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Lipopeptides of *Borrelia burgdorferi* Outer Surface Proteins Induce Th1 Phenotype Development in αβ T-Cell Receptor Transgenic Mice

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Induction of the appropriate T helper cell (Th) subset is crucial for the resolution of infectious diseases and the prevention of immunopathology. Some pathogens preferentially induce Th1 or Th2 responses. How microorganisms influence Th phenotype development is unknown. We asked if *Borrelia burgdorferi*, the spirochete which causes Lyme arthritis, can promote a cytokine milieu in which T cells which are not specific for *B. burgdorferi* are induced to produce proinflammatory cytokines. Using αβ T-cell receptor transgenic mice as a source of T cells with a defined specificity other than for *B. burgdorferi*, we found that *B. burgdorferi* induced Th1 phenotype development in ovalbumin-specific transgenic T cells. Small synthetic lipopeptides corresponding to the N-terminal sequences of *B. burgdorferi* outer surface lipoproteins had similar effects. *B. burgdorferi* and its lipopeptides induced host cells to produce interleukin-12. When the peptides were used in delipidated form, they did not induce Th1 development. These findings may be of pathogenic importance, since it is currently assumed that a Th2-mediated antibody response is protective against *B. burgdorferi*. Bacteria associated with reactive arthritis, namely, *Yersinia enterocolitica*, *Shigella flexneri*, and *Salmonella enteritidis*, had different effects. The molecular definition of pathogen-host interactions determining cytokine production should facilitate rational therapeutic interventions directing the host response towards the desired cytokine response. Here, we describe small synthetic molecules capable of inducing Th1 phenotype development.

T helper cells can be categorized into at least two groups according to their cytokine production. Th1 cells produce mainly interleukin-2 (IL-2), gamma interferon (IFN-γ), and lymphotoxin, whereas Th2 cells produce mainly IL-4, IL-5, and IL-13 (1, 28, 30). T-cell cytokine production is a major immunoregulatory mechanism determining the outcome of many infectious and autoimmune diseases (1, 30). Various factors, such as major histocompatibility complex genes (1, 6) and non-major histocompatibility complex "background genes" (19, 32, 42), different antigen-presenting cells (APC) (1, 6, 41), antigen dose or structure (6), availability of co-stimulation (1, 6), and cytokines present during T-cell priming (1, 6), can determine if a Th1 or a Th2 response develops. The major factors for the induction of a Th1 or Th2 response seem to be IL-12 and IL-4, respectively (1, 20, 43).

The T-cell response to some pathogens is biased towards a Th1 or Th2 pattern (30). The molecular mechanisms used by microorganisms to induce Th1 or Th2 development in the host are unknown. Understanding of these mechanisms is important if one wants to be able to manipulate the outcome of immune responses towards a particular Th phenotype. We have studied how the Lyme disease agent, *Borrelia burgdorferi*, and gram-negative bacteria associated with reactive arthritis, i.e., *Yersinia enterocolitica*, *Shigella flexneri*, and *Salmonella enteritidis*, might direct Th phenotype development.

Lyme arthritis is a tick-borne disease caused by the spirochete *B. burgdorferi* sensu lato (2). The clinical spectrum of Lyme arthritis ranges from a self-limiting disease to antibiotic-resistant chronic arthritis. The synovial lesion in patients with chronic Lyme arthritis is similar to that found in other forms of chronic inflammatory arthritis, including rheumatoid arthritis. Whereas most patients with Lyme arthritis can be cured with the appropriate antibiotic therapy, about 10% have persistent arthritis for months or even several years after antibiotic treatment (46, 47). The host response to *B. burgdorferi* is likely to play a role in the pathogenesis of Lyme arthritis (4, 22, 23, 27). Two major pathways, which are not mutually exclusive, are conceivable for *B. burgdorferi* induced immunopathology: T- or B-cell responses to *B. burgdorferi* could cause hypersensitivity or even autoimmunity (e.g., via molecular mimicry), and *B. burgdorferi*-induced cytokine production could induce or maintain chronic inflammation. Susceptibility to Lyme arthritis has been linked to Th1-like cytokine production in mice (25, 29, 36) and humans (53, 54).

