Identification of LFA-1 as a Candidate Autoantigen in Treatment-Resistant Lyme Arthritis

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Treatment-resistant Lyme arthritis is associated with immune reactivity to outer surface protein A (OspA) of *Borrelia burgdorferi*, the agent of Lyme disease, and the major histocompatibility complex class II allele *DRB1*0401*. The immunodominant epitope of OspA for T helper cells was identified. A homology search revealed a peptide from human leukocyte function–associated antigen-1 (hLFA-1) as a candidate autoantigen. Individuals with treatment-resistant Lyme arthritis, but not other forms of arthritis, generated responses to OspA, hLFA-1, and their highly related peptide epitopes. Identification of the initiating bacterial antigen and a cross-reactive autoantigen may provide a model for development of autoimmune disease.

Lyme disease is a multisystem illness caused by infection with the spirochete *Borrelia burgdorferi* (*1*). A prominent late manifestation of the disease is Lyme arthritis (*1, 2*). About 10% of patients with Lyme arthritis develop what we have termed antibiotic treatment–resistant Lyme arthritis, which typically affects one knee for months to years after multiple courses of antibiotics (*1*). Such patients have no detectable spirochetal DNA in joint fluid after antibiotic therapy, which suggests that the spirochete has been eliminated by this treatment (*3*). Because there is increased frequency of the *HLA-DRB1*0401* allele in these patients (*4*), an autoimmune etiology should be considered. The hypervariable 3 region (HVR3) at residues 67 to 74 of *DRB1*0401* is associated with susceptibility to rheumatoid arthritis (RA) and is contained in at least 15 different *DRB1* alleles (*5*). Most patients with prolonged treatment-resistant Lyme arthritis have one of these homologous alleles (*4*). What antigen are these class II molecules presenting?

Borrelia burgdorferi induces an immune response of expanding reactivity to an array of spirochetal proteins over months to years (*6*). Antibody reactivity to outer surface protein A (OspA) typically develops near the beginning of prolonged episodes of arthritis (*7*). T cell lines from patients with treatment-resistant Lyme arthritis preferentially recognize OspA, compared with patients with treatment-responsive disease. OspA-reactive type 1 T helper (T_H1) cells are detectable in the synovial fluid of individuals with treatment-resistant arthritis years after antibiotic treatment (*7*). Thus, these patients may have progressed into an autoimmune state by developing a cross-reactive response between OspA and a self-antigen.

We used the DRB1*0401 peptide-binding algorithm (*8*) to determine the scores for all nine-residue peptides in the OspA protein sequence that contained an appropriate pocket 1 anchor residue—F, I, M, L, T, V, or Y—necessary for binding in the DRB1*0401 peptide-binding cleft. According to this algorithm, only peptides with scores greater than 2 are likely to bind and be able to be presented by the DRB1*0401 molecule (*8*). The highest scoring peptide that was identified, $OspA_{165–173}$, had a predicted binding score of 6.5, and the next best scoring peptide, $OspA_{237–245}$, achieved a score of 3.7. To verify that these peptides can bind to DRB1*0401 in vitro, the binding of ^{125}I-labeled m1–7 (YRAMATL; predicted DRB1*0401 binding score = 5.9), which has the consensus binding motif for DRB1*0401 (*9*), was measured when in competition with unlabeled 20-residue peptides from OspA. Only $OspA_{154–173}$, which contains the DRB1*0401-predicted dominant epitope $OspA_{165–173}$, inhibited binding of the radiolabeled peptide m1–7 to purified DRB1*0401 (Table 1), confirming the algorithm's prediction.

To test for T cell reactivity in vivo, we made use of class II–deficient mice transgenic for a chimeric DRB1*0401 molecule (DRB1*0401-tg) (*9*). Any $CD4^+$ T cell response generated in these mice can be directly attributed to the presence of the DRB1*0401 molecule. The ElisaSpot assay was used for measuring anti-

