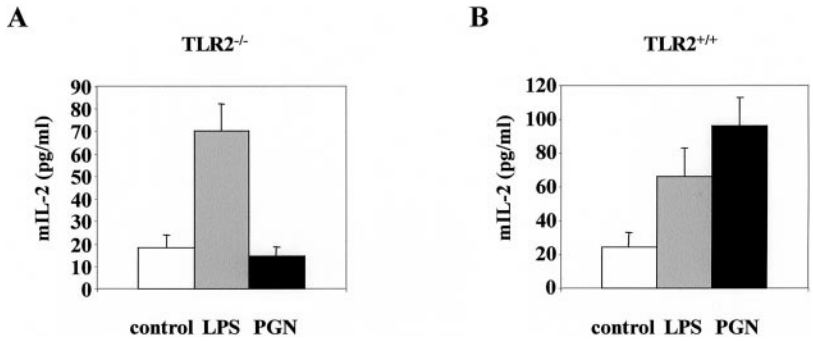

FIG. 4. **Antigen-presenting function of DCs derived from TLR2$^{-/-}$ mice in an MLR.** DCs from TLR2$^{-/-}$ (*A*) and wild-type mice (*B*) were cultured with either 5 μg/ml PGN or 10 ng/ml LPS for 24 h. Purified CD4+ T cells were added to the DCs at a DC:T cell ratio of 1:50 and cultured for an additional 3 days. Concentrations of IL-2 in the culture supernatants were measured by ELISA. Data shown represent the mean value of triplicate measurements with S.D. from one experiment representative of two separate experiments with similar results.

