An effective second-generation outer surface protein A-derived Lyme vaccine that eliminates a potentially autoreactive T cell epitope

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The antigenic component of a common Lyme disease vaccine is recombinant outer surface protein A (rOspA) of Borrelia burgdorferi (Bb), the causative agent of Lyme disease. Coincidentally, patients with chronic, treatment-resistant Lyme arthritis develop an immune response against OspA, whereas those with acute Lyme disease usually do not. Treatment-resistant Lyme arthritis occurs in a subset of Lyme arthritis patients and is linked to HLA-DRB1*0401 (DR4) and related alleles. Recent work from our laboratory identified T cell crossreactivity between epitopes of OspA and lymphocyte function-associated antigen 1α chain (LFA-1α) in these patients. We generated a form of rOspA, FTK-OspA, in which the LFA-1α/rOspA crosstable cell epitope was mutated to reduce the possible risk of autoimmunity in genetically susceptible individuals. FTK-OspA did not stimulate human or mouse DR4-restricted, WT-OspA-specific T cells, whereas it did stimulate antibody responses specific for WT-OspA that were similar to mice vaccinated WT-OspA. We show here that the protective efficacy of FTK-OspA is indistinguishable from that of WT-OspA in vaccination trials, as both C3H/HeJ and BALB/c FTK-OspA-vaccinated mice were protected from Bb infection. These data demonstrate that this rOspA-derived vaccine lacking the predicted cross-reactive T cell epitope, but retaining the capacity to elicit antibodies against infection, is effective in generating protective immunity.

Lyme disease is the most common vector-borne disease in the United States, with ~15,000 new cases each year (1). It is caused by the spirochete Borrelia burgdorferi sensu stricto (Bb), which is transmitted through the bite of infected ticks of the Ixodes family, primarily Ixodes scapularis in the Eastern and North central United States (2). Early symptoms of Lyme disease are nonspecific and can include malaise, fever, and chills. Because these symptoms are not specific to Lyme disease alone, combined with the fact that patients often do not recall being bitten (3), individuals may experience debilitating late manifestations of untreated Lyme disease weeks to months later, including musculoskeletal, cardiological, and neurologic dysfunctions before diagnosis (4–6). Prevention is the best method for avoiding infection and subsequent disease-related complications. However, personal protective strategies have not always proven to be successful (7, 8), indicating a need for an efficacious vaccine.

Lyme disease vaccine development has primarily targeted the outer surface protein A (OspA) of Bb (9). This surface-expressed lipoprotein is significantly up-regulated in the tick midgut, and anti-OspA antibodies can passively protect mice against Bb infection (10). OspA-based vaccines function by killing the bacteria in the tick midgut, thereby blocking transmission of Bb to the human host (11, 12). The first Food and Drug Administration-approved form of the vaccine, LYMErix (GlaxoSmithKline, Research Triangle Park, NC), consisted of a lipidated recombinant OspA (rOspA) from Bb strain ZS7 adsorbed with aluminum hydroxide adjuvant. Clinical trials in adults showed a 76% efficacy in preventing symptomatic Lyme disease after three doses (13). In children, vaccination efficacy has been shown to be close to 100% (14, 15). A nonadjuvant, lipidated form of rOspA Lyme vaccine, ImuLyme [Pasteur Merieux Connaught USA, Swiftwater, PA (16)], was found to have 92% efficacy after three injections. Thus, vaccination with rOspA is an effective preventative measure against Lyme disease.