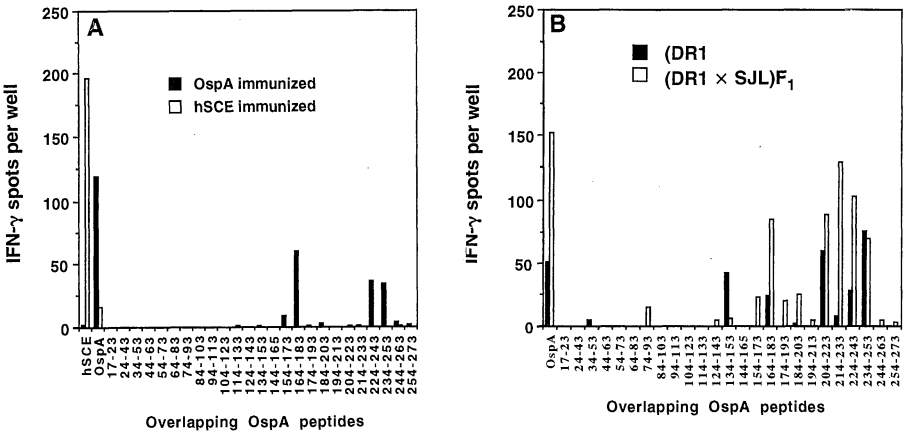

Fig. 1. IFN-γ ElisaSpot analysis demonstrates $OspA_{165–173}$ as the functional, immunodominant epitope of OspA in DR4-tg mice. (**A**) Class II–deficient, DRB1*0401-tg mice, immunized with whole OspA, but not a control protein, recall whole OspA and $OspA_{164–183}$ specifically. DRB1*0401-tg mice were immunized in both hind footpads with either 50 μl of OspA (44 μg/ml) or human spinal chord extract (hSCE; 100 μg/ml) in complete Freund's adjuvant. Eight days later, draining popliteal lymph nodes were isolated and 5×10^5 cells were cultured with either a positive control stimulant, CD3 antibody, mAb 145.2C11, or one of the following test antigens: hSCE (50 μg/ml), OspA (10 μg/ml), overlapping OspA 20-mer peptides (10 μg/ml each), or medium alone. IFN-γ production was analyzed 24 hours later by ElisaSpot (*10*). Values from wells with medium alone were subtracted from values from wells that contained antigen. Antigens are listed as overlapping 20-mer peptides spanning OspA, beginning with amino acid 17. Residues 1 to 17 contain the leader sequence and are therefore cleaved during export through the bacterial membrane. Representative experiments of six OspA-immunized and two hSCE-immunized mice are shown. (**B**) DRB1*0101-tg and (DRB1*0101-tg × SJL)F_1 mice immunized with whole OspA recall $OspA_{165–173}$ as well as other epitopes. In contrast to the DRB1*0401-tg mice, the DRB1*0101-tg mice express murine class II; therefore, a broader array of OspA epitopes is recognized. Experiments were performed as described above. One of three and one of two representative experiments are shown for DRB1*0101-tg and (DRB1*0101-tg × SJL)F_1 mice, respectively.

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gen-specific T cell reactivity, a sensitive and efficient technique that allows detection of cytokine production at the single cell level, which may occur in the absence of proliferation (*10*). We initially assayed for production of T_H1 and T_H2 cytokines, interferon-γ (IFN-γ), and interleukin-5 (IL-5), respectively. Both IFN-γ–producing and IL-5–producing cells were detected when cells were activated with a polyclonal stimulus [anti-CD3; monoclonal antibody (mAb) 145.2C11]. In contrast, when cells were stimulated with OspA antigen, IFN-γ production was dominant, with essentially no detectable IL-5 secretion (*11*). Therefore, detection of IFN-γ was used as the readout for antigen-specific T cell reactivity in all subsequent assays. DRB1*0401-tg mice were immunized with OspA and lymph node cells were stimulated with overlapping 20-residue peptides of OspA: the immunostimulatory epitopes correlated precisely with the epitopes predicted by the DRB1*0401 algorithm (Fig. 1A). Immunization of the DRB1*0401-tg mice with $OspA_{165–173}$ resulted in a recall response to whole OspA in vitro (*11*). Hence, we have identified the immunodominant epitope of OspA in the context of DRB1*0401. To test the ability of $OspA_{165–173}$ to be presented by *DRB1* alleles related to DRB1*0401 (*5*), we performed the same experiment in mice transgenic for DRB1*0101 (*12*). These transgenic mice possess a full complement of murine class II genes, thereby providing distinct major histocompatibility complex (MHC) alleles for OspA peptide presentation. ElisaSpot analyses of OspA-immunized DRB1*0101-tg or (DRB1*0101-tg × SJL)F_1 mice showed reactivity to $OspA_{165–173}$ as well as to an array of other epitopes (Fig. 1B). In contrast to DRB1*0401-tg mice, reactivity toward $OspA_{165–173}$ developed as a subdominant epitope, suggesting that alternative determinants are available for binding that could influence disease development. Interestingly, the F_1 mice had a response to $OspA_{165–173}$ that was three times the response of DRB1*0101-tg mice. This is likely because of expression of the murine I-Eβ^s chain, which is homologous in the HVR3 to DRB1*0401 (*5*), thereby providing twice the number of class II molecules for presentation of this particular peptide. Thus, we have identified the immunodominant OspA peptide recognized in the context of DRB1*0401 and found that DRB1 and murine class II alleles homologous to DRB1*0401 in their HVR3 can also present this epitope.

