Lipopolysaccharides from Distinct Pathogens Induce Different Classes of Immune Responses In Vivo

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The adaptive immune system has evolved distinct responses against different pathogens, but the mechanism(s) by which a particular response is initiated is poorly understood. In this study, we investigated the type of Ag-specific CD4\(^{+}\) Th and CD8\(^{+}\) T cell responses elicited in vivo, in response to soluble OVA, coinjected with LPS from two different pathogens. We used *Escherichia coli* LPS, which signals through Toll-like receptor 4 (TLR4) and LPS from the oral pathogen *Porphyromonas gingivalis*, which does not appear to require TLR4 for signaling. Coinjections of *E. coli* LPS + OVA or *P. gingivalis* LPS + OVA induced similar clonal expansions of OVA-specific CD4\(^{+}\) and CD8\(^{+}\) T cells, but strikingly different cytokine profiles. *E. coli* LPS induced a Th1-like response with abundant IFN-\(\gamma\), but little or no IL-4, IL-13, and IL-5. In contrast, *P. gingivalis* LPS induced Th and T cell responses characterized by significant levels of IL-13, IL-5, and IL-10, but lower levels of IFN-\(\gamma\). Consistent with these results, *E. coli* LPS induced IL-12(p70) in the CD8\(^{+}\) dendritic cell (DC) subset, while *P. gingivalis* LPS did not. Both LPS, however, activated the two DC subsets to up-regulate costimulatory molecules and produce IL-6 and TNF-\(\alpha\). Interestingly, these LPS appeared to have differences in their ability to signal through TLR4; proliferation of splenocytes and cytokine secretion by splenocytes or DCs from TLR4-deficient C3H/HeJ mice were greatly impaired in response to *E. coli* LPS, but not *P. gingivalis* LPS. Therefore, LPS from different bacteria activate DC subsets to produce different cytokines, and induce distinct types of adaptive immunity in vivo. *The Journal of Immunology*, 2001, 167: 5067-5076.

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**Materials and Methods**

**Mice**

OT-2 TCR transgenic mice (strain 426-6), generated by W. Heath (Walter & Eliza Hall Institute, Melbourne, Australia) and F. Carbone (Monash...
LPS was sonicated extensively, to ensure uniform mixing of micelles and observed to be below the detection limit. Before mixing with OVA, LPS, endotoxin activity in saline was measured by LAL QCL-1000 kit, analyzed by SDS-PAGE to confirm and stained for protein with Coomassie blue. Selected samples were also separated by SDS-PAGE against distilled water for 3 days, lyophilized, and stored at room temperature (density fractions between 1.42 and 1.52 g/ml) were pooled, dialyzed twice, and the resulting purity of CD11c+ cells after two rounds was >90%. The enriched DCs were stained with FITC-conjugated CD11c (BD PharMingen) and PE-conjugated CD8α (BD PharMingen) and sort-ed into the CD11c+CD8α+ and CD11c+CD8α− subsets, using a FACSVantage flow cytometer (BD Biosciences, San Jose, CA), equipped with Enterprise II laser (Coherent Radiatin, Palo Alto, CA). In some experiments, CD11c+ DCs were enriched from the spleens of C3H/HeN and CH/HeJ mice using the CD11c + microbeads; the purity of such enriched DCs was ~90%.

Induction of cytokines from DC subsets

DCs were cultured in RPMIc + 5% FBS with GM-CSF (20 ng/ml) + IFN-γ (20 ng/ml), in the presence of either E. coli LPS or P. gingivalis LPS for 24 or 48 h, and the cytokines were secreted in the supernatants assayed by ELISA.

Results

E. coli LPS and P. gingivalis LPS enhance Ag-specific Th responses in vivo

We examined whether LPS from E. coli and P. gingivalis could enhance Ag-specific Th responses against a soluble protein, such as OVA. To investigate this, we used OVA-specific, MHC class II-restricted (I-Aβ), αβ TCR transgenic mice (OT-2 mice) (42). In these mice, the CD4+ OVA-specific T cells express Vα2 and Vβ8. TCR transgenic T cells were adoptively transferred into Thy-1 congenic B6.PL.Thy-1+ (B6.PL) mice, such that they constituted a small, but detectable proportion of all T cells (43). In this system, the fate of OVA-specific, transgenic T cells was followed using the Thy-1.2 Ab, which stains the transferred cells, but not the host cells. Cells with the phenotype Thy-1.2+ CD4+ Vα2− Vβ8− are considered OVA-specific CD4+ T cells. In some of the experiments, we simply used Thy-1.2 in combination with CD4, to detect the OVA-specific T cells.

