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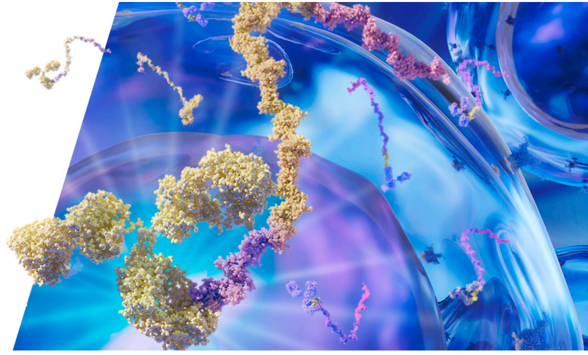

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Molecular Mimicry in Lyme Arthritis Demonstrated at the Single Cell Level: LFA-1α_L Is a Partial Agonist for Outer Surface Protein A-Reactive T Cells[1]

Christina Trollmo,[2]* Abbie L. Meyer,[2†] Allen C. Steere,[‡] David A. Hafler,* and Brigitte T. Huber[3†]

Antibiotic treatment-resistant Lyme arthritis is a chronic inflammatory joint disease that follows infection with *Borrelia burgdorferi* (*Bb*). A marked Ab and T cell response to *Bb* outer surface protein A (OspA) often develops during prolonged episodes of arthritis. Furthermore, cross-reaction between the bacterial OspA and human LFA-1α_L at the T cell level and the inability to detect *Bb* in the joint implicate an autoimmune mechanism. To analyze the nature of response to OspA and LFA-1α_L, we used OspA-specific T cell hybrids from DR4 transgenic mice, as well as cloned human cells specific for OspA$_{165-184}$, the immunodominant epitope, from five DRB1*0401$^+$ patients, using OspA-MHC class II tetramers. Although OspA$_{165-184}$ stimulated nearly all OspA-specific human T cell clones tested to proliferate and secrete IFN-γ and IL-13, LFA-1$\alpha_{L326-345}$ stimulated ~10% of these clones to proliferate and a greater percentage to secrete IL-13. Assays with LFA- or OspA-DR4 monomers revealed that higher concentrations of LFA-DR4 were needed to stimulate dual-reactive T cell hybrids. Our analysis at the clonal level demonstrates that human LFA-1$\alpha_{L326-345}$ behaves as a partial agonist, perhaps playing a role in perpetuating symptoms of arthritis. *The Journal of Immunology,* **2001, 166: 5286–5291.**

A variety of human autoimmune diseases are thought to be mediated by CD4 T cells (1, 2). Thus, it is of great interest to characterize Ag-specific T cells in individuals afflicted by these illnesses. Recently, MHC tetramer technology has been developed that allows the direct enumeration of Ag-specific T cells (3, 4). Although class I tetramers identified large populations of virus-specific CD8$^+$ cells, a different picture has emerged from the use of class II tetramers. Very low frequencies of collagen/matrix-specific CD4$^+$ cells could be isolated from the PBMC or synovial fluid (SF)[4] of patients with rheumatoid arthritis (RA), using class II tetramers that contained two joint-specific Ags (5). In an alternate model, influenza vaccine peptide-bearing tetramers were able to detect influenza-specific T cells only after PBMC from influenza-vaccinated individuals were expanded in vitro by a 7-day culture with Ag (6).

We have analyzed the Ag-specific CD4$^+$ T cells in treatment-resistant Lyme arthritis, using class II tetramers, and have shown that arthritis-associated *Borrelia burgdorferi* (*Bb*) sensu stricto outer surface protein A (OspA)-specific T cells can be identified with this technology. We were able to visualize and directly isolate these cells from the SF and PBMC of patients suffering from treatment-resistant Lyme arthritis (7). Infection of humans with *Bb* sensu stricto through the bite of an infected ixodes tick results in arthritis in 50–60% of untreated patients (8, 9). In most cases of Lyme arthritis, joint inflammation resolves with antibiotic therapy (8). However, in ~10% of patients, the arthritis persists despite multiple courses of antibiotics (10). These individuals often develop high titers of OspA-specific Abs during the treatment-resistant phase of disease (11, 12). There is an association with the presence of RA-associated alleles, including HLA-DRB1*0401 (13). We have recently reported that SF T cells from these individuals respond in bulk functional assays to bacterial OspA (14). Following analysis of overlapping peptides of OspA, an important HLA*0401-binding OspA peptide, aa 165–173, was identified. A GenBank search of that amino acid sequence revealed a homologous peptide within the α_L-chain of human LFA-1, aa 332–340 (hLFA-1α_L (14)). LFA-1α_L is expressed on T cells, macrophages, and many other cells and is up-regulated at the site of inflammation. Thus, sequence homology between bacterial and self antigenic epitopes may be the basis for the molecular mimicry between host and bacteria and may play an important role in the etiology of treatment-resistant Lyme arthritis (14, 15). In both a *Chlamydia* species infection model (16) and HSV-type 1 model (17), T cells specific for the microbial Ag induced tissue-specific destruction in the animal host.

