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The Effect of an Anti-HLA-B27 Immune Response on CTL Recognition of Chlamydia

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The interplay between triggering bacteria and HLA-B27 in the pathogenesis of the spondyloarthropathies remains one of the most active areas of investigation in the rheumatic diseases. This has proved difficult to study systematically in the clinical setting, and in this study we utilized a rat model to address the influence that B27-related immunity may have on the process of generating anti-Chlamydia immunity. When splenocytes from HLA-B27 DNA-immunized Lewis (LEW) animals received restimulation in vitro with Chlamydia-treated cells from B27-transgenic LEW rats, we observed that in addition to the expected CTL recognition of HLA-B27, there was also anti-Chlamydia CTL killing of Chlamydia-sensitized syngeneic fibroblast targets. This was not seen when responding cells in vitro were naive LEW splenocytes. To confirm the existence of CTLs recognizing both HLA-B27 and Chlamydia, LEW rats were immunized with B27-transgenic LEW cells, instead of the B27 DNA construct. Splenocytes from the immune rats were restimulated in vitro with Chlamydia-treated B27-transgenic LEW cells. In this instance, the CTLs retained the allele-specific recognition of HLA-B27, as well as recognition of Chlamydia-sensitized syngeneic fibroblasts. Thus, if there is prior expansion of an immune response against HLA-B27, then the resulting splenocytes demonstrate a reduced threshold for generating a primary anti-Chlamydia CTL response. These studies implicate a dynamic interrelationship between recognition of HLA-B27 and Chlamydia trachomatis. The results may have implications for deciphering the cellular basis of Chlamydia-induced reactive arthritis. The Journal of Immunology, 2001, 167: 3375–3382.

In human disease, the MHC class I allele HLA-B27 is strongly associated with a group of inflammatory rheumatic disorders termed spondyloarthopathies (SpA)3 (1, 2), which include ankylosing spondylitis, reactive arthritis (ReA), some forms of psoriatic arthritis, and inflammatory bowel disease-associated arthritis (3). One example of this family of diseases is a chronic, culture-negative inflammation of joints triggered by an infection with Chlamydia trachomatis at a site remote from the joint (4, 5). The mechanism by which the HLA-B27 mediates susceptibility to Chlamydia-induced arthritis remains unknown despite extensive investigation. The interplay between microbe and MHC in the pathogenesis of the SpA remains one of the most active areas of research into the fundamental mechanisms of arthritis (6). Since the canonical role for class I MHC molecules in the immune response is presentation of an antigenic peptide to cognate CD8+ CTLs, the interaction between CTL, peptide, and HLA-B27 has been invoked as the critical cellular event in the initiation of ReA. Yet it is still unresolved whether the contribution of HLA-B27 to disease pathogenesis is solely as a restriction element for an antigenspecific or clonotypic peptide, or whether HLA-B27 itself may modulate host immune response through other mechanisms.

CTL responses against class I HLA molecules may be broadly divided into two specificities: 1) those that recognize the HLA molecule as an alloantigen; and 2) those that recognize the HLA molecule presenting either a foreign or self peptide. Studies on allorecognition of HLA-B27 (7, 8) lend some support to the possibility that HLA-B27 itself shares peptide sequences in common with the triggering bacteria, or HLA-B27 itself mimics a clonotypic peptide important in disease regulation (9).

CTL recognizing intracellular bacteria-derived peptides presented in the context of self HLA-B27 molecules on the surface of infected cells are relevant in infection (10, 11). Such a HLA-B27-orchestrated event might figure critically in the host clearance of an arthritogenic pathogen. Alternatively, the CTL response might be directed against a self peptide presented by a MHC molecule, thereby initiating an autoimmune process (12). This response is possible since MHC class I molecules expressed on the surface of even uninfected cells are not empty, but are filled with self peptides derived from the regular pool of endogenous intracellular proteins (13–15). To avoid the induction of autoimmunity by CTLs with this specificity, CTLs undergo positive and negative selection in the thymus (16). This process is not complete, however, and T cells with specificity for self can be found in both health and disease (17). CTLs with either of these specificities (self or microbial Ags) may play a pathogenic role in HLA-B27-associated diseases. As suggested by the arthritogenic peptide model (18), cross-recognition of bacterial and self peptides might be favored by the Ag-presenting properties of the HLA-B27 and/or other host MHC molecules. In support of this hypothesis, it has been shown that HLA-B27-restricted CTLs with specificity for both self and bacterial Ags can be found in synovial fluid of ReA and ankylosing spondylitis patients (19).