The reconstituted mice were injected with 2 mg soluble OVA alone, or OVA + E. coli LPS, or OVA + P. gingivalis LPS, either i.p. or in the footpads (Fig. 1). Before the experiment, OVA was depleted of endotoxin contamination, using the Detoxi Columns, and tested for endotoxin using the QCL-1000 kit. The CD4+ OVA-specific T cell response either in the draining lymph nodes or in the spleen was monitored by flow cytometry (Fig. 2A). Injection of OVA elicited a significant clonal expansion of the Thy-1.2+ CD4+ T cells in the draining lymph nodes of mice, which received footpad injections (Fig. 2, A, B, and D). Similar expansions were observed with the i.p. route of injection (Fig. 2, C and E). Both E. coli LPS and P. gingivalis LPS could significantly enhance the percentage and absolute numbers of Thy-1.2+ CD4+ T cells, regardless of the route of injection (Fig. 2). However, P. gingivalis LPS was marginally better than E. coli LPS in enhancing the clonal expansion (7% OVA + E. coli LPS vs 9.4% OVA + P. gingivalis

Differential LPS Induce Distinct Immune Responses

Endotoxin-free OVA

Chicken OVA (Sigma, St. Louis, MO) was freshly prepared in PBS and depleted of the endotoxin activity (measured by LAL QCL-1000 kit from BioWhittaker, Walkersville, MD), using the Detoxi-Gel Affinity Pack Columns (Pierce). After depletion, the endotoxin level was below the limit of detection of the LAL QCL-1000 kit (<0.1 EU).

Injections

Reconstituted mice (three to five per group) were injected either i.p. or in the footpad, with either 2 mg OVA in saline (Baylor Hospital, Dallas, TX), or 2 mg OVA + 25 μg E. coli LPS, or 2 mg OVA + 25 μg P. gingivalis LPS. Endotoxin activity in saline was measured by LAL QCL-1000 kit, and observed to be below the detection limit. Before mixing with OVA, LPS was sonicated extensively to ensure uniform mixing of micelles. Footpad injections were given in a volume of 25 μl, i.p. injections were given in a volume of 100 μl.

Flow cytometry

For analyses of OT-2 cells, cell suspensions were prepared from the draining popliteal lymph nodes or spleens, and incubated on ice with PE-labeled anti-Thy-1.2 (BD PharMingen), followed by streptavidin aliphophycocyanin (ALPC; BD PharMingen). In some of the experiments, we simply used Thy-1.2 vs CD4. For analyses of OT-1 cells, cell suspensions of draining popliteal lymph nodes or spleens were stained with PE-labeled anti-Thy-1.2 vs CD4. For analyses of OT-1 cells, cell suspensions of draining popliteal lymph nodes or spleens were stained with PE-labeled anti-Thy-1.2 vs CD4. For analyses of OT-1 cells, cell suspensions of draining popliteal lymph nodes or spleens were stained with PE-labeled anti-Thy-1.2 vs CD4.
DAY 0
UNINJECTED
OVA
OVA + E.coli LPS
OVA + P.gingivalis LPS
E.coli LPS alone
P.gingivalis LPS alone

DAY 4
ANALYSES:
1. Clonal expansion of OVA-specific CD4+ T cells
2. In vitro proliferation of OVA-specific CD4+ T cells
3. Cytokine production by OVA-specific CD4+ T cells

FIGURE 1. Experimental design. B6.PL.Thy-1a (B6.PL) mice, or C57BL/6 mice that were reconstituted with OT-2 cells were injected with either soluble OVA, soluble OVA + E. coli LPS, soluble OVA + P. gingivalis LPS, E. coli LPS alone, or P. gingivalis LPS alone i.p. or in the footpad. Four days later, the spleens or draining lymph nodes were removed for phenotypic and functional analyses, including clonal expansion of OVA-specific CD4+ T cells, in vitro proliferation of OVA-specific CD4+ T cells, and cytokine production by the OVA-specific CD4+ T cells.