In this study, we quantitatively measured the nature of the OspA$_{165-184}$/LFA-1$\alpha_{L326-345}$ dual reactivity at the clonal level, examining whether a single T cell is able to recognize and respond

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[4] Abbreviations used in this paper: SF, synovial fluid; *Bb, Borrelia burgdorferi*; hLFA, human LFA; OspA, outer surface protein A; THy, T cell hybridoma.

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to both the bacterial and the self Ag presented in the context of HLA-DRB1*0401. About 10% of OspA-reactive T cell hybridomas (THy) generated from OspA-immunized DRB1*0401 transgenic mice secrete IL-2 in response to hLFA-1$\alpha_{L326-345}$. From a panel of human OspA-specific T cell clones, derived by single cell cloning of DRB1*0401/OspA tetramer-binding cells from patients with chronic Lyme arthritis, ~50% mount a response to hLFA-1α_L. However, the quality and quantity of the response to the two Ags are significantly different, as measured by cytokine production and proliferation, respectively. Although OspA is the full agonist that induces high proliferation and cytokine production, LFA-1α_L behaves as a partial agonist, reflecting the lack of high affinity LFA-1α_L-specific T cells due to self-tolerance.

Materials and Methods

Patients

Patients met the Centers for Disease Control and Prevention criteria for the diagnosis of Lyme disease (18). They had arthritis affecting one or both knees and a positive Ab response to *Bb* by ELISA and Western blot, interpreted according to the Centers for Disease Control and Prevention/Association of State and Territorial Public Health Laboratory Directors' criteria (19). One patient was selected for extensive study. He was determined to be HLA DR*0401 homozygous. HLA analysis was performed by Lee Ann Baxter-Lowe (20). Treatment consisted of at least 2 mo of oral doxycycline or at least 1 mo of i.v. ceftriaxone, or both. After antibiotic treatment, repeated PCR tests (21) for *Bb* DNA in joint fluid were negative.

Preparation of MHC II monomers and tetramers

Soluble MHC class II molecules were prepared using baculovirus, as previously described (3, 5, 7, 22). Briefly, using a two-promoter baculovirus transfer vector, the gene encoding the extracellular region of the DRA1*0401 chain was cloned behind the p10 promoter, and the gene encoding the extracellular portion of the DRB1*0401 chain was cloned behind the polyhedrin promoter. Sequence encoding either the *Bb* OspA$_{164-176}$ peptide, hLFA-1$\alpha_{L332-343}$ (for plate-bound assay), or the human cartilage glycoprotein, gp-39$_{263-275}$ peptide, and a 14-aa flexible linker was inserted between the leader and the N terminus of the DRB1*0401 domain. The C terminus of the DRB1*0401 chain carried a peptide tag for biotinylation by BirA (Avidity, Denver, CO). DR4 was purified from culture supernatant of infected insect cells by immunoaffinity and size exclusion chromatography. The purified proteins were biotinylated for use in plate-bound assays as monomers or incorporated into multimeric complexes with PE-streptavidin (BioSource International, Camarillo, CA), as previously described (3).

Production of THy

Eight-week-old DR4 transgenic mice lacking mouse MHC class II molecules (23) were immunized s.c. with 100 μg OspA$_{165-184}$ in CFA twice over the course of 3 mo. Lymph node cells were stimulated 48 h with 40 μg/ml OspA$_{165-184}$, washed extensively, mixed with BW-TCR$^{-/-}$ cells (American Type Culture Collection, Manassas, VA), and fused in 35% polyethylene glycol. Washed fused cells were plated at 1 cell/well in hypoxanthine/aminopterin/thymidine medium (Sigma, St. Louis, MO). Every 4 days, plates were fed with hypoxanthine/aminopterin/thymidine-supplemented 10% FCS-RPMI 1640, until unfused BW$^{-/-}$ control cells were dead. Subclones and lines were screened for Ag specificity by incubating THy, irradiated syngeneic spleen cells plus OspA$_{165-184}$, control OspA peptide, LFA-1$\alpha_{L326-345}$, or plate-bound anti-CD3 or medium alone for 48 h. Supernatants were harvested, and IL-2 was measured by IL-2 ELISA.

