Gorillas with Spondyloarthropathies Express an MHC Class I Molecule with Only Limited Sequence Similarity to HLA-B27 that Binds Peptides with Arginine at P2


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The human MHC class I gene, HLA-B27, is a strong risk factor for susceptibility to a group of disorders termed spondyloarthropathies (SpAs). HLA-B27-transgenic rodents develop SpAs, implicating HLA-B27 in the etiology of these disorders. Several nonhuman primates, including gorillas, develop signs of SpAs indistinguishable from clinical signs of humans with SpAs. To determine whether SpAs in gorillas have a similar HLA-B27-nonhuman primates, including gorillas, develop signs of SpAs indistinguishable from clinical signs of humans with SpAs. To determine whether SpAs in gorillas have a similar HLA-B27-related etiology, we analyzed the MHC class I molecules expressed in four affected gorillas. Gogo-B01, isolated from three of the animals, has only limited similarity to HLA-B27 at the end of the α1 domain. It differs by several residues in the B pocket, including differences at positions 45 and 67. However, the molecular model of Gogo-B*0101 is consistent with a requirement for positively charged residues at the second amino acid of peptides bound by the MHC class I molecule. Indeed, the peptide binding motif and sequence of individual ligands eluted from Gogo-B*0101 demonstrate that, like HLA-B27, this gorilla MHC class I molecule binds peptides with arginine at the second amino acid position of peptides bound by the MHC class I molecule. Furthermore, live cell binding assays show that Gogo-B*0101 can bind HLA-B27 ligands. Therefore, although most gorillas that develop SpAs express an MHC class I molecule with striking differences to the human MHC class I model, Gogo-B*0101 demonstrates that the specificity of peptides bound to B27 significantly influences the prevalence of arthritis in these animals (8). Thus, whether the disease mechanism involves B27 functioning in its conventional role of peptide binding molecule remains in question.

There are several features of B27 that make it unique among MHC class I molecules. Crystal structures and molecular models have demonstrated that B27 is unique in its possession of an unusually deep B pocket when compared with other MHC class I molecules (9). Indeed, many groups have now eluted and sequenced peptides bound to B27 and found that the peptides contained the bulky and positively charged amino acid, arginine, at the second position (10–12). Additionally, the combination of amino acids that make up the B27 B pocket is unique to and conserved in all B27 subtypes (13), all of which bind peptides with arginine at the second position. Of these residues, glutamic acid at position 45 (E45), and cysteine at position 67 (C67) have been shown to be critical for peptide binding, cell surface expression, and CTL recognition (13, 14). C67 has also been implicated in triggering autoimmunity (15) and in the formation of unique β2-microglobulin (β2m)-free B27 heavy chain homodimers (7).

Although B27 is the primary genetic factor determining susceptibility to SpAs, not all B27-positive individuals develop disease; this remains one of the mysteries of these disorders. In general, ~0.2% of the general population will develop ankylosing spondylitis (AS), whereas 2% of B27-positive individuals will develop the strongest known association between the MHC and disease susceptibility is that of HLA-B27 and inflammatory spondyloarthropathies (SpAs) in humans. Several theories have been proposed to explain the role of B27 in the development of SpAs and have been reviewed elsewhere (1–3). The primary function of MHC class I molecules is to present endogenously produced peptides to CTLs. Thus the most intuitive of these theories, the arthritogenic peptide hypothesis, postulates that B27 plays a direct role in pathogenesis by binding an arthritogenic peptide (or peptides) and presenting it to autoreactive CTLs. Recent transgenic mouse and in vitro studies suggest that the role of B27 in the mechanism of disease may be distinct from its primary function as an Ag presentation and CD8+ T cell restriction molecule (4–7). However, the transgenic rat model provides evidence that the specificity of peptides bound to B27 significantly influences the prevalence of arthritis in these animals (8). Thus, whether the disease mechanism involves B27 functioning in its conventional role of peptide binding molecule remains in question.

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the disease (16). Although there is no direct data showing what percentage of B27-positive individuals will develop reactive arthritis (ReA), extrapolation from data summarized by Keat indicate that 20–30% of B27-positive individuals that contract Shigella should develop ReA (17). Sixty to 80% of ReA patients are B27-positive, a lower association than is seen in AS where as many as 96% of patients are B27-positive. However, this allele is only present in ~7% of Caucasian populations.