FIGURE 2. E. coli LPS and P. gingivalis LPS enhance Ag-specific Th responses in vivo. B6.PL.Thy-1a (B6.PL) mice reconstituted with OT-2 transgenic T cells were immunized with either soluble OVA, soluble OVA + E. coli LPS, soluble OVA + P. gingivalis LPS, E. coli LPS alone, or P. gingivalis LPS alone i.p. or in the footpad. The absolute numbers of Thy-1.2+ CD4+ T cells expanded in vivo, by footpad injections or spleens (i.p. route) were removed, and the clonal expansion of OVA-specific CD4+ T cells was assessed by flow cytometry, by staining with Thy-1.2 vs CD4. A, Flow cytometry profiles from day 4 of the response in the popliteal lymph nodes, from a representative experiment. B and C, The percentage expansion of OVA-specific CD4+ T cells (Thy-1.2+, CD4+) in the draining lymph nodes (B) and the spleens (C) at day 4. Both E. coli LPS and P. gingivalis LPS significantly enhance the clonal expansion, regardless of the route of injection. Data represent the means of four mice per group from one representative experiment. SDs are indicated, and differences between OVA group and the OVA + LPS groups are highly significant (p < 0.01). D and E, The absolute numbers of Thy-1.2+ CD4+ T cells per popliteal lymph node (D) or per spleen (E) at day 4. Data represent the means of four mice per group from one representative experiment. SDs are indicated, and differences between OVA group and the OVA + LPS groups are highly significant (p < 0.01). F and G, In vitro restimulation of OVA-specific T cells expanded in vivo, by footpad (F), or i.p. (G) injections. Four days after priming, single cell suspensions from the draining popliteal lymph nodes (F) or spleens (G) were restimulated with varying concentrations of OVA for 72 h, and pulsed with 3H for 12 h. Note that injections of E. coli LPS alone or P. gingivalis LPS alone did not result in significant clonal expansion or in vitro proliferation. A–G, Representative of 10 independent experiments.

LPS; Fig. 2). Thus, both types of LPS, injected with OVA, enhanced the clonal expansion of OVA-specific T cells. No detectable clonal expansion was observed, when either type of LPS was injected alone (data not shown).

Previous work has shown that productive T cell immunity is elicited only when the Ag is injected with an adjuvant, and that injections of soluble Ags only result in a transient and abortive clonal expansion, in which Ag-specific T cells cannot be efficiently restimulated in vitro with protein or peptide (16, 43, 44). We thus examined the in vitro proliferative capacity of the OVA-specific T cells from the various cohorts of mice, by culturing single cell suspensions of the draining lymph nodes with varying concentrations of OVA. As shown in Fig. 2, F and G, mice that received an injection of OVA + E. coli LPS, or OVA + P. gingivalis LPS, had greatly enhanced responses compared with those that received OVA alone (Fig. 2, F and G). Either type of LPS alone did not result in any significant proliferation (data not shown).

E. coli LPS and P. gingivalis LPS induce distinct types of Ag-specific Th responses in vivo

Cytokine production by Ag-specific T cells was measured by assaying the culture supernatants from the cultures described above for IL-2, IFN-γ, IL-4, IL-10, and IL-5. Assessment of cytokine...
production in these cultures revealed significant differences between mice injected with OVA, OVA + E. coli LPS, and OVA + P. gingivalis LPS (Fig. 3). In cultures from mice injected with OVA alone, there was little, if any, IL-2, IFN-γ, IL-10, IL-4, or IL-5 produced. In contrast, in cultures from mice injected with OVA + E. coli LPS, there was significant IL-2, IL-10, and very high levels of IFN-γ produced by the Ag-specific T cells. Neither IL-4 nor IL-5 could be detected. However, in cultures from mice injected with OVA + P. gingivalis LPS, there was a striking diminution of IFN-γ production, despite significant production of IL-2 and the Th2 cytokines IL-10 and IL-5 (Fig. 3). In fact, the level of IFN-γ was as low as that observed with OVA alone. Therefore, while both types of LPS elicit potent clonal expansion of Ag-specific CD4+ T cells in vivo, E. coli LPS + OVA induces a Th1-like response, characterized by high levels of IFN-γ. In contrast, P. gingivalis LPS + OVA induces a response that is essentially devoid of IFN-γ, and characterized by significant levels of IL-10 and IL-5, regardless of the route of injection (Fig. 3). Either type of LPS alone did not result in any cytokine production in these cultures (data not shown). No significant levels of IL-4 could be detected in any of the conditions, and this may reflect the Th1 bias of the C57BL/6 strain studied. Failure to detect IL-4 was not a peculiarity of the transgenic system because similar cytokine profiles were observed in experiments using nontransgenic mice (data not shown).