A consequence of disseminated Bb infection in up to 10% of infected individuals is a condition described as treatment-resistant Lyme arthritis (TRLA) (17). Arthritis persists in TLR patients after long-term antibiotic therapy, suggesting that bacterial persistence is not necessary (18). Notably, in these patients, serum IgG reactivity to OspA correlates with the onset of prolonged episodes of arthritis (19). The incidence of TRLA has been correlated with the rheumatoid arthritis-associated MHC class II alleles, which include HLA-DRB1*0401 (DR4) (20). Our laboratory identified human lymphocyte function-associated antigen (hLFA)-1αL326–345 as a possible autoantigen in the context of DR4, based on sequence similarity to a region of OspA spanning amino acid residues 165–173 (OspA165–173) (21). By using an MHC class II DR4 tetramer specific for OspA164–176, we isolated T cells that were also hLFA-1αL326–345 reactive from the synovial fluid of DR4 homozygous patients with TRLA (22). These findings suggested that the pathogenesis of TRLA may be due to an autoimmune response triggered by OspA/hLFA-1αL crossreactivity. The observed T cell crossreaction between hLFA-1αL chain and OspA raised the concern that rOspA-based vaccines may trigger autoimmunity in susceptible individuals.

In this study, we report the generation of an rOspA vaccine, FTK-OspA, in which the putative cross-reactive T cell epitope, Bb OspA165–173, has been altered to resemble the corresponding peptide sequence of Borrelia afzelii. Human and mouse T cells specific for Bb OspA165–173 did not respond to FTK-OspA, whereas FTK-OspA was as effective as WT-OspA in generating protective antibody responses against Bb infection in both C3H/HeJ and BALB/c mice.

Materials and Methods

Mice. Six- to 8-week-old female C3H/HeJ and BALB/c mice were obtained from The Jackson Laboratory. HLA-DR4-IE chimeric transgenic mice (23) were a gift from T. Forsthuber (Case Western Reserve University, Cleveland) and were bred in our facility. All animal use protocols are approved by the accredited animal care and use committees at Tufts University.

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and Texas A&M University Health Science Center Institute of Biosciences and Technology, respectively.

**Bb.** Low-passage *Bb* N40 or B31 were cultured in complete Barbour–Stoenner–Kelly (BSK) medium (Sigma) at 34°C and counted under darkfield microscopy.

**Recombinant OspA Mutagenesis and Protein Production.** OspA was amplified from a plasmid containing the OspA sequence from *Bb* strain B31 by using published primers (24). The five' leader sequence and lipidation site were omitted as described (25), and the resulting truncated product was cloned into the pGEX4T.1 GST-fusion expression vector (Amersham Biosciences, Piscataway, NJ).

Site-directed mutagenesis was performed in two separate rounds by using the QuikChange PCR mutagenesis kit (Stratagene). First-round primers: P6K-Fwd gctatgttcttgaaggaAAGct-Tactgtgaaaaacaaacttt (HindIII); and second-round primers P1F2P2T-Fwd gaggctttaaggTTTACGcttgtaaggactc. Uppercase letters indicate substituted bases and underlined letters correspond to a restriction endonuclease site that was added. Recombinant FTK-OspA protein and empty vector control lysate (EV-Control) were produced in *Escherichia coli* BL-21 and purified by using GST-Sepharose 4B bead slurry (Amersham Biosciences). Cleavage with thrombin released the rOspA proteins of ~28 kDa from the beads and the GST moieties.

**Antibody Assays.** WT-OspA, FTK-OspA, or EV-Control lysate were plated at 100 ng per well in 50 μl of 0.1 M NaHCO₃, pH 8.2 overnight at 4°C. Plates were blocked with 1% BSA/0.5% Tween 20/0.02% sodium azide/PBS, pH 7.4 for 2 h at room temperature, or overnight at 4°C. Two-fold serial dilutions of mAb LA-2 or H5332 (26, 27) were plated at 100 μl per well and incubated at room temperature for 2.5 h. Antibody binding was detected with Zymax alkaline phosphatase-labeled goat anti-mouse IgG (Zymed), diluted 1:5,000 in 100 μl per well and allowed to bind for 1 h at 21°C. Plates were developed with 100 μl per well p-nitrophenyl phosphate (Pierce Biotechnology, Rockford, IL) substrate in diethanolamine buffer. Reactions were stopped with 0.1 M NaHCO₃, pH 8.2 overnight at 4°C. Serial twofold dilutions of mouse sera were plated in triplicate overnight at 4°C; serial twofold dilutions of unlabelled LA-2 (starting at 1 μg/ml) were placed on each plate as a standard curve. Plates were washed and biotinylated LA-2 was plated at 300 ng/ml for 30 min at room temperature. Each plate contained positive and negative control pooled sera samples. Plates were washed, and NeutrAvidin–alkaline phosphatase conjugate (Pierce Biotechnology) was added at 0.1 μg/ml/100 μl for at least 30 min at room temperature. Plates were developed with p-nitrophenyl phosphate substrate.