Although SpAs are a common phenomenon in nonhuman primates, very little is known about the relationship between disease occurrence and expression of MHC class I molecules in these species. Previously, other groups described AS in the gorilla, Beta (18), and post shigellosis ReA in Holli and Husani (19). Another gorilla, Harry, also developed ReA (D. Neiffer, manuscript in preparation). By examining skeletal remains, Rothschild and Woods have also observed SpAs in 20% of gorillas (20). Gorillas are one of humans closest relatives, last sharing a common ancestor 20% of gorillas (20). Gorillas are one of humans closest relatives, last sharing a common ancestor.

Materials and Methods

Animals

Whole blood was obtained opportunistically from venipuncture from gorillas anesthetized for routine exams (Gorilla gorilla) at the Yerkes Regional Primate Research Center (Atlanta, GA), the Bronx Zoo (Wildlife Conservation Society, Bronx, NY), the Brookfield Zoo (Chicago Zoological Society, Brookfield, IL), the Toledo Zoo (Toledo Zoological Society, Toledo, OH), and the Pittsburgh Zoo (Zoological Society of Pittsburgh, Pittsburgh, PA). Description of AS in Beta and ReA in Holli and Husani was reported previously (18, 19). The clinical report of ReA in Harry is in preparation (D. Neiffer et al., manuscript in preparation).

Cell culture

PBL were separated from whole blood using Ficoll/diatrizoate gradient centrifugation. These cells were cultured with 5 U/ml IL-2 (a gift from Dainippon, Osaka, Japan) and 20 U/ml rIL-2 (a gift from Roche, Nutley, NJ). PBLs from M. gloveri and M. mulatta were separated from whole blood using Ficoll/diatrizoate gradient centrifugation. These cells were cultured with 5 U/ml Con A (Sigma, St. Louis, MO) and 20 U/ml IL-2 (a gift from Roche, Nutley, NJ). PBLs from Beta and Harry were transformed with EBV by culturing PBL with supernatant of the B95-8 cell line (25) in the presence of cyclosporin A at 0.2 μg/ml using a protocol modified from Lawlor et al. (26). Transformed and activated lymphocytes were cultured at 1 × 10⁶ cells/ml in RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 15% heat-inactivated FBS (Sterile Systems, Logan, UT), 2 mM l-glutamine, 5 × 10⁻³ M 2-ME, 20 mM HEPES, 50 U/ml penicillin, and 50 μg/ml streptomycin.

DNA/RNA extraction, cDNA synthesis, and PCR

Total cellular RNA was extracted from 2–7 × 10⁶ lymphocytes using RNAzol (Tel-Test, Friendswood, TX). cDNA was synthesized from 0.1–1 g of RNA in a 20-μl reaction containing 50 mM Tris, pH 8.3, 5 mM MgCl₂, 1 mM each of dATP, dGTP, dCTP, and dTTP (Gene AMP-Perkin-Elmer, Foster City, CA). cDNA was synthesized at room temperature for 10 min, 42°C for 15 min, 99° for 5 min, and 5° for 5 min in a Perkin-Elmer Cetus 9600 thermocycler (Norwalk, CT). PCR was then conducted in a Perkin-Elmer Cetus 9600 using several sets of locus-specific primer. All of the loci were typed in Table III was conducted with the B locus-specific primer set GG5’-CGCCCGTCCTGATTGAGGCAGGCTGTCCG-3’, GG3’-CGGAGCTTCTCCATACACAGCTGGTCTT-3’, and GG2’-ATGCAGTGTGGGGAC-3’. The resulting model was subjected to 40 cycles of energy minimization refinement using X-PLOR (33) with a small harmonic constraint placed on the α carbon positions.

Stable transfection of Gogo-B*0101 into the 721.221 cell line

A clone containing the consensus cDNA for Gogo-B*0101 was subcloned into the pG5s expression vector (a gift from Andrew McMichael, Oxford University, Oxford, U.K.). This vector was then electroporated into the 721.221 cell line, a cloned EBV-transformed B lymphoblastoid cell line (BCLL) with homozygous deletions of the MHC class I loci (34). Cell line 721.221 cells (7.5 × 10⁶) were transfected in a 0.4-cm electroporation cuvette with 25 μg of plasmid DNA. Electroporation was conducted with a Bio-Rad Gene Pulser II Electroporation System (Bio-Rad, Hercules, CA) at 200 V and a capacitance of 950 μF. The cells were then put into 50 ml of RPMI 1640 culture medium supplemented with 50 U/ml penicillin, 50 μg/ml streptomycin, and subcultured twice before being used.