Fluorescent cell sorting and T cell cloning

As previously reported, a frozen aliquot of patient cells was thawed and washed just before staining with tetramer (7). Staining reactions were set up at 300–400 μl final volume in complete RPMI 1640 containing 6–8 × 10^6 SF cells, 20 μg/ml OspA, or gp39 tetramer. Reactions were incubated at 37°C for 1.5–2.5 h. Anti-CD4 FITC and anti-CD64 CyChrome (BD PharMingen, San Diego, CA) were added to the reaction mixture, and the cells were incubated for an additional 30 min at room temperature. The cells were washed twice and resuspended in 5% human serum-RPMI 1640 plus additives. To exclude monocytes from analysis, only CD64$^-$ cells (monocyte-macrophage negative (24)) were included in the sort. OspA tetramer$^+$ CD4$^+$CD64$^-$ cells were single-cell sorted into 96-well round-bottom plates containing 100 μl 5% human serum-supplemented RPMI 1640 complete medium, 150,000 irradiated human PBMC (5000 rad), plus 2 μg/ml PHA, and incubated at 37°C. As control, OspA tetramer-negative, CD4$^+$CD64$^-$ were also sorted. At 48 h, human rIL-2 (Teceleukin; National Cancer Institute, Frederick, MD) in complete medium was added for a final concentration of 20 U/ml in each well. Clones were fed or expanded every 3 days with 100 μl of rIL-2-containing medium. After 14 days, 168 OspA tetramer-positive and 8 OspA tetramer-negative clones were established; the cloning efficiency was 33%. A total of 2 × 10^4 resting cells from each clone was restimulated with 2 μg/ml PHA and 150,000 irradiated PBMC. At 48 h, rIL-2 was added to wells at a final concentration of 20 U/ml. After an additional 2 wk of feeding, the clones could be used for functional assays.

Plate-bound MHC monomer assay

ELISA plates were coated overnight with 50 μl of a 100 μg/ml solution of extrAvidin (Sigma) in 50 mM Tris, 100 mM NaCl, pH 7.4. After blocking with 2% FBS/HBSS for 2 h, followed by four washes with the same solution, biotinylated soluble monomeric class II molecules (OspA-DR4, LFA-DR4, and gp39-DR4) were added in decreasing concentrations, beginning with 20 μg/ml, and plates were incubated for 2 h. Following four washes, 1 × 10^5 THy cells were added to wells and incubated overnight at 36°C in 5% CO_2. Serial 2-fold dilutions of duplicate culture supernatants were analyzed for IL-2 secretion in the murine IL-2 ELISA, as described below.

Proliferation assays

The human T cell clones were tested for their Ag specificity in a split well assay. Autologous EBV-transformed B cells (lymphoblastoid cell lines) were treated with mitomycin C in <0.5% human serum for 1 h at 37°C for use as APC. Following three washes, 2 × 10^4 APC were added to each well of round-bottom 96-well plates. Ag was added to one-half of the wells to a final concentration of 10 μg/ml OspA$_{165-184}$ or LFA-1$\alpha_{L326-345}$. A total of 2 × 10^4 cells of each T cell clone was added to all wells. At 48 h, one-half of the medium was removed for later cytokine analysis, and plates were pulsed with 0.5 μCi [^{3}H]thymidine for 18 h. Plates were harvested and counted on a beta-plate scintillation counter (EG&G Wallac, Gaithersburg, MD). Data are expressed as Δ-cpm, which is equal to the average counts obtained after culture with Ag minus average counts with medium alone.