We searched the Genetics Computer Group gene bank for human proteins containing sequences homologous to $OspA_{165–173}$. Of the 20 peptides retrieved with the highest identity and homology scores, two were of human origin: hLFA-1 (CD11a/CD18, integrin $\alpha_L\beta_2$) and 40*S* ribosomal protein. Only the peptide contained in hLFA-1, hLFA-$1\alpha_{L332–340}$, attained a significant DR4-binding score (7.3), with six–amino acid identity (YVIEGT*SKQ*; nonconserved residues in italics), suggesting hLFA-1 as a potential autoantigen. The peptide contained within the 40*S* ribosomal protein sequence (YVLEG*KELE*) attained a DR4-binding score of 0, mostly because of Lys at position p6, which is not tolerated in the DR4-HVR3 (*13*). The hLFA-$1\alpha_{L332–340}$ peptide is located extracellularly in the interactive or I-domain that mediates the binding interaction between LFA-1 and its ligand, intercellular adhesion molecule–1 (ICAM-1) (*14*). When the DR4-binding algorithm was applied to the entire I-domain (amino acids 170 to 349), hLFA-$1\alpha_{L332–340}$ achieved the highest predicted bind-

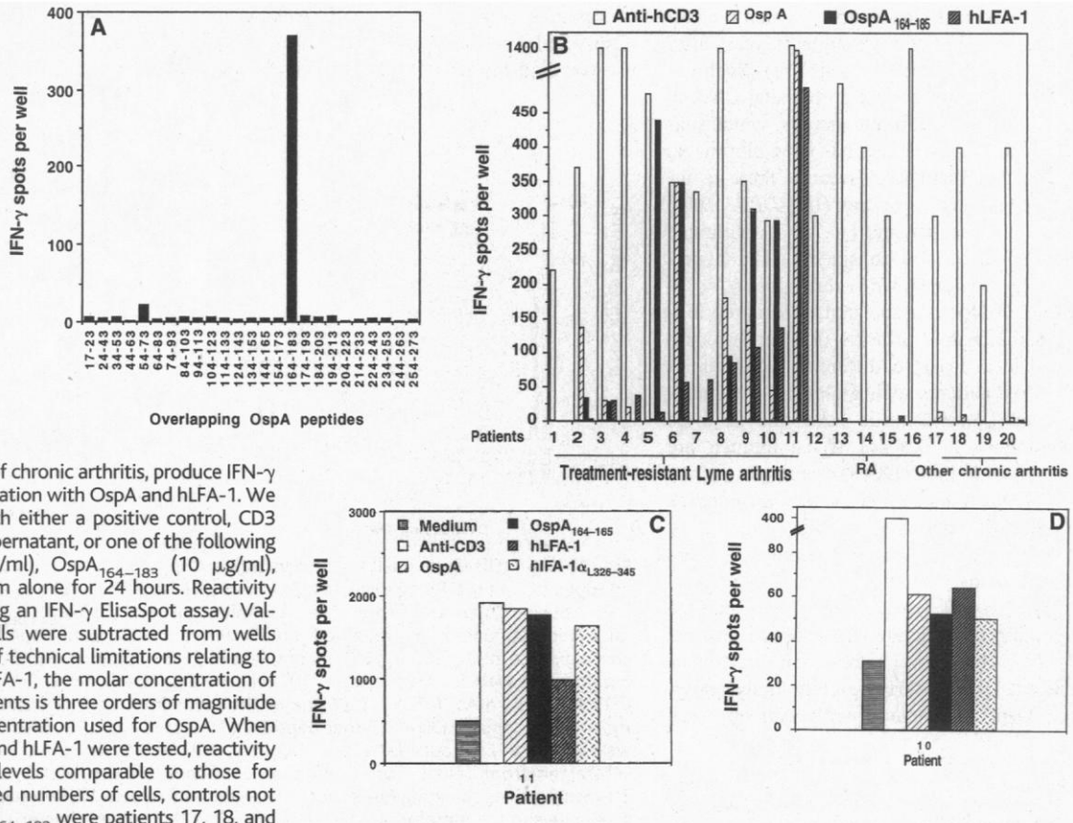

Fig. 2. SF T cells from patients with treatment-resistant Lyme arthritis generate a response to hLFA-1. (**A**) IFN-γ ElisaSpot analysis of 3×10^5 SF T cells per well, from patient 4, cultured with each of the overlapping OspA peptides at 10 μg/ml, revealed $OspA_{164–183}$ as the immunodominant epitope (*10, 15*). Reactivity to whole OspA was positive as determined by proliferation assay (medium, 2552 177 248 cpm; OspA, 24,497 177 2079 cpm) (*16*). (**B**) SF T cells from patients with treatment-resistant Lyme arthritis, but not other forms of chronic arthritis, produce IFN-γ in response to in vitro restimulation with OspA and hLFA-1. We cultured 3×10^5 SF cells with either a positive control, CD3 antibody hybridoma OKT3 supernatant, or one of the following test antigens: OspA (10 μg/ml), $OspA_{164–183}$ (10 μg/ml), hLFA-1 (70 ng/ml), or medium alone for 24 hours. Reactivity was determined by performing an IFN-γ ElisaSpot assay. Values from medium-alone wells were subtracted from wells containing antigen. Because of technical limitations relating to the purification process of hLFA-1, the molar concentration of hLFA-1 used in these experiments is three orders of magnitude lower than the optimal concentration used for OspA. When equimolar amounts of OspA and hLFA-1 were tested, reactivity to OspA was depressed to levels comparable to those for hLFA-1 (*11*). Because