

Neisserial Porin-Induced Dendritic Cell Activation Is MyD88 and TLR2 Dependent¹

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Neisserial porins have been shown to act as B cell mitogens and immune adjuvants. PorA and PorB are the major outer membrane porin proteins of the human pathogen *Neisseria meningitidis*. We have shown that the mechanism of the immunopotentiating capability of porin involves up-regulation of the T cell costimulatory ligand, CD86. Due to neisserial porin's ability to activate B cells and potentiate immune responses, we hypothesized that porin also employs the potent immune stimulatory function of dendritic cells (DC). We examined the ability of purified *N. meningitidis* PorB to induce maturation of murine splenic and bone marrow-derived DC. PorB treatment induced DC maturation, as demonstrated by increased expression of CD86 and class I and II MHC molecules. In addition, PorB not only enhanced the allostimulatory activity of DC, but also augmented the ability of DC to stimulate T cells in an Ag-specific manner. PorB-matured DC secreted the inflammatory cytokine IL-6, which may have implications for the adjuvant property of porin. Induction of IL-6 by PorB is also significant because IL-6 is one of a number of cytokines produced during infection with *N. meningitidis* and may be involved in the inflammatory process observed during infection and disease. We previously demonstrated the requirement of MyD88 and TLR2 for PorB-induced B cell activation. In the present study, MyD88 and TLR2 were also essential for PorB-induced DC activation. This work is significant for elucidating the mechanism(s) of neisserial porin's immune stimulatory activity. *The Journal of Immunology*, 2005, 174: 3545–3550.

Due to their potent stimulation of naive, Ag-specific T cells, dendritic cells (DC)³ have been described as professional APCs (1). Although immature DC localize in nonlymphoid peripheral tissues where they act as sentries that capture and process Ag, mature DC reside in the secondary lymphoid organs where their main function is to stimulate Ag-specific T cells. Through this process of maturation, which involves peripheral tissue surveillance, Ag uptake and processing, and subsequent Ag presentation to naive T cells, DC serve to link the innate and adaptive immune systems (1–3). DC play this role so well that they have been referred to as “nature's adjuvant” (4).

Neisseria meningitidis, a Gram-negative human pathogen, is the leading worldwide cause of bacterial meningitis and fatal sepsis (5). Neisserial porins are the major outer membrane proteins expressed on the surface of *Neisseria* species and comprise >60% of the outer membrane protein content (6). Neisserial porins act as B cell mitogens (7) and are immunogenic in the absence of exogenous adjuvant, thereby enhancing immune responses to poorly immunogenic substances, such as small peptides (8) and capsular polysaccharides (9). Neisserial porins have thus been used as adjuvants in a number of vaccine preparations (8–13). Our group has

reported that neisserial porins are able to induce both B cell proliferation and Ig secretion as well as induce increased surface expression of CD86 and class II MHC molecules on B cells (14, 15). The mechanism of the adjuvant activity of neisserial porins involves up-regulation of the T cell costimulatory ligand, CD86, on the surface of APCs. In a murine vaccine model in which *N. meningitidis* PorB was directly conjugated to meningococcal capsular polysaccharide, administration of CD86 blocking Abs abrogated the ability of porin to induce anticapsular polysaccharide IgG (9).

We have previously reported the requirement of both TLR2 and MyD88 for PorB-induced up-regulation of CD86 and class II MHC on murine B cells (16). We have also demonstrated the requirement of TLR2 expression for PorB-induced NF- κ B nuclear translocation in both Chinese hamster ovary and human embryonal kidney reporter cell lines (16). We have thus established *N. meningitidis* porin as a pathogen-associated molecular pattern (17, 18) recognized by TLR2.

Given the potential involvement of CD86 expression in the adjuvant effect of neisserial porins and the fact that porin enhances the T cell-costimulatory capacity of B cells, we asked whether other APC, namely DC, were also affected by neisserial porin. We hypothesized that the immunopotentiating capability of neisserial porin employs the potent immune stimulatory function of DC. This work is not only significant for understanding the mechanism of the adjuvant activity of neisserial porins, but also for gaining insight into how porin signals activation of immune cells.