Cytokine ELISA

Wells were coated with purified Abs to human IL-4, IL-5, IL-13 (BD PharMingen), or IFN-γ (Endogen, Woburn, MA) in 0.1 M $NaHC0_3$ buffer overnight. Plates were blocked with 1% BSA-PBS in 200 μl for 2 h at room temperature. Serial 2-fold dilutions of human recombinant standards were made in 5% human RPMI 1640 complete medium starting at 2000 pg/ml (rIL-4 and rIL-5, Boehringer Mannheim, Indianapolis, IN; rIL-13, R&D Systems, Minneapolis, MN; IFN-γ, Life Technologies, Gaithersburg, MD). After washing plates, 50-μl samples (diluted 1/1 in medium) and 50 μl appropriate standards were added. Following a 2-h incubation, plates were washed before adding biotinylated secondary Abs (b-αIL-4, b-αIL-5, b-αIL-13; BD PharMingen), diluted 1/1000 in 1% BSA-PBS. Plates were incubated for 1 h at room temperature. Avidin-peroxidase conjugate (Sigma) at 1/5,000 to 1/10,000 dilution was added to all wells for 45 min. In the IFN assay, the anti-IFN-γ secondary Ab (Endogen) was not biotinylated. Following a 1-h incubation with 1/1000 dilution of anti-IFN-γ and washes, a 1/10,000 dilution of peroxidase goat anti-rabbit IgG (BioSource) was added for 1 h. All plates were washed extensively, and 3,3′,5,5′-tetrametylbenxidine (Kirkegaard & Perry Laboratories, Gaithersburg, MD) substrate was added. Substrate development was stopped using 0.1 M H_3PO_4, and plates were read at 450 nm. Values were determined by comparing OD_{450} values to the standard curve generated for each plate. For measurement of murine IL-2, anti-mouse IL-2 and biotinylated anti-mouse IL-2 (BD PharMingen) and mouse rIL-2 (R&D Systems) were used.

Results

Cross-reactivity between OspA and LFA-1α_L in THy from DR4 transgenic mice

We prepared a panel of THy from the lymph node cells of DR4 transgenic mice (23) that had been immunized and boosted with OspA in vivo. Because these mice do not express murine class II molecules, all their CD4$^+$ T cells are restricted to HLA-DRB1*0401. As expected from previous results obtained with

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bulk T cell cultures (14, 25), the majority of these THy were specific for $OspA_{165-184}$. An initial screen revealed that ~10% of these OspA-reactive THy also secreted IL-2 in response to the LFA-$1\alpha_L$ cross- reactive peptide, LFA-$1\alpha_{L326-345}$ (results not shown). When these dual-reactive THy were tested for their ability to bind tetramers, nearly the whole clonal population stained with $OspA_{165-184}$-DR4 tetramer (7), but were negative for staining with the LFA-$1\alpha_{L326-345}$-DR4 tetramer (results not shown). Because these clones had responded to LFA-$1\alpha_{L326-345}$ peptide in vitro, we analyzed the reactivity pattern of these cloned T cells in more detail. THy cells were cultured in the presence of increasing concentrations of monomeric OspA-, LFA-1-, or control gp39-DR4 molecules (Fig. 1, *A*, *B*, or *C*, respectively), bound to wells via streptavidin, and measured secretion of IL-2. Two representative THy lines, 4–41 and 4–38, secreted large amounts of IL-2 in response to plate-bound OspA. Plate-bound LFA monomers were also able to stimulate these clones, although significantly higher concentrations of reagent were required. In contrast, control gp39-DR4 monomer did not stimulate any IL-2 production in these THy. These results indicate that the LFA tetramer is able to bind TCR and stimulate OspA-specific cloned THy.

Cross-reactivity between OspA and LFA-1α_L peptides in patient samples

SF and/or PBMC from eight individuals with treatment-resistant Lyme arthritis (six patients were DRB1*0401$^+$, two patients were DRB1*0401$^-$) and two non-Lyme disease controls (DRB1*0401$^+$) were stained with $OspA_{165-184}$-DR4-PE tetramer (as reported in Ref. 7). OspA-responsive PBMC are not common in treatment-responsive patients. In Lyme arthritis patients, 0.01 to 3.05% of SF cells bound the OspA tetramer, while lower percentages, <0.005 to 0.11%, were identified in peripheral blood. To analyze the cross-reactivity, T cell clones were generated from SF and/or PBMC from three treatment-resistant Lyme arthritis patients by stimulating OspA-DR4 tetramer-sorted cells with PHA in the presence of irradiated allogeneic feeder cells and feeding with IL-2-containing medium, as described previously (7). No Ag-specific clones could be generated from the controls. Although dual-reactive clones were identified in two patients (from SF and/or PBMC), in one patient who was DRB1*0401 homozygous, about one-half of the OspA-reactive T cell clones also respond to LFA-$1\alpha_{L326-345}$. In this patient, the vast majority (93%) of clones generated by sorting OspA tetramer$^+$ SF cells had strong responses to OspA, as measured by proliferation, while the strength of their responses to LFA-$1\alpha_{L326-345}$ varied greatly (Fig. 2). CD4$^+$ OspA

