Breakdown of CTL Tolerance to Self HLA-B*2705 Induced by Exposure to Chlamydia trachomatis

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There is a strong association between seronegative arthritis and HLA B27, but it is still unresolved whether the contribution of B27 to disease pathogenesis is solely as a restriction element for an arthritogenic peptide, or whether B27 itself serves as an autoantigen. This study uses transgenic rats to address the question as to whether exposure to an arthritogenic pathogen can alter tolerance to B27. Unlike their nontransgenic counterparts, B27-transgenic rats are tolerant of B27 immunization using either B27+ splenocytes or plasmid DNA and do not develop anti-B27 CTL. However, if splenocytes from such immunized animals are exposed to Chlamydia in vitro, CTL are generated that lyse B27+ targets. No killing was seen with targets transfected with control B7, B14, B40, or B44. This phenomenon was not observed with immunization by nontransgenic splenocytes, or HLA-A2 DNA alone. Using targets expressing mutated B27, we show that the epitope for autoreactive CTL recognition of B27 involves the Lys 70 amino acid residue in the α1 domain of the MHC class I molecule. The generation of CTL with specificity for B27 under these conditions demonstrates that tolerance to B27 can be subverted by Chlamydia. This indicates a dynamic interrelationship between the pathogen and B27, which may have important implications for B27-related spondyloarthropathies triggered by intracellular bacteria. The Journal of Immunology, 2002, 169: 4033–4038.

Although the presence of the HLA class I allele B27 predisposes humans to the spondyloarthropathies (SpA) (1), it is unresolved whether the contribution of B27 to disease pathogenesis is solely as a restriction element for an arthritogenic peptide, or whether B27 itself may serve as an autoantigen. Besides genetic predisposition, a role for environmental factors appears very likely (2, 3). This is highlighted by the triggering of reactive arthritis, a subset of SpA, by a type of bacterial pathogen and B27, which may have important implications for B27-related spondyloarthropathies triggered by intracellular pathogens (4, 5). One central aspect of SpA pathogenesis that remains to be clarified is the mechanism by which the B27 genetic background can specifically influence the immune response to such arthritogenic pathogens (6).

It is known that Ag derived from intracellular bacteria may lead to activation of CD8+ lymphocytes via class I-mediated pathways (7), thereby invoking CD8+ CTL in a central role in resistance to these bacteria (8). CTL recognizing bacteria-derived peptides presented by self B27 are relevant in host defense (9, 10). Such a B27-dependent event might figure critically in the clearance of an arthritogenic agent. Alternatively, the CTL response might be directed to a self peptide, thereby initiating an autoimmune process (11). Presentation by HLA class I molecules of closely related peptides from B27 and bacteria could lead to T cell responses to self B27 or inactivation of antibacterial T lymphocytes (12). The peptide specificity of B27 also seems to figure critically in the spontaneous arthritis seen in certain lines of B27 transgenic (Tg) rats (13). Although the role of CTL in recognizing MHC class I-restricted Chlamydia peptides has been studied in both clinical and experimental systems (14–17), these studies have generally not sought to address the interrelationship with concurrent B27-related immune responses. Kuon et al. (18) recently identified B27-restricted peptides from the Chlamydia proteome that were demonstrated specificity for CTL derived from B27-Tg mice. Addressing this issue in the current study, we used a Tg rat model involving both Chlamydia and B27 to study the influence that host genetic background may have on the process of generating anti-Chlamydia and anti-B27 CTL.

Materials and Methods

Animals

The Tg rat line 21-4L, bearing six copies each of the B*2705 and human β2-microglobulin (hβ2-m) on the inbred Lewis (LEW) background (RT11), was a kind gift of J. Taurog (University of Texas Southwestern Medical Center, Dallas, TX) (19). Non-Tg inbred LEW rats, 2–3 mo old, were purchased (Harlan Sprague Dawley, Indianapolis, IN). Rats were maintained in microisolators, and screened by FACS of their PBMC to confirm appropriate MHC expression.

Antibodies

For flow cytometry, mAbs were derived from different hybridomas (American Type Culture Collection (ATCC), Manassas, VA): HB24 (anti-H-2Db), HB95 (W6/32, reacting to all HLA-I in association with hβ2-m), was a kind gift of J. Taurog (University of Texas Southwestern Medical Center, Dallas, TX) (19). Non-Tg inbred LEW rats, 2–3 mo old, were purchased (Harlan Sprague Dawley, Indianapolis, IN). Rats were maintained in microisolators, and screened by FACS of their PBMC to confirm appropriate MHC expression.