**OspA Immunization/Vaccination and Bb Infection.** Six- to 8-week-old Female C3H/HeJ and BALB/c mice were immunized s.c. with 100 μl of containing adjuvant plus WT-OspA, FTK-OspA, EV-Control, or adjuvant alone. Primary immunizations were performed in complete Freund's adjuvant (CFA), whereas subsequent immunizations were made by using incomplete Freund's adjuvant (IFA). C3H/HeJ mice were immunized on days 0, 14, and 28 with 10 μg of each protein or EV-Control. Two weeks after the last immunization, mice were infected with 10⁵ *Bb* N40 (passage 2) in the right hind footpad. BALB/c mice were immunized with 20 μg of each protein or EV-Control on days 0 and 28, and were infected with 10⁴ *Bb* B31 (passage 2) in 100 μl through intradermal injection at the base of the tail 2 weeks after the last immunization (31). Mock-infected controls were injected with 100 μl of BSK media and were not immunized with OspA.

Over the course of three weeks after *Bb* infection, ankle swelling was monitored by means of caliper measurements of the anterior-posterior tibiotalar joint thickness. Mice were killed on the fourth week after infection. *Bb* in the blood or bladder of C3H/HeJ mice were detected by culturing two drops of aseptically harvested blood or whole bladders in 1.5 ml of complete BSK medium (Sigma) at 34°C for 2 weeks and then examining culture supernatant by darkfield microscopy. In experiments using BALB/c mice, vaccine efficacy was determined by harvesting and culturing the blood and tissues (ear, heart, bladder, and joints) 7 and 14 days post infection, respectively. Briefly, 50 μl of blood or tissue samples were cultured in 6 ml of BSK for 2 weeks at 34°C in a CO₂-enriched atmosphere by using a GasPak chamber (Becton Dickinson and Company, Franklin Lakes, NJ) and were assessed for the presence of *Bb* by darkfield microscopy (30).

**DNA Isolation and PCR.** Genomic DNA extractions were performed on ear tissues harvested from infected C3H/HeJ mice by using a protocol adapted from Morrison et al. (32). Briefly, ear tissue was digested at 37°C for 2–4 h in 300 μl of 0.1% collagenase A in PBS, pH 7.4, then were digested overnight at 55°C after adding 300 μl of 0.2 mg/ml protease K/200 mM NaCl/20 mM Tris-HCl/50 mM EDTA/1% SDS. Extraction, ethanol precipitation, and resuspension were followed by digestion with RNase A, and an additional extraction and ethanol precipitation with a final resuspension in 10 mM Tris-HCl, pH 8.5. Primers used for amplification of *Bb recA* and murine nitrogen DNA were as published (32). PCR was performed by using 200, 20, and 2 ng of DNA, 0.5 μM recA primers, or 0.05 μM nitrogen primers, and 27 μl of Platinum Taq Supermix (Invitrogen) in a final volume under the following conditions: at 95°C, 5 min to denature, followed by 33 cycles of 95°C for 15 sec, 60°C for 30 sec, and 72°C for 30 sec. Products were visualized on ethidium bromide-stained agarose gels.

