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HLA–DR Alleles Determine Responsiveness to *Borrelia burgdorferi* Antigens in a Mouse Model of Self-Perpetuating Arthritis

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Objective. **Arthritis is a prominent manifestation of Lyme disease, which is caused by infection with *Borrelia burgdorferi* (Bb). Chronic Lyme arthritis persisting even after antibiotic treatment is linked to HLA–DRB1*0401 (DR4) and related alleles. In contrast, patients whose Lyme arthritis resolves within 3 months postinfection show an increased frequency of HLA–DRB1*1101 (DR11). The aim of this study was to analyze the underlying mechanism by which HLA–DR alleles confer genetic susceptibility or resistance to antibiotic-refractory Lyme arthritis.**

Methods. **We generated DR11-transgenic (DR11-Tg) mice on a murine MHCII$^{-/-}$ background and compared their immune response to Bb antigens with the response of DR4-Tg mice after immunization with Bb outer surface protein A (OspA) or infection with live Bb.**

Results. **T cells from OspA-immunized and Bb-infected DR11-Tg mice had defective production of interferon-γ as compared with those from DR4-Tg mice. In contrast, DR11-Tg mice developed higher titers of anti-OspA and anti-Bb antibodies, respectively, than did DR4-Tg mice. Consistent with this observation, we found that the Bb-infected DR11-Tg mice had a decreased spirochetal burden as compared with the DR4-Tg mice, as measured by quantitative polymerase chain reaction.**

Conclusion. **This study provides direct evidence that in the presence of HLA–DR11, the immune response against Bb antigens is directed toward a protective antibody response. In contrast, an inflammatory Th1 response is induced in the presence of DR4. These observations offer an explanation for the differential genetic susceptibility of DR4+ and DR11+ individuals to the development of chronic Lyme arthritis and, eventually, the progression to antibiotic-refractory Lyme arthritis.**

Lyme disease is a debilitating infection transmitted via the bite of ticks infected with *Borrelia burgdorferi* (Bb). One of the most prominent clinical manifestations of Lyme disease is the development of chronic Lyme arthritis. Generally, a 1–2-month course of oral doxycycline or a 2–4-week course of intravenous ceftriaxone resolves the joint inflammation associated with the presence of the spirochetes (1). However, some patients continue to experience persistent joint inflammation despite antibiotic treatment, a condition referred to as antibiotic-refractory Lyme arthritis (1–3). This inflammatory response is characterized by proliferative synovitis, and it may persist for months or even several years.

One of the factors that confer susceptibility to antibiotic-refractory Lyme arthritis is the presence of certain HLA–DR alleles. Indeed, patients presenting with joint inflammation after antibiotic therapy have a higher frequency of HLA–DRB1*0401 (DR4) and related alleles (4–7). Interestingly, these alleles, which share a sequence in the third hypervariable region of the DRB1 chain, have also been associated with susceptibility to rheumatoid arthritis (RA) (8). In contrast, Lyme arthritis patients in whom the arthritis resolves within 3 months of the infection show an increased frequency of the HLA–DRB1*1101 (DR11) allele (6,7,9). This HLA–DR linkage prompted the hypothesis that antibiotic-refractory Lyme arthritis represents an autoimmune disease, where the inflammatory response is perpetuated by a self protein after elimination of the

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causative agent, Bb (6,10,11). This notion is supported by the fact that certain DR alleles are strongly associated with diseases that have an autoimmune basis, such as RA, multiple sclerosis (MS), and type 1 diabetes mellitus (DM) (12,13).

HLA alleles affect positive and negative selection of immature T cells in the thymus by presenting a range of self peptides. In addition, upon exposure to foreign antigens, the various HLA alleles present peptides with different affinities to the peripheral mature T cells, thereby determining the type of cellular immune response that is initiated. By analyzing the crystal structure of disease-associated DR alleles in complex with peptides, it has been shown that the properties of the peptide-binding groove define the selection of peptides presented and, thus, confer susceptibility to disease (8). Structural comparison of DR alleles associated with the risk of, or protection against, type 1 DM, RA, and MS has revealed that the properties of the P1, P4, P6, and P9 pockets of the DRB1 allele, such as volume, hydrophobicity, and electrostatic charge, constitute the disease-determining factors (8).