Antibodies

W6/32 hybridoma was obtained from the American Type Culture Collection (ATCC) (Manassas, VA). This mouse mAb is directed against human MHC class I proteins. W6/32 from hybridoma supernatant was purified over a Sepharose CL4B column (Pharmacia) for use in purifying MHC class I molecules. The 949 (anti-class II Ab) hybridoma obtained from ATCC was used to generate mouse 949 ascites according to established protocols (29).
μg/ml streptomycin, 2 mM t-glutamine, 5% defined FBS (HyClone, Logan, UT), and 10% defined/supplemented bovine calf serum (HyClone) and plated at 1 ml/well in 24-well plates. The cells were incubated for 2 days at 37°C. On day 3, the cells were placed under selection by adding 1 ml of culture medium containing G418 (Life Technologies, Gaithersburg, MD) for a final concentration of 650 μg/ml. About 4 wk later, viable transfectants were tested for MHC class I surface expression by flow cytometry with the W6/32 mAb directly conjugated to FITC (Sigma). The transfectant with the highest level of MHC class I expression was selected to be grown up for peptide elution studies.

We also produced soluble Gogo-B*0101 transfectants to produce higher amounts of bound peptides for sequencing. This method was described previously (35).

**Affinity purification of Gogo-B*0101**

MHC class I molecules were purified from 721.221 transfectants according to a modified protocol as previously described (36, 37). Briefly, 6 × 10⁶ cells were washed in cold HBSS (Life Technologies), harvested, and frozen until needed. Thawed cells were then resuspended in 100 ml of 1% Nonidet P-40 lysis buffer containing 0.25% sodium deoxycholate, 174 μg/ml PMSF, 5 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 20 μg/ml iodoacetamide, 0.2% sodium azide, and 0.003 μg/ml EDTA. Cell lysates were incubated at 4°C for 1 h, centrifuged at 100,000 × g at 4°C to remove cellular debris, and then filtered sequentially through 0.8- and 0.22-μm Nalgene filters to remove any remaining lipids. Filtered lysates were then passed twice over a 549-coupled protein A-Sepharose column to preclude the lysate. The flowthrough was passed twice over two consecutive W6/32-coupled columns to specifically bind MHC class I molecules. The protein A beads of the W6/32 columns were then washed separately, twice with lysis buffer (without protease inhibitors), twice with a high salt buffer (1 M NaCl, 20 mM Tris pH 8.0), and twice with no salt buffer (20 mM Tris, pH 8.0). Purification of soluble Gogo-B*0101 was as described previously (35).

**Purification of Gogo-B*0101-bound peptides**

Peptides were eluted from Gogo-B*0101 as described previously (36). Briefly, the protein A beads were incubated in 0.2 N acetic acid. The beads were then briefly centrifuged and transferred to a new tube and the process was repeated. Glacial acetic acid (100 ml) was added to each tube to allow for dissociation of the MHC heavy chain/β2m/peptide complexes. MHC class I heavy chains, β2m, and W6/32 Abs were then separated from the peptides by centrifugation through an Ultrafree-CL filter (500 MWL; Millipore, Bedford, MA). Peptide yields were determined by quantitation of Gogo-B*0101 heavy chain using SDS-PAGE. Purification of peptides from soluble Gogo-B*0101 was as described (35).

**HPLC fractionation and automated Edman degradation sequencing of peptides**

The filtered peptide eluate was purified by reverse phase HPLC on a 1.0 × 150 mm C18 column (Michrom Bioresources, Auburn, CA) using the following gradient conditions at a flow rate of 40 μl/min: 2–10% acetonitrile in 0.02 min, 10–60% acetonitrile in 2 min. The entire region corresponding to uv absorbance at 215 nm was collected during the gradient and subjected to pooled Edman degradation on a model 492A pulsed liquid phase protein sequencer (Perkin-Elmer Applied Biosystems Division, Norwalk, CT) with underivatized cysteine. Nonpeptide material, which copurified with the peptides in the first experiment (from the cell-bound molecules), eluted as two peaks on the HPLC analysis. One of these peaks observed in Edman degradation for the majority of the cycles and eluted for the first three cycles. Only data from the second run (peptides eluted from soluble molecules) is used for these amino acids. Raw data analysis was performed according to established protocols (38–40). The average relative frequency table was generated according to Kubo et al. (38).

