Activation of Human Monocytic Cells by *Borrelia burgdorferi* and *Treponema pallidum* Is Facilitated by CD14 and Correlates with Surface Exposure of Spirochetal Lipoproteins

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Here we examined the involvement of CD14 in monocyte activation by motile *Borrelia burgdorferi* and *Treponema pallidum*. *B. burgdorferi* induced secretion of IL-8 by vitamin D$_3$-matured THP-1 cells, which was inhibited by a CD14-specific mAb known to block cellular activation by LPS and the prototypic spirochetal lipoprotein, outer surface protein A. Enhanced responsiveness to *B. burgdorferi* also was observed when THP-1 cells were transfected with CD14. Because borreliae within the mammalian host and in vitro-cultivated organisms express different lipoproteins, experiments also were performed with “host-adapted” spirochetes grown within dialysis membrane chambers implanted into the peritoneal cavities of rabbits. Stimulation of THP-1 cells by host-adapted organisms was CD14 dependent and, interestingly, was actually greater than that observed with in vitro-cultivated organisms grown at either 34°C or following temperature shift from 23°C to 37°C. Consistent with previous findings that transfection of Chinese hamster ovary cells with CD14 confers responsiveness to LPS but not to outer surface protein A, *B. burgdorferi* failed to stimulate CD14-transfected Chinese hamster ovary cells. *T. pallidum* also activated THP-1 cells in a CD14-dependent manner, although its stimulatory capacity was markedly less than that of *B. burgdorferi*. Moreover, cell activation by motile *T. pallidum* was considerably less than that induced by treponemal sonicates. Taken together, these findings support the notion that lipoproteins are the principle component of intact spirochetes responsible for monocyte activation, and they indicate that surface exposure of lipoproteins is an important determinant of a spirochetal pathogen’s proinflammatory capacity. The *Journal of Immunology*, 1999, 163: 2049–2056.

*B. burgdorferi* and *Treponema pallidum* subspecies *pallidum* are the etiologic agents of Lyme disease and venereal syphilis, respectively (1, 2). Although transmitted differently (tick bite vs sexual transmission), these multisystem diseases share many clinical and histopathological features; moreover, in both disorders, host injury is presumed to result from the inflammatory response to intrinsic spirochetal components (1, 2). It is well established that spirochetes lack the potent proinflammatory molecule LPS found in the outer membranes of Gram-negative bacteria (3–5). However, they do possess abundant lipoproteins (3, 4, 6, 7), and there is now a substantial body of evidence that these molecules act as major proinflammatory agonists with the ability to influence both innate and adaptive immune responses during spirochetal infection (8–15).

The well characterized LPS receptor, CD14, has been described as a pattern recognition molecule for diverse bacterial components that trigger innate immune responses (16–18). CD14 exists as a 55-kDa glycosylphosphatidylinositol-linked protein (membrane CD14 (mCD14)) on the surface of macrophages and neutrophils (19–21) and as a soluble protein (sCD14) in serum (22). The soluble form of CD14 facilitates the activation of cells, such as endothelial cells, which lack mCD14 (23, 24). Recent in vitro studies implicate both forms of CD14 in the inflammatory response to spirochetal lipoproteins, although there appear to be distinctions between the CD14-dependent pathways used by LPS and spirochetal lipoproteins (25–27). The lipid moiety of lipoproteins is crucial for binding to CD14 as well as for cell activation (12, 27).

However, an apparent paradox arises from the fact that these lipid components are sequestered within the spirochetal membrane (28), where they should be inaccessible for binding to CD14. Topological concerns are even more pertinent with *T. pallidum*, as evidence to date indicates that it has a paucity of lipoproteins on its surface (29, 30). Nevertheless, motile *B. burgdorferi* and *T. pallidum* do activate innate immune effector cells (31–33), although it remains to be proven that these effects are lipoprotein-mediated. As an initial step toward addressing this issue, and to employ in vitro conditions that more closely resemble the in vivo situation, we examined monocyte activation by motile *B. burgdorferi* and *T.
**Materials and Methods**