Another cytokine that is a strong hallmark of Th2 responses is IL-13 (4–7). We wondered whether P. gingivalis LPS induced IL-13 from Ag-specific Th cells in vivo. In the cultures described above, P. gingivalis LPS induced much higher levels of IL-13 than E. coli LPS (Fig. 4). Interestingly, although there is a titration of IL-13 production with the dose of OVA, even when no OVA was used there is some basal level of IL-13 production. Whether this is due to IL-13 production from other cell types (e.g., NK T cells) is presently under investigation. Therefore, in this system, P. gingivalis LPS, while shifting the balance toward the Th2 pathway, does not appear to elicit a classic Th2 response that is characterized by IL-4 production.

E. coli LPS and P. gingivalis LPS enhance Ag-specific CD8+ T cell responses in vivo

The strikingly different Th responses induced by E. coli LPS and P. gingivalis LPS suggested that there may be differences in Ag-specific CD8+ T cell responses. We investigated this using OT-1 mice (H-2Kb-restricted, OVA-specific TCR transgenic mice) (45, 46). A total of 2.5 × 10^6 or 5 × 10^6 spleen cells from OT-1 mice (B6.PL, Thy-1.2) was adoptively transferred into B6.PL (Thy-1.1) hosts. Cohorts of host mice were injected with either OVA, OVA + E. coli LPS, or OVA + P. gingivalis LPS. Clonal expansion of OVA-specific CD8+ T cells (CD8+ Thy-1.2+) was assessed by flow cytometry (Fig. 5, A–E). Both E. coli LPS + OVA and P. gingivalis LPS + OVA enhanced the clonal expansion of OVA-specific CD8+ T cells, >60-fold (Fig. 5, A–E). LPS alone had no detectable effect (data not shown).

We then examined the in vitro proliferative capacity of the OVA-specific CD8+ T cells from the various cohorts of mice, by culturing single cell suspensions of the draining lymph nodes (s.c. route) or spleen (i.p.) with varying concentrations of OVA. As
FIGURE 5. E. coli LPS and P. gingivalis LPS enhance Ag-specific CD8\(^{+}\) T cell responses in vivo. C57BL/6 mice or B6.PL-Thy-1\(^{-}\) (B6.PL) mice reconstituted with OT-1 transgenic T cells were immunized with soluble OVA, OVA + E. coli LPS, or OVA + P. gingivalis LPS, either in the footpad (s.c., B, D, and F) or i.p. (C, E, and G). Four days later, the draining popliteal lymph nodes (footpad injections) or spleens (i.p. route) were removed, and the clonal expansion of OVA-specific CD8\(^{+}\) T cells was assessed by flow cytometry, by staining with CD8 vs V\(\alpha\)2, or CD8 vs Thy-1.2 vs V\(\alpha\)2 (A). B and C. The percentage expansion of OVA-specific CD8\(^{+}\) T cells (CD8\(^{+}\) Thy-1.2\(^{+}\)) in the draining lymph nodes (B) and the spleens (C) at day 4. Both E. coli LPS and P. gingivalis LPS significantly enhance the clonal expansion, regardless of the route of injection. Data represent the means of four mice per group from one representative experiment. SDs are indicated, and differences among groups are highly significant (p < 0.001). D and E. The absolute numbers of OVA-specific CD8\(^{+}\) T cells per popliteal lymph nodes (D) or per spleen (E) at day 4. Data represent the means of four mice per group from one representative experiment. SDs are indicated, and differences between OVA group and the OVA + LPS groups are highly significant (p < 0.001). F and G. In vitro restimulation of OVA-specific CD8\(^{+}\) T cells expanded in vivo, by footpad (F), or i.p. (G) injections. Four days after priming, single cell suspensions from the draining popliteal lymph nodes (F) or spleens (G) were restimulated with varying concentrations of OVA for 72 h, and pulsed with \(^{3}\)H for 12 h. Note that injections of E. coli LPS alone or P. gingivalis LPS alone did not result in significant clonal expansion, or in vitro proliferation. Representative of three independent experiments.