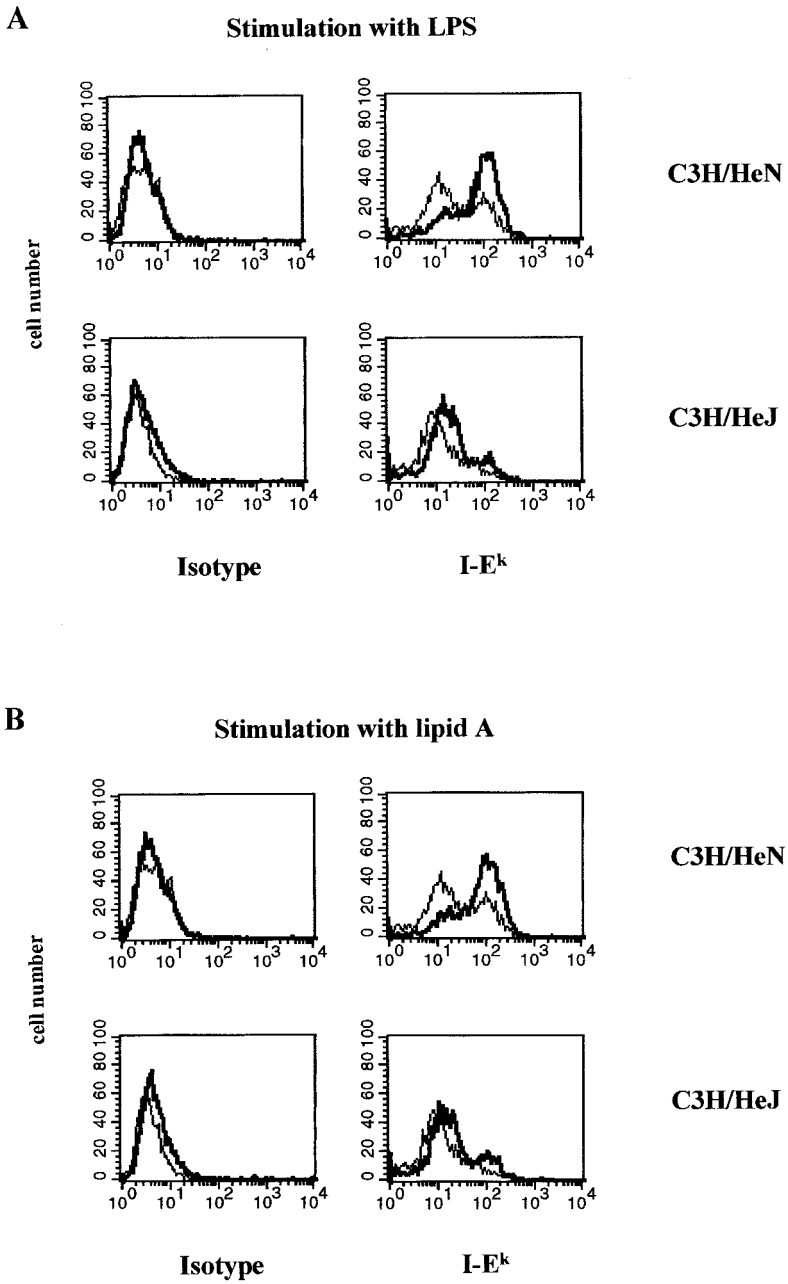

FIG. 5. **Expression of MHC class II molecules in C3H/HeJ and C3H/HeN DCs upon LPS and lipid A stimulation.** DCs from C3H/HeJ and C3H/HeN mice were cultured in the presence of 10 ng/ml *S. minnesota* Re 595 LPS (*A*) or 4 ng/ml synthetic lipid A (*B*) for 24 h. MHC class II (I-E^k) expression was analyzed by FACScan. Unstimulated DCs (*thin lines*) were compared with LPS and lipid A-stimulated DCs (*bold lines*). Data shown are representative of three separate experiments with identical results.

berg, Germany). FITC-dextran (40,000 kDa) was purchased from Molecular Probes (Göttingen, Germany).

Animals—C3H/HeJ and C3H/HeN mice were purchased from Charles River Germany (Sulzfeld, Germany). TLR2 knockout mice were generated by homologous recombination by Deltagen (Menlo Park, CA) and kindly provided by Tularik Inc. (South San Francisco, CA). Heterozygous mice were backcrossed on C57BL/6 for several generations before interbreeding yielded TLR2-deficient mice. Age- and sex-matched TLR2-deficient mice and their heterozygous and wild-type littermates from these intercrosses were used for DC generation.

Bone Marrow-derived Murine DCs—DCs were isolated from murine bone marrow according to a modified version of a protocol previously described (19). Briefly, bone marrow cells were flushed from femurs and tibias of mice by complete medium (RPMI 1640, 2 mM L-glutamine, 10% fetal calf serum, 50 μM 2-mercaptoethanol, 100 units/ml penicillin, 100 μg/ml streptomycin). After 24 h of incubation of the obtained cells with

mGM-CSF-containing (30 ng/ml) complete medium, non-adherent cells were discarded, and the remaining cells were fed with mGM-CSF-containing complete medium. This step was repeated on day 3 of incubation. On day 5 of culture, cells were fed with mGM-CSF-containing medium, and non-adherent cells were used for experiments on day 6 when they were positive for CD11c.

Stimulation of DCs—Bone marrow-derived DCs were washed with PBS and resuspended in medium plus 5% fetal calf serum. Stimulations were performed with 200,000 cells/ml or as indicated with PGN (0.1–10 μg/ml), LPS (0.1–100 ng/ml), LTA(10 μg/ml), and synthetic lipid A (0.4–4 ng/ml) for 24 h as indicated. After 4 and 24 h of stimulation supernatants were harvested for cytokine analysis, and cells were processed for flow cytometry.

Flow Cytometric Analysis—Cells were pre-blocked using rat anti-mouse CD32 F(ab′)$_2$ antibody (PharMingen) at 5 μg/ml in PBS plus 2% rat serum at room temperature for 30 min. Next, cells were washed once in PBS and incubated with rat anti-mouse I-A^b-FITC or I-E^k-FITC, CD86-FITC, and CD11c-FITC. Finally, cells were fixed in 1% paraformaldehyde/PBS and analyzed by FACScan (Becton Dickinson, Heidelberg, Germany) using CellQuest software (Becton Dickinson).