**Reagents**

*Salmonella minnesota* R5 LPS (Sigma, St. Louis, MO) was suspended in 0.1% triethylamine adjusted to pH 8.0 with 100 mM Tris·HCl. Dilutions of LPS were made with HNEB buffer (20 mM HEPES, pH 7.4, 150 mM NaCl, 0.1 mM EDTA, and 0.03% BSA). 1.25-di-hydroxyvitamin D3 was obtained from Biomol Research Laboratories (Plymouth Meeting, PA). G-418 sulfate was purchased from Mediatech (Herndon, VA). Great care was taken during the preparation of all buffers and reagents to minimize contamination with environmental LPS by using baked glassware, disposable plasticware, and pyrogen-free H2O.

**Bacterial strains**

Low-passage *B. burgdorferi* 297 (34) was obtained from Russell Johnson (University of Minnesota). The B31-type strain of *B. burgdorferi* MedImmune clone (4) was provided by Raju Lathigra (MedImmune, Gaithersburg, MD). Spirochetes were cultivated in vitro at 23°C, 34°C, or 37°C after temperature shift from 23°C as described previously (35). All experiments were performed with spirochetes that had been passaged no more than five times in Barbour-Stoenner-Kelly (BSK)-H medium (Sigma). With both strains, infectivity was periodically assessed by low-dose (1 × 105 borreliae) intradermal needle inoculation of 3-wk-old C3H/HeJ mice (The Jackson Laboratory, Bar Harbour, ME) and culture of ear punch biopsies 2 wk later. *T. pallidum* (Nichols) was propagated by intratesticular inoculation of adult New Zealand White rabbits as previously described (36). Treponemes were extracted from infected testes in RPMI 1640 medium (Mediatech) supplemented with 10% heat-inactivated FBS (heated 30 min at 56°C (Mediatech) and separated from testicular debris by low-speed centrifugation (350 × g for 10 min). In all experiments, spirochetes were enumerated by dark-field microscopy using a Petroff Hauser counting chamber (Hauser Scientific Company, Horsham, PA).

**Cultivation of B. burgdorferi**

*B31 within dialysis membrane chambers (DMCs) implanted into rabbits*

To generate increased quantities of host-adapted spirochetes, we modified the procedure of Akins et al. (37). Briefly, Spectra/Per 6 dialysis membrane chambers (Fisher Scientific, Pittsburgh, PA) with a molecular mass cut-off of 8000 Da were prewashed with Milli-Q water and sterilized by boiling in 1 mM EDTA and then in Milli-Q water for 20 min each. Dialysis bags were submerged in BSK-H medium overnight at 4°C. After tying one end, each bag was filled with 25 ml of BSK-H medium containing 2.5 × 107 organisms from a mid-log phase culture grown at 23°C. Tubing was then tied and excess membrane was removed from both ends. New Zealand White rabbits were anesthetized with 3.5% isoflurane, and three DMCs per rabbit were implanted into the peritoneal cavities using strict aseptic technique. Eight to 10 days postimplantation, the chambers were harvested. The outside of each chamber was washed extensively with environmental LPS by using baked glass, disposable plasticware, and pyrogen-free H2O.

**Purification of native outer surface protein A (nOspA) from**

*B. burgdorferi**

nOspA was affinity purified from *B. burgdorferi* strain THI-EV as previously described (38) and stored in 33 mM Tris, pH 7.4, 1.6 mM NaCl, and 20 mM n-octyl-β-glucoside at −70°C. Preparations of nOspA contained ≤12 pg LPS/µg protein as measured by the QCL-1000 quantitative, chromogenic *Lactobacillus* amnobeocyte lysate assay (BioWhittaker, Walkersville, MD).