of limited numbers of cells, controls not tested for reactivity to $OspA_{164–183}$ were patients 17, 18, and 20; and, for hLFA-1, patients 17 and 18. (**C**) Treatment-resistant Lyme arthritis patient 11, who is homozygous for *DRB1*0401*, demonstrates SF T cell reactivity to the 20-mer containing the OspA homologous, *DRB1*0401*-defined dominant epitope within the I-domain, hLFA-$1\alpha_{L326–345}$. We cultured 3×10^5 SF cells with hLFA-$1\alpha_{L326–345}$ (25 μg/ml). IFN-γ ElisaSpot assay was performed as described above. (**D**) Treatment-resistant Lyme arthritis patient 10, who is heterozygous for an RA-associated allele (*DRB1*0102*), demonstrates SF T cell reactivity to the 20-mer containing the OspA homologous, *DRB1*0401*-defined dominant epitope within the I-domain, hLFA-$1\alpha_{L326–345}$. We cultured 3×10^5 SF cells with equimolar amounts of $OspA_{164–183}$, hLFA-1, and hLFA-$1\alpha_{L326–345}$. IFN-γ ElisaSpot assay was performed as described above.

ing score (7.3), nearly twice that of the next highest scoring peptide, hLFA-1$\alpha_{L196-204}$ (binding score = 4.3), and higher than that of OspA$_{165-173}$. We determined, by performing the peptide binding competition assay [median inhibitory concentration (IC_{50}) = 0.7825 mM], that hLFA-1$\alpha_{L331-345}$, a 15-mer containing the core residues 332 to 340, was capable of binding DRB1*0401 in vitro.

To test the hypothesis that hLFA-1 is an autoantigen in patients with treatment-resistant Lyme arthritis, but not in other forms of chronic inflammatory arthritis, we mapped the immunodominant epitope of OspA in synovial fluid (SF) cells from a patient (4) with treatment-resistant Lyme arthritis (Fig. 2A) (*10*). As in the DRB1*0401-tg mouse, OspA$_{164-183}$ was immunodominant. We then analyzed the antigen reactivity profile of SF T cells from patients with treatment-resistant Lyme arthritis as well as patients with other forms of chronic arthritis (*15*). ElisaSpot for IFN-γ production (*10*) and proliferation assays (*16*) showed that people in a panel consisting of only those with treatment-resistant Lyme arthritis have varying degrees of SF T cell reactivity to whole OspA, OspA$_{164-183}$ as well as hLFA-1 (Fig. 2B). Reactivity to hLFA-1 is due to recognition of hLFA-1$\alpha_{L326-343}$, the region homologous with OspA$_{164-183}$ (Fig. 2, C and D). This reactivity appears to develop over time, as patients who initially showed no response to hLFA-1 had marked reactivity when tested 1 to 3 months later (*11*).