Materials and Methods

Mice

Female, 5- to 6-wk-old C3H/HeJ (LPS-hyporesponsive (19)), C57BL/6, and BALB/c mice were purchased from The Jackson Laboratory. Female and male DO11.10 TCR transgenic mice (20) (specific for OVA peptide 323–339 in the context of H-2^b) were a gift from Dr. A. Rothstein (Department of Microbiology, Boston University School of Medicine, Boston, MA). MyD88 knockout (KO), TLR2 KO, and TLR4 KO mice (all on C57BL/6 background) were provided by Dr. S. Akira (Research Institute

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³ Abbreviations used in this paper: DC, dendritic cell; KO, knockout; BM, bone marrow; LOS, lipo-oligosaccharide.

for Microbial Diseases, Osaka University, Osaka, Japan). Mice were maintained at the Laboratory Animal Science Center (Boston University Medical Campus, Boston, MA) under germfree conditions.

Monoclonal Abs

FITC-conjugated rat anti-mouse CD86, mouse anti-mouse class II (I-A^k), mouse anti-mouse class I (H-2K^b), rat IgG2a isotype control, and mouse IgG2a isotype control mAbs were purchased from Caltag Laboratories. FITC-conjugated hamster anti-mouse CD80, hamster anti-mouse CD40, mouse anti-mouse class II (I-A^b), mouse anti-mouse class I (H-2K^b), hamster anti-mouse CD11c (integrin α_x chain), and hamster IgG1a isotype control mAbs were purchased from BD Pharmingen. Vectashield fluorescence mounting medium was purchased from Vector Laboratories.

Generation of splenic DC

Splenic CD11c-positive DC were generated from C3H/HeJ mice. Single-cell splenocyte suspensions were obtained using a sterilized metal mesh and 10-cc syringe plunger. Erythrocytes were lysed with NH₄Cl (pH 7.2), and cell pellet was washed one time with HBSS (BioWhittaker). Cells were labeled with CD11c magnetic beads and purified by MACS (Miltenyi Biotec) following the manufacturer's protocol. RPMI 1640 (BioWhittaker) was supplemented with 10% FBS (Gemini Bio-Products), 100 U/ml penicillin, 100 μ g/ml streptomycin, 2 mM L-glutamine (all from BioWhittaker), and 55 μ M 2-ME (Invitrogen Life Technologies) and designated R10 medium. R1 medium was prepared by supplementing R10 with 30% 0.22- μ m-filtered NIH 3T3 supernatant (fibroblast cell line secreting murine IL-4) and 3% 0.22- μ m-filtered J558L supernatant (B cell line secreting murine GM-CSF). Cells were cultured in R1 medium and fed every 3–4 days. DC were >95% pure based on flow cytometric analysis of expression of the integrin CD11c.

Generation of bone marrow (BM)-derived DC

BM-derived DC were generated from femurs of C3H/HeJ, BALB/c, C57BL/6, TLR4 KO, MyD88 KO, and TLR2 KO mice according to the method described by Inaba et al. (21) with modifications. Following removal of muscle tissue, marrow was flushed from bone with RPMI 1640. Erythrocytes were lysed with NH₄Cl (pH 7.2), and B cells were depleted using anti-class II MHC hybridoma supernatants from TIB229 and TIB120 cell lines (American Type Culture Collection) and rabbit complement (purified from rabbit serum). Remaining cells were seeded in 24-well plates (10⁶/ml) in R1 medium. Cells were fed every 2 days by gently swirling the plate, removing old medium, and adding fresh R1 medium. DC were harvested on day 6 by dislodging aggregates with gentle pipetting.