**SDS-PAGE and two-dimensional nonequilibrium pH gel gel electrophoresis (2D-NEPHEG)**

For SDS-PAGE, samples were boiled for 5 min in final sample buffer (62.5 mM Tris·HCl, 2% SDS, 10% glycerol, 5% 2-ME, 0.001% bromphenol blue) and electrophoresed through 4.5% stacking and 12.5% resolving gels before staining with silver. 2D-NEPHEG was performed as described by O’Farrell et al. (39). Briefly, whole-cell lysates were subjected to first dimension NEPHGE for 5 h at 400 V in 11-cm tube gels consisting of 4% pH 6.3 and 1% pH 3.5–10 amorpholines (Bio-Rad, Hercules, CA). For the second dimension, tube gels were overlaid on top of 2.4% stacking and 12.5% separating SDS-polyacrylamide slab gels. Apparent isoelectric point (pI) values were determined by adding pl standards (Bio-Rad) to one tube gel and subjecting it to first- and second-dimension electrophoresis under the same conditions.

**Cell lines**

The human promyelomonocytic cell line THP-1 (40) was maintained in RPMI 1640 medium (Mediatech) containing 2 mM L-glutamine and supplemented with 10% heat-inactivated FBS, 100 U of penicillin per ml, and 100 µg of streptomycin per ml. Cells were differentiated by preincubation with 100 nM vitamin D3 for 72–96 h before stimulation by LPS, nOspA, *B. burgdorferi*, and *T. pallidum*. CD14- and Rous sarcoma virus vector-transfected THP-1 cells (26, 41), kindly provided by Richard Ulevitch (Scripps, La Jolla, CA), were cultured in the continuous presence of 0.5 µg/ml (active drug) of the aminoglycoside G-418 sulfate to ensure the maintenance of stably transfected DNA conferring neomycin resistance. G-418 sulfate was removed 24 h before experimentation. Chinese hamster ovary (CHO) K1 cells stably cotransfected with pCEP4 (Invitrogen, San Diego, CA), which constitutively expressed the gene for human CD14 from the CMV promoter, and pUM5/ELAM1-Tac, which contains the gene for CD25 under the control of an NF-κB-inducible E-selectin promoter (3E10 cells) (42), were grown in Ham’s F12 medium containing 10% heat-inactivated FBS and 400 U of hygromycin B per ml.

**Cell activation experiments**

THP-1 cells were seeded in 24-well flat-bottom tissue culture plates (Becton Dickinson Labware, Lincoln Park, NJ) at a density of 2.5 × 105 cells/ml and were maintained at 37°C in a humidified atmosphere of 5% CO2 and air. Cells were then stimulated for 3 h with LPS, nOspA, *B. burgdorferi*, or *T. pallidum*, and culture supernatants were assayed for IL-8 using the Duoset ELISA development system from R&D Systems (Minneapolis, MN). For Ab blocking experiments, vitamin D3-matured THP-1 cells (2.5 × 105 cells in 0.1 ml) were preincubated at room temperature for 15 min with 10 µg of murine mAb directed against CD14 (60bcA and 603d; both IgG1) before the addition of agonists. For sonication experiments, *T. pallidum* were washed twice in PBS, pH 7.2, and ruptured with a sonic dismembrator (Fisher Scientific); the efficiency of sonication was verified by dark-field microscopy. For analyzing cell activation by *B. burgdorferi* and *T. pallidum* based upon total protein content, equivalent numbers of organisms were sonicated as described above and protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad). 3E10 cells were seeded at a density of 2 × 105 cells per well in 24-well plates; the following day cells were washed twice with medium and stimulated for 8 h with 100 ng/ml of LPS, 1 µg/ml of nOspA, or graded ratios of *B. burgdorferi* B31. The cells were then washed and incubated with PE-conjugated mAb directed against human CD25 (Becton Dickinson Immucytochemistry Systems, San Jose, CA) for FACS analysis. In all activation and blocking experiments, spirochetes were ≥90% viable (i.e., motile) at the end of the experiment, as assessed by dark-field microscopy.

**Statistics**

The two-tailed Student’s *t* test or a one-way ANOVA with Tukey-Kramer multiple comparisons post test was performed using GraphPad InStat version 3.00 for Windows 95 (GraphPad Software, San Diego, CA). A value of *p* ≤ 0.05 was used as the *α* value to determine statistical significance for all analyses.

**Results**

CD14 expression enhances the responsiveness of human monocytic cells to motile *B. burgdorferi*.