In an effort to elucidate the mechanisms of antibiotic-refractory Lyme arthritis manifestations in humans, we recently developed a mouse model of self-perpetuating arthritis induced by Bb infection of $DR4^{+}CD28^{-/-}$ mice and subsequent eradication of the spirochetes by antibiotic treatment (14). This model was based on the use of $CD28^{-/-}$ mice, which lack most regulatory T cells and which manifest chronic Lyme arthritis upon Bb infection (14,15). Persistent inflammation in the joints of the $DR4^{+}CD28^{-/-}$ mice following antibiotic treatment required the previous establishment of chronic joint inflammation in the presence of the DR4 allele. These data provide direct evidence that the HLA–DR4 allele is indispensable for the development of antibiotic-refractory Lyme arthritis.

In the present study, we analyzed the underlying mechanism by which DR alleles confer genetic susceptibility or resistance to antibiotic-refractory Lyme arthritis. By generating DR11-transgenic (DR11-Tg) mice, we were able to directly compare the immune response to Bb antigens mediated by the DR11 allele, which protects against antibiotic-refractory Lyme arthritis, and the DR4 allele, which predisposes individuals to the development of these symptoms. We found that DR11-Tg mice mounted a vigorous antibody response, but had defective interferon-γ (IFNγ) production. In addition, Bb-infected DR11-Tg mice had a decreased spirochete burden compared with DR4-Tg mice, as measured by quantitative reverse transcription–polymerase chain reaction (RT-PCR) analysis of Bb DNA. This is in contrast to DR4-Tg mice, which produced an inflammatory response characterized by a high level of IFNγ production, a finding consistent with our previously published results (10). Furthermore, the antibody response in DR4-Tg mice to Bb antigens was significantly lower than that in DR11-Tg mice, which is consistent with the higher spirochete burden observed in DR4-Tg mice after Bb infection.

Thus, our data provide a possible explanation for the differential regulation of the immune response in DR4+ and DR11+ patients upon Bb infection, namely, that HLA–DR4 would predispose individuals to the development of chronic Lyme arthritis by generating an inflammatory milieu against Bb infection, while HLA–DR11 would exert a protective role through the production of antispirochete antibodies.

MATERIALS AND METHODS

Mice. DR4-Tg mice produced on a mouse $MHCII^{-/-}$ C57BL/6 (B6) background were a gift of T. Forsthuber (Case Western Reserve University, Cleveland, OH) and were bred in our facility. These mice were generated with HLA–DRA-I-Eα and HLA–DRB1*0401-I-Eβ chimeric genes (16). The DR11 transgene was generated as follows: the mouse I-Eβ^{d} genomic construct with exon 2 from human DRB1*0401 (a generous gift from Dr. Kouichi Ito [16]), was used as a starting reagent. Exon 2 was then substituted with the intron-flanked exon 2 of DRB1*1101. The DRB1*1101 exon 2 was cloned from peripheral blood mononuclear cells (PBMCs) from a DR11-homozygous subject, using a nested PCR strategy. DR11-specific PCR primers described by Kotsch et al (17) were used in the first PCR reaction on PBMC genomic DNA template: for 5′ DRA, AAT-GCC-CGG-GTA-AAG-AAA-GT; for 3′ DRA, GCA-GGA-AGT-GGT-GGA-GAG-AG; for 5′ DRB11, CCG-GTT-AAG-GTT-CCC-AGT-G; and for 3′ DRB11, AAG-TCC-TTC-TGG-CTG-TTC-CA. The second PCR used internal primers containing an *Eco* RI site for cloning and yielded a single product that was confirmed via sequencing to correspond to DRB1*1101. The *Eco* RI–digested nested PCR product was ligated to the mouse I-Eβ^{d} construct after *Eco* RI–mediated release of the DRB1*0401 exon.