Despite the strong circumstantial evidence implicating CTLs in ReA by the HLA-B27 association, little is known about the CTL immune response in SpA. There is evidence that CD4+ T cells

1 Abbreviations used in this paper: SpA, spondyloarthropathies; EB, elementary bodies; hβ2m, human β2-microglobulin; LEW, Lewis; ReA, reactive arthritis; tf, transfected; tg, transgenic.

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play an important role in disease pathogenesis (4, 20). Nevertheless, it is known that Ag derived from intracellular bacteria may lead to activation of CD8\(^+\) lymphocytes via class I-mediated mechanisms (21), and CD8-positive T-cells are now considered to play an important role in resistance to intracellular bacteria (22). To date, MHC class I presentation has been documented to occur against several intracellular pathogens, including *Listeria*, *Mycobacterium*, *Salmonella*, and *Chlamydia* (23–25).

These complex interrelationships have proved difficult to resolve in the clinical setting. In the present work, we utilize a rat model (26) to address the influence that HLA-B27-related immunity may have on the process of generating anti-Chlamydia CTLs.

**Materials and Methods**

**Animals**

Inbred female Lewis (LEW) rats (RTI\(^\text{1}\)), 2–3 mo old, were purchased (Harrlan Sprague-Dawley, Indianapolis, IN) and maintained in microisolation housing in the Toronto Western Hospital animal facility. The transgenic (tg) rat line 21-4L, bearing six copies each of the HLA-B\(^*\)2705 and human \(\beta_2\)-microglobulin (h\(\beta_2\)m) on the inbred LEW background, was originally produced at University of Texas Southwestern Medical Center (Dallas, TX) (27). These rats do not develop a spontaneous SpA. We are currently breeding and maintaining them in our animal colony. Animals for these experiments were bred by repeated brother-sister mating, and individual offspring were screened by flow cytometry of their PBMCs to confirm appropriate MHC expression using mAbs.

**Abs for flow cytometry and anti-CD4/CD8 treatment**

We purified mAbs, using a protein G-Sepharose column, from culture supernatants of different hybridomas (American Type Culture Collection (ATCC), Manassas, VA): HB24 (anti-H-2D\(^\text{a}\)), specific for murine class I H-2, HB95 (W6/32, reacting to all class I HLA in association with h\(\beta_2\)m), HB119 (ME-1, recognizing HLA-B27 as well as HLA-B7 and HLA-Bw22), and HB82 (BB7.2, anti-HLA-A2).

For flow cytometry analysis, \(10^5\) cells were incubated with predetermined saturating amount of a mAb for 30 min on ice. Isotype-matched murine IgG of no known specificity was used as a control. Cells were washed; pellets were resuspended in 40 \(\mu\)l of FITC-conjugated secondary Ab and incubated for 30 min on ice. The washed cells were then fixed, and cells were analyzed using FACS\(^\text{\textregistered}\)Scan research software (BD Biosciences, Mountain View, CA) with 10\(^5\) cells being analyzed per sample.

The mAbs purified from hybridoma W3/25 (MCA55G, specific to rat CD4) and MRC OX-8 (MAC48G, rat CD8 specific) (Serotec, Raleigh, NC) were used for an in vitro blocking of the appropriate T-cell subsets, as described below.

**Isolation of genes**

Full-length HLA-A2 and HLA-B27 cDNAs were generated by standard RT-PCR using mRNA isolated from splenocytes of HLA-A2 and HLA-B27-tg mice, respectively. To amplify the HLA-A2 and HLA-B27 genes, the sequences of the PCR primers were used as previously described (28). The HLA-A2 and HLA-B27 primers contained restriction sites, HindIII and XhoI at the 5' end to allow for directional cloning.