Analysis of Endocytosis—To assess endocytosis of the DC preparations, FITC-dextran was used according to the method described previously (20). Briefly, cells were incubated in complete medium with FITC-dextran at a final concentration of 1 mg/ml at 37 °C for 30 min. Background staining at 4 °C was used as reference. After washing the cells four times with ice-cold PBS, we analyzed DCs by FACScan, measuring FITC-dextran uptake of immature DCs.

Quantification of Cytokine Production of Murine Dendritic Cells—ELISA plates were coated with anti-murine TNF-α antibody (PharMingen). Samples and recombinant murine TNF-α (R & D Systems, Wiesbaden-Nordenstadt, Germany) were detected using a biotin-labeled monoclonal anti-murine TNF-α antibody (PharMingen). For quantification of IL-6, plates were coated with anti-murine IL-6 antibody (PharMingen). Samples and recombinant murine IL-6 (BioConcept) were detected using a biotin-labeled monoclonal anti-murine IL-6 antibody (PharMingen). The detection limits for TNF-α and IL-6 were 31 and 16 pg/ml, respectively. MIP-2, IL-2, and IL-12p40 were measured by ELISA according to the manufacturer's instructions (R & D Systems for MIP-2 and PharMingen for IL-2 and IL-12p40).

Mixed Lymphocyte Reaction (MLR)—Spleens from BALB/c mice were homogenized to obtain single cell suspensions, and Ficoll was overlaid with this suspension (Biochrom, Berlin, Germany). Mononuclear cells obtained from the interphase were washed twice with PBS. Allogeneic CD4$^+$ T cells were isolated employing CD4 microbeads (Milteny Biotech, Bergisch-Gladbach, Germany) and an Auto-Macs separation system (Milteny Biotech). Purity of the CD4$^+$ T cells was greater than 80% as assessed by flow cytometric staining for CD3. Bone marrow-derived DCs from C3H/HeJ and TLR2$^{-/-}$ and corresponding wild-type mice were stimulated with 10 ng/ml LPS and 5 μg/ml PGN for 24 h. DCs were mixed with 5×10^4 CD4$^+$ T cells at a ratio of 1:50 to assess their T cell stimulatory capacity. After 3 days of coculture, supernatants were taken and immediately examined for IL-2 secretion.

Statistics—Data are presented as means ± S.D. Statistical differences were determined by the Mann-Whitney-U test. $p < 0.05$ is considered significant and is indicated by asterisks.

RESULTS

PGN Induces the Maturation of Bone Marrow-derived Murine DCs in a Dose-dependent Manner—We investigated whether PGN of Gram-positive bacteria is able to induce the maturation of bone marrow-derived murine DCs. Stimulation of murine DCs with 0.1, 1, and 10 μg/ml PGN for 24 h resulted in a dose-dependent up-regulation of the costimulatory molecule CD86 and MHC class II molecules and thereby maturation of DCs (Table I).

Expression of Costimulatory Molecules and MHC Class II Molecules Is Impaired in TLR2-deficient Mice upon Stimulation with PGN—To assess whether PGN-induced maturation of DCs is initiated via TLR2, we examined the responsiveness of DCs from TLR2-deficient mice to PGN and, as control, to LPS. First, we examined the expression of MHC class II molecules and CD86 on DCs from TLR2-deficient and control mice by flow cytometry. PGN induced an increase in the expression of MHC class II molecules on wild-type DCs (Fig. 1*A*). In contrast, induction of MHC class II and CD86 by PGN was impaired in