**Nanoelectrospray tandem mass spectrometry (nanoES-MS/MS)**

Sequencing of individual peptide ligands was as previously described (41). Typical nanoES-MS/MS runs involved gating for an ion with the first quadrupole and scanning a range with the third quadrupole of 30–1200 m/z using a step size of 0.2 atomic mass units and a dwell time of 1.5 ms with underivatized lysine; the collision gas (Ar) was adjusted in each case to optimize fragmentation for the ion examined. Nano-ES-MS/MS data were evaluated and interpreted using the Predict Sequence algorithm (BioMul-ti, Boston, MA) as well as PeptideSearch 3.0.2 (42) in instances of low ligand ionization/concentration or poor fragmentation. Advanced Basic Local Alignment Search Tool searches were performed against databases available through the National Center for Bio-

**Results**

**Gorillas that develop SpAs express an MHC class I molecule with limited similarity to B27**

Gorillas develop signs of SpAs indistinguishable from clinical signs seen in humans with SpAs. The case description of Beta’s AS was the first in a nonhuman primate (18) (Table I). She was initially diagnosed with arthritis in 1979 when radiographs were taken due to a stiffness in her gait. Later her condition worsened further; she moved with great difficulty and used an awkward, rigid gait. Under anesthesia, board-like rigidity of the dorsal spine and extreme bilateral contractions of the hips were noted. There was also evidence of chronic synovitis of the larger peripheral joints, and swelling and/or contractions involving the shoulders, elbows, wrist, knees, and ankles in a symmetric fashion. Radiographs showed hallmark and progressive changes typical of AS, including advanced sacroiliitis and lumbar spondylitis. Beta’s son, Kwashi developed inflammatory synovitis of the right wrist (18) (Table I). Holli and Husani developed signs very typical of ReA (19). Both developed Shigella flexneri enteritis and subsequently developed inflammatory joint disease. Harry also developed signs of ReA following shigellosis (Table I).

Given the remarkable similarity between SpAs in gorillas and humans and the close association of B27 with these diseases in humans, we cloned and sequenced all of the MHC class I molecules isolated from these animals, Gogo-B01 was the most similar to B27 (Figs. 1 and 2). However, at the amino acid level, Gogo-B*0101 is most similar to HLA-B*1513, differing by 21 residues. HLA-B*2702 is the next most similar human class I molecule, differing from Gogo-B*0101 by 22 aa (Fig. 1 and data not shown). Fifteen of those differences occur in the polymorphic α1 and α2 domains that comprise the peptide binding region (PBR). Interestingly, Gogo-B01 is identical with HLA-B*2702 from residues 71 to 90 at the end of the α1 domain (Figs. 3336)
The ability of B27 to bind peptides with arginine at position 2 is diminished by the substitution of E45 for M45, the substitution present in Gogo-B01, greatly diminishing the ability of B27 to bind peptides with arginine at the second position. Moreover, previous studies have shown that substitution of arginine at position 2 for histidine makes up the P2 environment (47) in other HLA molecules that have been shown to accommodate arginine at the second position resolved after 4 wk of treatment; swelling in stifle and lameness recurred within 3 days after medication was discontinued; right stifle was then enlarged and firm; radiography showed increased soft tissue swelling; medication was changed, symptoms resolved and no further occurrences were observed.

We found that Gogo-B01 has only limited similarity to B27, primarily at the end of the α1 domain. Additionally, there are key differences between the B pocket residues of this molecule and B27. Moreover, previous studies have shown that substitution of E45 for M45, the substitution present in Gogo-B01, greatly diminished the ability of B27 to bind peptides with arginine at the second position.
exhibiting relative variation in N-proximal amino acids and overall peptide length (41). Additionally, one of the P9 anchor residues of B*2702 is W (12). Thus, Gogo-B*0101 shares both a P2 and a C-terminal anchor residue with B27.