Reactive arthritis is triggered by infection either of the urogenital tract with *Chlamydia trachomatis* or of the enteric tract with *Yersinia*, *Salmonella*, *Shigella*, or *Campylobacter* (4). All of these microbes are either obligate or facultative intracellular bacteria. It seems likely that the host's immune response is important in determining whether reactive arthritis becomes chronic or resolves within a few months. A Th1 response seems to be necessary for protection against these pathogens (4).

We investigated whether *B. burgdorferi* can promote a cytokine milieu in which T cells that are not specific for *B. burgdorferi* are induced to secrete proinflammatory cytokines. To examine such bystander effects, a source of naive T cells with defined specificities other than for *B. burgdorferi* is needed. In T-cell receptor (TCR) transgenic mice, most of the peripheral T cells express the transgenic TCR. Furthermore, these T cells can be primed in vitro (11). Depending on the conditions during in vitro priming, TCR transgenic T cells develop either a Th1 or Th2 phenotype (1, 20, 43). Therefore, we used T cells transgenic for a TCR recognizing an ovalbumin peptide bound

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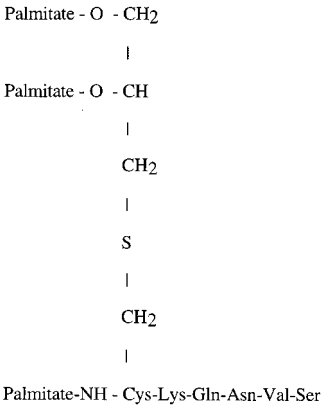

FIG. 1. Structure of the synthetic OspA lipopeptide. Cys is the cysteine at position 17 of the OspA molecule.

to I-A^d (31) to investigate whether *B. burgdorferi* exerted effects on non-*B. burgdorferi*-specific bystander T cells.

We show here that *B. burgdorferi* and synthetic lipopeptides derived from its outer surface lipoproteins induce Th1 phenotype development in naive TCR transgenic cells.

MATERIALS AND METHODS

Animals. Mice expressing the transgene for the DO11.10 TCR (31) were kindly provided by D. Loh (Washington University, St. Louis, Mo.). The DO11.10 TCR recognizes residues 323 to 339 of chicken ovalbumin (OVA peptide) in association with I-A^d. Transgenic mice were maintained on the BALB/c background in our animal facility. Genotypes were determined by using a PCR protocol previously described (11). Mice were housed in our animal facilities in microisolator cages under pathogen-free conditions, and all procedures were performed in accordance with institutional and state guidelines.

Cell culture and assay conditions. All cultures and assays were done in RPMI with 10% fetal calf serum, 10 mM glutamine, 100 U of penicillin/ml, 100 μg of streptomycin/ml, and 2×10^{-5} M 2-mercaptoethanol (complete medium) at 37°C in 5% CO_2 as described previously (11). Mice were sacrificed, and spleens were taken. A single-cell suspension was prepared by pressing the spleens through a fine wire mesh. For some experiments T cells were purified with nylon wool as described previously (24). The purity of nylon wool-separated populations was routinely >90% T cells. T cells (10^6) were stimulated in 2-ml cultures with 0.3 μM OVA peptide presented by 2×10^6 irradiated (26 Gy) syngenic splenocytes. For other experiments 4×10^6 splenocytes were cultured in 2 ml with 0.3 μM OVA. In some experiments cytokines, antibodies, *B. burgdorferi* sonicates, or synthetic antigens were added to the primary culture. On day 3 the T cells were expanded by adding new medium and IL-2 (100 U/ml), and on day 7 the cells were harvested, washed twice, counted, and restimulated with 0.3 μM OVA peptide alone. Forty-eight hours later, the supernatants were taken and analyzed for cytokine content.

Antigen. The antigenic OVA peptide was synthesized by conventional Merrifield solid-phase chemistry and purchased from the Department of Biochemistry of the Charité Hospital, Berlin, Germany.

***B. burgdorferi* sonicates and synthetic peptides.** The low-passage N40 and B31-4 strains of *B. burgdorferi* sensu stricto were propagated in BSK medium (Sigma, St. Louis, Mo.) with 6% rabbit serum (Sigma), and sonicates were made as described before (27). *B. burgdorferi* N40 is an infectious strain isolated from ticks (3) and was provided by J. Leong (Tufts University, Boston, Mass.). N40 expresses the outer surface proteins OspA, -B, -C, and -D. *B. burgdorferi* B31-4 is an infectious mutant of the tick isolate B31 (ATCC 35210), from which it was derived by growth in antibody-containing medium (39), and was provided by A. Barbour (University of Texas, San Antonio). B31-4 does not express OspA, -B, and -D but does still express OspC. Unless otherwise indicated, sonicates of *B. burgdorferi* N40 were used in all experiments. *B. burgdorferi* sonicates were used at concentrations ranging from 0.1 to 100 μg/ml. The sonicate of 10^6 *B. burgdorferi* organisms yields approximately 3 μg of protein (reference 26 and our unpublished data).