The chimeric I-Eα/DRA1*0101 (a generous gift of Dr. Kouichi Ito) and I-Eβ^{d}/DRB1*1101 constructs were purified according to the CsCl method and linearized prior to microinjection into C3H/HeJ embryos at the Tufts Core Transgenic Facility. Positive progeny were screened by chimeric α- and β-chain–specific PCRs and confirmed by immunophenotyping using anti-DR (L243 clone; PharMingen, San Diego, CA) monoclonal antibody. One positive progeny was selected to generate the transgenic mouse colony, which is kept in the heterozygous state. The mice were then backcrossed onto B6 $\times$ 129 mixed $MHCII^{-/-}$ background for 10 generations and then further backcrossed to pure B6 $MHCII^{-/-}$ background for another 3 generations. No differences in the immune response

against Bb antigens were detected between mice carrying DR4 on the B6 or the B6/129 background, implying that the background genes do not contribute to the outcome of the immune response. (Data obtained from our analyses of IFNγ production by popliteal lymph node cells from rOspA-immunized DR11-Tg mice compared with DR4-Tg mice generated on a B6/129 background are available upon request from the corresponding author.)

Recombinant OspA (rOspA) immunization of DR11-Tg and DR4-Tg mice. $DR4^{+/+}MHC\text{-}II^{-/-}$ mice and $DR11^{+/+}MHC\text{-}II^{-/-}$ mice (ages 6–8 weeks) were immunized in the footpad with rOspA (50 μg/mouse) in Freund's complete adjuvant (CFA). Two weeks later, the draining popliteal lymph node cells were harvested, and single-cell suspensions were prepared at a concentration of 10^6 cells/ml. Cells were then restimulated for 72 hours in vitro with rOspA (10 μg/ml) as well as with plate-bound anti-CD3 in 96-well round-bottomed tissue culture plates (Becton Dickinson, Franklin Lakes, NJ). Following incubation, cells were centrifuged, and the supernatant was collected and stored at –20°C, until further processing by enzyme-linked immunosorbent assay (ELISA).

ELISAs for IFNγ, interleukin-17 (IL-17), and IL-4. ELISAs for IFNγ and IL-4 were performed according to the manufacturer's instructions using murine IFNγ and IL-4 ELISA kits (BD Biosciences, San Jose, CA), respectively. To assess IL-17 cytokine production, plates were coated overnight with 3 μg/ml of capture anti-mouse IL-17 antibody (R&D Systems, Minneapolis, MN) in phosphate buffered saline (PBS) and then blocked with 2% bovine serum albumin and 5% sucrose in PBS for 1 hour at room temperature. Recombinant mouse IL-17 (standard curve) and the supernatants from the in vitro restimulation assays were added in duplicate to the ELISA plates and incubated for 45 minutes at 37°C. Plates were washed and incubated for 1 hour at 37°C with biotinylated anti-mouse IL-17, followed by another wash and incubation for 30 minutes at room temperature with NeutrAvidin-AP (Pierce, Rockford, IL). Plates were then developed with alkaline phosphatase substrate and were read at 405 nm in a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA).

ELISAs for anti-OspA and anti-Bb antibodies. Flat-bottomed Immulon 2HB plates (Fisher Scientific, Pittsburgh, PA) were coated overnight with either 10 μg/ml of Bb lysate or 5 μg/ml of rOspA in coating buffer (0.1*M* Na_2HPO_4, pH 9). Uncoated wells served as non-antigen controls. ELISAs were performed as previously described (15).

Bacterial cultures. Low-passage (passage 2) infectious Bb organisms (N40 clone D10E9A1-E; a kind gift of Jenifer Coburn [18,19]) were used for all infections. Bb were cultured in complete Barbour-Stoenner-Kelley medium (Sigma, St. Louis, MO) at 34°C until mid-log phase (5×10^7 Bb organisms/ml) and were counted by darkfield microscopy.

Bb infections. Infection with Bb as well as antibiotic treatment of Bb-infected $DR11^+$ $CD28^{-/-}$ and $CD28^{-/-}$ mice upon establishment of chronic Lyme arthritis were performed as previously described (20).

Determination of Bb burden. DNA was extracted from ear punch and ankle tissue, and the Bb burden was determined by real-time quantitative RT-PCR, as previously described (20).