**Plasmid DNA immunogens**

Human HLA-A2 and HLA-B27 PCR products were cloned into the expression vector pcdNA3 (Invitrogen, San Diego, CA). Two eukaryotic expression vectors (Fig. 1) encoding class I HLA molecules were constructed: pcdNA3-A2 and pcdNA3-B27 (28). The vector constructions were characterized by restriction enzyme analysis and confirmed by sequencing with T7 polymerase (Pharmacia, Piscataway, NJ). The completed plasmid DNA were amplified in the JM109 Escherichia coli and purified using EndoFree Megaprep Kit (Qiagen, Chatsworth, CA). In vitro cell surface expression of HLA-A2 and HLA-B27 proteins encoded by the respective plasmid DNA constructs was confirmed by COS-7 cell transfections, according to previously described methods (29). Transfection of COS-7 cells with these vectors resulted in high level of surface expression of the HLA-A2 and HLA-B27 proteins, as detected by Ag-specific Abs (BB7.2 and ME-1, respectively) and assessed by flow cytometry.

**Immunization of rats**

Groups of four LEW rats were immunized with each of the following immunogens: B27-tg LEW splenocytes, non-tg LEW splenocytes, HLA-B27 DNA, HLA-A2 DNA or vector DNA alone, and *C. trachomatis* (LGV type II, strain 434) elementary bodies (EB) (Microbix Biosystems, Toronto, Ontario, Canada). The splenocytes were irradiated with 2000 rad, and 2 \(\times\) \(10^6\) cells per animal, in 1.5 ml sterile PBS, were injected i.p. The DNA vector constructs were injected by i.m. route. A total of 200 \(\mu\)g of each lyophilized closed circular plasmid construct DNA in 150 \(\mu\)l of sterile PBS was injected into the anterior tibialis muscle of a single hind leg of a Forane (Zenoca Pharma, Mississauga, Ontario, Canada)-anesthetized rat. Chlamydial EB were injected i.p. (10\(^8\) EB per animal in 1.5 ml of PBS). All the rats were immunized at days 0 and 21, and subsequently boosted at day 42 with the same cell, DNA, or EB doses via the same routes of immunization. Spleens from the immunized animals were harvested for CTL activity measurements 3–4 wk postboost.

**Mixed lymphocyte culture**

Spleen cells harvested from individual immunized rats were restimulated in vitro. Specifically, immune spleen cells were cocultured in vitro for 5 days with stimulator splenocytes from naive B27-tg LEW rats or non-tg LEW ones at 5:1 responder:stimulator cell ratio, in complete medium (RPMI 1640, 10% FCS (v/v), 2 mmL-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, nonessential amino acids, and sodium pyruvate) supplemented with 50 \(\mu\)M 2-ME. Before starting mixed lymphocyte culture, 10–20 \(\times\) \(10^6\) of some stimulator splenocytes were treated with 10\(^8\) chlamydial EB in 1 ml of complete RPMI 1640 medium for 4 h at 37\(^\circ\)C and under conditions of 5% CO\(_2\)/95% air. Finally, before adding to responders, stimulators were irradiated with 2000 rad.