**Murine T Cell Hybridomas (Thy).** OspA-specific DR4 (DR4–51 and DR4–38) and DR1 (L3C4)-restricted THY were generated as described (22, 33). For stimulation studies, 3 × 10⁵ Thy cells per well were cultured for 48 h in complete RPMI medium 1640/10% FCS with an equal number of antigen-presenting cells (APCs): DAP-DR4 and DAP-DR1 for DR4- and DR1-restricted hybridomas, respectively. Culture supernatants were harvested and relative quantities of IL-2 secretions were determined by sandwich ELISA using standard protocols (BD Pharmingen). Recombinant mouse IL-2 standards were used to determine the linear range of the assay.

**Testing of Human T Cell Clones.** T cells were isolated and cloned from synovial fluid of a DR4 homozygous patient as described (22, 33). For proliferation assays, autologous Epstein–Barr virus-transformed B cells were treated with mitomycin C (Sigma) for use as APCs. APCs and T cell clones were added at 2 × 10⁴ cells with antigen as indicated. At 48 h, 100 μl of the medium was removed for cytokine analysis, and plates were pulsed with 0.5 μCi (1 Ci = 37 GBq) of [³H]-labeled thymidine for 18 h. Plates were counted on a β-plate scintillation counter (Perkin–Elmer, Willett et al.
phosphatase-coupled detecting antibodies were plated at 100 µg/ml. Mouse sera were plated at room temperature for 2 h. Alkaline phosphatase, as described (33). Values were determined by comparing OD_{405} values to the standard curve generated for each plate.

Results

Generation of rOspA. The immunodominant DR4-restricted T cell epitope of Bb OspA, OspA165–173, is located in the thirteenth β-pleat of the central β-sheet structure (34). We designed a modification of this epitope to prevent its association with DR4 and thereby inhibit the activation of T cells specific for this region, by using a DR-binding algorithm (35). FTK-OspA was generated by making the substitutions Y165 to F, V166 to T, and T170 to K in WT-Bb OspA, thus resembling the peptide sequence found in B. afzelii (36), Borrelia strain not associated with chronic arthritis (Fig. 1a). The DR-binding algorithm predicted that the MHC-binding score would drop from 6.5 for WT-OspA, and to +0.2 in the FTK-OspA mutant (Fig. 1a).

OspA from Bb strain B31, designated WT-OspA, and FTK-OspA, were expressed as GST-fusion proteins and were purified by thrombin cleavage from the GST moiety. The resulting recombinant protein preparations were of the anticipated mass (28 kDa, data not shown).

Because the efficacy of OspA-derived vaccines is contingent on a potent anti-OspA humoral response, it was critical to demonstrate that the changes made in the T cell-reactive domain of FTK-OspA did not significantly alter the protective antibody response. We determined whether FTK-OspA was able to bind a panel of OspA-specific mAbs by ELISA. mAbs specific for either the OspA N terminus, H5332 (27), or the C terminus, LA-2 (26), 9B3D (37), and CIII.78 (38) bound to both the WT-OspA and mutated FTK-OspA in a similar fashion. No reactivity to EV-Ctrl lysate was detected (Fig. 1b and data not shown).

WT-OspA_{165–173}–specific T Cells Do Not Respond to FTK-OspA. We used a panel of DR4-restricted murine Th1 and human T cell lines to determine whether we had eliminated the DR4-restricted T cell response to FTK-OspA by mutating the amino acids 165–173 epitope. Th1 were generated from OspA-immunized murine MHC class II-deficient mice transgenic for chimeric mouse MHC I-E^k with human DR4 α- and β-chains (23). DR4-restricted Th1 specificity for OspA_{165–173} produced significant levels of IL-2 in response to WT-OspA in a dose-dependent manner, but no IL-2 was detected in response to FTK-OspA or to EV-Ctrl (Fig. 2a). To determine whether FTK-OspA could bind overnight at 4°C. Plates were blocked with 200 µl of 0.5% BSA/0.05% Tween 20/0.02% sodium azide/PBS, pH 7.4 for 2 h at 21°C. Threefold serial dilutions of preinfection-immunized mouse sera were plated at room temperature for 2 h. Alkaline phosphatase-coupled detecting antibodies were plated at 100 µl per well for 1 h. Total IgG was detected with Zymax goat anti-mouse IgG (Zymed) at 1:2,000; isotype-specific detection antibodies were used at 1:500 (clonotyping system, Southern Biotechnology Associates). Plates were developed with p-nitrophenyl phosphate as described above. Data are expressed as OD_{405} with the plate background values subtracted.