Quantitative RT-PCR analysis of messenger RNA (mRNA) for IFNγ, IL-17, and FoxP3. Mouse ankles were harvested and immediately frozen in liquid nitrogen. Frozen tissue was pulverized using a mortar and pestle that had been precooled in liquid nitrogen. Popliteal lymph nodes were collected, and single-cell suspensions were prepared by disrupting the tissue on a cell strainer with the help of the flat side of a 3-ml syringe plunger. The cells were then washed twice in RPMI 1640 (Sigma) containing 10% fetal bovine albumin, 2 m*M* L-glutamine, and 100 units/ml of penicillin/streptomycin (1,300 revolutions per minute for 5 minutes in a Sorvall RT7 Plus centrifuge). A minimum of 500,000 cells were frozen in RNA stabilization buffer (Qiagen, Valencia, CA) and were stored at –80°C until further processing for RNA extraction.

RNA from pulverized ankles and popliteal lymph node cells was extracted using an RNeasy Mini kit (Qiagen) according to the manufacturer's instructions. Quantitative RT-PCR was performed as previously described (20). Primers and FAM-labeled probes specific for murine IL-17 (Mm00439619-m1), murine INFγ (Mm00801778-m1), and murine FoxP3 (Mm00475156-m1), as well as VIC-labeled probes specific for murine 18S, were purchased from Applied Biosystems (Foster City, CA).

Statistical analysis. Statistical analysis was performed using GraphPad Prism software (San Diego, CA). All data were tested for Gaussian distribution using the Shapiro-Wilk normality test. Quantitative differences between groups were assessed by Student's 2-tailed *t*-test for normally distributed data or by Mann-Whitney test for skewed data. The significance level of each correlation was addressed by the *P* value. Significance was declared at a 2-sided 0.05 level for all statistical analyses.

RESULTS

Generation of DR11-Tg mice on a murine $MHCII^{-/-}$ B6 background. To understand the mechanism that regulates the immune response to Bb in DRB1*1101-positive individuals, we generated DR11-Tg mice on a murine $MHCII^{-/-}$ B6 background (see Materials and Methods). A schematic representation of the chimeric human/mouse DR11/I-E^d construct that was used for the DR11-Tg mouse is shown in Figure 1A. The phenotype of the resulting DR11-Tg mice was determined by fluorescence-activated cell sorting (Figure 1B). DR11-Tg mice had slightly higher class II expression on their B cells than did DR4-Tg mice. No differences in the basal levels of antibody or cytokine production were detected between DR4-Tg and DR11-Tg mice. In addition, the size and the absolute number of lymph node cells in the 2 groups of mice were similar, implying that there is no difference in the lymphoid tissue composition (data not shown).

Decreased levels of IFNγ produced by lymph node cells from rOspA-immunized DR11-Tg mice compared with DR4-Tg mice. In order to determine whether the T cell immune response generated against Bb anti-

mixed B6/129 background of the DR11-Tg mice. To exclude this possibility, we generated DR4-Tg mice on a B6/129 MHCII$^{-/-}$ background and compared their immune responses to those of the DR11-Tg mice upon rOspA immunization. Lymph node cells from rOspA-immunized DR11-Tg mice still produced decreased levels of IFNγ as compared with DR4-Tg mice generated on the mixed B6/129 background, implying that background genes do not contribute to the outcome of this particular immune response. (Data obtained from this analysis of IFNγ production by the 2 groups of mice are available upon request from the corresponding author.)

In contrast, no significant difference in IL-17 production was observed between DR11-Tg and DR4-Tg mice (Figure 2B). These data imply that the DR11 allele negatively influences the development of OspA-specific Th1, but not Th17, responses. The production of IL-4 was also investigated in rOspA-immunized DR11-Tg and DR4-Tg mice. However, this cytokine was not detectable under the experimental conditions used (data not shown).

Higher titers of anti-OspA antibody in rOspA-immunized DR11-Tg mice than in rOspA-immunized DR4-Tg mice. The observation that OspA-specific T cell immunity was influenced by the presence of a particular HLA–DR allele prompted us to question whether the humoral response would be similarly affected. We therefore measured the OspA-specific serum antibody level by ELISA in DR11-Tg and DR4-Tg mice 2 weeks after immunization with rOspA in CFA. Surprisingly, DR11-Tg mice showed significantly higher titers of anti-OspA antibodies than did DR4-Tg mice (P = 0.0005) (Figure 3A). To further investigate the anti-OspA humoral response in these mice, we determined the isotype of the OspA-specific antibodies. We observed that the most prominent isotype in DR11-Tg mice was IgG1, followed by IgG2b and IgG2c. There was no difference in the IgM and IgG3 isotypes between the DR11-Tg and DR4-Tg mice (Figure 3B).