Flow cytometry

Immature splenic C3H/HeJ DC were seeded in 24-well plates (10⁵/ml) in R10 medium and R10 containing either 10 μ g/ml purified *N. meningitidis* PorB, isolated from the bacterial outer membrane as previously described (22), or 100 ng/ml murine rTNF α (BD Pharmingen). BM-derived C3H/HeJ, C57BL/6, TLR4 KO, MyD88 KO, and TLR2 KO DC were seeded in either 24- or 96-well plates (10⁵–10⁶/ml) in R1 medium and R1 containing 10 μ g/ml PorB, 100 ng/ml rTNF α , or 50 μ g/ml poly(I:C) (Amersham Biosciences). Poly(I:C) was used for stimulation of C57BL/6, TLR4 KO, MyD88 KO, and TLR2 KO DC. Upon 24-h incubation at 37°C (5% CO₂), cells were detached with either PBS or PBS containing 3 mM EDTA, incubated for 45 min in PBS/2% FBS containing the appropriate FITC-conjugated mAb and 10 μ l of mouse IgG (Sigma-Aldrich) to block non-specific Fc γ binding of primary mAbs. mAbs were used at either 1/5 or 1/50 dilution. Cells were washed one time with PBS, resuspended in PBS/2% FBS, and analyzed on a FACScan flow cytometer using CellQuest software (BD Biosciences).

Confocal microscopy

Immature splenic or BM-derived C3H/HeJ DC were seeded (10⁴ or 10⁵/ml) in chamber slide wells (Fisher Scientific) in R1 medium. Nonadherent cells were forced to adhere by centrifugation (1200 rpm; 5 min). R1 medium was removed, and cells were incubated for 24 h in R10 medium and R10 containing 10 μ g/ml PorB. All cell washes were performed three times for 5 min each with PBS and gentle rocking. All incubations were conducted with gentle rocking. Cells were washed, fixed with 4% paraformaldehyde/5% sucrose (15 min), washed, permeabilized with 0.025% SDS (4 min), washed, blocked with 1% BSA (15 min), and incubated for 1 h with FITC-conjugated MHC class II mAb (1/50 or 1/100 dilution in 1% BSA). Cells were washed and mounted in 1:1 Vectashield:Tris-Cl (0.075 M; pH 8.9). Cells were analyzed on a Bio-Rad Radiance 2000 confocal microscope (Bio-Rad Laboratories).

Mixed lymphocyte reaction

Immature splenic C3H/HeJ DC were seeded in 96-well plates (10⁵/ml) in R10 medium and R10 containing either 10 μ g/ml PorB or 100 ng/ml rTNF α . Upon 24-h incubation, cells were irradiated at a dose of 2000 rad (GC40 cesium-137 irradiator; Atomic Energy of Canada Limited) and cocultured with allogeneic, primary, lymph node-derived (inguinal and mesenteric nodes) C57BL/6 T cells (10⁶/ml) for 4 days. T cells were purified by Abs and complement-mediated depletion of B cells, as described above. Cells were pulsed with [³H]thymidine (5 μ Ci/ml) for 18 h. Cells were harvested, and counts were measured using a beta scintillation counter (Beckman Coulter). Negative controls included T cells incubated alone and DC incubated alone as well as T cells incubated with PorB only or TNF- α only. PHA (Sigma-Aldrich) was used at 8 μ g/ml as a positive control for T cell proliferation.

Activation of Ag-specific T cells

Immature BM-derived BALB/c DC were seeded in 96-well, flat-bottom plates (5 \times 10⁴/ml) in R10 medium and R10 containing chicken egg OVA protein alone (10, 50, or 100 μ g/ml) (Sigma-Aldrich), 10 μ g/ml PorB alone, or both OVA (10, 50, or 100 μ g/ml) and PorB (10 μ g/ml). Upon 24-h incubation, DO11.10 CD4⁺ lymph node-derived T cells (purified from inguinal and mesenteric nodes as described above) were added directly to wells containing OVA-treated, PorB-treated, and OVA/PorB-treated DC. DC were cocultured with DO11.10 T cells (2.5 \times 10⁵/ml) for 5 days. Cells were pulsed for 19 or 20 h with [³H]thymidine (5 μ Ci/ml), and counts were measured using a beta scintillation counter.