Isolation of genes

Full-length A2 and B27 cDNAs were generated by standard RT-PCR using mRNA isolated from splenocytes of Tg mice. To amplify the A2 and B27 genes, PCR was conducted, as previously described (20).
Plasmid DNA immunogens

A2 and B27 PCR products were cloned into the expression vector pcDNA3 (Invitrogen, San Diego, CA). Two eukaryotic expression vectors were constructed: pcDNA3-A2 and pcDNA3-B27 (18). The vector constructs, encoding class I HLA, were characterized by restriction enzyme analysis and confirmed by sequencing with T7 polymerase (Pharmacia, Piscataway, NJ). The completed plasmid DNA was amplified in the JM109 Escherichia coli and purified using EndoFree Megaprep Kit (Qiagen, Chatsworth, CA).

In vitro cell surface expression of A2 and B27 proteins was confirmed by COS-7 cell transfections, according to previously described methods (21).

Immunization of rats

Groups of four B27-Tg rats were immunized with each of the following immunogens: B27-Tg splenocytes, non-Tg splenocytes, B27 DNA, A2 DNA. Cells and DNA were irradiated with 2000 rad, and 2 × 10^6 cells/animal, in 1.5 ml PBS, were i.p. injected. A total of 200 μCi of each lympholyzed closed circular plasmid construct DNA in 150 μl PBS was injected into the anterior tibialis muscle of a single hind leg of a rat anesthetized with Forane (Zeneca Pharma, Mississauga, Ontario, Canada). The rats were immunized at days 0 and 21, and subsequently boosted at day 42. Splenocytes were harvested for CTL activity measurements 3–4 wk postboost.

Mixed lymphocyte culture

Spleen cells harvested from individual immunized rats were cocultured in vitro for 5 days with stimulator splenocytes from naive B27-Tg rats at 5:1 responder/stimulator ratio. In some experiments, stimulators (1–2 × 10^4) were treated with 10^3 of Chlamydia trachomatis (Ct; LGV type II, strain 434) elementary bodies (EB) (Microbix Biosystems, Toronto, Ontario, Canada) washed 1 ml of complete medium for 4 hr at 37°C under 5% CO_2/95% air. Finally, stimulators were irradiated with 2000 rad.

CTL 51 Cr release assay

A total of 10^10–10^11 target cells was resuspended in 1 ml of complete medium containing 100 μCi Na_2[51]CrO_4 (Amersham Pharmacia Biotech U.K. Limited, Little Chalfont, U.K.), and incubated under 5% CO_2/95% air for 1.5 h at 37°C with occasional agitations (once in 5 min). Then the cells were washed and added into 96-well round-bottom microtiter plates (Falcon 3077; BD Biosciences, Lincoln Park, NJ) (10^4 cells/well in 100 μl medium). A total of 100 μl/well of serial dilutions of in vitro restimulated effectors was also added. Spontaneous 51 Cr release was measured in wells with targets and nonimmune spleen cells. Plates were centrifuged at 1000 g/11000 for 1.5 h at 37°C and nonimmune spleen cells. The data shown in the figure is representative of three to five experiments. We had previously demonstrated that both the plasmid DNA and cellular strategies were effective in generating CTL against B27 in non-Tg LEW rats (26).

To address the influence that self B27-related immunity may have on the process of generating anti-Chlamydia CTL, splenocytes from either syngeneic cell-immunized or B27 DNA-immunized rats underwent a 5-day restimulation in vitro with Chlamydia-treated B27-Tg spleen cells. We observed that splenocytes from Tg animals immunized with either syngeneic cells or the plasmid DNA construct were now shown to generate an anti-Chlamydia CTL response. There was CTL killing of Chlamydia-sensitized non-Tg LEW fibroblast targets, but this was observed only when the effectors had been restimulated in vitro with Chlamydia-treated B27-Tg splenocytes. In contrast, in vitro generation of anti-Chlamydia CTL could be induced if animals had first been primed in vivo with B27-Tg cells or B27 DNA, but not with non-Tg splenocytes, A2 DNA, or vector DNA alone (data not shown).