Decreased IFNγ mRNA expression in lymph nodes and ankles from Bb-infected DR11-Tg mice as compared with Bb-infected DR4-Tg mice. In order to investigate the role of HLA–DR in regulating the immune response against Bb infection, DR11-Tg and DR4-Tg mice were inoculated with 2×10^4 Bb organisms/mouse. No difference in the development of acute Lyme arthritis was observed in these 2 strains, as assessed by measures of ankle swelling (data not shown). This was expected, because the acute inflammatory reaction is mainly induced by the innate immune response, and only a minimal adoptive immune response develops in mice of the B6 background.

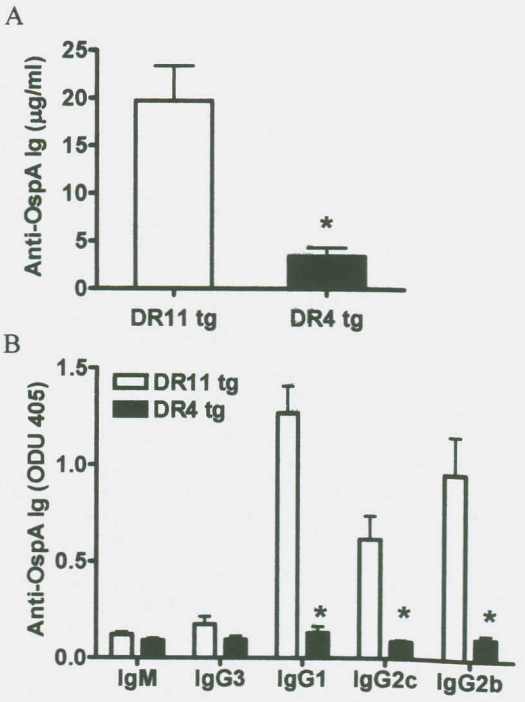

Figure 3. Higher titers of anti-OspA antibody in recombinant OspA (rOspA)–immunized DR11-transgenic (DR11-Tg) mice. DR11-Tg and DR4-Tg mice (n = 10 per group) were immunized in the footpad (50 μg/mouse) with rOspA in Freund's complete adjuvant. **A,** Anti-OspA antibody in the serum 2 weeks following rOspA immunization, as assessed by enzyme-linked immunosorbent assay. **B,** Isotypes of anti-OspA antibodies. Values are the mean and SEM and include the pooled results of 4 independent experiments. * = $P < 0.0005$ by Student's unpaired 2-tailed t-test. ODU = optical density units.

On day 40 postinfection, mice were killed, and RNA was extracted from the draining popliteal lymph nodes and the ankles. Levels of mRNA for the proinflammatory cytokines IFNγ and IL-17, as well as for the regulatory T cell–specific transcription factor FoxP3, were assessed by quantitative RT-PCR. As shown in Figure 4A, popliteal lymph nodes from DR11-Tg mice had significantly lower expression of IFNγ mRNA than did those from DR4-Tg mice (P = 0.03). Decreased IFNγ mRNA expression was also observed in the ankles of Bb-infected DR11-Tg mice as compared with DR4-Tg mice (P = 0.04) (Figure 4B). These results are consistent with the decrease in IFNγ production observed in OspA-immunized DR11-Tg mice upon rOspA-restimulation in vitro, implying that DR11 regulates the immune response similarly in the Bb infection system.

To determine whether the expansion of Th17 cells and FoxP3+ regulatory T cells is differentially influenced by the 2 HLA–DR alleles in response to Bb

As shown in Figure 6, both groups of mice developed chronic joint inflammation over the course of 70 days as a result of persistent Bb infection. Upon establishment of chronic Lyme arthritis, ceftriaxone was administered (day 75 postinfection), and the mice were monitored for 50 days following antibiotic therapy. We observed that after antibiotic treatment, joint inflammation resolved in both the $DR11^+CD28^{-/-}$ and the $CD28^{-/-}$ mice. This is in contrast with the fact that arthritis continued in a significant proportion of $DR4^+CD28^{-/-}$ mice even after antibiotic treatment (20).