Detection of IL-6

Immature BM-derived C57BL/6, MyD88 KO, and TLR2 KO DC were seeded in 96-well, flat-bottom plates (10⁶/ml) in R1 medium and R1 containing either 10 μ g/ml PorB or 100 ng/ml *N. meningitidis* lipo-oligosaccharide (LOS) (a gift from M. Apicella, Department of Microbiology, University of Iowa, Iowa City, IA). Upon 24-h incubation (37°C; 5% CO₂), cell-free supernatants were harvested by centrifugation (1200 rpm; 10 min) and stored at –20°C before determination of IL-6 concentration (OptEIA Mouse IL-6 Set; BD Pharmingen).

Results

PorB induces DC morphological changes and up-regulation of surface MHC and CD86 molecules

We have previously shown that neisserial porins induce up-regulation of CD86 and class II MHC molecules on murine B cells (14), thereby enhancing the T cell-costimulatory capacity of these cells. To further investigate the effect of neisserial porins on APCs, we examined the ability of PorB to induce maturation of DC. Splenic-derived C3H/HeJ DC treated for 24 h with purified *N. meningitidis* PorB displayed changes in morphology consistent with cell activation (3, 23). Although R10 medium-treated cells were small and round, cells treated with either PorB or the proinflammatory cytokine, TNF- α , had many dendritic prolongments and were large and granular, as evidenced by phase contrast microscopy (Fig. 1).

We then investigated the effect of neisserial porins on the surface expression of class I and II MHC molecules as well as the T cell-costimulatory molecules CD40, CD80, and CD86 on DC.

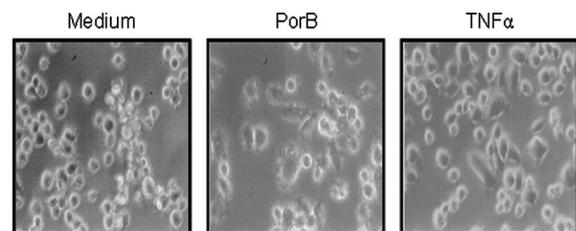


FIGURE 1. *N. meningitidis* PorB induces DC activation. Splenic-derived C3H/HeJ DC were treated for 24 h with R10 medium alone or R10 medium containing either 10 μ g/ml PorB or 100 ng/ml TNF- α . Phase contrast micrographs are representative of one of several experiments.

Treatment of splenic or BM-derived C3H/HeJ DC for 24 h with PorB induced up-regulation of surface CD86, class I MHC and class II MHC molecules, similar to levels induced by 24-h treatment with the proinflammatory cytokine TNF- α , as evidenced by flow cytometry (Fig. 2). Treatment of splenic DC with PorB had no effect on levels of CD80 or CD40. Although CD80 expression by BM-derived DC was also not affected by PorB treatment, levels of CD40 on PorB-treated BM-derived DC were greater than that of cells incubated with medium alone.

PorB induces surface localization of class II MHC molecules on DC

In immature DC, class II MHC molecules reside within an intracellular compartment known as the MHC class II-enriched compartment (24). During DC maturation, peptide-loaded class II molecules localize at the cell surface where they engage the TCR on naive, Ag-specific T cells. We examined whether PorB-induced up-regulation of class II MHC molecules could be visualized as a rearrangement of cellular class II molecules. Following 24-h PorB treatment, class II MHC molecules localized at the DC surface, as compared with 24-h treatment with medium in which class II molecules were present in an intracellular, perinuclear compartment, as evidenced by confocal microscopy (Fig. 3). This experiment was also conducted with C3H/HeJ BM-derived DC with identical results.