In a previous study (26), functional blockade of CD14 by polyclonal anti-CD14Abs was one of several strategies used to demonstrate that monocytic cell activation by spirochetal lipoproteins is facilitated by CD14. To correlate lipoprotein- and spirochete-mediated activation, we reasoned that it would be useful to determine whether both interact with the same domain on CD14. Recently, two groups have independently shown that anti-CD14 mAb 60bcA, which is known to block LPS-mediated monocytic and endothelial cell activation, also blocks cellular activation by boreal...
lipoproteins (25, 27). In preliminary studies, we also observed that this Ab blocked secretion of the neutrophil chemoattractant IL-8 by vitamin D$_3$-matured THP-1 cells over a range of nOspA concentrations, whereas 63D3, a known LPS-nonblocking mAb (43), was without effect (data not shown). We next examined the ability of virulent *B. burgdorferi* to stimulate vitamin D$_3$-matured cells. As shown in Fig. 1A, *B. burgdorferi* induced the secretion of IL-8 in a dose-dependent fashion; significant cellular activation occurred with a single spirochete per cell. Interestingly, activation of undifferentiated cells was observed at spirochete-to-cell ratios of 100:1 and 1000:1, although the IL-8 levels were significantly less than those produced by vitamin D$_3$-matured cells under the same conditions. Thus, cellular activation by *B. burgdorferi* also may have a CD14-independent component, a phenomenon previously observed at relatively high concentrations of spirochetal lipoprotein/lipopeptide agonists (26). Activation of vitamin D$_3$-matured cells by *B. burgdorferi* was substantially blocked by 60bca, whereas 63D3 was noninhibitory (Fig. 1B), thereby demonstrating that the cellular response at this spirochete-to-cell ratio (10:1) was largely CD14 dependent. The inability of the Ab to completely block IL-8 production also was consistent with the notion of the existence of a minor CD14-independent component. We previously demonstrated that transfection of undifferentiated THP-1 cells with CD14 confers enhanced responsiveness to spirochetal lipoproteins as well as to LPS (26). To confirm the Ab blocking results, we investigated whether CD14 transfected THP-1 cells also manifest enhanced responsiveness to live spirochetes. Fig. 2 shows that at spirochete-to-cell ratios above 1:1, the CD14-transfected cells produced significantly greater amounts of IL-8 than did cells transfected with the empty vector. As before, a lesser degree of stimulation was observed in the absence of CD14 expression.

**B. burgdorferi** cultivated in DMCs also activate vitamin D$_3$-matured THP-1 cells in a CD14-dependent manner

Because the antigenic composition of *B. burgdorferi* during mammalian infection differs from that of in vitro-cultivated organisms (35, 44), experiments performed with in vitro-cultivated spirochetes may not accurately reproduce pathogen-host cell interactions occurring in vivo. Recently, we showed that cultivation of spirochetes within DMCs implanted into the peritoneal cavities of rats generates spirochetes that more closely resemble the host-adapted phenotype (37). Therefore, it was of considerable interest to compare monocytic cell activation by chamber- and in vitro-grown organisms. To obtain the numbers of host-adapted spirochetes required for these studies, we modified our original system by cultivating borreliae within DMCs implanted into rabbits. Fig. 3 demonstrates that rabbit DMC-cultivated *B. burgdorferi* B31 expressed greatly diminished amounts of OspA and OspB and greatly increased amounts of OspC when compared with organisms grown in BSK-H at 34°C or following temperature shift from 23°C to 37°C (a control for the higher ambient temperature within the rabbit peritoneal cavity). Furthermore, as previously described for *B. burgdorferi* 297 (37), other differences in polypeptide composition were readily discernible by both SDS-PAGE (Fig. 3) and 2D-NPHGE analysis (data not shown). Spirochetes cultivated at 34°C or following temperature shift induced similar levels of IL-8 secretion by vitamin D$_3$-matured THP-1 cells, and these levels were roughly equivalent to those observed in earlier experiments with in vitro-cultivated *B. burgdorferi* 297 (compare Fig. 1A and Fig. 4A). In contrast, DMC-cultivated organisms stimulated significantly greater IL-8 secretion than either population of in vitro-cultivated organisms (Fig. 4A). To assess the contribution of CD14 to this response, blocking experiments were performed with mAbs 60bca and 63D3. Fig. 4B shows that 60bca reduced IL-8 secretion to the levels typically observed with undifferentiated cells, whereas 63D3 was noninhibitory.
parts. To address this issue, we examined the ability of motile T. pallidum lipoproteins are predominantly subsurface (29, 30) and pallidum to stimulate THP-1 cells. Activation of vitamin D₃-matured THP-1 cells. As shown in Fig. 7, B. burgdorferi whole-cell lysates from B. burgdorferi B31 (2 × 10⁷) grown in vitro at 23°C, after temperature shift to 37°C, within DMCs or in vitro at 34°C were separated by SDS-PAGE and stained with silver. Several well-characterized borrelial proteins are indicated on the right and molecular mass markers in kilodaltons are shown on the left.