Serum OspA-specific Antibody Response. WT-OspA was plated at 1 µg/ml in 50 µl of 0.1 M NaHCO_3, pH 8.2 and were allowed to bind overnight at 4°C. Plates were blocked with 200 µl of 1% BSA/0.5% Tween 20/0.02% sodium azide/PBS, pH 7.4 for 2 h at 21°C. Total IgG was detected with Zymax goat anti-mouse IgG (Zymed) at 1:2,000; isotype-specific detection antibodies were used at 1:500 (clonotyping system, Southern Biotechnology Associates). Plates were developed with p-nitrophenyl phosphate as described above. Data are expressed as OD_{405} with the plate background values subtracted.
FTK-OspA stimulates anti-OspA serum antibody responses similar to those stimulated by WT-OspA. C3H/HeJ mice were immunized with FTK-OspA, WT-OspA, adjuvant alone (mock), or EV-Ctrl in three doses. Sera were collected 2 weeks after the final vaccine boost, and serial dilutions were assessed for WT-OspA-specific antibodies by isotype-specific ELISA. Data are expressed as OD405. (a) Total IgG. (b) IgG1. (c) IgG2a. (d) IgG2b. Data are combined from two experiments.

Fig. 3. FTK-OspA stimulates anti-OspA serum antibody responses similar to those stimulated by WT-OspA. C3H/HeJ mice were immunized with FTK-OspA, WT-OspA, adjuvant alone (mock), or EV-Ctrl in three doses. Sera were collected 2 weeks after the final vaccine boost, and serial dilutions were assessed for WT-OspA-specific antibodies by isotype-specific ELISA. Data are expressed as OD405. (a) Total IgG. (b) IgG1. (c) IgG2a. (d) IgG2b. Data are combined from two experiments.

FTK-OspA responses equally well to OspA234–253, WT-OspA, FTK-OspA, or EV-Ctrl plus APC. L3C4 DR4-restricted T cells were sufficient to abrogate the response of WT-OspA-specific, OspA165–173, THy cells were stimulated with FTK-OspA or WT-OspA, or EV-Ctrl. Both WT- and FTK-OspA-induced equivalent levels of WT-OspA-specific total IgG, IgG1, IgG2a, and IgG2b (Fig. 3). Negligible antibody responses were detected after vaccination with EV-Ctrl (Fig. 3a).

Table 1. WT-OspA- and FTK-OspA-vaccinated C3H/HeJ mice are protected against Bb infection

<table>
<thead>
<tr>
<th>Group</th>
<th>Ankles swelling total</th>
<th>Protection, %</th>
<th>Day of onset (n)</th>
<th>Bb outgrowth</th>
<th>Bb1 PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>4/16</td>
<td>75</td>
<td>14 (3), 17 (1)</td>
<td>2/7</td>
<td>4/16</td>
</tr>
<tr>
<td>FTK</td>
<td>2/16</td>
<td>86</td>
<td>14 (2)</td>
<td>0/6</td>
<td>2/16</td>
</tr>
<tr>
<td>EV-Ctrl</td>
<td>11/11</td>
<td>0</td>
<td>11 (4), 12 (5), 14 (2)</td>
<td>6/6</td>
<td>11/11</td>
</tr>
<tr>
<td>Mock</td>
<td>0/11</td>
<td>—</td>
<td>—</td>
<td>0/5</td>
<td>0/6</td>
</tr>
</tbody>
</table>

*The number of mice showing ankle swelling of >300 μm over 3 weeks of observation over total mice per group; C3H/HeJ mice were vaccinated and challenged as described above.
†The percent of mice in each group that had no ankle swelling during 3 weeks of observation.
‡The day after infection that ankle swelling reached at least 300 μm.
§The outgrowth of Bb from blood or bladder, expressed as number mice positive for Bb per total mice per group.
¶Genomic DNA from ear tissue was amplified with primers specific for Bb recA and scored on ethidium bromide-stained agarose gels, expressed as number of mice with recA product per total mice per group.