We found it curious that substitutions that were shown to abrogate binding of R2 peptides in B27 (13, 14) did not result in this effect on Gogo-B*0101. Therefore, we sought to determine through computer modeling how the B pocket might accommodate R at P2. The molecular model of the Gogo-B*0101 molecule suggests that changes in other residues of the B pocket compensate for the lack of E45 such that the B pocket should still preferentially bind peptides with positively charged residues at P2 (Fig. 3). The substitutions in and around the B pocket of Gogo-B*0101 are similar in charge and size to those found in B27, such that the charge of the B pocket remains predominantly negative like that of B27 (Fig. 3, A–C). At a more detailed level, the E45/M45 substitution changes the charge of that side of the B pocket from negative to neutral. This substitution appears to make the pocket smaller on that side, pushing the P2 residue away from M45 toward the aspartate (D) at position 9 (Fig. 3, D and E). However, the change in Gogo-B*0101 from H9 to D9 at this position results in a smaller and now positively charged residue that can interact with P2. The substitution of I66 for the smaller and more polar T66, may also allow a positively charged P2 residue to interact more closely with D9 (Fig. 3, D and E). Thus, compensatory changes in and around the B pocket of Gogo-B*0101 allow for binding of peptides with R at P2.

Table II. Molecular typinga of the MHC class I loci in gorillas that developed AS or ReA

<table>
<thead>
<tr>
<th>Name</th>
<th>Clinical Symptomsb</th>
<th>Gogo-A</th>
<th>Gogo-B</th>
<th>Gogo-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta</td>
<td>AS</td>
<td>A<em>0401, A</em>0501</td>
<td>B<em>0101, B</em>0301</td>
<td>C<em>0101, C</em>0103</td>
</tr>
<tr>
<td>Husani</td>
<td>ReA</td>
<td>A<em>0401, A</em>0501</td>
<td>B<em>0101, B</em>0201</td>
<td>C<em>0101, C</em>0202</td>
</tr>
<tr>
<td>Holli</td>
<td>ReA</td>
<td>A<em>0101, A</em>0501</td>
<td>B<em>0201, B</em>0401</td>
<td>C<em>0202, C</em>0204</td>
</tr>
<tr>
<td>Harry</td>
<td>ReA, 404F*  ?</td>
<td>B<em>0101, B</em>0201</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Kwashi</td>
<td>Signs of peripheral arthritis</td>
<td>ND</td>
<td>B<em>0101, B</em>0103</td>
<td>ND</td>
</tr>
<tr>
<td>(Beta’s male offspring)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a MHC class I cDNAs from affected gorillas were amplified by PCR and cloned as described in Materials and Methods.
b See Table I for references.
c Only one copy isolated by cloning and sequences.

FIGURE 1. Predicted amino acid translations of MHC class I A, B, and C locus cDNAs isolated from gorillas have limited sequence similarity to B27. Predicted amino acid sequences of the a1 and a2 domains of gorilla MHC class I A, B, and C locus cDNAs are aligned to a gorilla consensus sequence. HLA-B*2702 and B*2705 are included for comparison. B pocket residues are denoted by the symbol . The critical B pocket residues E45 and C67 are boxed. The GenBank accession numbers for other sequences used in this paper are: HLA-B*2702 L20086, HLA-B*2702 X03664, Gogo-B*0101 X06255, Gogo-B*0102 X06693, Gogo-B*0103 X06254, Gogo-B*0201 X06253, Gogo-C*0101 X06252, Gogo-C*0202 X06249, Gogo-A*0101 X06258, Gogo-A*0401 X54376, and Gogo-A*0501 X60256.
Gogo-B*0101 can bind peptides known to be bound by B27

Given the similarity in the peptide binding motifs of Gogo-B*0101 and B27, we asked whether Gogo-B*0101 could bind peptides that are known to bind to B27. Live cell binding assays using a variety of B27 and B27-like ligands demonstrated that Gogo-B*0101 can bind B27 ligands (Table VII). Furthermore, when we tested binding of peptides with substitutions at P2, we found that the arginine at P2 is crucial for binding to Gogo-B*0101 (Table VII).