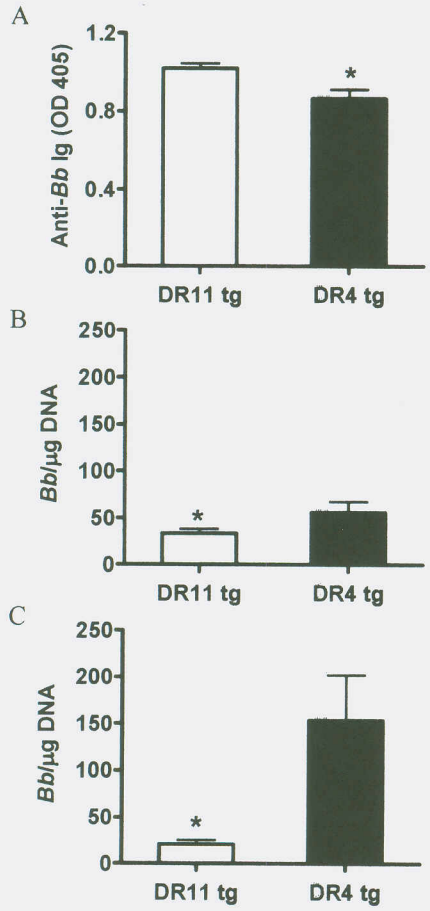

Figure 5. Increased anti–*Borrelia burgdorferi* (anti-Bb) Ig and decreased Bb burden in Bb-infected DR11-transgenic (DR11-Tg) mice. DR11-Tg (n = 5) and DR4-Tg (n = 8) mice were infected with 2×10^4 Bb organisms/mouse. **A,** Anti-OspA antibodies in the serum at 40 days postinfection were measured by enzyme-linked immunosorbent assay. **B,** Ear punch and **C,** ankle tissues were collected, DNA was extracted, and the Bb RecA gene was amplified by quantitative reverse transcription–polymerase chain reaction (20). Values are the mean and SEM and are representative of 5 independent experiments. * = $P < 0.01$ by Student's unpaired 2-tailed *t*-test.

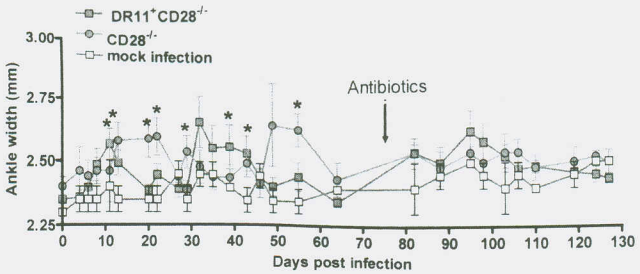

Figure 6. Lack of development of antibiotic-refractory Lyme arthritis in *Borrelia burgdorferi* (Bb)–infected $DR11^+CD28^{-/-}$ mice. $DR11^+CD28^{-/-}$ mice, $CD28^{-/-}$ mice, and mock-infected mice (n = 5 per group) were infected with 2×10^4 Bb organisms/mouse, and arthritis was assessed by ankle joint measurements. On day 75 postinfection, mice were treated with antibiotics and were monitored for an additional 50 days thereafter. Inflammation resolved in the $DR11^+CD28^{-/-}$ and the $CD28^{-/-}$ mice following antibiotic treatment. Values are the mean ± SEM. * = $P < 0.05$ versus mock-infected mice, by Student's unpaired 2-tailed *t*-test.

These data provide independent confirmation of the genetic basis of antibiotic-refractory Lyme arthritis. More specifically, in our murine model of chronic Lyme arthritis, as well as in human patients, a genetic predisposition to antibiotic refractory Lyme arthritis is linked to the presence of the DR4 allele, whereas the DR11 allele is associated with antibiotic-responsive Lyme arthritis.