The synthetic peptide OspA-4, derived from the published sequence of *B. burgdorferi* ZS7 (GenBank accession number X16467), was synthesized by using conventional Merrifield solid-phase chemistry and purchased from BioTeZ (Berlin-Buch, Germany). The sequence of the OspA-4 peptide is CKQNVSSLDEKN SVSVDLP. OspA-4 was used at a final concentration of 1 μM.

The structure of the synthetic OspA lipopeptide is shown in Fig. 1. The structure of the OspB lipopeptide is identical except that the peptide sequence is Cys-Ala-Gln-Asn-Val-Ser, where Cys is the cysteine at position 16 of the OspB molecule (34). The lipopeptides were made by solid-phase synthesis as described previously (34) and purchased from the Department of Biochemistry of the Charité Hospital, Berlin, Germany. Lipopeptides were used at final concentrations ranging from 0.1 to 100 μM.

***Y. enterocolitica*, *S. flexneri*, and *S. enteritidis*.** *Y. enterocolitica* P108 and patient isolates of *S. flexneri* and *S. enteritidis* were grown under standard conditions in tryptic soy broth. Heat-killed (90 min at 70°C) bacteria were kindly provided by M. Mielke (Institut für Medizinische Mikrobiologie, Freie Universität Berlin). Heat-killed bacteria were used at a bacterium/T-cell ratio of 1:1, 10:1, or 100:1.

Cytokines. Recombinant murine IL-2 was obtained from Eurocetus (Amsterdam, The Netherlands) and used at a final concentration of 100 U/ml. Recombinant murine IL-4 was obtained from Biosource International (Camarillo, Calif.) and used at a final concentration of 200 U/ml. Recombinant murine IL-12 was obtained from M. K. Gately (Hoffman-La Roche, Nutley, N.J.) and used at a final concentration of 1 ng/ml.

Antibodies. Polyclonal anti-murine IL-12 (49) was obtained from M. K. Gately (Hoffman-La Roche) and used at a final concentration of 100 ng/ml. Polyclonal goat immunoglobulin G was purchased from Dianova (Hamburg, Germany).

Cytokine assays. Seven days after in vitro priming, 10^6 transgenic T cells were cultured with 2×10^6 APC (irradiated syngeneic spleen cells) and 0.3 μM OVA. Culture supernatants were taken 48 h later. The IL-4 and IFN-γ levels were analyzed with a sandwich enzyme-linked immunosorbent assay (ELISA) (Genzyme, Cambridge, Mass.).

RESULTS

Induction of Th1 phenotype development by *B. burgdorferi*. We used BALB/c mice transgenic for the DO11.10 TCR (31). In initial experiments we used purified $CD4^+$ T cells which were stimulated with irradiated syngeneic splenocytes and OVA peptide with or without further additions of cytokines, antibodies, or *B. burgdorferi* N40 sonicate. In control experiments we used either purified $CD4^+$ T cells plus irradiated splenocytes or unfractionated splenocytes from TCR transgenic mice in the primary stimulation. Fluorescence-activated cell sorter analysis showed that 7 days after initiation of the culture, the percentage of $CD4^+$ transgenic T cells (identified with the clonotypic monoclonal antibody KJ 1-26.1) was >95% under both conditions (data not shown). Furthermore, the cytokine patterns obtained 48 h after restimulation were the same with either condition. Thus, we used unfractionated spleen cells for the in vitro priming. Seven days later the transgenic T cells were harvested and restimulated with irradiated syngeneic splenocytes and OVA peptide. The T cells produced both IFN-γ and IL-4 in the secondary culture when no additional cytokines were added during the in vitro priming (Fig. 2). When IL-12 or IL-4 was added during in vitro priming, the T cells developed a Th1 or Th2 phenotype, respectively (Fig. 1), as previously described by others (19, 33). The addition of various doses of *B. burgdorferi* sonicate during in vitro priming induced Th1 phenotype development with a 3-fold increase in IFN-γ production and a greater-than-10-fold decrease in IL-4 production compared to those for T cells primed with OVA peptide alone in the absence of *B. burgdorferi* sonicate (Fig. 2). The *B. burgdorferi*-mediated Th1 induction was dose dependent. At 0.1 μg/ml there were minimal effects on IL-4 and IFN-γ production. IFN-γ production increased with higher doses of *B. burgdorferi* sonicate added to the culture, and a plateau was reached at a concentration of 10 μg/ml. The IL-4 production by transgenic T cells decreased with higher doses of *B. burgdorferi* sonicate, reaching a nadir at a concentration of 100 μg/ml (Fig. 2). *B. burgdorferi* sonicate alone induced neither T-cell proliferation nor production of IL-4 or IFN-γ (data not shown).