Gogo-B01 is present in nearly every gorilla

Because such a high rate of SpAs are found in gorillas, we reasoned that a candidate susceptibility allele would be similarly...
present at a high frequency. Therefore, we determined the MHC type of 10 additional unrelated, unaffected gorillas and found that Gogo-B01 is expressed in nearly every animal (Table VIII). These results could account for the high rate of cross-reactivity between gorilla lymphocytes and human B27 heteroantisera previously observed by other groups (18, 23). We also tested human B27 antisera against PBLs from β and T cell blasts from another gorilla, Pattycake, and observed similar cross-reactivity (data not shown).

**Discussion**

In this study, we show that most gorillas that develop SpAs express an MHC class I molecule, Gogo-B01, that has only limited sequence similarity to B27. Gogo-B01 and HLA-B27 differ at several residues that make up the B pocket. However, compensatory substitutions change the B pocket of Gogo-B*0101 such that it can accommodate arginine at P2. In fact, Gogo-B*0101 has a remarkably similar peptide binding motif to that of B27; furthermore, it can bind B27 ligands. This molecule is expressed in the majority of gorillas.

We have not shown that B01 is associated with the development of disease in gorillas. Due to the small number of affected gorillas and the high frequency of this allele in the gorilla population, this kind of study was not feasible. However, the high prevalence of disease in these animals (20) suggests that an associated MHC class I molecule would also have to be present at a high frequency. If 20% of *Shigella*-infected humans were to develop SpAs, all of them would have to be B27-positive (17). If Gogo-B01 is associated with disease, the fact that a very small percentage of Gogo-B01-positive animals develop disease suggests that other genes may be involved in susceptibility, as is also observed in humans. Studies currently under way in humans to discover the nature of non MHC genes associated with development of SpAs (55) may provide further insight into the mechanism of disease in humans and gorillas.

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**Table III. Amino acid residues of the P2 environment for alleles with motifs that show arginine occupancy at P2**

<table>
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<tr>
<th>Amino Acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<th>7</th>
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<td>354.74</td>
<td>103.26</td>
<td>68.96</td>
<td>61.29</td>
<td>46.25</td>
<td>31.19</td>
<td>29.69</td>
<td>27.72</td>
<td>13.55</td>
</tr>
<tr>
<td>S</td>
<td>82.54</td>
<td>15.52</td>
<td>22.69</td>
<td>28.93</td>
<td>28.76</td>
<td>34.57</td>
<td>32.32</td>
<td>28.79</td>
<td>19.14</td>
<td>11.45</td>
</tr>
<tr>
<td>T</td>
<td>119.91</td>
<td>20.56</td>
<td>22.42</td>
<td>17.21</td>
<td>24.39</td>
<td>32.26</td>
<td>33.90</td>
<td>88.97</td>
<td>49.32</td>
<td>22.27</td>
</tr>
<tr>
<td>V</td>
<td>16.89</td>
<td>5.16</td>
<td>24.21</td>
<td>27.82</td>
<td>40.25</td>
<td>36.40</td>
<td>27.68</td>
<td>24.33</td>
<td>12.59</td>
<td>6.57</td>
</tr>
<tr>
<td>W</td>
<td>3.15</td>
<td>7.32</td>
<td>8.47</td>
<td>8.45</td>
<td>8.79</td>
<td>10.01</td>
<td>8.05</td>
<td>2.12</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>Y</td>
<td>15.30</td>
<td>35.72</td>
<td>73.83</td>
<td>34.85</td>
<td>31.00</td>
<td>13.71</td>
<td>29.58</td>
<td>19.88</td>
<td>26.86</td>
<td>13.30</td>
</tr>
</tbody>
</table>

---

a One letter code for residues.

b Raw data of pool sequencing of peptides extracted from Gogo-B*0101 interpreted according to previously outlined methods (39). Values increasing (≥50% or 0.02 SDs) are underlined. Values increasing >100% are bolded.

c See Materials and Methods for formula used to calculate average relative frequency values. Underlined values indicate those which exceed 1.00 by at least 2 SDs. Average SD = 0.26 ± 0.26.

d Average relative frequencies for aspartate (D), glycine (G), and threonine (T) could not be calculated because signals were obscured in the first experiment by a nonpeptide contaminating peak. Values shown for these amino acids are the relative frequencies from the second experiment. Cysteine residues were not modified prior to pool sequencing and, therefore, were not detected.
The question of whether HLA-B27 acts as an Ag-presenting molecule in the mechanism of SpAs remains a central and, as yet, unanswered question. The peptide binding capabilities of the various B27 subtypes either associated with disease susceptibility or not associated with disease susceptibility have been described (Reviewed in Refs. 3 and 48). Although the results presented here do not show that Gogo-B01 is associated with susceptibility to disease, they are consistent with recent data from transgenic rats (8) that suggest that the peptides bound by the B27 molecule play a role in the mechanism of disease.