Induction of autoreactive anti-B27 CTL follows in vitro exposure to Chlamydia

We next addressed the specificity of CTL obtained from B27-Tg rats primed in vivo with either syngeneic cells or B27 DNA and thereafter restimulated in vitro with Chlamydia-treated B27-Tg splenocytes. As expected, killing was observed, with recognition of both Chlamydia-sensitized B27+ rat fibroblasts (Fig. 1A) and murine L cells (Fig. 1C). When cytotoxicity against nonsensitized B27-Tg rat and B27+ murine cell targets was examined, we observed killing of all of the B27+ targets. Killing was comparable, whether (Fig. 1, B and D) or not (Fig. 1, A and C) the targets had first been sensitized with Chlamydia. No cytotoxicity was found on non-Tg LEW synovial fibroblasts and murine L cells transfected with third party B44. Furthermore, no such killing was seen with splenocytes from control animals immunized with HLA-A2, vector DNA alone, or non-Tg LEW splenocytes (data not shown). These observations point toward the existence of autoreactive anti-B27 CTL.

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

We evaluated whether CD4+ or CD8+ cells were responsible for the lysis of Chlamydia+ and B27+ targets. Splenocytes from B27-Tg animals challenged in vivo with B27 DNA, then stimulated in vitro with Chlamydia-treated B27+ cells were incubated

Results

Prior exposure to self B27 reduces the threshold for generating anti-Chlamydia CTL

Irradiated naive B27+ Tg LEW spleen cells were injected i.p. into syngeneic rats. The animals were boosted as described, and the primed splenocytes were restimulated in vitro with the Tg cells used for the in vivo immunization. In contrast to our previous studies using non-Tg rats, B27+ Tg animals were tolerant of immunization and restimulation with the self B27+ cells, as reflected in the absence of any CTL killing of B27+ targets. The T cell tolerance was also observed when a plasmid DNA construct encoding full-length B27 was used, instead of the splenocytes, for in vivo immunization via an i.m. route (Fig. 1, A and C). The figure is representative of three to five experiments. We had previously demonstrated that both the plasmid DNA and cellular strategies were effective in generating CTL against B27 in non-Tg LEW rats (26).

TOLERANCE TO HLA-B27 SUBVERTED BY Chlamydia

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

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with anti-CD4 or anti-CD8 mAb. Lysis of non-Tg fibroblasts sensitized with *Chlamydia* was decreased by 61% with the anti-CD8 treatment of effector cells. For the corresponding experiment using B27-Tg rats injected either with syngeneic splenocytes or with B27 DNA, and restimulated in vitro with B27-Tg spleen cells either treated with Ct EB (Ct+) or untreated (Ct-).

**FIGURE 1.** Autoreactive anti-B27 CTL response following in vitro exposure to Ct. Lysis of B27-Tg LEW fibroblasts (A and B) and B27-transfected L cells (C and D) either sensitized with Ct EB (B and D) or unsensitized (A and C), measured in a standard $^{51}$Cr release assay. CTL were obtained from B27-Tg rats injected either with syngeneic splenocytes or with B27 DNA, and restimulated in vitro with B27-Tg spleen cells either treated with Ct EB (Ct+) or untreated (Ct-).
expressing B27.AKA69–71TNT mutant molecules (Fig. 3, lane 4). Indeed, the single residue mutant B27.K70Q was not recognized, indicating that mutation of B27 Lys at position 70 alone was sufficient to abrogate the autoreactive B27-specific CTL recognition (Fig. 3, lane 3).

Discussion

We have recently shown that prior expansion of an in vivo immune response against B27 in LEW rats reduced the threshold for generating a primary anti-Chlamydia CTL response (26). To address the role of reactivity to self-B27 vs nonself, in this study we examined this phenomenon in B27-Tg animals.

Rats Tg for B27 and hβ2,m have proved useful for investigating the basis of B27-associated SpA. Tg rat lines expressing high levels of B*2705/β2,m develop a spontaneous disease with striking resemblance to human SpA, and this disease is critically dependent on B27-bound peptides (18). Tg rats from lines that express lower copy numbers of B27 develop no spontaneous disease. In the present study, we have used the 21-4L, one such low-copy line on LEW background.

As was seen in the xenogeneic B27 response observed with our non-Tg animals (24), we have also observed in this study that prior exposure to self B27 results in a reduced threshold for generating a primary in vitro CTL response to Chlamydia. Unexpectedly, this precondition of in vitro exposure to Chlamydia led to induction of the autoreactive CTL with specificity for B27, in the animals otherwise tolerant to self B27, by a single exposure in vitro to Chlamydia following the in vivo priming with self B27. These data implicate a dynamic interrelationship between immune recognition of Chlamydia and breakdown of tolerance to self B27. This phenomenon might be explained by cross-reactive recognition of self and microbial peptides. CTL with either of these two specificities may play a pathogenic role in B27-associated SpA. The arthritogenic peptide model (27) for the pathogenesis of SpA postulates that the B27-binding motif sets the stage for presentation of peptides to potentially pathogenic CTL. These peptides might be of endogenous origin or exogenous origin or both, and cross-recognition of such peptides might be favored by B27-restricted T cells.