Induction of Th1 phenotype development by synthetic lipopeptides. The molecular mechanisms by which some pathogens influence Th phenotype development are hitherto unknown. In order to elucidate these mechanisms, we asked if defined *B. burgdorferi* antigens could exert the Th1-inducing effect seen with whole borreliae. *B. burgdorferi* strongly induces

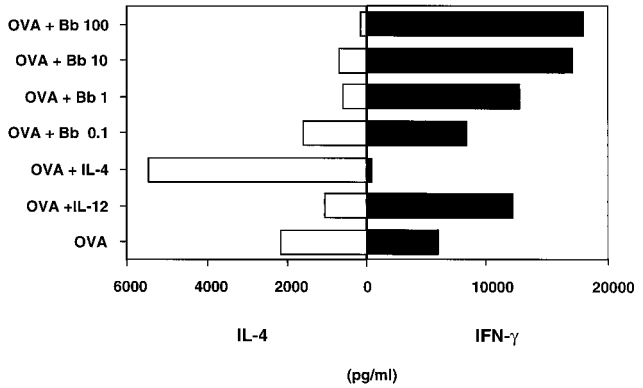

FIG. 2. Induction of Th1 phenotype development by *B. burgdorferi* N40 sonicate. Splenocytes from unimmunized TCR transgenic mice were primed in vitro with the OVA peptide either alone (OVA) or in the presence of IL-12 (OVA + IL-12), IL-4 (OVA + IL-4), or *B. burgdorferi* sonicates at concentrations ranging from 0.1 to 100 μg/ml (OVA + Bb), as described in Materials and Methods. Seven days later T cells were harvested and restimulated with APC and OVA. Supernatants were taken 48 h later and assayed by ELISA for IL-4 and IFN-γ to examine the effect of the different priming conditions on Th phenotype development. The experiment was performed more than three times with similar results.

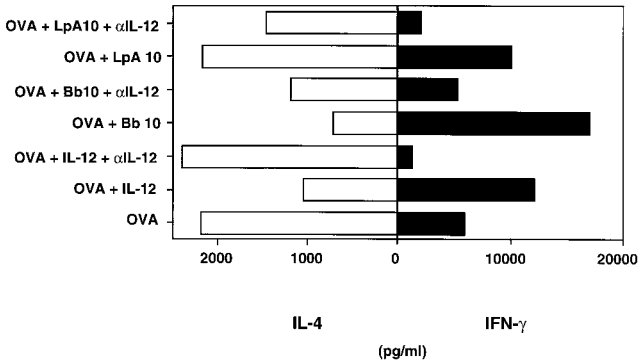

FIG. 4. Inhibition of Th1 development by anti-IL-12. Splenocytes from unimmunized TCR transgenic mice were primed in vitro as described in the legend to Fig. 2. Priming was with the OVA peptide either alone (OVA) or in the presence of IL-12 (OVA + IL-12), *B. burgdorferi* sonicate at 10 μg/ml (OVA + Bb 10), or OspA-derived lipohexapeptide at 10 μM (OVA + LpA 10), each in the presence or absence of anti-IL-12 (αIL-12) (49), as described in Materials and Methods. Seven days later T cells were harvested and restimulated with APC and OVA. Supernatants were taken 48 h later and assayed by ELISA for IL-4 and IFN-γ. The experiment was performed more than three times with similar results.