shown in Fig. 5, F and G, mice that received an injection of either E. coli LPS + OVA or P. gingivalis LPS + OVA had greatly enhanced responses, compared with those that received OVA alone. Identical results were obtained when class I-restricted OVA peptide (SIINFEKL) was used to restimulate the cells in vitro, suggesting that it was indeed the CD8\(^{+}\) OVA-specific T cells that proliferated in culture (data not shown). LPS alone did not result in any significant proliferation (data not shown).

E. coli LPS and P. gingivalis LPS induce distinct types of Ag-specific CD8\(^{+}\) T cell responses in vivo

We next examined the cytokines produced in these cultures by ELISA. As observed with the CD4\(^{+}\) OT-2 cells, CD8\(^{+}\) OT-1 cells stimulated with OVA alone did not secrete significant levels of IL-2, IFN-\(\gamma\), IL-10, or IL-5 (Fig. 6). Cells from mice injected with E. coli LPS + OVA produced very high levels of IFN-\(\gamma\) and significant IL-10, but no IL-5 (Fig. 6). In contrast, cells from mice injected with P. gingivalis LPS + OVA produced strikingly lower levels of IFN-\(\gamma\), and significant levels of IL-10 and IL-5 (Fig. 6), consistent with the cytokine profiles observed with OT-2 cells (Fig. 3). Identical results were obtained when SIINFEKL peptide was used to restimulate the cells in vitro (data not shown). LPS alone did not result in any significant cytokine production (data not shown). No significant levels of IL-4 were detected, and this may reflect the Th1 bias of the C57BL/6 strain.

Both E. coli LPS and P. gingivalis LPS activate CD8\(^{alpha}\) and CD8\(^{alpha^-}\) DC subsets in vivo

Adjuvants and certain microbial products, such as LPS, are known to activate DCs, thereby enhancing T cell immunity (47–51). In this study, we investigated whether both types of LPS were capable of activating DC subsets in vivo. C57BL/6 mice were injected with 25 \(\mu\)g E. coli LPS, or P. gingivalis LPS, either s.c., i.p., or i.v., and sacrificed 6 h later. Spleens and lymph nodes were collected, and the expression of activation markers (CD80, CD86, and CD40) on DCs was determined. CD8\(^{alpha}\) and CD8\(^{alpha^-}\) DC subsets are well characterized (8–13, 47, 50–53), and may derive from different lineages or may simply reflect different developmental stages of the same lineage (8, 9, 13). CD8\(^{alpha}\) and CD8\(^{alpha^-}\) DCs from the spleens of PBS-treated, control mice express significant levels of CD80, CD86, and CD40, as reported previously (50). However, upon injection of either type of LPS, there was a significant up-regulation of CD80, CD86, and CD40 on both DC subsets (Fig. 7). Therefore, both types of LPS appear to activate
FIGURE 6. E. coli LPS and P. gingivalis LPS induce distinct types of Ag-specific CD8⁺ T cell responses in vivo. Culture supernatants from the cultures described in Fig. 5, E and F, were assayed for IL-2, IFN-γ, IL-10, IL-5, and IL-5 with ELISA. Note that injections of E. coli LPS alone or P. gingivalis LPS alone did not result in significant cytokine production. Each data point represents mean ± SD of triplicate wells, from a typical experiment. Differences in IL-2 production between the E. coli LPS and the P. gingivalis LPS groups are not significant; IL-5 production in the P. gingivalis LPS group at 500 µg/ml OVA is significant (p < 0.05; detection limit of ELISA assay is 6 pg/ml). Note that injections of E. coli LPS alone or P. gingivalis LPS alone did not result in significant cytokine production. Representative of three independent experiments.