**CTL \(^{51}\text{Cr} \) release assay**

To assess CTL effect functions, in vitro restimulated spleen cells were used in a standard \(^{51}\text{Cr} \) release assay by incubation with target cells. For labeling, target cells were resuspended in 1 ml of complete medium containing...
100 μCi Na$^{51}$CrO$_4$ (Amersham Pharmacia Biotech U.K. Limited, Little Chalfont, U.K.) adjusted to 10$^9$–10$^7$ cells/ml. Then the cells were incubated under 5% CO$_2$/95% air for 1.5 h at 37°C with an occasional agitation (once in 5 min). After labeling, the target cells were washed three times to remove free $^{51}$Cr, and added to experimental wells of 96-well round-bottom microtiter plates (Falcon 3077; BD Biosciences) in a volume of 10$^4$ cells/100 μl complete medium. The experimental wells contained 100 μl serial dilutions of in vitro restimulated splenocytes. Spontaneous $^{51}$Cr release was measured in wells with target cells and nonimmune spleen cells. Total release was determined by incubating the target cells with 1% Triton X-100 solution. Effectors and targets in the plates were centrifuged at 100 × g for 5 min, and the plates were incubated for 6–8 h at 37°C under 5% CO$_2$/95% air and, then, centrifuged at 1000 × g for 10 min. A supernatant from each well was harvested, and radioactivity was measured using a gamma counter (Beckman, Fullerton, CA). Percent specific lysis was calculated using the mean of triplicate samples, as follows: experimental $^{51}$Cr release cpm − spontaneous $^{51}$Cr release cpm/total $^{51}$Cr release cpm × 100%. All experimental assessments were repeated from two to five times with different rats as donors and recipients of immune spleen cells. The data shown in the figures represent from three to five separate experiments. The ratio of spontaneous to total $^{51}$Cr release from lysed targets was routinely >20% and averaged >14% at any E:T ratio. SD among triplicate assays was always <5% of specific lysis.

**Target cells**

Synovial fibroblast lines were generated in non-tg LEW and B27-tg LEW rats using induced synovitis, as described (30). These are the stable cell lines in long-term culture conditions. The transgene gives high surface expression of HLA-B27 molecule over time, as we regularly confirm by FACS.

The murine L cell line J26 transfected with hβ2m (ATCC) was previously cloned. Its clone J26.6 was transfected with genome DNA encoding HLA-A2, HLA-B27, or HLA-B44, as described (31). HLA-A2 and HLA-B44 were selected as allele controls having no known relationship to SpA. HLA-A2, HLA-B27, or HLA-B44, as described (31). HLA-A2 and HLA-B44, as described (31).

**Results**

**Generation of HLA-B27-specific CTL response in LEW rats**

To examine whether the LEW rats could raise a CTL response to HLA-B27, we injected the irradiated naive B27-tg spleen cells into the LEW rats i.p. The animals were boosted as described, and the immune splenocytes were restimulated in vitro with the same tg cells used for the in vivo immunization. Syngeneic LEW splenocytes were included in the immunization protocol as negative controls both for in vivo immunization of LEW rats and for subsequent in vitro restimulation of the spleen cells. Lysis was obtained with two cell targets only: the tg rat synovial fibroblasts and the transfected murine L cells, both of which expressed HLA-B27 and hβ2m (Fig. 2). No cytotoxicity was found using as targets syngeneic synovial fibroblasts or murine L cells transfected with third-party HLA-B44 and hβ2m, as well as in all negative controls (data not shown). CTL killing of the L cells appeared to be primarily a recognition of HLA-B27, since recognition of HLA-B44 or of the endogenous murine class I molecule (H-2$k$) would have resulted in killing of the HLA-B44-transfected (tf) L cells, and this was not observed.

**Plasmid HLA-B27 DNA immunization of rats induces a xenospecific CTL response restricted by host MHC**

To assess the influence of the restricting element in the recognition of HLA-B27, we contrasted the generation of CTL by either cellular immunization or DNA immunization. Plasmid DNA construct encoding full-length HLA-B27 was used, instead of the cellular immunogen, for in vivo immunization of LEW rats via an

**FIGURE 2.** CTL response against HLA-B27 in rats. Lysis of B27-tg LEW fibroblast and B27-tf murine L cell targets by HLA-B27-specific CTLs measured in a standard $^{51}$Cr release assay. CTL effectors were generated from LEW rats injected i.p. with irradiated naive HLA-B27-tg LEW spleen cells, and restimulated in vitro with the same cells used for the in vivo immunization.