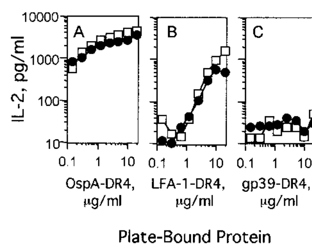

FIGURE 1. Plate-bound OspA-DR4 and LFA-DR4 monomers stimulate OspA-specific THy. Representative THy 4-41 (□) and 4-38 (●) were generated from lymph node cells of OspA-immunized DR4 transgenic mice and were screened for their ability to secrete IL-2 in response to $OspA_{165-184}$. Dual reactivity was tested by culturing the THy (10^5) for 24 h with increasing concentrations of OspA-DR4 (*A*), LFA-DR4 (*B*), or gp39-DR4 (*C*) monomeric molecules, bound via extrAvidin to ELISA plates. Supernatants were harvested and analyzed for IL-2 content by ELISA. Values were determined by comparison with standard curve.

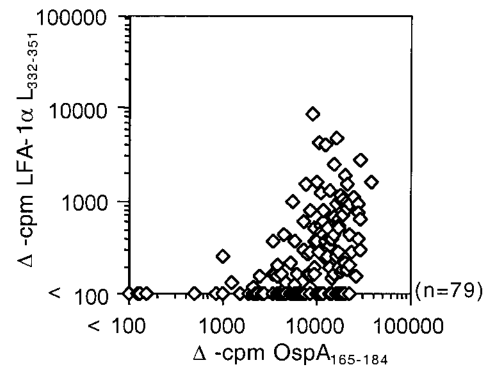

FIGURE 2. T cell clones generated from OspA tetramer-sorted cells proliferate in response to $OspA_{165-184}$ and LFA-$1\alpha_{L326-345}$. A total of 5×10^4 cloned T cells from a DRB1*0401$^+$ homozygous patient was cultured with 20 μg/ml $OspA_{165-184}$, LFA-$1\alpha_{L326-345}$, or medium alone plus 5×10^4 autologous EBV-transformed B cells, treated with mitomycin C, in 200 μl in duplicate. Following a 48-h incubation at 37°C, plates were pulsed with 0.5 μCi [^{3}H]thymidine. Cells were harvested 18 h later and counted for [^{3}H]thymidine incorporation. Data, OspA vs LFA response (◇), are expressed in Δ-cpm (cpm of wells with Ag − cpm of wells with medium). Background counts from wells containing T cell clones and APC in the absence of Ag ranged from 50 to 230 cpm. Clones generated from non-tetramer-staining CD4 populations had average Δ-cpm <400. Similar results were observed in repeat experiments of the same clones and in clones derived from other patients (n = 3).

tetramer$^-$ cells were also sorted into single wells and expanded. Although 1 of 20 sort-negative clones responded minimally to OspA in vitro (1500 cpm), further analysis proved that the response was not significant (results not shown).

OspA tetramer-selected clones respond differentially to OspA and LFA-1α_L peptides

To begin identifying characteristics of OspA/LFA-$1\alpha_L$-reactive vs OspA-only-reactive T cell clones, we measured the amount of cytokines secreted in response to stimulating Ag. Cloned T cells plus autologous EBV-transformed lymphoblastoid cell lines were incubated with peptide Ags. Supernatants were removed from cultures for cytokine ELISA at 48 h. Stimulation with $OspA_{165-184}$ induced strong proliferation and led to the secretion of large amounts of IFN-γ and IL-13 in nearly all clones tested and lesser amounts of IL-4 and IL-5 in a subset of clones (Fig. 3*A*, ○). In contrast, when the stimulating Ag was LFA-$1\alpha_{L326-345}$, proportionally more IL-13 was made than IFN-γ at 10 μg/ml peptide tested (Fig. 3*A*, ●). The amount of IFN-γ produced seemed to reflect the level of proliferation, with OspA-stimulated clones secreting more IFN-γ than LFA-$1\alpha_L$-stimulated clones (Fig. 3*A*). OspA-stimulated clones secreted more of each cytokine tested than LFA-$1\alpha_L$-stimulated clones. When cytokine secretion patterns were compared with each other, the OspA-stimulated clones could be divided in two groups, reflecting higher and lower levels of IL-13, IL-4, and IL-5 secretion (Fig. 3*B*). Additionally, IFN-γ, a Th1 cytokine, was not secreted to the exclusion of Th2 cytokines such as IL-4 and IL-5. OspA-responsive clones were very sensitive to OspA stimulation in dose-response experiments. They proliferated and secreted high amounts of IFN-γ and somewhat less IL-13 in response to as little as 0.1 ng/ml OspA. When the clones were stimulated with $OspA_{164-185}$, 0.1 ng/ml was enough to induce half-maximal proliferation or cytokine secretion, while clones required ~10 μg/ml LFA-$1\alpha_{L326-345}$ to reach half-maximal response. Interestingly, when LFA-$1\alpha_{L326-345}$ concentration was increased to 40 μg/ml, it also induced a low, but measurable IFN-γ