DISCUSSION

Numerous studies have established an association between HLA–DR alleles and infectious and autoimmune diseases (12,13,28). The various DR alleles, which are expressed on thymic epithelial cells as well as on antigen-presenting cells, have different peptide-binding requirements. The ability of the DR alleles to accommodate self- or disease-associated peptides depends on the properties of the respective peptide-binding groove (8). Therefore, the immune response against infectious agents, as well as the susceptibility to autoimmune diseases, varies from one individual to another. However, DR is highly polymorphic, and direct associations with autoimmune diseases have been difficult to make in the presence of other confounding genetic and environmental factors. To circumvent this problem, DR-Tg $MHCII^{-/-}$ mice have been generated, which present a unique opportunity to study how the T cell repertoire and immune response are affected by a single factor, HLA–DR (29,30). The use of such in vivo models has been invaluable in understanding the patho-

genesis of certain diseases, such as RA, MS, and type 1 DM (30). In this study, we have constructed DR11-Tg mice and compared them with DR4-Tg mice in order to identify the mechanism by which HLA–DR determines responsiveness to Bb antigens and, subsequently, disease outcome.

A strong correlation between DR alleles and disease severity has been documented in Lyme arthritis (4–7). The DR4 allele is the main risk factor for antibiotic-refractory Lyme arthritis, whereas DR11 is associated with antibiotic-responsive Lyme arthritis (6,7,9). These clinical observations suggest that CD4+ T cells are directly involved in the disease pathogenesis. It has been reported that there is a direct correlation between arthritis severity and the magnitude of the T cell response against OspA of Bb (31), namely, that OspA reactivity is significantly greater in antibiotic-refractory Lyme arthritis patients than in treatment-responsive arthritis patients and that this response is targeted against specific OspA epitopes (31). According to a DR/peptide-binding algorithm designed by Hammer et al (32), the immunodominant OspA epitope presented by HLA–DR4 is $OspA_{165–173}$ (33). This was confirmed using DR4-Tg mice (10), as well as using in vitro DR/peptide-binding assays (9). For example, the DR4 allele associated with antibiotic-refractory Lyme arthritis was shown to bind strongly to the $OspA_{165–173}$ peptide, whereas the DR11 allele, which is associated with antibiotic-responsiveness, bound weakly to this peptide. The significance of the T cell response to the $OspA_{165–173}$ immunodominant epitope in the pathogenesis of chronic Lyme arthritis was accentuated by the observation that $OspA_{165–173}$-reactive T cells could be directly isolated from the joint fluid of DR4+ antibiotic-refractory Lyme arthritis patients with the use of MHC tetramer technology (34).

The data gained from the current study provide a possible mechanism for the differential association of DR4 and DR11 with antibiotic-refractory Lyme arthritis. We show that upon rOspA immunization and in vitro restimulation, CD4+ T cells from DR11-Tg mice produced decreased levels of IFNγ as compared with the DR4-Tg mice. Similarly, decreased expression of mRNA for IFNγ was observed in the lymph nodes and ankles of DR11-Tg mice upon Bb infection. Therefore, in the presence of DR11, the CD4+ T cell immune response to Bb antigens was less inflammatory than in the presence of DR4.

It has been reported that Th1 cells are expanded in the synovial fluid of antibiotic-refractory Lyme arthritis patients, which directly correlates with arthritis severity (25). In addition, IFNγ production in the synovial tissue has been associated with antibiotic-refractory Lyme arthritis (25–27). Using cytometric bead array and flow cytometry techniques, it was recently shown that during the period of antibiotic-refractory Lyme arthritis, synovial fluid samples had higher levels of the Th1 chemoattractants CXCL9 and CXCL10 and the proinflammatory cytokines IFNγ, TNFα, and IL-1β as compared with those in patients with antibiotic-responsive arthritis (26). This is indicative of a strong proinflammatory response that takes place during antibiotic-refractory Lyme arthritis. At a median of 9 months following antibiotic therapy, antibiotic-refractory Lyme arthritis patients continued to demonstrate a Th1 proinflammatory phenotype as compared with patients in whom the arthritis had resolved (26). In addition, in a murine model of antibiotic-refractory Lyme arthritis that we recently established, we observed increased levels of IFNγ mRNA expression in the joints of mice with persistent arthritis following antibiotic treatment, implying that IFNγ contributes to the disease pathogenesis (20).