PorB induces allostimulatory activity in DC

PorB-matured DC expressed high levels of surface CD86 and MHC molecules and hence should be primed to induce activation of T cells. To determine the functional consequences of PorB-induced DC maturation, we investigated the allostimulatory activity of PorB-treated DC. In the MLR, MHC-restricted T cells are mixed with APC from a different MHC background, and the proliferation of alloreactive T cells (T cells specific for allogeneic MHC) is measured (25). PorB-matured, C3H/HeJ (H-2^k) splenic-derived DC were cocultured with primary, C57BL/6 (H-2^b-restricted) T cells. Incubation of DC with PorB for 24 h induced

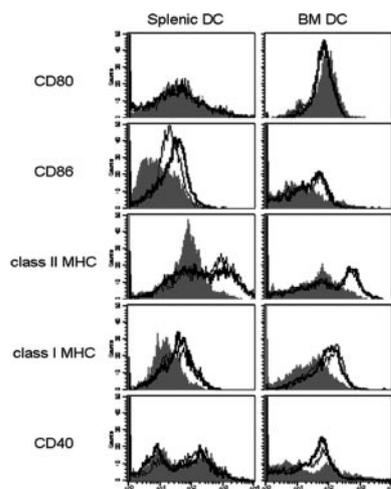


FIGURE 2. *N. meningitidis* PorB induces up-regulation of surface MHC and CD86 molecules on DC. Splenic C3H/HeJ DC were treated for 24 h with R10 medium alone (filled histograms) or R10 medium containing either 10 μ g/ml PorB (thick line) or 100 ng/ml TNF- α (thin line). BM-derived C3H/HeJ DC were treated as above except R1 medium was used in place of R10. Cells were analyzed for expression of surface CD80, CD86, class I and II MHC, and CD40. This experiment was conducted more than three times with similar results. Histograms display results from one representative experiment.

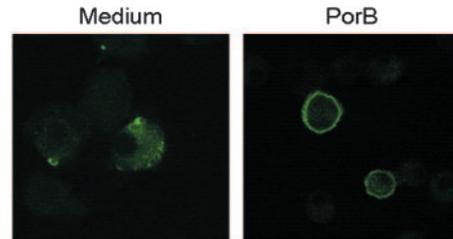


FIGURE 3. *N. meningitidis* PorB induces surface localization of class II MHC molecules on DC. Splenic C3H/HeJ DC were treated for 24 h with R10 medium alone or R10 medium containing 10 μ g/ml PorB. Cells were fixed, permeabilized, and stained with anti-I-A^K (class II MHC) mAb. Localization of class II molecules was visualized by confocal microscopy. This experiment was conducted three times with identical results.

allostimulatory activity in DC, greater than that induced by 24-h treatment with TNF- α , as shown by T cell [³H]thymidine uptake (Fig. 4). As expected, medium-treated DC did not exhibit allostimulatory activity. Also, DC incubated alone or T cells incubated alone did not proliferate. Additional negative controls included T cells incubated with either PorB or TNF- α in the absence of DC. These T cells also did not proliferate (data not shown).

PorB-matured DC induce activation of Ag-specific T cells

Because the MLR occurs in the absence of Ag, we next examined the ability of PorB to enhance presentation of the model protein Ag, chicken egg OVA, to DO11.10 TCR transgenic CD4⁺ T cells (recognize OVA_{323–339} peptide in the context of H-2^d). BALB/c BM-derived DC were treated for 24 h with R10 medium or R10 containing one of the following: OVA protein alone, PorB alone, or both OVA and PorB. DC were then cocultured with primary, DO11.10 CD4⁺ T cells. DC cotreated with OVA and PorB induced proliferation of DO11.10 T cells 4-fold over levels seen when DC were treated with OVA protein alone. DC treated with either R10 medium or PorB alone did not induce proliferation of DO11.10 T cells (Fig. 5).