Finally, to prove the in vivo protective efficacy of the FTK-OspA vaccine, C3H/HeJ mice were immunized with FTK-OspA, WT-OspA, or EV-Ctrl followed by intradermal challenge with Bb strain N40. Protection from Bb challenge was determined by maximal ankle thickness after challenge (Fig. 4b). Ankle thickness >300 μm was considered positive ankle swelling. The ability of FTK-OspA to protect mice from Bb infection was similar to that of recombinant WT-OspA, with 86% and 75% of mice protected from ankle swelling, respectively (Table 1).
Borrelia cultures for each pretreatment group. BSK to detect the outgrowth of crosshatched bar), and ear tissue (open bar) were harvested and cultured in test.

recombinant OspA molecule modified from Bb responses, although lacking a potentially autoreactive T cell

FTK-OspA is as effective as WT-OspA in the prevention (Fig. 5)

There were no positive cultures from FTK-OspA-vaccinated mice, compared with 75% of cultures from adjuvant only or infection only control mice.

Whereas /H11022/P

Bb heterologous immunity across different genetic backgrounds, BALB C3H

immunity will be compromised. Although the C3H/Hen strain of mice has been well established for testing the efficacy of Lyme disease vaccine candidates, this strain cannot address this issue, because the primary T cell epitope in the context of H-2k is OspA179–193 (41). Thus, we used the class II-deficient, HLA-DR4 chimeric transgenic mice to determine whether OspA-specific antibody titers were equivalent with FTK-OspA and WT-OspA vaccination. Our findings that both total IgG and IgG1 subclass titers were similar after WT- and FTK-OspA immunization indicate that the T helper mediated class switching in these mice does not depend on OspA165–173 presentation (Fig. 6, which is published as supporting information on the PNAS web site). In addition, our previous data indicate that the T cell response to OspA165–173 is predominantly an inflammatory Th1 response (21, 42). Thus, the FTK-OspA modification ablates a proinflammatory epitope in the context of DR4, while preserving structural integrity and remaining Th2 epitopes for the generation of protective immunity.

We generated this modified rOspA vaccine in response to concerns that the WT-OspA sequence used in previously developed vaccines contained an epitope that was cross-reactive with a region of hLFA-1αL. Vaccination of individuals who possess rheumatoid arthritis-associated MHC class II alleles, such as DR4, could potentially elicit autoreactive T cells after WT-OspA exposure. Because LFA-1αL is up-regulated at sites of inflammation, this self-antigen would be readily available to stimulate OspA-primed T cells at various sites in the body, including the vaccination site. Individuals in the clinical vaccine trial reported an increased incidence of localized pain after vaccine administration versus placebo administration; however, this reaction subsided in most recipients within 30 days (13). Further, the broad expression pattern of LFA-1αL could lead to a variety of adverse reactions that are not limited to arthritis.

Several studies have demonstrated an association between immunity to OspA and crossreactivity to hLFA-1αL in individuals of a select MHC background (22, 43, 44). In one report, the data did not support a link between reactivity to hLFA-1αL and clinical status (arthritis); unfortunately, the patient genetic backgrounds were not determined (45). However, in another study, arthritis was reported after vaccination in four males: two adults, and two children (46). By using data compiled from the Vaccine Adverse Events Reporting System database (Food and Drug Administration and Centers for Disease Control and Prevention) to identify any unusual patterns of adverse events in OspA-vaccinated individuals, one report indicated a possible link between the DR4 allele and individuals suffering from serious Lyme vaccine-related illnesses (47). This connection may be confounded by bias in self-reporting. In an interesting development, the LYMErix vaccine has been removed from the market.