Borrelia burgdorferi sensu stricto is the only spirochetal strain associated with treatment-resistant Lyme arthritis (*17*) and the sole strain that contains the OspA$_{165-173}$ sequence that is highly related to hLFA-1$\alpha_{L332-340}$. Murine LFA-1a differs significantly from hLFA-1 at this particular epitope, providing an explanation for why chronic Lyme arthritis does not develop in DRB1*0401-tg mice exposed to *B. burgdorferi* (*12*).

Our demonstration of autoreactivity against hLFA-1 (in particular, the predicted cross-reactive epitope) in patients with treatment-resistant Lyme arthritis suggests that this disease involves an autoimmune process. However, although the genetic predisposition for development of treatment-resistant Lyme arthritis has been correlated with DR4, we cannot rule out other genetic, environmental, and infectious factors that might be involved. As mentioned above, the HVR3 of the DRB1 chains associated with RA possesses a shared epitope at residues 67 to 74 (*5*). Most patients with severe RA carry at least one allele that contains the shared epitope sequence of DRB1*0401, henceforth referred to as an RA-associated allele (*5*). Individuals who develop the most severe form of RA typically have two RA-associated alleles (*18*). HLA typing of our panel of 11 treatment-resistant Lyme arthritis patients revealed that 7 possessed at least one RA-associated allele (*15*), and 9 made a response to hLFA-1. Patient 11, who was homozygous for DRB1*0401, responded four times more vigorously to both OspA and hLFA-1 than the next highest responder. In patients with other forms of arthritis, the presence of an RA-associated allele by itself was not sufficient for induction of an OspA or hLFA-1 response, as at least five of the nine control patients possessed an RA-associated allele (*15*) yet made no response to OspA or hLFA-1. Thus, priming by *B. burgdorferi* infection or at least with OspA may be required for development of an autoimmune response to hLFA-1. Other factors may also be involved in development of treatment-resistant Lyme arthritis, as some treatment-resistant patients who do not possess an RA-associated allele make a response to hLFA-1 and some patients with treatment-resistant Lyme arthritis do not respond to either OspA or hLFA-1 (Fig. 2B).

On the basis of our DRB1*0401-restricted OspA T cell epitope mapping data, as well as previous work on immune reactivity and cytokine production in response to infection with *B. burgdorferi* (*7*), we propose a model on how an immune reaction to *B. burgdorferi* might result in development of an autoimmune response against hLFA-1: *B. burgdorferi* enters the host via a tick bite and disseminates to multiple tissues. Months later, a highly inflammatory immune response develops in the joint, and this response is dominated by T_H1 IFN-γ–producing cells that contain OspA reactive cells. We propose that the high local concentration of IFN-γ up-regulates expression of ICAM-1 (*19*) on synoviocytes and synovial fibroblasts as well as of MHC class II molecules on the local professional and nonprofessional antigen-presenting cells (APCs) (*19*). This enhanced ICAM-1 expression leads to recruitment of LFA-1 expressing cells, in particular activated T_H1 cells. The combination of elevated LFA-1 expression on T cells and macrophages plus MHC class II up-regulation on APCs may result in increased LFA-1 peptide presentation by macrophages and synoviocytes that have processed either endogenous or phagocytosed LFA-1 (*20*). Hence, a vicious cycle is initiated so that, even after elimination of the spirochetes by antibiotic therapy, the OspA-primed T cells remain activated by stimulation with LFA-1. The release of inflammatory cytokines by these activated T cells and macrophages may then result in tissue damage and joint destruction (*21*).

Table 1. Inhibition of m1–7 peptide binding to DRB1*0401 (*15*) by 20-residue peptides of OspA.

OspA peptide*	IC_{50} (μM)	Nine-residue peptides with appropriate p1 anchor residue†	DR4-algorithm scores for peptides with an appropriate p1 anchor residue‡
154–173	4.381	161, 162, 165, 166	(−) 0.4, (−) 0.8, (+) 6.5, (−) 5.4
54–73	>100	54, 55, 58, 61, 63	(−) 1.1, (−) 4.1, (−) 6.3, (−) 2.8, (−) 0.1
74–93	>100	75, 76, 79, 86	(−) 6.6, (+) 1.1, (+) 2.4, (−) 1.9
124–143	>100	126, 132, 136, 137	(−) 4.7, (−) 4.3, (−) 3.3, (−) 2.4

*Testing was limited to peptides with both sufficient quantity of material and a broad range of DR4-predicted binding scores. OspA$_{164-183}$ was not available for testing. †The number of potential DRB1*0401-binding 9-residue peptides contained within a 20-residue sequence was determined by the presence of an appropriate p1 anchor residue (F, I, L, M, V, T, or Y). p1 anchor residue amino acid numbers are listed for each candidate peptide. ‡Scores were calculated for OspA nine-residue peptides beginning with F, I, L, M, V, T, or Y (*9*). Scores are listed, respectively, for each 9-mer peptide contained within the 20-mer peptide tested.