Transfection of CHO cells with CD14 fails to confer responsiveness to B. burgdorferi

Unlike THP-1 cells, transfection of CHO cells with CD14 does not confer responsiveness to spirochetal lipoproteins as it does to LPS (26). This important observation was one of several lines of evidence suggesting that spirochetal lipoproteins and LPS use disparate transmembrane signaling elements immediately downstream from CD14 (11, 12, 38). In light of the above findings with CD14-transfected THP-1 cells, we next sought to determine the effect of CD14 transfection on the responsiveness of CHO cells to live spirochetes. For these experiments, we used a CHO cell line cotransfected with CD14 (expressed constitutively) and the Tac Ag (CD25) under the control of the NF-κB-inducible E-selectin promoter (3E10 cells) (42). In contrast to LPS, both B. burgdorferi and nOspA failed to induce the expression of CD25 in 3E10 cells (Fig. 5).

Motile T. pallidum activate vitamin D₃-matured THP-1 cells in a CD14-dependent manner but are markedly less stimulatory than B. burgdorferi

T. pallidum is similar to B. burgdorferi in that it contains abundant lipoproteins capable of activating monocytic cells in a CD14-enhanced fashion (11, 12, 26, 38). However, unlike B. burgdorferi, T. pallidum lipoproteins are predominantly subsurface (29, 30) and thus may not be as accessible to CD14 as their borrelial counterparts. To address this issue, we examined the ability of motile T. pallidum to stimulate THP-1 cells. Activation of vitamin D₃-matured cells was observed only at 1000 treponemes per cell (Fig. 6A); blocking with mAb 60bca confirmed that this response was facilitated by CD14 (Fig. 6B). Comparison of Figs. 1A and 6A highlights the much lower stimulatory capacity of T. pallidum as compared with B. burgdorferi. We next considered the possibility that the difference in IL-8 secretion induced by the two spirochetes was due to the larger size of B. burgdorferi rather than to their different molecular architectures. To evaluate this, experiments were conducted in which the input spirochete-to-cell ratios were plotted by protein content. As shown in Fig. 7A, B. burgdorferi was more stimulatory than T. pallidum over the entire range of protein concentrations evaluated. If the relatively poor stimulatory activity of T. pallidum were due to its paucity of surface-exposed lipoproteins, one would predict that T. pallidum sonicates would be more stimulatory than intact organisms. As shown in Fig. 7B, this was indeed the case when IL-8 secretion induced by intact treponemes and equivalent cell lysates was directly compared. It is noteworthy that the cellular response to treponemal sonicate plateaus at ratios above 10 organisms/cell; a plateau in responsiveness also was observed when purified lipoproteins or synthetic/lipopeptides were used in stimulation assays (26).