The incidence of Lyme disease in the United States remains high and this fact underlines the need for additional pharmaceutical approaches to controlling infection. OspA vaccines used in humans have demonstrated a 76% efficacy and function by inducing anti-OspA antibodies that block the transmission of Bb from the tick to the mammalian host. However, if Bb escape

Willett et al.

Fig. 5. FTK-OspA vaccination abolishes outgrowth of Bb in BALB/c mice. After vaccination with FTK-OspA (n = 15), adjuvant (CFA/IFA, n = 10), or no treatment (infection only, n = 10), BALB/c mice were challenged with Bb B31. (a) At day 14 postinfection, blood was harvested and diluted 1:100 (filled bar) or 1:1,000 (open bar) and cultured in BSK medium to detect the outgrowth of Bb. (b) Joint (diagonal crosshatched bar), bladder (filled bar), heart (vertical crosshatched bar), and ear tissue (open bar) were harvested and cultured in BSK to detect the outgrowth of Bb. Data are expressed as percent positive Borrelia cultures for each pretreatment group. *P < 0.0001 by Fisher’s exact test.

Discussion

In this report, we describe the generation of FTK-OspA, a recombinant OspA molecule modified from Bb strain N40. FTK-OspA retained its ability to induce protective antibody responses, although lacking a potentially autoreactive T cell epitope. The T cell domain of WT-OspA was modified by substituting three residues from the N40 sequence with residues contained in a nonarthritogenic Bb strain, B. afzelii (Fig. 1a). These amino acid changes in the newly formed FTK-OspA epitope resulted in a significant reduction in antigen presentation by the DR4 APC, as predicted by a published DR4-binding algorithm (35). FTK-OspA did not stimulate epitope-specific, potentially autoreactive human or mouse T cells in vitro, yet it was as effective as WT-OspA in inducing protection against Bb infection (Fig. 2).

The modification of a major T cell epitope in a vaccine agent raises the concern that the necessary T cell help for humoral immunity will be compromised. Although the C3H/Hen strain of mice has been well established for testing the efficacy of Lyme disease vaccine candidates, this strain cannot address this issue, because the primary T cell epitope in the context of H-2k is OspA179–193 (41). Thus, we used the class II-deficient, HLA-DR4 chimeric transgenic mice to determine whether OspA-specific antibody titers were equivalent with FTK-OspA and WT-OspA vaccination. Our findings that both total IgG and IgG1 subclass titers were similar after WT- and FTK-OspA immunization indicate that the T helper mediated class switching in these mice does not depend on OspA165–173 presentation (Fig. 6, which is published as supporting information on the PNAS web site). In addition, our previous data indicate that the T cell response to OspA165–173 is predominantly an inflammatory Th1 response (21, 42). Thus, the FTK-OspA modification ablates a proinflammatory epitope in the context of DR4, while preserving structural integrity and remaining Th2 epitopes for the generation of protective immunity.

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killing in the tick and are successfully transferred to the human host, they may escape elimination in the host because Bb down-regulate OspA expression as they make their way from the tick midgut to the salivary gland and into the host (48, 49). To avoid immune evasion in the host, an effective approach is to design a multivalent vaccine. Decorin-binding protein A, in combination with OspA, has been shown to confer a synergistic enhancement of protection against Bb infection (50). Additionally, developing a OspA-based combination vaccination with decorin-binding protein A may allow for an amnestic response in the event of infection, because decorin-binding protein A is significantly up-regulated in the mammalian host (51). Other Bb proteins examined for vaccine efficacy include OspC (52), p35/Bb352 (53), and VraA/BB116 (54). Multivalent vaccines may also result in increased protection against heterologous Bb strains (55), because crossprotection with unmodified OspA proteins has been difficult to achieve due to Bb heterogeneity (56). These combined data suggest that an optimal second generation Lyme disease vaccine could consist of FTK-OspA and one or two other vaccine candidates.

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