PorB-induced DC activation is MyD88 and TLR2 dependent

We have previously reported the requirement of members of the TLR-signaling pathway for PorB-induced B cell activation. We demonstrated that expression of both MyD88 and TLR2 was essential for the activation of B cells by neisserial porin (16). To determine the role of these signaling molecules in PorB-induced DC activation, we examined the effect of PorB on MyD88 KO and TLR2 KO DC. BM-derived C57BL/6, TLR4 KO, MyD88 KO, and

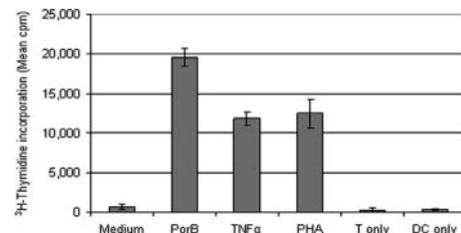


FIGURE 4. *N. meningitidis* PorB induces allostimulatory activity in DC. Splenic-derived C3H/HeJ (H-2^k) DC (10^5 /ml) were treated for 24 h with R10 medium alone or R10 medium containing either 10 μ g/ml PorB or 100 ng/ml TNF- α . Lymph node-derived C57BL/6 (H-2^b) T cells (10^6 /ml) were cocultured with DC for 4 days before 18-h pulse with [³H]thymidine. PHA (8 μ g/ml) was used as a positive control for T cell proliferation. Negative controls included T cells incubated alone and DC incubated alone. Results are represented as mean cpm \pm SD of triplicate values. This experiment was repeated three times with similar results.

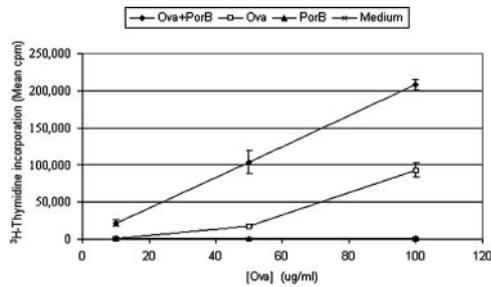


FIGURE 5. *N. meningitidis* PorB induces Ag-specific T cell activation. BM-derived BALB/c DC (5×10^4 /ml) were treated for 24 h with R10 medium alone or R10 medium containing one of the following: OVA protein alone (10, 50, or 100 μ g/ml), PorB alone (10 μ g/ml), or both OVA protein (10, 50, or 100 μ g/ml) and PorB (10 μ g/ml). At 24 h, DO11.10 lymph node-derived T cells (2.5×10^5 /ml) were added to the wells of pretreated DC. T cells and DC were cocultured for 5 days before 19- to 20-h pulse with [3 H]thymidine. Results are represented as mean cpm \pm SD of nine replicate values. This experiment was repeated four times with similar results.

TLR2 KO DC were treated for 24 h with either PorB or poly(I:C) (a synthetic dsRNA mimic known to induce cell activation through TLR3 (26), thus providing a control for both MyD88 KO and TLR2 KO DC). High baseline levels of CD86, class II MHC, class I MHC, and CD40 were observed in C57BL/6 DC (Fig. 6, gray histograms). Despite these high levels, PorB induced up-regulation of CD86, class II MHC, and CD40 molecules, similar to levels induced by poly(I:C). TLR4 KO DC resembled C57BL/6 DC in that baseline levels of activation markers were high and PorB induced increased levels of CD86, class II MHC, and CD40. As expected, treatment of MyD88 KO DC with PorB did not induce up-regulation of CD86, MHC molecules, or CD40. Poly(I:C) induced up-regulation of these markers in MyD88 KO cells. Although treatment of TLR2 KO DC with PorB did not lead to up-regulation of CD86 or CD40, there were potential minor increases in class I and II MHC molecules.