Table I. Blocking of CTL specific to Ct and self B27 by anti-CD8 mAb

<table>
<thead>
<tr>
<th>Target Cells</th>
<th>B27-Tg LEW Cells</th>
<th>B27 DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+Anti-CD8 (%)</td>
<td>+Anti-CD4 (%)</td>
</tr>
<tr>
<td>LEW fibroblasts</td>
<td>Ct⁺ 40</td>
<td>-9⁺</td>
</tr>
<tr>
<td>B27-Tg LEW fibroblasts</td>
<td>Ct⁺ 49</td>
<td>12⁺</td>
</tr>
<tr>
<td></td>
<td>Ct⁺ 44</td>
<td>-7⁺</td>
</tr>
<tr>
<td>B27-transfected murine</td>
<td>Ct⁺ 55</td>
<td>15⁺</td>
</tr>
<tr>
<td>L cells</td>
<td>Ct⁺ 43</td>
<td>-11⁺</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.
This notion of potentially cross-reacting peptides is supported by the observation that B27-restricted CTL with specificity for both self and bacterial peptides can be found in synovial fluid of SpA patients (28). The notion of such cross-reactivity has been proposed to be a central factor in the pathogenesis of B27-related SpA (29).

The interrelationships of infection and autoimmunity are complex, and may invoke a variety of pathways in addition to molecular mimicry (12). It has been argued that mimicry may arise as a secondary phenomenon caused by alteration of host antigenic determinants through tissue injury and the creation of neoepitopes. This process of tissue injury uncovering cryptic epitopes does not seem pertinent to our present observation of in vitro stimulation. Activation of otherwise anergic autoreactive T cells can result from up-regulation of costimulatory molecules on APCs, being a necessary precondition for T cell activation in conjunction with cognate binding of the MHC-peptide complex by TCRs. It is evident that autoreactive T cells do survive thymic deletion strategies to some extent, as must be the case for our B27-Tg rats. Peripheral tolerance mechanisms (30) then become the means of keeping such cells in check and preventing an ongoing autoimmune assault on host tissues.

Our studies on the mutant B27 targets implicate Lys at position 70 as being critical for an epitope recognized by the autoreactive CTL. In the studies of Lopez de Castro and colleagues (31, 32), the residue Lys70 plays a key role in the specificity of CTL. It was of interest that in our study the Cys67Tyr mutation did not affect autoreactive CTL recognition of B27. A Cys67Ser mutation of B*2705 has been felt to still predispose Tg animals to arthritis in vivo (33), and to weaken B pocket interactions affecting alloreactivity (34). Studies in progress in our laboratory are addressing whether the epitope of xenoreactive anti-B27 CTL differs from that of autoreactive anti-B27 CTL.

Activation of quiescent autoreactive T cells might implicate a sequence homology between the respective peptides from Chlamydia and B27, but this may not necessarily be the precondition for cross-reacting T cell responses. Studies by Wucherpfennig and Strominger (35) demonstrate that T cell clones may show significant stimulatory responses to peptides that are widely divergent in their amino acid sequences. Tertiary structure, hydrophobicity, and electrostatic charges are all potential contributors to interactions with cross-reacting TCRs. It is of interest that the residue Lys70 does not point upward, in a way that would reflect direct contact with the TCR, but forms a salt bridge with residue Asp74, which in turn figures critically in peptide binding (28, 29). The elution studies by Jardetzky et al. (36) indicate that a significant population of peptides normally resident in the B27 peptide-binding cleft is derived from B27 itself. It remains a distinct possibility that the B27-mediated sequelae of Chlamydia infection such as reactive arthritis are fundamentally autoimmune processes. Antibiotic therapy does not alter the natural course of this arthritis (37), nor has recent experience with anti-TNF therapies unmasked any occult or subclinical infectious arthritis (38). Thus, if the persistent inflammation is maintained by a sustained source of microbial peptides, the source of such exogenous peptides is obscure. Our results provide experimental evidence of a break in tolerance to B27 occasioned by exposure to Chlamydia. This may provide a mechanistic bridge between what begins as a septic event, but which evolves into an autoimmune process. It also raises the possibility that peptide therapy may be a feasible approach to treating this condition in the future.

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References