References and Notes

1. A. C. Steere *et al.*, *Ann. Int. Med.* **90**, 896 (1979); A. C. Steere, R. T. Schoen, E. Taylor, *ibid.* **107**, 725 (1987); A. C. Steere, *N. Engl. J. Med.* **321**, 586 (1989); A. C. Steere *et al.*, *Arthritis Rheum.* **37**, 878 (1994).
2. A. C. Steere, E. Dwyer, R. Winchester, *N. Engl. J. Med.* **323**, 219 (1990).
3. J. F. Bradley, R. C. Johnson, J. L. Goodman, *Ann. Int. Med.* **120**, 487 (1994); J. J. Nocton *et al.*, *N. Engl. J. Med.* **330**, 229 (1994).
4. A. C. Steere and L. A. Baxter-Lowe, unpublished data.
5. P. K. Gregersen, J. Silver, R. J. Winchester, *Arthritis Rheum.* **30**, 1205 (1987); G. T. Nepom and H. Erlich, *Annu. Rev. Immunol.* **9**, 493 (1991). DRB1 alleles with the shared-epitope amino acids 67 to 74 of DRB1*0401: *0101, *0102, *0104, *0404, *0405, *0408, *0409, *0413, *11011, *1402, *1406, *1409, *1413, *1417, and murine H-2 I-Eβ^s.
6. J. E. Craft *et al.*, *J. Clin. Invest.* **78**, 934 (1986).
7. R. A. Kalish, J. M. Leong, A. C. Steere, *Infect. Immun.* **61**, 2774 (1993); T. Kamradt, B. Lengl-Janssen, A. F. Strauss, G. Bansal, A. C. Steere, *ibid* **64**, 1284 (1996); R. Lahesmaa *et al.*, *J. Immunol.* **150**, 4125 (1993); B. Lengl-Janssen, A. F. Strauss, A. C. Steere, T. Kamradt, *J. Exp. Med.* **180**, 2069 (1994); D. M. Gross, A. C. Steere, B. T. Huber, *J. Immunol.* **160**, 1022 (1998).
8. J. Hammer *et al.*, *J. Exp. Med.* **180**, 2353 (1994); K. W. Marshall *et al.*, *J. Immunol.* **154**, 5927 (1995). Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
9. In vitro binding studies were performed as described, and generation of DR4-tg mice are documented in [K. Ito *et al.*, *J. Exp. Med.* **183**, 2635 (1996)]. Overlapping 20-mer OspA peptides were synthesized by R. Woods and were a generous gift from M. Hanson (MedImmune, Gaithersburg, MD). OspA 15-mer (SYVLEGTLTAEKTTL) and 9-mer (YVLEGTLTA) peptides, as well as hLFAα_L 15-mer (IYVIEGTSKQDLTSF) and mLFA-1 15-mer (IYAIEGTNRQDLTSF) peptides were purchased from Bio-Synthesis. The hLFAα_L 20-mer (ELQKKIYVIEGTSKQDLTSF) was purchased from Research Genetics.
10. Single-cell suspensions of popliteal lymph node cells from immunized mice, or Ficoll-Hypaque (Sigma) centrifugation isolation of human lymphocytes from peripheral blood mononuclear cells or SF, were prepared and coincubated with appropriate antigen [5 × 10^5 cells per well (mouse) or 3 × 10^5 cells per well (human) and OspA or OspA peptides (10 μg/ml), hLFA-1 (70 ng/ml), or anti-CD3 supernatant] to T-