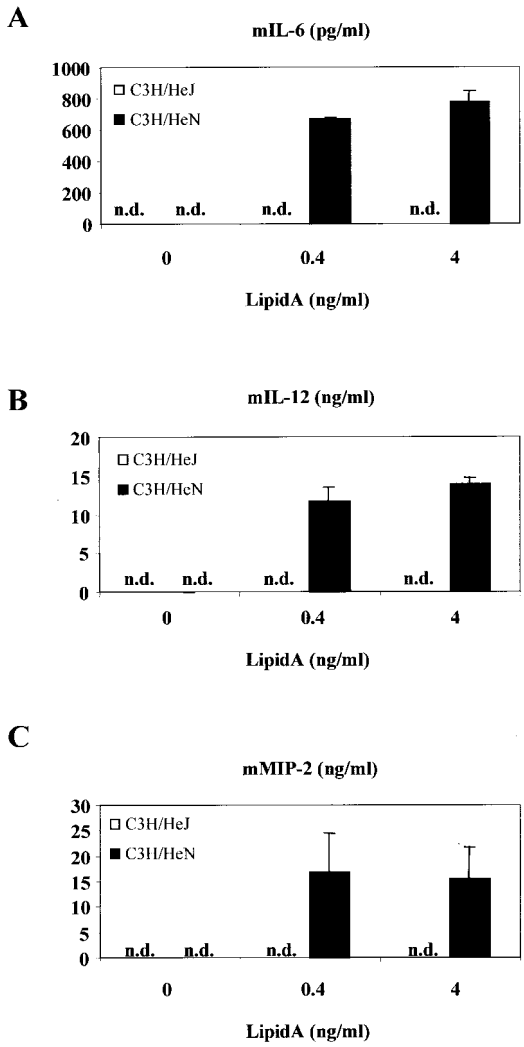

FIG. 6. **Expression of cytokines and chemokines in DCs derived from C3H/HeJ and C3H/HeN mice upon stimulation with lipid A.** DCs from C3H/HeJ and C3H/HeN mice were cultured with the indicated concentrations of lipid A for 24 h. Concentrations of IL-6 (*A*), IL-12p40 (*B*), and MIP-2 (*C*) in the culture supernatants were measured by ELISA. Mean values ± S.D. of two independent experiments are shown. *n.d.*, not detectable.

TLR2$^{-/-}$ DCs. TLR2 heterozygous littermates exhibited an intermediate response (data not shown). Expression of CD86 and MHC class II in response to LPS was up-regulated in TLR2-deficient and wild-type mice (Fig. 1*B*).

Expression of Proinflammatory Cytokines, T_H1 Cytokines, and Chemokines Is Impaired in DCs Derived from TLR2-deficient Mice upon Stimulation with PGN—Next, the production of IL-6, TNF-α, IL-12p40, and MIP-2 by DCs upon stimulation with increasing concentrations of PGN was determined (Fig. 2). Whereas stimulation of wild-type DCs with PGN resulted in a dose-dependent response for all cytokines, stimulation of TLR2-deficient mice led to an impaired response for IL-6, IL-12, and MIP-2 and no response at all for TNF-α. Production of IL-6, TNF-α, MIP-2, and IL-12p40 in response to stimulation with LPS, in contrast, was strongly induced in DCs from TLR2-deficient mice, as expected (data not shown).

Antigen Uptake of Immature Dendritic Cells Derived from TLR2$^{-/-}$ Mice—To confirm the observed lack of DC maturation in TLR2$^{-/-}$ mice stimulated with PGN by a functional assay, we tested the capacity of antigen capture by DCs as measured by FITC-dextran uptake. For this purpose DCs were incubated with FITC-dextran at 4 and 37 °C (Fig. 3). Unstimu-

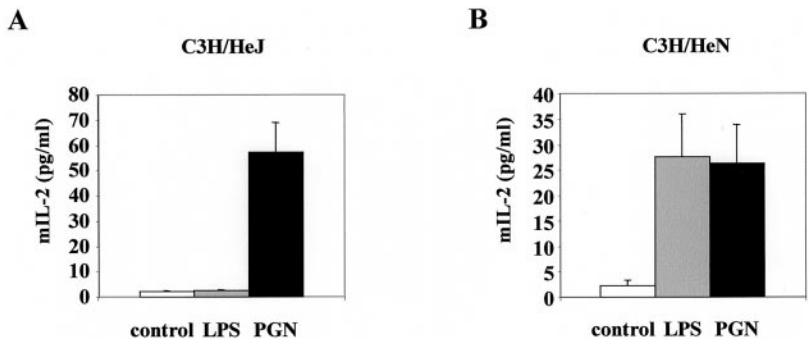

FIG. 7. **Antigen-presenting function of DCs derived from C3H/HeJ and C3H/HeN mice in an MLR.** DCs from C3H/HeJ (*A*) and C3H/HeN mice (*B*) were cultured with either 5 μg/ml PGN or 10 ng/ml LPS for 24 h. Purified CD4+ T cells were added to the DCs at a DC:T cell ratio of 1:50 and cultured for an additional 3 days. Concentrations of IL-2 in the culture supernatants were measured by ELISA. Data shown represent the mean value of triplicate measurements with S.D. from one experiment representative of two separate experiments with similar results.