Discussion

CD14 has been designated a pattern recognition receptor by virtue of its ability to mediate monocytic and endothelial cell activation in response to myriad bacterial cell wall products in addition to LPS (17, 18, 45). From this work has arisen the notion that CD14-mediated interactions are a critical first step in the elicitation of innate immune responses to bacterial pathogens (18). However, because of the complexity of bacterial cell wall architecture, it cannot be assumed that all cell wall components are equally accessible to CD14. For this reason, in vitro studies using purified components eventually must be validated using the intact organism from which the cell wall component was derived. Along these lines, studies from several laboratories have demonstrated that spirochetal lipoproteins and/or synthetic lipopeptides have proinflammatory activities (8–15, 32, 38), while others have shown that motile spirochetes also are capable of activating innate immune effector cells (31–33). The recent finding that spirochetal lipoproteins/lipopeptides activate cells via a distinctive CD14-dependent
pathway (26) provided us with a convenient starting point for relating these two sets of experimental observations.

Here we used two different approaches to demonstrate that induction of IL-8 secretion by monocytic cells during incubation with viable spirochetes is facilitated by CD14. The first used vitamin D3-matured THP-1 cells. Previous studies have shown that vitamin D3 maturation induces expression of surface-bound CD14 and that this results in enhanced cellular responsiveness to both LPS and spirochetal lipoproteins (26, 38, 46, 47). Because the effects of vitamin D3 are pleiotropic (48, 49), we confirmed the CD14 dependence of the response with a LPS-blocking anti-CD14 mAb, which also antagonized cellular activation by the prototypical spirochetal lipoprotein OspA (25, 27). Although the epitope on CD14 recognized by 60bca has not been mapped to a specific region of CD14 required for LPS-mediated signaling (50–52), this result is consistent with the finding that LPS and nOspA appear to bind to similar domains on CD14 (27). To confirm the blocking studies, we also examined THP-1 cells transfected with CD14; as previously observed with lipoproteins/lipopeptides (26), responsiveness was significantly enhanced in the transfected cells. Lastly, we noted that CHO cells transfected with CD14, while sensitive to LPS, failed to respond to either spirochetes or nOspA, indicating that both spirochetes and nOspA use a CD14-dependent signaling pathway(s) different from that used by LPS. Although these results do not constitute definitive evidence that lipoproteins are the principal stimulatory component of live spirochetes, they are entirely consistent with this notion.

Lipid modification is essential for CD14-dependent lipoprotein activation and probably reflects binding of the agonist to an amphipathic pocket within the receptor. However, because the lipid moieties of lipoproteins are stably embedded within the spirochetal membrane (28), it is not immediately clear how this binding interaction occurs. At least three testable scenarios, which are not

FIGURE 5. CD14-transfected CHO cells are activated by LPS but not by nOspA or B. burgdorferi. 3E10 cells were incubated for 8 h with LPS (100 ng/ml), nOspA (1 μg/ml), or B. burgdorferi B31 at a 1000:1 spirochete-to-cell ratio. The cells were harvested, stained with PE-CD25 mAb, and analyzed for reporter gene expression by FACS. Thin and thick lines represent unstimulated and stimulated cells, respectively. Shown are results representative of three independent experiments.

FIGURE 6. Activation of monocytic cells by T. pallidum is facilitated by CD14. A, IL-8 production by undifferentiated and vitamin D3-matured THP-1 cells incubated with increasing numbers of motile T. pallidum. Shown are the mean ± SE of duplicate determinations for three independent experiments. B, IL-8 production by undifferentiated cells and vitamin D3-matured cells preincubated with medium alone, 10 μg/ml 60bca, or 10 μg/ml 63D3 before the addition of LPS (10 ng/ml) or spirochetes at a 1000:1 ratio. Shown are the means ± SE of duplicate determinations for three independent experiments. Asterisks indicate results that are significantly different from their undifferentiated counterparts.
with respect to spirochetal lipoproteins could have broader implications for other disorders, including Gram-negative bacterial sepsis and septic shock (57).