IL-1β and tumor necrosis factor alpha (TNF-α) production, as well as IL-12 mRNA (10, 15, 34, 35). *B. burgdorferi* outer surface lipoproteins A and B are sufficient for the induction of IL-1β and TNF-α in host macrophages (34, 50). IL-1β and TNF-α are necessary cytokines for the IL-12-induced early IFN-γ production (12, 21). We therefore asked if *B. burgdorferi* outer surface lipoproteins could induce Th1 phenotype development in TCR transgenic T cells. Since it had been shown earlier that macrophages can be activated by bacterial lipoproteins (16, 18) or synthetic lipopeptides (34), we used synthetic lipopeptides derived from the *B. burgdorferi* OspA and OspB sequences in our experiments. Figure 3 shows that the synthetic OspA lipopeptide (LpA) induced Th1 phenotype development in a dose-dependent manner. At 0.1 μg/ml, LpA influenced neither IFN-γ nor IL-4 production. At 1 and 10 μg/ml, LpA induced a fourfold increase in IFN-γ production. At 100 μg/ml, LpA no longer had an effect on IFN-γ production. Only at 100 μg/ml, the highest dose tested, did LpA induce a decreased IL-4 production (Fig. 3). The synthetic lipopeptide derived from the *B. burgdorferi* OspB sequence had similar effects (data not shown).

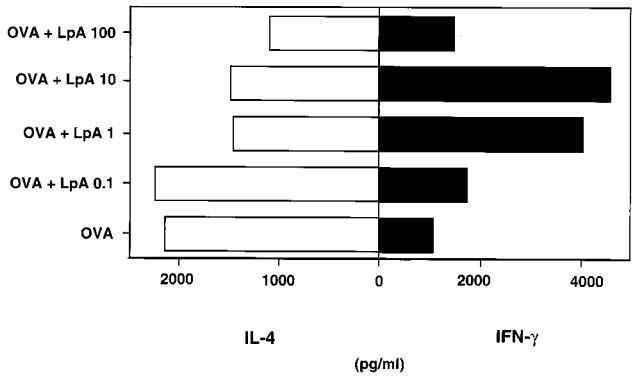

FIG. 3. Synthetic lipopeptides derived from OspA or OspB induce Th1 phenotype development. Splenocytes from unimmunized TCR transgenic mice were primed in vitro as described in the legend to Fig. 2. Priming was with the OVA peptide alone (OVA) or in the presence of the OspA-derived lipohexapeptide (OVA + LpA). Lipopeptides were used at final concentrations ranging from 0.1 to 100 μM, as described in Materials and Methods. Seven days later T cells were harvested and restimulated with APC and OVA. Supernatants were taken 48 h later and assayed by ELISA for IL-4 and IFN-γ. The experiment was performed more than three times with similar results.

***B. burgdorferi*-induced Th1 phenotype development is mediated by IL-12.** Since IL-12 is a strong inducer of Th1 phenotype development (1), we examined whether *B. burgdorferi* sonicate- or LpA-induced Th1 development was caused by IL-12. When *B. burgdorferi* sonicate or LpA was added simultaneously with anti-IL-12 (49), Th1 phenotype development was inhibited (Fig. 4); the increase in IFN-γ production was completely blocked. However, the *B. burgdorferi* sonicate-mediated decrease in IL-4 production was only partially reversed (Fig. 4). An isotype-matched control antibody did not block Th1 phenotype development (data not shown).

Induction of Th1 phenotype development by other *B. burgdorferi* antigens. *B. burgdorferi* lacks lipopolysaccharide (LPS) but is rich in lipoproteins other than OspA and OspB (45, 48). We therefore asked whether a mutant *B. burgdorferi* strain lacking OspA, -B, and -D (39) could induce Th1 phenotype development in TCR transgenic T cells. Figure 5 shows that in the absence of OspA, -B, and -D, *B. burgdorferi* sonicate still strongly induced Th1 phenotype development. The mutant *B. burgdorferi* strain induced a 10-fold increase in IFN-γ production, indicating that other lipoproteins, such as OspC, which is still expressed by the mutant, or antigens which are not lipoproteins contribute to the *B. burgdorferi*-induced Th1 phenotype development. Lipidation of the synthetic lipopeptides was necessary for the induction of Th1 phenotype development. Addition of unlipidated OspA peptides did not change the spontaneously developing cytokine pattern (Fig. 5).