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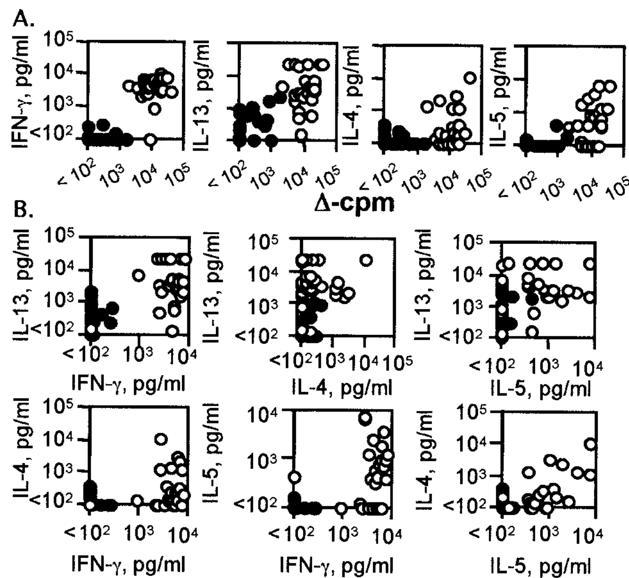

FIGURE 3. Differential cytokine production profile of T cell clones stimulated with $Osp_{165-184}$ (○), vs LFA-1$\alpha_{L326-345}$ (●). Twenty-eight sort-positive clones were cultured with Ag plus APC and tested for Th1 and Th2 cytokine production 48 h after stimulation, using standard capture ELISA. Purified anti-cytokine mAbs (IFN-γ, IL-13, IL-4, and IL-5) were bound to ELISA plates in bicarbonate buffer and blocked with 1% BSA. A total of 25 μl of each supernatant tested was added to duplicate wells (IL-13 and IFN-γ) or single wells (IL-4 and IL-5) and incubated for 2 h. Biotinylated secondary Abs (IL-4, IL-5, and IL-13) were added, and plates were incubated 1 h. Streptavidin HRP conjugate was added, followed by substrate. For measurement of IFN-γ, peroxidase-conjugated anti-rabbit IgG was added before substrate. Mean OD_{450} values were calculated, and concentrations were determined from a standard curve. Data are expressed as a comparison between (*A*) Δ-cpm vs cytokines and (*B*) Th1 and Th2 cytokines. Data are representative of the range of values seen in analyses of different subsets of clones.

production (data not shown). Neither OspA nor LFA-1α_L responses were measurable in sort-negative control clones.

Discussion

Treatment-resistant Lyme arthritis in the United States is a chronic disease, in which joint inflammation persists after the apparent eradication of the spirochete *Bb* sensu stricto from synovium and SF by antibiotic therapy. No residual OspA has yet been identified in synovial tissue (21, 26). Gross et al. (14, 25) demonstrated that bulk SF T cells of these patients mount a strong response to bacterial OspA, predominantly to the epitope $OspA_{165-184}$, as well as to a homologous human peptide, LFA-1$\alpha_{L326-345}$. These results led to the hypothesis that inflammation at the initial site of *Bb*-induced arthritis is perpetuated via molecular mimicry between a bacterial Ag and a self-determinant (14, 15, 17). The observed HLA-DRB1 restriction of these patients to *0401 and related alleles (13) led us to postulate that a self-response is initiated in the chronic phase of the disease. Thus, it is vital that we carefully evaluate the nature of the *Bb* OspA immune response in individuals with treatment-resistant Lyme arthritis. Chronic treatment-resistant Lyme arthritis is not prevalent in Europe. This may be due to the fact that *B. garinii* and *B. afzelii,* which are primarily responsible for the infections there, have different sequences in the cross-reactive position of OspA than *Bb* sensu stricto strains, which cause the infection in the U.S.