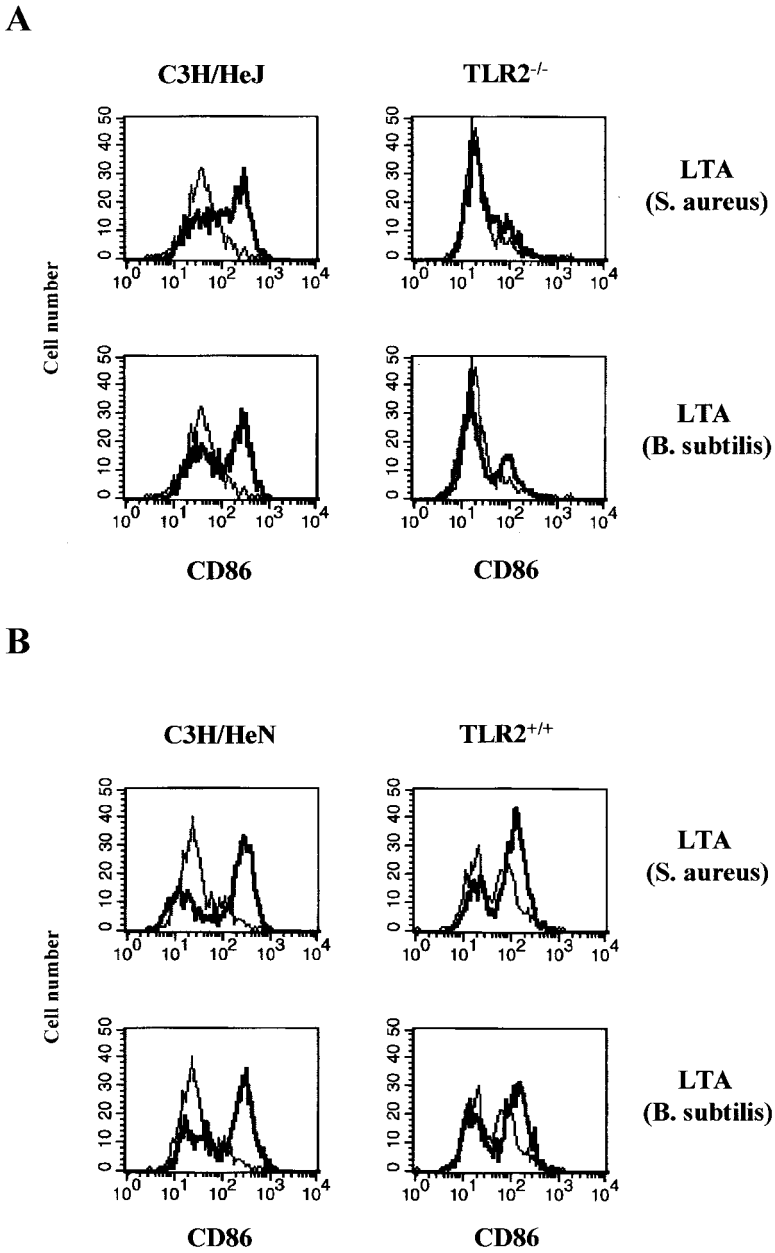

FIG. 8. **Expression of costimulatory molecules in TLR2$^{-/-}$ and C3H/HeJ DCs upon LTA stimulation.** DCs from TLR2$^{-/-}$, TLR2$^{+/+}$, C3H/HeJ, and C3H/HeN mice were cultured in the presence of 10 μg/ml *S. aureus* or *B. subtilis* LTA for 24 h. CD86 expression of DCs from deficient mice (*A*) or corresponding wild-type mice (*B*) were analyzed by FACScan. Unstimulated DCs (*thin lines*) were compared with LTA-stimulated DCs (*bold lines*).

lated immature DCs derived from TLR2$^{-/-}$ mice displayed an uptake of FITC-dextran at 37 °C, which was completely lost upon maturation induced by LPS. TLR2$^{-/-}$ DCs stimulated with PGN displayed an FITC-dextran uptake similar to that of the control DCs, indicating that these cells are functionally still immature.