Spot plates (Autoimmune Diagnostika) precoated with capture monoclonal antibody to IFN-γ (4 μg/ml) and blocked with Dulbecco's modified Eagle's medium containing 10% fetal calf serum. Plates were washed at 24 hours and probed with a sandwich biotinylated antibody to IFN-γ. Spots were detected with an anti-biotin alkaline phosphatase (AP) (murine ElisaSpot) or streptavidin–horseradish peroxidase (human ElisaSpot) with detection enzyme reactions of either NBT/BCIP (Pierce) or 3-amino-9-ethylcarbzole and *N,N*-dimethylformamide (Pierce/Fisher), generating purple or red spots, respectively. Scores were determined by the Series I T-Spot Image analyzer (Autoimmune Diagnostika) as the difference between the number of spots produced with and without antigen. OspA protein was a kind gift from B. Lade and J. Dunn (Brookhaven National Lab) and purified hLFA-1 was a kind gift from D. Staunton (ICOS Corporation). Human spinal chord extract was prepared according to standard procedures. The following antibodies were used for murine in vitro assays: 145.2C11 (murine antibody) or OKT3 (human antibody), CD3 antibody (hybridoma supernatant); R4-6A2, coat, IFN-γ antibody and XMG1.2, capture, biotinylated IFN-γ antibody (PharMingen); biotin-AP antibody (Vector). The following antibodies were used for human in vitro assays: OKT3, CD3 antibody (hybridoma supernatant); coat, IFN-γ antibody, and capture, biotinylated IFN-γ antibody (Endogen); streptavidin–horseradish peroxidase (Zymed).

11. D. Gross *et al.*, unpublished results.
12. B10.m/Sn mice transgenic for DRB1*0101 were a kind gift from D. Zaller (Merck Research Laboratories); S. Feng, S. W. Barthold, L. K. Bockenstedt, D. M. Zaller, E. Fikrig, *J. Infect. Dis.* **172**, 286 (1995).
13. J. Hammer *et al.*, *Cell* **74**, 197 (1993); J. Hammer *et al.*, *J. Exp. Med.* **181**, 1847 (1995).
14. S. D. Marlin and T. A. Springer, *Cell* **51**, 813 (1987).
15. We studied 11 patients (7 male, 4 female; between 12 and 40 years old) with treatment-resistant Lyme arthritis and 9 control patients (4 male, 5 female; between 17 and 78 years old) with RA or other forms of chronic inflammatory arthritis. All Lyme patients met the case definition of the U.S. Centers for Disease Control and Prevention for diagnosis of Lyme disease. They had arthritis affecting the knee and serologic reactivity with *B. burgdorferi* by ELISA and protein blotting. The 11 Lyme arthritis patients and 5 of the control patients were evaluated in the Lyme Disease Clinic at New England Medical Center (NEMC). The remaining 3 RA (patients 12, 13, and 15) and 1 psoriatic (patient 19) control patients' samples were a generous gift from R. Schumacher (Department of Medicine, University of Pennsylvania Medical School). The protocol was approved by the Human Investigations Committee, and informed consent was obtained from each subject. Patients with Lyme arthritis were treated with both oral and intravenous antibiotic regimens. The duration of arthritis after antibiotic therapy ranged from 2 to 33 months. High-resolution HLA-DR typing with sequence-specific amplification was performed by the Clinical Laboratory of Immunology (NEMC) and by Lee Ann Baxter-Lowe (University of South Carolina, Columbia, SC). Patient *DRB1* alleles are as follows: 10, *0102* and *1501*; 6, *0102* and *1501*; 5, *0401* and *1501*; 7, *0701* and *1601*; 2, *0301* and *1201*; 1, *1* and *11*; 4, *14* and *15*; 11, *0401* and *0401*; 8, *0402* and *7*; 9, *0301* and *1302*; 3, *0404* and *13*, 12, *0401* and *1*; 13, *15* and *7*; 16, *4*; 15, *0401* and *7*; 17, *4* and *17*; 18, *11*, *3*, or *13*; 20, *1* and *13*. Insufficient DNA was available from patients 14 and 19, so *DR* typing was not performed on them.
16. Patient SF cells were plated in 96-well U-bottomed plates (Costar) at a density of 2×10^5 cells per 200 μl in complete RPMI medium (Sigma). Cells were stimulated for 5 days with antigen (2 days with phytohemagglutinin), pulsed with 0.5 μCi of [^{3}H]thymidine during the final 16 to18 hours, and harvested for scintillation counting. Insufficient cells were available from patients 5 and 11; therefore proliferation assays were not performed. All Lyme arthritis patients' cells responded to OspA (except for patient 1) and $OspA_{164-183}$ (except for patients 1 and 2). Responses ranged from 254 to 2552 cpm (background) and from 2275 to 56,725 cpm (antigen).
17. B. Wilske *et al.*, *Res. Microbiol.* **143**, 583 (1992); J. Welsh *et al.*, *Int. J. System. Bacteriol.* **42**, 370 (1992); G. Baranton *et al.*, *ibid.*, p. 378; W. T. Golde, *Infect. Med.* **15**, 38 (1998).
18. J. S. Lanchbury *et al.*, *Hum. Immuol.* **32**, 56 (1991).
19. P. S. Steeg, R. N. Moore, H. M. Johnson, J. J. Oppenheim, *J. Exp. Med.* **156**, 1780 (1982); M. B. Sztein, P. S. Steeg, H. M. Johnson, J. J. Oppenheim, *J. Clin. Invest.* **73**, 556 (1984).
20. J. Moreno, *J. Immunol.* **147**, 3306 (1991); S. Salemi, A. P. Caporossi, L. Boffa, M. G. Longobardi, V. Barnaba, *J. Exp. Med.* **181**, 2253 (1995); A. K. Barlow, X. He, C. Janeway Jr., *ibid.* **187**, 1403 (1998).
21. M. L. Corcoran *et al.*, *J. Biol. Chem.* **267**, 515 (1992).
22. We thank L. Glickstein, J. Coburn, K. Yardley, N. Sutkowski, C. Tay, and R. Seward for assistance in preparation of the manuscript; R. Schumacher, E. Massaroti, R. Kalish, and A. Vaz for acquisition of control patients' synovial fluid; D. Zaller for providing the DRB1*0101-tg mice; and D. Staunton for provision of purified hLFA-1. Supported by Biological Research Grants from The Arthritis Foundation (B.T.H. and A.C.S.), NIH grant RO1 AR20358, The Mathers Foundation, and the Eshe Fund (A.C.S.).