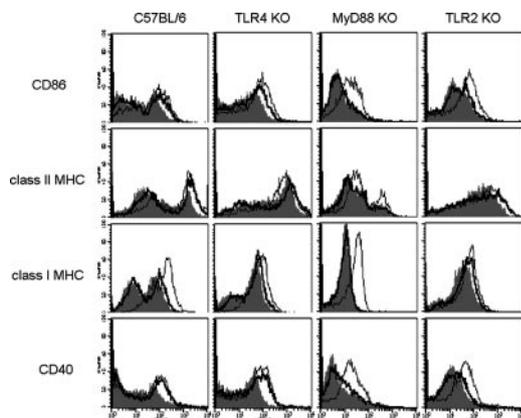


FIGURE 6. *N. meningitidis* PorB does not induce up-regulation of surface MHC, CD86, and CD40 molecules in MyD88- and TLR2-deficient DC. BM-derived C57BL/6, TLR4 KO, MyD88 KO, and TLR2 KO DC were treated for 24 h with R1 medium alone (filled histograms) or R1 medium containing either 10 μ g/ml PorB (thick line) or 50 μ g/ml poly(I:C) (thin line). Cells were analyzed for expression of surface CD86, CD40, and class I and II MHC molecules. This experiment was repeated three times with similar results. Histograms display results from one representative experiment.

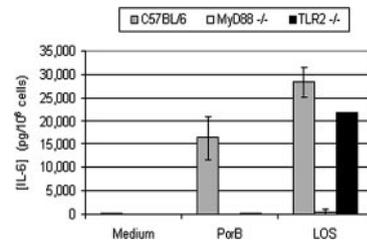


FIGURE 7. *N. meningitidis* PorB induces IL-6 production in DC that is dependent on expression of MyD88 and TLR2. BM-derived C57BL/6, MyD88 KO, and TLR2 KO DC were treated for 24 h with R1 medium alone or R1 medium containing either 10 μ g/ml PorB or 100 ng/ml *N. meningitidis* LOS. Cell-free supernatants were collected, and IL-6 concentration was analyzed by ELISA. Results are represented as mean \pm SD of triplicate measurements.

PorB-induced IL-6 secretion by DC is MyD88 and TLR2 dependent

To further characterize the functionality of PorB-matured DC, we examined the ability of PorB to induce cytokine production in DC. BM-derived C57BL/6 DC were treated for 24 h with R1 medium and R1 containing either PorB or *N. meningitidis* LOS. Cell-free supernatants were harvested and examined by ELISA for IL-6 concentration. Although control C57BL/6 DC did not produce IL-6 in response to medium, PorB-treated cells secreted nearly 15 ng/ml IL-6 (Fig. 7). LOS-induced IL-6 secretion in C57BL/6 DC was roughly 2-fold that elicited by PorB. We next examined the requirement of both MyD88 and TLR2 for PorB-induced IL-6 production. PorB-induced IL-6 secretion was completely abrogated in both MyD88 and TLR2 KO DC (Fig. 7). LOS treatment induced IL-6 secretion in TLR2 KO DC, but not in MyD88 KO DC, indicative of the known requirement of MyD88 for LOS-induced cytokine production in DC (27).

Discussion

Neisserial porins have long been used as vaccine adjuvants, although the exact mechanism of their immunopotentiating properties has remained largely unexamined. The present study demonstrates the ability of *N. meningitidis* PorB to activate DC, thereby implicating a potential role for DC in the adjuvant effect of neisserial porins.

N. meningitidis PorB induced maturation of murine DC, as demonstrated by cell morphology consistent with activation; increased levels of surface CD86 and class I and II MHC molecules; and movement of class II MHC from intracellular compartments to the cell surface. Hence, PorB augmented the T cell-costimulatory capacity of DC. In fact, PorB-matured DC displayed enhanced allostimulatory activity and increased ability to stimulate naive, Ag-specific T cells. Thus, the immunopotentiating ability of PorB may use the potent immune stimulatory function of DC, which could prove to be a useful tool for manipulation of immune responses.

The ability of PorB to induce activation of DC was not due to lipopeptide contamination, because previous studies comparing purified H8 and PorB demonstrated that the ability of PorB to induce CD86 up-regulation in B cells was unrelated to the presence of lipopeptide (16). Moreover, a new purification method was used to obtain PorB for all experiments, which allowed for isolation of PorB free of H8 lipopeptide contamination (P. Massari, unpublished observations).