Antigen Presentation of Mature Dendritic Cells Derived from TLR2$^{-/-}$ Mice in MLRs—To further examine the functional consequences of TLR2 deficiency, we investigated the capacity of DCs derived from TLR2$^{-/-}$ mice stimulated with PGN and LPS to stimulate T cells in an allogeneic MLR (Fig. 4). Unstimulated DCs and PGN- and LPS-stimulated DCs derived from TLR2$^{-/-}$ and TLR2$^{+/+}$ mice were cocultured with allogeneic T cells at a DC:T cell ratio of 1:50 for 3 days. T cell activation was assessed by IL-2 release. Because IL-2 is not produced by DCs, activated T cells in the cocultures are the only source of IL-2.

DCs derived from TLR2$^{-/-}$ mice stimulated with PGN were not able to induce IL-2 secretion from T cells similar to untreated cells (Fig. 4*A*), whereas DCs from TLR2$^{+/+}$ mice stimulated with PGN induced substantial IL-2 secretion of T cells (Fig. 4*B*). In contrast, DCs from TLR2$^{-/-}$ and TLR2$^{+/+}$ mice were both found to be effective inducers of IL-2 secretion when stimulated with LPS, as expected.

Expression of MHC Class II Is Impaired in C3H/HeJ Mice upon Stimulation with LPS and Lipid A—To determine the role of TLR4 in DC maturation, we examined the maturation of DCs from C3H/HeJ and C3H/HeN mice upon stimulation with LPS. Stimulation of DCs of C3H/HeN mice with LPS resulted in an up-regulation of MHC class II molecules. In contrast, LPS stimulation of DCs derived from C3H/HeJ mice led to an impaired up-regulation of MHC class II molecules (Fig. 5*A*). Similar results were obtained using a synthetic lipid A preparation (Fig. 5*B*). Additionally, FITC-dextran uptake in C3H/HeJ mice stimulated with LPS confirmed the lack of maturation of DCs in C3H/HeJ mice (data not shown).

Expression of Proinflammatory Cytokines, T_H1 Cytokines, and Chemokines Is Impaired in C3H/HeJ Mice upon Stimulation with Lipid A—We next examined the cytokine secretion of DCs during maturation upon stimulation with lipid A, the bioactive component of LPS. Whereas stimulation with lipid A resulted in a response in DCs from C3H/HeN mice, in C3H/HeJ mice no IL-6 (Fig. 6*A*), IL-12 (Fig. 6*B*), and MIP-2 (Fig. 6*C*) response upon lipid A stimulation could be observed. Additional experiments with LPS led to a similar lack of cytokine and chemokine response in DCs from C3H/HeJ mice (data not shown).

Antigen Presentation of Mature Dendritic Cells Derived from C3H/HeJ and C3H/HeN Mice in MLRs—We also investigated the capacity of C3H/HeJ- and C3H/HeN-derived DCs stimulated with PGN and LPS to induce IL-2 production of allogeneic T cells (Fig. 7). LPS-stimulated DCs derived from C3H/HeJ mice were unable to increase IL-2 release from T cells as compared with untreated cells (Fig. 7*A*). DCs from C3H/HeN mice, in contrast, enhanced the IL-2 production of T cells after stimulation with LPS (Fig. 7*B*). DCs from both mice strains were effective in inducing IL-2 secretion when stimulated with the TLR2 ligand PGN, as expected.