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Pioneer Axon Guidance by UNC-129, a *C. elegans* TGF-β

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The *unc-129* gene, like the *unc-6* netrin gene, is required to guide pioneer motoraxons along the dorsoventral axis of *Caenorhabditis elegans*. *unc-129* encodes a member of the transforming growth factor–β (TGF-β) superfamily of secreted signaling molecules and is expressed in dorsal, but not ventral, rows of body wall muscles. Ectopic expression of UNC-129 from ventral body wall muscle disrupts growth cone and cell migrations that normally occur along the dorsoventral axis. Thus, UNC-129 mediates expression of dorsoventral polarity information required for axon guidance and guided cell migrations in *C. elegans*.

Axon guidance along the dorsoventral (D/V) axis of animals of diverse phyla involves secreted, laminin-related, UNC-6/netrin guidance cues (*1*). The signaling pathways activated by these molecules require the UNC-5 and UNC-40/DCC transmembrane receptor families (*2*–*4*). In *C. elegans*, mutations in *unc-129* (*5*) cause defects in the dorsally oriented trajectories of motoraxons that resemble those present in *unc-5*, *unc-6*, and *unc-40* mutants (*5*, *6*).

A 6.5-kb genomic subclone of cosmid C53D6 was able to rescue the uncoordinated phenotype of *unc-129* mutants after germline transformation (*7*, *8*) (Fig. 1A). Sequence analysis by the *C. elegans* genome-sequencing consortium (*9*) revealed a single open reading frame on this fragment that encodes a protein related to the TGF-β superfamily. The corresponding 1.5-kb cDNA (*10*) includes 5 exons, 34 base pairs (bp) of 5′ untranslated region (UTR), and 281 bp of 3′ UTR and is predicted to encode a protein of 407 amino acids (Fig. 1B). Northern (RNA) analysis of wild-type mRNA revealed a single transcript (*11*) consistent with the size of the cDNA. The 6.5-kb rescuing genomic fragment includes 3 kb of 5′ promoter sequence. A minigene containing 4.5 kb of 5′ promoter sequence fused to the *unc-129* cDNA was able to rescue the phenotype of *unc-129* mutants, indicating that there are no essential regulatory elements in introns or the 3′ sequence (*12*).

UNC-129 shares features with the TGF-β superfamily, including a signal sequence, a prodomain, and a COOH-terminal region that contains seven conserved cysteines (*13*). The UNC-129 COOH-terminal sequence identity ranges from 33% with human BMP-7 to 24% with TGF-β2. Thus, *unc-129* likely represents a subfamily of the TGF-β superfamily.

Sequence analysis revealed the absence of residues in UNC-129 that would be expected between the α-helical region and β sheet of TGF-β molecules (Fig. 1C) (*14*). This interdomain region forms a β turn with a protruding loop accessible to solvent. The three-dimensional structures of TGF-β1 and TGF-β2 differ at this site, which may promote their differing receptor-binding affinities (*15*). Deletion of the loop in TGF-β1 abolishes certain TGF-β1–mediated responses (*16*). Without knowledge of the crystal structure of UNC-129, it remains unclear whether the missing residues form the COOH-terminal end of the long α-helix or affect receptor specificity.

In *C. elegans*, TGF-β signaling pathways

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