To directly test the molecular mimicry hypothesis, we analyzed whether the cross-reaction between OspA and LFA-1α_L could be seen at the single cell level. Two approaches were used: 1) generation of THy from OspA-immunized DRB1*0401 transgenic mice, and 2) single cell cloning of OspA-specific T cells from chronic treatment-resistant Lyme arthritis patients, using DRB1*0401/$OspA_{165-184}$ tetramers (7). A subpopulation of $OspA_{165-184}$-specific human T cells or mouse THy responded to the LFA-1α_L mimic peptide, although we identified proportionally more dual-responsive human clones with methods used. In preliminary TCR Vβ sequence analysis, multiple clones reactive with both OspA and LFA-1α_L used single unique TCR β-chains (data not shown). This indicates that the same TCR can respond to the two related peptides in the context of DRB1*0401.

In these dual-reactive clones, the OspA response differs quantitatively and qualitatively from the LFA-1α_L response, as measured by proliferation and cytokine production, respectively. Typically, the human T cell clones proliferated vigorously in response to OspA and produced large amounts of IFN-γ, IL-4, IL-5, and IL-13, even at low concentrations of Ag. In contrast, dual-reactive clones proliferated less vigorously in response to higher concentrations of LFA-1α_L and secreted less IFN-γ, yet still produced significant amounts of IL-13, but not other Th2 cytokines, IL-4 or IL-5. When clones were tested with high doses, 40–200 μg/ml, of LFA-1α_L peptide, IFN-γ was secreted, although at much lower amounts than following OspA stimulation. All dual-reactive THy produced significantly less IL-2 in response to LFA-1α_L compared with OspA, as tested over a wide dose range. These results indicate that the nature of the peptide determines the quality and quantity of the signal delivered through the TCR. Perhaps IL-13 is a cytokine that characterizes weak TCR/Ag interactions. The Hammer algorithm predicts that both the $OspA_{165-184}$ and LFA-1$\alpha_{L326-345}$ peptides bind to the HLA-DRB1*0401 molecule with similar affinities (27, 28), suggesting that the two peptides are presented to the T cell with equal efficiency. Despite this similarity in Ag presentation, the three-dimensional interaction of the peptide/MHC complex with the TCR may differ. The OspA peptide is strongly agonistic and induces a vigorous response, even at low concentrations of Ag (data not shown), indicative of high affinity TCR recognition. In contrast, the LFA-1α_L peptide induces a less efficient interaction, reminiscent of a partial agonist. Stimulation of the TCR with a partial agonist induces a different set of downstream effects that may result in minimal proliferation and, in this case, a different pattern of cytokine secretion at a given concentration of peptide. Furthermore, given more efficient endogenous Ag presentation in situ, it is possible that it is not necessary to achieve LFA-1α_L concentrations equal with those needed in vitro to maintain chronic stimulation in the joint.

Nearly all OspA-reactive T cells tested produce both Th1-type inflammatory and Th2-type B cell-stimulatory cytokines, IFN-γ, IL-4, IL-5, and IL-13, respectively. This lack of clearly defined Th1/Th2 response seems to be a common feature of human Ag-specific T cell lines and contrasts with the pattern seen in murine T cell lines (29). Of special interest is that stimulation with LFA-1α_L results predominantly in IL-13 production, with little IFN-γ secretion only. The role of IL-13 has not been fully established yet (30). IL-13 is strongly associated with allergic reactions, but it has also been shown to play a role in fibrosis following Schistosome infection (31). Thus, IL-13 from LFA-1$\alpha_{L326-345}$-stimulated T cells could be contributing to the persistent joint inflammation in chronic treatment-resistant Lyme patients.

The degeneracy of TCR specificity was not fully appreciated until the advent of combinatorial peptide libraries, which allow the probing of the full spectrum of a TCR's binding requirements (32–34). These studies have revealed that the TCR is flexible and can