FIGURE 7. Both E. coli LPS and P. gingivalis LPS activate CD8α⁺ and CD8α⁻ DC subsets in vivo. C57BL/6 mice were injected with either PBS (open red histograms) E. coli LPS, or P. gingivalis LPS (open green histograms), either i.v. or i.p., and 6 h later, the expression of CD80, CD86, and CD40 was assessed on gated, splenic CD11c⁺CD8α⁺ and CD11c⁺CD8α⁻ DC subsets by flow cytometry. Isotype controls are filled purple histograms. Data are representative of three experiments.
E. coli LPS, but not P. gingivalis LPS, induces IL-12(p70) in CD8α⁺ DCs

It has been previously shown that splenic CD8α⁺ DCs can be induced to secrete IL-12 by various microbial products (8–10, 50–52), and that this IL-12 is influential in the elicitation of Th1 responses by the CD8α⁺ DC subset (10). In this study, we wished to determine whether both types of LPS could induce biologically active IL-12(p70) in CD8α⁺ DCs. Splenic CD11c⁺ CD8α⁺ and CD11c⁺ CD8α⁻ DC subsets were isolated by flow cytometry, and cultured for 48 h with either E. coli LPS, P. gingivalis LPS, or alone. Then the supernatants were assayed for IL-12, IL-6, and TNF-α by ELISA. Both types of LPS induced IL-6 and TNF-α in both DC subsets (Fig. 8). However, only E. coli LPS induced IL-12 in the CD8α⁺ DC subset (Fig. 8). Therefore, while both types of LPS could activate both DC subsets, only the E. coli LPS could elicit the Th-1-inducing cytokine IL-12, this being consistent with the strikingly different Th responses induced by E. coli LPS and P. gingivalis LPS in vivo. Whether P. gingivalis LPS induces Th2 responses by simply failing to elicit IL-12 in DCs, or whether it actually stimulates the production of a Th2-inducing cytokine is not known. Good candidates for Th2-inducing cytokines are IL-10 and IL-4. However, significant levels of IL-10 or IL-4 could not be consistently detected in these cultures (data not shown).

P. gingivalis LPS is less dependent on TLR4 signaling than E. coli LPS

While E. coli LPS mediates its effects by signaling though TLR4 (14, 15), P. gingivalis LPS is reported signal through a TLR4-independent mechanism (18–25). Thus, we examined the effect(s) of either type of LPS on proliferation of splenocytes from C3H/HeJ mice, which have a point mutation in the TLR4 gene, and wild-type (C3H/HeN) mice. C3H/HeJ splenocytes cultured with E. coli LPS were greatly impaired in their proliferative capacity, compared with the C3H/HeN controls (Fig. 9A). In contrast, C3H/HeJ splenocytes cultured with P. gingivalis LPS were only modestly impaired in their proliferative capacity, compared with the C3H/HeN controls (Fig. 9B). Consistent with this, production of IL-6 induced by E. coli LPS was greatly impaired in C3H/HeJ splenocytes, compared with C3H/HeN splenocytes (Fig. 10). However, production of IL-6 induced by P. gingivalis LPS was not impaired in C3H/HeJ mice (Fig. 10). Furthermore, induction of IL-12 and other cytokines, by E. coli LPS, from enriched CD11c⁺ splenic DCs was severely impaired in C3H/HeJ mice; in contrast, cytokine production induced by P. gingivalis LPS was only moderately affected (Fig. 11). Therefore, while E. coli LPS signaling is largely dependent on TLR4, P. gingivalis LPS appears less dependent on this pathway.

Discussion

Our current study demonstrates that LPS from different bacteria elicit distinct types of adaptive immune responses against an exogenous soluble Ag, such as OVA. E. coli LPS induces OVA-specific T cell responses characterized by very high levels of the Th1 cytokine IFN-γ. In contrast, P. gingivalis LPS induces OVA-specific T cell responses, characterized by significant levels of the Th2 cytokines IL-13, IL-5, and IL-10, but lower levels of IFN-γ. Consistent with these findings, E. coli LPS induces IL-12 production from CD8α⁺ DCs, while P. gingivalis LPS does not. These
observations are consistent with several previous reports implicating a central role for IL-12 in IFN-γ induction by E. coli LPS (54, 55). Despite their strikingly different effects on IL-12 production, both LPS molecules do activate the CD8α+ and CD8α−/CD11c+ DC subsets, as judged by the production of IL-6 and TNF-α, and the up-regulation of costimulatory molecules.