Expression of Cytokines and CD86 in C3H/HeJ Mice upon Stimulation with LTA—To assess LTA-induced maturation of DCs, we examined the responsiveness of DCs from TLR2- and TLR4-deficient mice to LTA from *S. aureus* and *B. subtilis*. Stimulation of DCs derived from C3H/HeJ and C3H/HeN mice with highly purified LTA from *S. aureus* and *B. subtilis* resulted in the up-regulation of CD86 in both strains (Fig. 8, *A* and *B*, *left panels*). Furthermore, no differences in secretion of IL-6 (Fig. 9*A*) in LTA-induced DC maturation could be observed in either mouse strain. In contrast, up-regulation of CD86 in DCs from TLR2-deficient mice was severely impaired upon LTA stimulation compared with wild-type mice (Fig. 8, *A* and *B*, *right panels*). Additionally, production of IL-6 (Fig. 9*B*) was not detectable in DCs from TLR2-deficient mice in response to LTA from *S. aureus* and *B. subtilis*. Similar results have been obtained for the production of IL-12 upon LTA stimulation (data not shown).

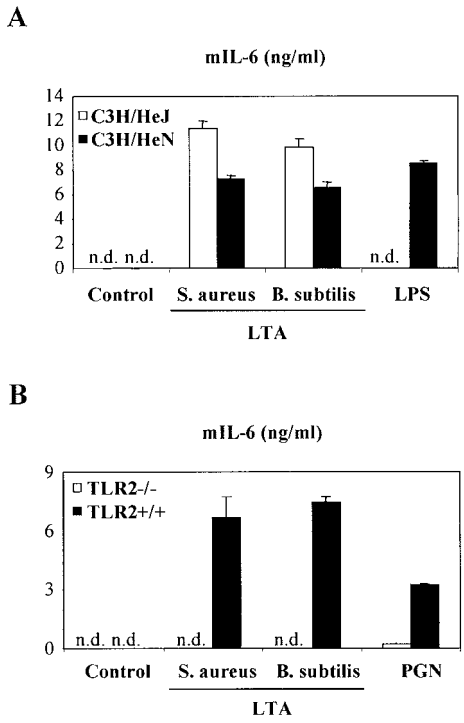

FIG. 9. **Expression of IL-6 in TLR2$^{-/-}$ and C3H/HeJ DCs upon stimulation with LTA.** DCs (4×10^5 cells/ml) from TLR2$^{-/-}$, TLR2$^{+/+}$, C3H/HeJ, and C3H/HeN mice were cultured in the presence of 10 μg/ml *S. aureus* or *B. subtilis* LTA, 100 ng/ml *S. minnesota* Re 595 LPS (*A*), or 10 μg/ml PGN (*B*) for 24 h. Concentrations of IL-6 in the culture supernatant were measured by ELISA. Mean values ± S.D. of two independent experiments are shown. *n.d.*, not detectable.

DISCUSSION

Dendritic cells are antigen-presenting cells with the unique ability to initiate both innate immune responses and a highly specific acquired immunity (1). Therefore, these cells have been the focus of intensive investigations in biomedical research, *i.e.* in the area of cancer research for developing DC-based cancer vaccines as a novel immunotherapy. DCs activate both naïve and memory CD4+ and CD8+ T cells and seem to meet all requirements as a strong vaccine for antitumor immunity or immunity against microbial infection (21). Located in most host tissues, DCs function as mobile sentinels for infectious agents and inflammatory products of bacteria. In contrast to macrophages, the phagocytosis of bacteria by DCs is limited (3). After challenge with bacteria, DCs enter the process of maturation and migrate to lymphoid organs. During this process DCs not only lose the ability of phagocytosis, but they also produce large amounts of cytokines and chemokines (3). Simultaneously, MHC class II molecules are translocated to the membrane, and costimulatory molecules such as CD86 and CD40 are up-regulated (1, 22).