### Table I. FACS analysis of mAb binding to HLA-transfected cell lines

<table>
<thead>
<tr>
<th>HLA</th>
<th>Cell Line Description</th>
<th>Anti-HLA I + β2m</th>
<th>Anti-B27</th>
<th>Anti-D^*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B27</td>
<td>pSV2neo/B27-transfected J26.6.2</td>
<td>30</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>A2</td>
<td>pSV2neo/A2-transfected J26.6</td>
<td>40</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>B44</td>
<td>pSV2neo/B44-transfected J26.6</td>
<td>25</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>Non</td>
<td>pSV2neo-transfected J26.6.neo.1</td>
<td>1</td>
<td>1</td>
<td>30</td>
</tr>
</tbody>
</table>

^* RFI, Relative fluorescence intensity determined by using the formula: $2(a − b)/(25.6)$ where $a = $ mean fluorescence intensity with specific mAb, $b = $ mean fluorescence intensity with control Ab, and 25.6 = a number of channels doubling of fluorescence intensity.
i.m. route. For in vitro restimulation of immune LEW splenocytes, the same B27-tg rat cells were used. HLA-A2 DNA immunogen and syngeneic LEW cells were selected for in vivo and in vitro negative controls, respectively. In a standard $^{51}$Cr release assay, cytotoxicity was observed only on B27-tg LEW fibroblasts, as expected (Fig. 3). There was no killing of B27-tg murine L cells, in contrast to the cellular immunization of LEW rats. These findings support the notion that the bulk culture of immune cells contains at least a population of anti-HLA-B27 CTLs that are restricted by endogenous rat MHC class I molecule.

**Influence of anti-CD4 and anti-CD8 mAb treatment of effector cells for HLA-B27-specific CTL killing**

We evaluated whether CD4$^+$ or CD8$^+$ cells were responsible for the lysis of targets expressing HLA-B27. In vitro restimulated splenocytes from B27-tg cell-immunized animals were treated with anti-CD4 or anti-CD8 mAb to assess their effects on CTL lysis of B27-tg LEW fibroblasts and murine HLA-B27 transfectants. Lysis of rat and murine HLA-B27-positive targets was decreased by 41 and 49%, respectively, with the anti-CD8 treatment of immune cells. In contrast, the anti-CD4 treatment of effector cells did not result in decreased activity against either target population, and indeed showed slight enhancement of killing by 12 and 15%, respectively (Table II). Thus, the cytotoxicity can be predominantly attributed to CD8$^+$ effector cell population. Some small degree of lysis of target may be due to nontarget or cross-reactive killing by other activated spleen cells.

The cytotoxicity of in vitro restimulated cells from HLA-B27 DNA-immunized rats could also be predominantly attributed to CD8$^+$ splenocytes. Lysis of B27-tg LEW cell targets was decreased by 48% with anti-CD8 treatment, and enhanced by 19% with an anti-CD4 one (Table II).

![Figure 3](http://www.jimmunol.org/) CTL response against HLA-B27 restricted by rat MHC class I molecule. Lysis of B27-tg LEW fibroblast targets by HLA-B27-specific CTL measured in a standard $^{51}$Cr release assay. CTL effectors were generated from LEW rats injected i.m. with plasmid DNA construct encoding full-length HLA-B27, and restimulated in vitro with irradiated native HLA-B27-tg LEW spleen cells.

**Prior expansion of anti-HLA-B27 CTL reduces the threshold for generating an anti-Chlamydia response**

To approach the question as to whether the contribution of HLA-B27 to SpA pathogenesis is solely as a restriction element for an arthritogenic peptide, or whether HLA-B27 may modulate host immune response through other mechanisms, we used *C. trachomatis*, as a representative arthritogenic pathogen, for different immunization protocols. First, in vivo exposure alone to *Chlamydia* following immunization with chlamydial EB did not generate any evidence of anti-*Chlamydia* CTL (data not shown). Second, splenocytes from naive LEW rats were unable to generate *Chlamydia*-specific CTL killing of any *Chlamydia*-sensitized targets after 5 days of in vitro mixed lymphocyte culturing LEW responders with *Chlamydia*-treated syngeneic or B27-tg LEW stimulators (data not shown). Thus, under these conditions, we were unable to demonstrate a primary CTL effect using naive splenocytes. Finally, splenocytes from LEW rats immunized i.p. with chlamydial EB were shown to generate specific anti-*Chlamydia* CTLs after in vitro restimulating with either *Chlamydia*-treated syngeneic or tg cells (Fig. 4, A and B). Thus, in the LEW rats, the combination of in vivo and in vitro exposure to *Chlamydia* within appropriate time intervals was both necessary and sufficient for the generation of anti-*Chlamydia* CTLs.