Our data also suggest that P. gingivalis LPS is less dependent on TLR4 signaling than E. coli LPS. This is consistent with several previous reports, and is attributed mainly to the unique lipid A motif of P. gingivalis LPS, which contains unusually branched and relatively long fatty acids, compared with lipid A from enteric bacterial LPS (19, 20, 23, 24). Unlike enteric LPS, P. gingivalis LPS has been reported to induce endotoxic shock in C3H/HeJ mice (19, 20, 23, 24). These data are consistent with recent findings from Vogel’s group (25), which suggest that P. gingivalis LPS activates murine peritoneal macrophages through a TLR4-independent mechanism to elicit the production of IL-6 and TNF-α, but not IL-12(p70); in contrast, E. coli LPS does induce IL-12(p70) in macrophages, in addition to IL-6 and TNF-α (25).

There could be several mechanisms by which E. coli LPS and P. gingivalis LPS interact with DCs to elicit distinct adaptive immune responses, but in principle two opposite kinds of mechanisms can be envisaged. First, a single DC subset may interpret distinct microbial or environmental signals differently to yield distinct types of adaptive immune responses (56–58). This instructive model is based on recent reports, which suggest that apparently homogeneous DC subsets can differentially transduce signals from distinct microbial products or cytokines, to elicit distinct Th cytokines in vitro (59–62). In this model, a given DC subset can elicit virtually any Th response, depending on the environmental stimulus. In the second selective model, functionally distinct DC subsets may express distinct repertoires of pattern recognition receptors that recognize different classes of microbial products (62). Thus, recognition of a particular microbial product by a given DC subset will

**FIGURE 10.** IL-6 production by splenocytes stimulated with E. coli LPS or P. gingivalis LPS in C3H/HeN and C3H/HeJ mice. Splenocytes from C3H/HeJ mice and C3H/HeN mice were cultured with varying concentrations of E. coli LPS (A and C) or P. gingivalis LPS (B and D) for 12 h (A and B) or 48 h (C and D). IL-6 was measured by cytokine ELISA. Representative of three independent experiments.

**FIGURE 11.** Cytokine production by splenic DCs from C3H/HeJ and C3H/HeN mice stimulated in vitro with E. coli LPS or P. gingivalis LPS. CD11c+ DCs, enriched from the spleens of mice, were cultured with either type of LPS for 24 h, and secretion of IL-12, IL-6, and TNF-α was measured by cytokine ELISA. Representative of three separate experiments.
result in a given Th response, different from that induced by an other microbe that activated a different DC subset. At present, we have limited evidence to discriminate between these scenarios, with respect to the differential effects of *E. coli* and *P. gingivalis* LPS. Clearly, both types of LPS activate the CD8α− and CD8α+ DC subsets (Fig. 8), suggesting that the signaling receptors for the two types of LPS are present on both subsets. *E. coli* LPS activates both DC subsets, most likely through TLR4, and *P. gingivalis* LPS activates both DC subsets, perhaps through a different TLR, expressed on both DC subsets. In this context, our additional data suggest that mRNA for TLR4 is expressed on both the CD8α+ and the CD8α− DC subsets, as measured by PCR (data not shown). Further studies are however needed to ascertain surface expression of the various TLRs on the different DC subsets. Whatever receptors through which they signal, our data suggest that two distinct LPS can elicit strikingly different patterns of adaptive immunity, most likely by stimulating different cytokines in DC subsets. These observations highlight a central role for distinct DC subsets for differentially interpreting signals from different microbial products and instructing the adaptive immune response to mount distinct patterns of immunity. Although the present study compares the effect of a single molecule (LPS) from different microbes, it is tempting to speculate that this may represent a model for how different microorganisms stimulate distinct types of immunities. Thus, the overall type of response against the whole microorganism may well be an integration of all microbial signals through all receptors and all APC subsets. Whether live pathogens or their extracts do indeed obey the rules observed for individual microbial products remains to be established. Finally, these data also point to the use of *P. gingivalis* LPS, or its derivatives such as lipid A or synthetic analogues in the elicitation of therapeutic Th2-like responses in clinical settings.

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**References**