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recognize a surprisingly wide array of amino acid sequences. Hemmer et al. (28) applied a positional scanning peptide combinatorial library to delineate peptide recognition of a T cell clone generated from the cerebrospinal fluid of a patient with chronic neuroborreliosis, another form of chronic Lyme disease. This clone preferentially recognizes peptides of *Bb* bacterial origin, but also responds to CNS-derived human peptides from myelin-associated oligodendrocyte basic protein and somatostatin receptor, as well as peptides from human TGF-β3. Similar to our observation, they found that higher concentrations of human self Ags than *Bb* Ags were necessary to obtain optimal proliferative responses in this T cell clone. Using peptide spot synthesis analysis, Maier et al. (35) determined a TCR-binding motif of peptide recognition of THy generated from a *Bb* OspA-immunized DR4 transgenic mouse. Database searches revealed a large number of both bacterial and human peptides with this binding motif, including hLFA-1α_L. Not all peptides identified were functional, as tested on a small panel of these DR4-restricted THy. Curiously, the LFA-1$\alpha_{L326-345}$ peptide, which stimulates a significant number of our human HLA-DRB1*0401-restricted and DR4 transgenic mouse-derived OspA-specific T cells, was not recognized by their hybridomas. This discrepancy may be explained by significant differences between the two DR4 transgenic mouse lines used by them (35) and us (23). Additionally, given that we see LFA-1α_L cross-reactivity in ~1/10 OspA-specific THy, they may have missed this reactivity in their small panel of OspA-specific hybridomas tested.

Possible mechanism of treatment-resistant Lyme arthritis

It is expected that high affinity LFA-1α_L-reactive T cells are eliminated in the thymus during negative selection. However, lower affinity LFA-1α_L-reactive cells may remain. Some of these same T cells have a higher affinity for and are fully responsive to the homologous peptide of OspA. In the absence of OspA, the activation threshold of these self-responsive T cells can only be overcome by a high local level of the self Ag, LFA-1α_L, as would be expected in an inflammatory environment such as an affected joint. During the course of Lyme arthritis, macrophages in the synovium present *Bb* Ags to immune cells, including those of OspA. In genetically susceptible individuals, this may include OspA$_{165-184}$ (Fig. 4*A*). As the immune response proceeds, OspA-specific T cells become stimulated and cytokines such as IFN-γ are secreted. An increased level of IFN-γ enhances the general state of activation in the synovium, while local levels of LFA-1α_L expressed on T cells, macrophages, and dendritic cells increase. Although *Bb* organisms are thought to be cleared eventually during antibiotic treatment, presentation of the abundantly expressed self Ag LFA-1α_L continues. Ultimately, LFA-1α_L peptides from endogenous production and scavenged cells are most likely presented by MHC class II expressed on macrophages and dendritic cells at the site (Fig. 4*B*). Then, OspA-expanded T cells can cross-react with MHC-presented self LFA-1α_L peptide, a partial agonist for the same TCR, inducing a slightly different cytokine milieu, including more IL-13. This unintentional but likely presentation of LFA-1α_L and the continued stimulation of T cells at the site of chronic inflammation could explain why the LFA-1α_L response in treatment-resistant Lyme arthritis patients is apparently limited to the site of intense inflammation. Although the role of IL-13 in the joint is unknown, it is possible that LFA-1α_L-induced IL-13 may contribute to persistent joint inflammation, and if the amount of LFA-1α_L is high enough, secretion of IFN-γ supports the inflammatory environment.

In summary, the present data support a role for molecular mimicry in treatment-resistant Lyme arthritis, because cells derived from a single OspA-specific T cell are able to proliferate and secrete cytokines in response to a self Ag, LFA-1α_L.

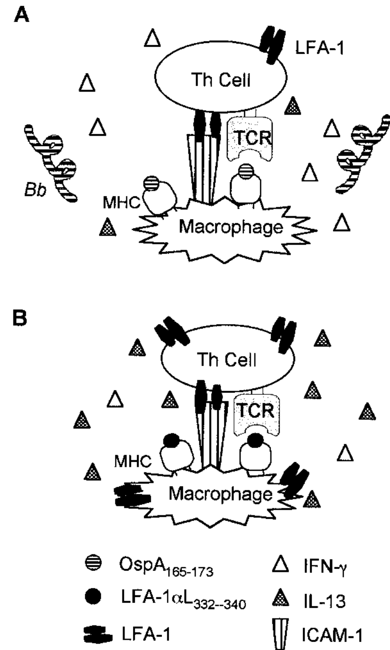

FIGURE 4. The role of LFA-1α_L in chronic Lyme arthritis. *A*, In the inflamed joint, OspA is presented to T cells in the context of class II expressed by macrophages and dendritic cells. OspA-reactive T cells produce high levels of IFN-γ. *B*, IFN-γ production leads to up-regulation of LFA-1 on macrophages and T cells. A critical threshold is reached that allows cross-reactivity of the OspA-specific T cells with the homologous LFA-1α_L epitope. This autoresponse continues after eradication of the spirochete by antibiotic treatment. Responses to LFA-1α_L are characterized by higher IL-13 production, which may contribute to the pathology of the inflamed joint.

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