Influence of intracellular gram-negative bacteria on Th phenotype development. We next asked if other arthritis-associated bacteria are also capable of Th1 phenotype induction. We analyzed the effects of *S. enteritidis*, *Y. enterocolitica*, and *S. flexneri* on Th phenotype development in our transgenic system. Heat-killed bacteria were used at a bacterium/T-cell ratio of 1:1, 10:1, or 100:1. *Salmonella* induced increased IFN-γ production at all bacterium/T-cell ratios tested, but not to the same extent as *B. burgdorferi* (Fig. 6). At a bacterium/T-cell

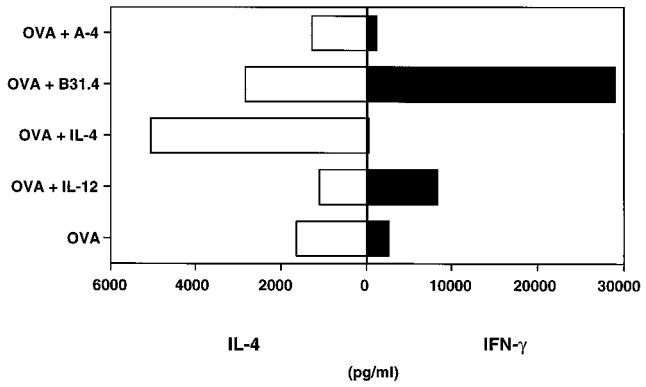

FIG. 5. A mutant strain which lacks OspA, -B, and -D induces Th1 phenotype development, but unlipidated OspA peptide does not. Splenocytes from unimmunized TCR transgenic mice were primed in vitro as described in the legend to Fig. 2. Priming was with the OVA peptide alone (OVA) or in the presence of IL-12 (OVA + IL-12), IL-4 (OVA + IL-4), mutant *B. burgdorferi* B31-4 sonicate at 10 μg/ml (OVA + B31-4), or unlipidated N-terminal OspA peptide at 1 μM (OVA + A-4). B31-4 lacks OspA, -B, and -D but still expresses OspC, as described in Materials and Methods. Seven days later T cells were harvested and restimulated with APC and OVA. Supernatants were taken 48 h later and assayed by ELISA for IL-4 and IFN-γ. The experiment was performed more than three times with similar results.

ratio of 1:1 or 10:1, *Salmonella* induced decreased IL-4 production, similar to what was observed with *B. burgdorferi* sonicates. At a bacterium/T-cell ratio of 1:100, IL-4 production was similar to that with the OVA peptide only (Fig. 6). Addition of *Shigella* to the primary cultures had dose-dependent effects similar to those observed with *B. burgdorferi* sonicates. At a bacterium/T-cell ratio of 1:100, IFN-γ production was about 3-fold increased and IL-4 production was about 15-fold decreased compared to those after stimulation with the OVA peptide alone (Fig. 6). At a bacterium/T-cell ratio of 1:1 or 10:1, *Yersinia* induced a slight increase in IFN-γ production combined with a slight decrease in IL-4 production (Fig. 6). At a bacterium/T-cell ratio of 1:100, IFN-γ production was similar to that with the OVA peptide only, while IL-4 production was enhanced (Fig. 6). Thus, the effects of *Shigella* were similar to those observed with *B. burgdorferi* sonicates, while *Salmonella* and *Yersinia* exerted different effects.

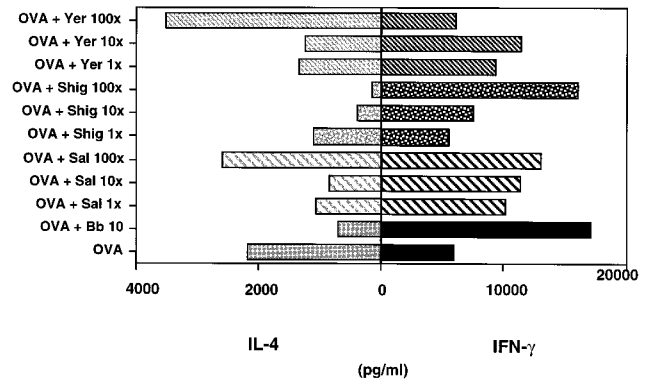

FIG. 6. Effects of gram-negative bacteria on Th phenotype development. The experiment was performed as described in the legend to Fig. 2. Priming was with the OVA peptide either alone (OVA) or in the presence of *S. enteritidis* (OVA + Sal), *S. flexneri* (OVA + Shig), or *Y. enterocolitica* (OVA + Yer). Heat-killed bacteria were used at a bacterium/T-cell ratio of 1:1 (1×), 10:1 (10×), or 100:1 (100×), as described in Materials and Methods. Seven days later T cells were harvested and restimulated with APC and OVA. Supernatants were taken 48 h later and assayed by ELISA for IL-4 and IFN-γ. The experiment was performed more than three times with similar results.