To address the influence that HLA-B27-related immunity may have on this process of generating anti-*Chlamydia* CTL, splenocytes from HLA-B27 DNA-immunized LEW animals received an in vitro 5-day restimulation with *Chlamydia*-treated cells from either syngeneic or tg rats. We observed that in addition to the expected anti-HLA-B27 CTL killing, there was also anti-*Chlamydia* CTL killing of *Chlamydia*-sensitized LEW fibroblasts, but this was observed only when effectors had been restimulated with *Chlamydia*-treated B27-tg cells in vitro (Fig. 5A). This was not seen when the restimulating cells in vitro were *Chlamydia*-treated syngeneic LEW splenocytes (data not shown). Thus, in contrast to naive spleen cell populations, in vitro generation of anti-*Chlamydia* CTL could be induced if a splenocyte population had first been challenged in vivo with HLA-B27 DNA. This *Chlamydia*-specific response was most likely restricted by the rat MHC class I

### Table II. Inhibition of HLA B27-specific CTL killing by anti-CD8 mAb

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>CTL (%) Induced by Anti-CD8</th>
<th>B27-tg LEW cells</th>
<th>HLA B27 DNA</th>
<th>Anti-CD8</th>
<th>Anti-CD4</th>
</tr>
</thead>
<tbody>
<tr>
<td>B27-tg LEW fibroblasts</td>
<td>41</td>
<td>+</td>
<td>48</td>
<td>−19</td>
<td></td>
</tr>
<tr>
<td>B27-tg murine L cells</td>
<td>49</td>
<td>+</td>
<td>Not applicable</td>
<td>49</td>
<td>−15</td>
</tr>
</tbody>
</table>

*Percent inhibition = [1 − (% lysis with specific mAb by immune cells − % lysis with specific mAb by naive cells)/(% lysis with control Ab by immune cells − % lysis with control Ab by naive cells)] × 100%. E:T ratio was 100:1. − refers to increase in CTL killing.*
molecule, because we observed a comparable degree of killing of *Chlamydia*-treated B27-tg and non-tg LEW cell targets only, but not murine ones (data not shown). Most importantly, the in vivo immunizing protocol in this instance was with DNA alone.

We next addressed this system using LEW rats in vivo immunized with B27-tg splenocytes, instead of HLA-B27 DNA, again followed by restimulation in vitro with *Chlamydia*-treated B27-tg cells. In this instance, anti-HLA-B27 bulk populations could still recognize HLA-B27-positive *Chlamydia*-sensitized murine targets (Fig. 6), as well as *Chlamydia*-sensitized syngeneic cells (Fig. 5B). HLA-B27-positive rat and murine cell targets were recognized whether or not the targets had first been treated with

FIGURE 4. CTL response against *C. trachomatis* in rats. Lysis of *Chlamydia*-sensitized non-tg and B27-tg LEW fibroblast targets by Chlamydia-specific CTLs measured in a standard 51Cr release assay. CTLs were obtained from LEW rats i.p. primed with chlamydial EB, and restimulated in vitro with *Chlamydia*-treated, irradiated syngeneic (A) or B27-tg (B) LEW splenocytes.

FIGURE 5. Evidence for CTLs of two specificities: anti-HLA-B27 and anti-*Chlamydia*. *Chlamydia*-specific lysis of *Chlamydia*-sensitized non-tg LEW fibroblasts and HLA-B27-specific lysis of B27-tg LEW fibroblasts by the same population of CTLs, measured in a standard 51Cr release assay. A. CTLs were obtained from i.m. HLA-B27 DNA-primed LEW rats, and restimulated in vitro with *Chlamydia*-treated, irradiated B27-tg LEW spleen cells. B. CTLs were obtained from LEW rats i.p. primed with irradiated B27-tg LEW splenocytes, and restimulated in vitro as described here in the legend to A.
Chlamydia. These observations confirm the existence of CTL of at least two specificities: first, anti-HLA-B27, and second, anti-Chlamydia CTLs.