When the humoral response was compared in DR4-Tg and DR11-Tg mice upon rOspA-immunization or Bb infection, we observed that DR11-Tg mice developed higher titers of anti-OspA or anti-Bb antibodies, respectively. The strong humoral response against Bb antigens in DR11-Tg mice is particularly intriguing in light of the decreased Th1 response observed in these mice. Thus, this finding might provide an explanation about why DR11+ Lyme arthritis patients do not present with antibiotic-refractory Lyme arthritis, namely, that it is possible that antibodies generated in these individuals have strong borreliacidal activity that efficiently controls Bb infection. It has been reported that IFNγ can suppress the production of borreliacidal antibody in an in vitro Bb culture system (35). Decreased levels of IFNγ may favor increased borreliacidal activity in DR11-Tg mice, thus controlling Bb infection. Interestingly, when we assessed the Bb burden after infection, DR11-Tg mice had reduced spirochete levels, both systemically and locally in the joints, as compared with Bb-infected DR4-Tg mice.

Based on these data, the cellular and humoral immune responses against Bb antigens are differentially regulated by DR4 and DR11 alleles. This pattern has been observed for other inflammatory and autoimmune diseases. For example, an increased frequency of DR11 has been documented in thymoma patients with myasthenia gravis, whereas DR4 has been negatively associated with the disease (36). In addition, peptides associ-

ated with systemic lupus erythematosus bind strongly to DR4, which is positively associated with the disease, as compared with DR11 (37). Furthermore, in the case of thyroid carcinoma, the DR11 allele is positively associated with disease development, as opposed to the DR4 allele, which is negatively associated (38). Finally, DR4 confers susceptibility to RA, MS, type 1 DM, and Crohn's disease (39), while no such associations with DR11 have been documented thus far.

The fact that DR4 and DR11 alleles are not genetically linked to the same diseases is partly due to differences in their peptide-binding groove. Each allele has a different peptide-binding specificity, which determines the array of the presented peptides and, subsequently, the immune response that will take place. Indeed, when DR4 and DR11 are compared, they differ by 2 amino acids at positions 67 and 71 of the HLA–DRB1 chain, which contributes to the P4 pocket. The DR4 allele has Leu67B1 and Lys71B1, whereas DR11 has Phe67B1 and Arg71B1 (8,40,41). The binding properties of the P4 pocket of DR4 have been specifically associated with susceptibility to RA, and even a single Lys-to-Glu exchange at position 71 of the DRB1 chain alters the peptide-binding specificity and, subsequently, the disease association (41). In addition, it has been reported that amino acid substitution at positions 67, 71, and 86 of the DR11 chain alters its peptide-binding specificity and influences DR–peptide interactions (40). Thus, we would like to propose that the differential peptide-binding properties of the P4 pocket in HLA–DR4 and HLA–DR11 account for the differential susceptibility to antibiotic-refractory Lyme arthritis.

In this study, we have shown in a murine model that in the presence of DR11, the immune response against Bb antigens is directed toward a protective antibody response that is reflected in a lower Bb burden after infection with the spirochete. In contrast, in the presence of DR4, Th1 cells dominate the immune response against Bb antigens, providing a proinflammatory environment, but not affecting the spirochete burden. Thus, our data may unravel a mechanism for the positive and negative association of DR4 and DR11, respectively, with antibiotic-refractory Lyme arthritis. It is likely that the excessive inflammation induced in the synovium of DR4+ patients upon Bb infection predisposes to autoimmune phenomena, which can be attributed to either cross-reactivity due to molecular mimicry and/or bystander activation of autoreactive cells. Indeed, we have demonstrated that chronic joint inflammation constitutes a prerequisite for the development of antibiotic-refractory Lyme arthritis (20). The data from our recently established murine model of antibiotic-refractory Lyme arthritis directly indicate that the DR4 allele is indispensable for the development of this disease. Furthermore, the data presented here indicate that Bb-infected $DR11^{+}CD28^{-/-}$ B6 mice do not develop antibiotic-refractory Lyme arthritis, a finding that is consistent with our working hypothesis. Therefore, our data from our animal model complement the clinical data demonstrating that there is a genetic predisposition to the development of antibiotic-refractory Lyme arthritis and identifies for the first time the underlying mechanism.

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All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Huber had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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