The topological considerations for T. pallidum are even more complex given its paucity of surface-exposed lipoproteins (29, 30). In head-to-head comparisons, purified T. pallidum cell lysates, lipoproteins, and synthetic lipopeptides are similar in potency to each other and to their borrelial counterparts (11, 12, 26). On the other hand, the in vitro experiments reported here clearly demonstrate that intact treponemes are markedly less stimulatory than either treponemal sonicates or intact B. burgdorferi and that differences in cell activation between T. pallidum and B. burgdorferi could not be attributed to the larger size of the latter. We believe that the disparate outer membrane architectures of the two organisms is the most straightforward explanation for their inherently different stimulatory capacities. It should be noted that these findings also lend additional, albeit indirect, support to the conjecture that lipoproteins are responsible for monocyte activation by intact spirochetes. Two additional implications of the disparity between T. pallidum and B. burgdorferi need to be mentioned. First, compared with B. burgdorferi, cellular activation by T. pallidum should be relatively dependent upon uptake and processing by professional phagocytes. Second, although constitutional manifestations occur with both syphilis and Lyme disease (1, 2), there is a general impression that they are more common and severe in Lyme disease despite its paucibacillary nature (2, 34, 58). A greater ability of intact borreliae to induce cytokine production by innate immune effector cells could underlie, at least in part, this clinical observation. T. pallidum’s ability to evade phagocytosis by macrophages, even in the presence of opsonizing Abs (30, 59, 60), would further serve to diminish cytokine production during syphilitic infection.

T. pallidum exists only within a mammalian host and, therefore, is perpetually host adapted. Consequently, one can be highly certain that in vitro experiments with the syphilis spirochete are examining the bacterium in the antigenic state actually encountered by the host during infection. B. burgdorferi, on the other hand, undergoes major shifts in antigenic composition as it cycles between arthropod and mammal (35, 44). Based upon their expression of proteins such as OspA, OspB, and Lp6.6 and their decreased expression of OspC, in vitro-cultivated organisms appear to more closely resemble the phenotype within ticks. These considerations sound a cautionary note regarding tissue culture studies conducted with in vitro-cultivated organisms. To circumvent this problem, we also conducted studies with DMC-grown organisms; surprisingly, we found that spirochetes cultivated in this manner were actually more stimulatory than their in vitro-grown counterparts. Although these host-adapted organisms expressed much lower levels of OspA and OspB, the up-regulation of other lipoproteins, and synthetic lipopeptides are similar in potency to each other and to their borrelial counterparts (11, 12, 26). On the other hand, the in vitro experiments reported here clearly demonstrate that intact treponemes are markedly less stimulatory than either treponemal sonicates or intact B. burgdorferi and that differences in cell activation between T. pallidum and B. burgdorferi could not be attributed to the larger size of the latter. We believe that the disparate outer membrane architectures of the two organisms is the most straightforward explanation for their inherently different stimulatory capacities. It should be noted that these findings also lend additional, albeit indirect, support to the conjecture that lipoproteins are responsible for monocyte activation by intact spirochetes. Two additional implications of the disparity between T. pallidum and B. burgdorferi need to be mentioned. First, compared with B. burgdorferi, cellular activation by T. pallidum should be relatively dependent upon uptake and processing by professional phagocytes. Second, although constitutional manifestations occur with both syphilis and Lyme disease (1, 2), there is a general impression that they are more common and severe in Lyme disease despite its paucibacillary nature (2, 34, 58). A greater ability of intact borreliae to induce cytokine production by innate immune effector cells could underlie, at least in part, this clinical observation. T. pallidum’s ability to evade phagocytosis by macrophages, even in the presence of opsonizing Abs (30, 59, 60), would further serve to diminish cytokine production during syphilitic infection.

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cells constitutively express the LPS-specific TLR but not that used by spirotcheral lipoproteins, thereby accounting for the lack of responsiveness of CD14-transfected CHO cells to lipoproteins and spirochetes. Furthermore, the consistently greater responsiveness of vitamin D3-matured THP-1 cells, as compared with CD14-transfected THP-1 cells, could reflect vitamin D3-induced expression of TLRs as well as CD14. Lastly, the CD14-independent activation observed with either higher dosages of lipoproteins/ lipopolipids (26) or higher spirochete-to-cell ratios could reflect the bypassing of CD14 in favor of a direct interaction with one or more TLRs. On the other hand, the possibility also exists that activation of undifferentiated THP-1 cells reflects a distinct signaling pathway. Examination of the role of TLRs in these signaling events will enable us to address the above issues while further exploring the relationship(s) between lipoprotein- and spirochete-mediated cellular activation.

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References