Inhibition of CTLs specific to Chlamydia and HLA-B27 by anti-CD8 mAb

In a final experiment, we addressed the question of whether Chlamydia and HLA-B27-specific CTLs, obtained from animals primed in vivo with both B27-tg cells and HLA-B27 DNA and restimulated in vitro with Chlamydia-treated B27-tg LEW splenocytes, could also be inhibited by incubation with anti-CD8 mAb. As shown in Table III, lysis of all Chlamydia-sensitized targets, caused by these two effector populations from tg cells and DNA-immunized rats, was inhibited by the Ab. We also observed slight enhancement of CTL killing from 6 to 16% caused by anti-CD4 mAb.

Table III. Inhibition of killing of CTL specific to HLA B27 and Chlamydia by anti-CD8 mAb

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>CTL (%) Induced by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B27-tg LEW cells</td>
</tr>
<tr>
<td></td>
<td>+ Anti-CD8</td>
</tr>
<tr>
<td>LEW fibroblasts + Chlamydia</td>
<td>64</td>
</tr>
<tr>
<td>B27-tg LEW fibroblasts − Chlamydia</td>
<td>35</td>
</tr>
<tr>
<td>− Chlamydia</td>
<td>50</td>
</tr>
<tr>
<td>B27-tf murine L cells − Chlamydia</td>
<td>62</td>
</tr>
<tr>
<td>+ Chlamydia</td>
<td>59</td>
</tr>
</tbody>
</table>

* Percent inhibition = [1 − (% lysis with specific mAb by immune cells − % lysis with specific mAb by naive cells)/(% lysis with control antibody by immune cells − % lysis with control antibody by naive cells)] × 100%. The E:T ratio was 100:1. − refers to increase in CTL killing.

Discussion

HLA-B27 remains one of the strongest genetic associations in all the rheumatic diseases, with the relative risk being over 300 for Caucasian individuals who carry this allele. HLA-B*2705 is the most common of the various allele subtypes that together make up this family of class I molecules. Studies on genetic polymorphism suggest that certain subtypes (e.g., B*2705) predispose to development of SpA, while other subtypes (e.g., B*2706) do not (32). Since these subtypes differ in very few residues, which themselves are clustered in the peptide-binding domains of the molecule, this subtype-differential susceptibility has been cited to implicate differential binding of arthritogenic peptides as critical in the pathogenesis of disease. Of several proposed mechanisms regarding involvement of B*2705 in the disease process (33, 34), one postulates a role for T cell cross-reactivity, whereby CTL recognize cross-reacting peptides of endogenous and exogenous origin. Underlying this cross-reactivity is the phenomenon of molecular mimicry, in which MHC class I/exogenous peptide complexes form antigenic structures that are similar in conformation or charge to MHC class I/endogenous peptide complexes (35, 36). Unique among the HLA-B molecules, the hypervariable regions of HLA-B27 surprisingly share a number of short peptide sequences with the proteins from intracellular bacteria (37). Presentation by host MHC class I molecule of such closely related peptides from HLA-B27 and bacterial Ag could lead to cross-reactive T cell responses against self HLA-B27 or inactivation cross-reactive antibacterial T cells, inducing autoimmune or persistent infection, respectively (38). This has proved difficult to study systematically in the clinical setting, and in the current study we use a rat model involving both HLA-B27 and C. trachomatis to address the influence that anti-HLA-B27 immunity may have on a process of generating anti-Chlamydia CTLs.

We have demonstrated for the first time in rats that a specific CTL response to HLA-B27 can be induced. The generation of CTLs was mediated by either cellular immunization with HLA-B27-expressing splenocytes or plasmid immunization with DNA encoding full-length HLA-B27. A comparable event was not observed with immunization with A2 DNA, but we cannot state at present that these cross-reactive events are unique to B27.