Because of the important role of DCs as a mediator between innate and adaptive immunity, it is of great importance to elucidate the regulation of their maturation and to analyze the molecules involved. Here we show the involvement of TLR2 and TLR4 in the maturation of DCs. Both TLR2 and TLR4 have been implicated in the recognition of bacterial cell wall products of Gram-positive (14, 23, 24) and Gram-negative bacteria (9, 25), spirochetes (26), yeast (23), and mycobacteria (23, 27) by the innate immune system. Although certain bacterial stimuli are well known as inducers of DC maturation, the receptor mechanism involved in this induction of maturation has not yet been elucidated. Besides induction by bacterial stimuli, DC maturation can also be induced by proinflammatory cytokines (TNF-α) and CD40 cross-linking by CD40 ligand on activated T cells. A very recent report, however, notes that for CD40-induced IL-12 production of murine DCs additional microbial stimuli are required for obtaining an optimal response (28). Our findings of the involvement of TLRs in bacteria-induced DC maturation complement theories on bridging innate and acquired immunity by pattern recognition receptors such as the TLRs (29).

Common understanding defines DCs as cells lacking

mCD14. By employing blocking CD14 antibodies, it was demonstrated that LPS-induced responses of human DCs are dependent on soluble CD14 (30). Utilizing murine CD14 antibodies a down-regulation of the LPS-induced response of DCs was also detected (31). Our present study provides substantial evidence that bacteria-induced maturation of DCs depends on TLR interaction; PGN-induced maturation of DCs is severely impaired in TLR2$^{-/-}$ mice. LPS- and lipid A-induced DC maturation is initiated via TLR4 occupation, because DCs from C3H/HeJ mice were unable to undergo maturation. Lack of TLR2 or -4 abolished up-regulation of MHC class II and CD86 and led to an impaired secretion of proinflammatory cytokines, chemokines, and T_H1 cytokines upon stimulation with the appropriate ligand. To address the biological relevance of these findings we employed two different functional assays for DC maturation. Antigen presentation was found to be severely impaired in TLR2$^{-/-}$ and C3H/HeJ DCs when stimulated with the appropriate ligand, as measured by IL-2 secretion of cocultured T cells. In addition, FITC-dextran antigen uptake, a characteristic function of immature DCs, could not be down-regulated by the maturation-inducing ligands PGN or LPS in DCs derived from TLR2- and TLR4-deficient mice. These findings led us to conclude that DCs deficient in TLR2 or TLR4 do not undergo maturation upon stimulation with the TLR2 ligand PGN and the TLR4 ligands LPS and lipid A, respectively. The question whether LTA signals via TLR2 or -4 has been controversial (14, 32, 33). Schwandner *et al.* (32) demonstrated that HEK293 cells overexpressing TLR2 regained the ability to react upon LTA stimulation with NF-κB translocation. This HEK293 system has been questioned recently in the context of LPS-induced signal transduction due to a lack of expression of MD-2, a molecule associated with TLR4 and absolutely required for LPS signaling via TLR4 (34). In the context of TLR2-mediated signal transduction, however, this system still may be valuable.[2] In contrast, it was recently reported that TLR4$^{-/-}$ macrophages, but not wild-type or TLR2$^{-/-}$ macrophages, failed to respond to LTA from *S. aureus* (14). Our results presented here clearly demonstrate that DCs derived from C3H/HeJ mice respond to LTA from *B. subtilis* and *S. aureus* to a similar extent as DCs from C3H/HeN mice regarding their secretion of cytokines as well as up-regulation of CD86. The results obtained with DCs from TLR2$^{-/-}$ mice furthermore support the view that TLR2 is the signal transducer for LTA. These discrepancies between our data and the report mentioned above may be due to differences in the cell type employed (DCs *versus* macrophages) or to differences in the purification procedure applied for the LTA used. We observed a slightly higher expression of IL-6 (Fig. 9*A*) and IL-12 (data not shown) in DCs derived from C3H/HeJ mice compared with C3H/HeN mice when stimulated with highly purified LTA from *S. aureus* as well as *B. subtilis*. An up-regulation of TLR2 or other TLRs to compensate for the loss of TLR4 may be an explanation for this phenomenon.

In summary, our results demonstrate that recognition of cell wall compounds of Gram-positive and Gram-negative bacteria by TLRs on the surface of DCs is essential for bacteria-induced DC maturation and thus for initiation of innate and acquired immune responses. Gaining further insight into DC maturation may increase understanding of DC biology and of how to utilize these cells in clinical applications.

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