The ability of PorB to up-regulate expression of CD86, CD40, and MHC molecules on DC was dependent on the expression of both MyD88 and TLR2, because DC from mice deficient in these signaling molecules did not respond to PorB. This finding was not

entirely surprising, because we have previously demonstrated the requirement of MyD88 and TLR2 for PorB-induced B cell activation (16), but does demonstrate that *N. meningitidis* PorB carries out its effect on both B cells and DC through identical receptor-signaling pathways.

It was interesting that the PorB-treated TLR2 KO DC displayed a slight increase in both class I and II MHC molecules, whereas CD86, CD40, and CD54 (Fig. 6 and data not shown) expression were completely unaffected by PorB treatment. This pattern (no change in levels of CD86, CD40, or CD54 and slight increase in class I and II MHC) was also seen when TLR2 KO DC were treated with a different TLR2 ligand, the synthetic lipoprotein Pam₃CSK₄ (data not shown). It has been reported that signaling via TLR2 occurs through heterodimers with either TLR1 or TLR6 (28–30). It is possible that the slight up-regulation of class I and II MHC molecules on PorB-treated TLR2 KO cells may be due to the presence of these additional signaling molecules.

It has recently been shown that a recombinant form of *N. meningitidis* PorA induces up-regulation of CD80, CD86, CD40, and class II MHC molecules on human DC (31). This is consistent with the ability of PorB in the present study to induce activation of murine DC. In addition, consistent with our findings that PorB enhanced both the allostimulatory activity of murine DC and the ability of murine DC to stimulate Ag-specific T cell responses, Al-Bader et al. (31) reported similar findings for rPorA in human DC.

N. meningitidis infection and resulting disease (meningitis and/or septicemia) leads to production of the proinflammatory cytokines TNF- α , IL-1, and IL-6, and the chemokine IL-8 (32, 33). It has been generally accepted that this response is induced by meningococcal LOS (34). A number of investigators have demonstrated that human DC can produce TNF- α , IL-1 β , IL-6, and IL-8 upon in vitro treatment with *N. meningitidis* (32, 33), suggesting that DC may contribute to the pathology of meningococcal disease. In addition, it has been demonstrated that *N. meningitidis* deficient in expression of outer membrane LOS is still capable of inducing production of TNF- α , IL-1 β , IL-6, and IL-8 by human DC, although with diminished levels compared with wild-type bacteria (33, 34). These findings not only implicate non-LOS bacterial components in the pathology of *N. meningitidis* infection and disease, but also identify DC as a potential source of inflammatory cytokines. In the current study, *N. meningitidis* PorB induced production of IL-6 in murine DC (Fig. 7), demonstrating that neisserial porins are capable of eliciting inflammatory cytokines. Consistent with this finding, Al-Bader et al. (31) showed that rPorA induced secretion of IL-6, IL-12p40, and TNF- α in human DC. The ability of PorB to elicit IL-6 secretion from DC has implications for the adjuvant effect of porin. Because IL-6 is known to promote B cell proliferation and the production of Igs (35), as well as regulate the differentiation of Th cells (36), it may play a role in the immunopotentiating ability of neisserial porin. In the present study, PorB-induced IL-6 secretion was dependent on expression of both MyD88 and TLR2, further demonstrating a requirement for these signaling molecules in PorB-induced DC activation.

The ability of PorB to induce functional maturation of professional APC sheds light on the mechanism of the adjuvant effect of neisserial porins. This is significant for the design of effective vaccines that use porin as an adjuvant. This work also demonstrates the potential contribution of neisserial porins to host inflammation seen during *N. meningitidis* infection. Moreover, we have characterized PorB as a pathogen-associated molecular pattern (18) recognized by DC via TLR2, thereby gaining insight into how neisserial porins induce activation of immune cells. Current studies focus on determining the ability of PorB to induce production of

additional inflammatory cytokines, such as IL-1 β , IL-12, and TNF- α in murine DC, as well as examining the type of T cell response (Th1 vs Th2) elicited by PorB.

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Disclosures

The authors have no financial conflict of interest.

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