In the case of the cellular immunogen, we demonstrated CTL-mediated lysis of B27-tg LEW target cells, but also B27-tf murine L cells as targets. These killing events are most likely induced by xenoreactive CTLs recognizing intact donor HLA-B27 molecules at the surfaces of both the injected splenocytes and target cells (direct recognition). Lysis of B27-tg LEW fibroblast targets in this
system would be attributed to possibly two different CTL populations: 1) T cells recognizing intact HLA-B27 (peptide-dependent or nonpeptide-dependent recognition), and 2) T cells specific for processed HLA-B27 peptides presented by the self MHC molecule (indirect recognition).

In the course of DNA immunization, the plasmid enters a host cell after i.m. injection of the animal. The exogenous DNA remains in the nucleus as an episome and is not integrated into the host cell DNA. Using the host cell metabolic pathways, the plasmid DNA in the episome directs the synthesis of the Ag that it encodes. B27 peptides synthesized under the direction of the plasmid DNA are brought to the surface of rat cells and displayed by MHC class I molecules, setting the stage for recognition by cognate CTLs (39). In addition, bacterial plasmid vector contains immunostimulatory nucleotide sequences: unmethylated cytidine phosphate guanosine motifs, which are absent in the mammalian genome and induce strong cellular immunity in animals (40). We observed HLA-B27-specific lysis of IgG LEW fibroblasts following HLA-B27 DNA immunization of LEW rats. In contrast to the cellular immunogen protocol, we did not observe CTL killing of the B27-tf L cell targets following DNA immunization. Thus, the specificity of the anti-HLA-B27 CTLs in this instance appeared to be HLA-B27 peptides presented by the self MHC class I molecule due to an endogenous expression of plasmid DNA-encoded Ags. If non-HLA-B27 peptide-reactive recognition was taking place, we would have seen killing of the HLA-B27-positive murine L cells, and this was not observed.

Because of the putative links between anti-Chlamydia CTLs and HLA-B27-dependent responses suggested on clinical grounds, we examined the capacity of the same LEW rats to mount a CTL response against C. trachomatis, a representative arthritogenic factor. The role of CD8+ CTL in the clearance of intracellular bacterial infections has traditionally been attributed to the ability of bacteria to escape from the phagolysosome, gain entrance to the cytosol in which proteolytic degradation takes place, and hence for microbial peptides to gain access to the class I MHC-processing pathway (41, 42). In the case of Listeria, some strains of bacteria with mutations in the hemolysin genes have significantly reduced virulence (43). However, it has recently been shown that macrophages infected either with wild-type or with a hemolysin-negative mutant Listeria are equally recognized by specific CD8+ CTL (44). Thus, while hemolysin is clearly a virulence factor, class I-mediated presentation of bacterial Ags is not dependent on its presence or production. This observation implies that intracellular bacteria need not have a mechanism of escape into the cytosol to access this presentation pathway. Using our immunization protocol, we were able to demonstrate for the first time in rats, the generating of a specific anti-Chlamydia CD8+ CTL capable of lysing Chlamydia-sensitized syngeneic LEW fibroblasts. The role of CTLs in host defense against Chlamydia has been studied in both clinical and experimental systems (45–48). While these studies support the notion of CTL recognizing MHc class I-restricted Chlamydia peptides, they have not addressed the interrelationship with concurrent HLA-B27-dependent immune responses.

It is generally held that it is difficult to demonstrate a primary in vitro generation of specific CTLs. In agreement with this, we were unable to generate in vitro expansion of anti-Chlamydia CTLs using naive splenocytes as the responder cells. In contrast, however, if the immune response against B27 was expanded in vivo first, then the resulting splenocytes demonstrated a reduced threshold for generating anti-Chlamydia CTLs. This implies a dynamic interrelationship between T cell recognition of HLA-B27 peptides and Chlamydia peptides. At present we cannot attribute this phenomenon to cross-reactive recognition of HLA and microbial peptides or to possible more indirect mechanisms such as up-regulation of costimulatory molecules on APC. But the results do point toward an interesting interaction of B27 and Chlamydia, which may have implications for deciphering the cellular basis of Chlamydia-induced ReA. These studies are ongoing and may shed light on the unresolved question of whether the ReA is indeed an autoimmune disease, rather than an atypical septic arthritis. This would have important implications for defining new treatment strategies for this disease.

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References