DISCUSSION

We have shown that *B. burgdorferi* sonicate can induce Th1 phenotype development in TCR transgenic murine T cells. This is mediated at least in part by the induction of IL-12 production in host cells. Whereas the *B. burgdorferi*-induced increase in IFN-γ production was completely blocked with an antibody against IL-12, this antibody did not completely restore the decreased IL-4 production (Fig. 4). It is therefore possible that *B. burgdorferi*-induced IL-12 acts in concert with other cytokines to induce Th1 phenotype development. Others have shown that *L. monocytogenes* can induce Th1 phenotype development in TCR transgenic T cells via the induction of IL-12 (20). Mycobacteria (7, 12) and *Toxoplasma gondii* (13) also induce IL-12. However, the induction of a Th1 response would seem beneficial to combat intracellular pathogens such as *Listeria monocytogenes*, *Mycobacterium bovis*, or *T. gondii*, whereas it might be harmful when dealing with *B. burgdorferi* (25, 29, 36).

LPS, which is expressed by gram-negative bacteria, is a potent inducer of IL-12 (9, 17). Recently, lipoteichoic acid, which is expressed by gram-positive bacteria, was found to induce IL-12 and thereby Th1 phenotype development (5). We wished to determine how *B. burgdorferi*, which does not possess LPS (45, 48), induces IL-12 production and Th1 phenotype development. We found that *B. burgdorferi* outer surface lipoproteins OspA and OspB induce Th1 phenotype development. Our data show that small synthetic lipopeptides corresponding to the N-terminal sequence of *B. burgdorferi* OspA or OspB can substitute for *B. burgdorferi* and induce Th1 phenotype development. *B. burgdorferi* sheds membrane blebs which contain Osp proteins (51). Therefore, the Th1-inducing effect in the infected host might occur at sites where intact *B. burgdorferi* or its DNA is not found.

The induction of a Th1 response might help *B. burgdorferi* to subvert the host's immune response, since a Th2 response seems to be necessary to successfully overcome *B. burgdorferi* infection (25, 29, 36, 53). It might also help explain the chronic synovial inflammation seen in some patients with Lyme borreliosis, the maintenance of a strong inflammatory response in the presence of only very few spirochetes (4, 22), and the slow development of a humoral immune response against *B. burgdorferi* (8). Furthermore, *B. burgdorferi*-induced IFN-γ production by T cells which are not specific for *B. burgdorferi* antigens might be partly responsible for the immunopathology that seems to be characteristic of treatment-resistant Lyme arthritis. Prolonged local overexpression of IFN-γ can cause immunopathology (14) independently of the specificity of the IFN-γ-producing cell (40).

The ability to induce Th1 phenotype development via IL-12 induction is not a general feature of microorganisms. *Leishmania major* promastigotes had no effect on Th phenotype development in TCR transgenic mice (19), *Mycoplasma fermentans* induces IL-10 but not IL-12 in host monocytes (37), and we report here that *Y. enterocolitica* and *S. enteritidis* differ from *B. burgdorferi* in their effects on Th phenotype development.

A microorganism's influence on Th phenotype development is likely to be the sum of many different, sometimes opposing, effects. *B. burgdorferi* induces not only IL-12 but also a variety of other cytokines, including IL-6 (44, 52). IL-6 has recently been reported to induce Th2 development (38). Thus, *B. burgdorferi*-induced Th1 development is the net effect of different

mechanisms that may antagonize each other. This may therefore be a much more physiologically relevant way to study T-cell differentiation than the commonly used systems in which IL-12 or IL-4 is added in vitro to Th cells.

In summary, we have demonstrated that the Lyme disease spirochete *B. burgdorferi* and synthetic lipopeptides corresponding to *B. burgdorferi* outer surface lipoproteins induce Th1 phenotype development in TCR transgenic murine T cells. This is not a general feature of all bacteria. The characterization of small synthetic molecules which potently direct Th phenotype development not only helps further understanding of microbial pathogenesis but also will be useful for future therapeutic manipulation of Th phenotype development.

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