Table V. Peptide binding motif of Gogo-B*0101

<table>
<thead>
<tr>
<th>Residue</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>C Terminus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gogo-B*0101</td>
<td>R</td>
<td>P</td>
<td>E</td>
<td>N</td>
<td>F</td>
<td>M</td>
<td>A</td>
<td>V</td>
<td>G</td>
<td>L</td>
<td>Y</td>
</tr>
<tr>
<td>Strong'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak''</td>
<td>Y</td>
<td>H</td>
<td>F</td>
<td>Q</td>
<td>I</td>
<td>N</td>
<td>E</td>
<td>H</td>
<td>K</td>
<td>E</td>
<td>Q</td>
</tr>
<tr>
<td>HLA-B*2702'</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other preferred residues</td>
<td>K</td>
<td>F</td>
<td>G</td>
<td>I</td>
<td>I</td>
<td>Y</td>
<td>K</td>
<td>L</td>
<td>P</td>
<td>K</td>
<td>V</td>
</tr>
<tr>
<td>d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

' C terminus preference was evident from sequence of individual ligands. See Table VI.
'' Determination of peptide binding motif according to previously established methods (39–41). Dominant: >3.5-fold increase over the previous cycle; anchor residue is shown in bold and is applied if a position reveals a strong signal for only one residue as is the case with R at P2 (see Table IV); Strong: >2-fold increase over the previous cycle; Weak: 1.5- to 2-fold increase over the previous cycle.

The question of whether HLA-B27 acts as an Ag-presenting molecule in the mechanism of SpAs remains a central and, as yet, unanswered question. The peptide binding capabilities of the various B27 subtypes either associated with disease susceptibility or not associated with disease susceptibility have been described (Reviewed in Refs. 3 and 48). Although the results presented here do not show that Gogo-B01 is associated with susceptibility to disease, they are consistent with recent data from transgenic rats (8) that suggest that the peptides bound by the B27 molecule play a role in the mechanism of disease.

Table VI. Peptides bound by Gogo-B*0101

<table>
<thead>
<tr>
<th>Source</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>No match</td>
<td>S D T C S M D T W</td>
</tr>
<tr>
<td>Human uridine nucleotide receptor, gi 1117913</td>
<td>P E D S S C R W</td>
</tr>
<tr>
<td>No match</td>
<td>P V G P G S M P T W</td>
</tr>
<tr>
<td>Human 6-6 fatty acid desaturase, gi 4406528</td>
<td>I R F L E S H W</td>
</tr>
<tr>
<td>Human serine/threonine protein kinase pLK-1 506–514, gi423046</td>
<td>A R L P Y L R T W</td>
</tr>
<tr>
<td>No exact match</td>
<td>R P M A F Y S W</td>
</tr>
<tr>
<td>No exact match</td>
<td>S D A Q P A L/I T S W</td>
</tr>
<tr>
<td>Human tubulin α-4 chain, AA722099</td>
<td>S R C H L G A</td>
</tr>
<tr>
<td>Chromosome 7, HELA cDNA library AA078075.1</td>
<td>K R C S P Y</td>
</tr>
<tr>
<td>Similar but not exact match to serine threonine kinase</td>
<td>T R F E G Y F</td>
</tr>
<tr>
<td>No exact match</td>
<td>T R F E G Y F</td>
</tr>
<tr>
<td>Homo sapiens hgsc, working draft AC007683.2</td>
<td>W P G P A V S Z W</td>
</tr>
</tbody>
</table>
Interestingly, a strong C-terminal W residue was revealed in the sequence of individual ligands eluted from Gogo-B*0101. This observation is consistent with results from peptides sequenced from HLA-B15 allotypes that showed that a S116 residue correlated with a strong C-terminal anchor residue (41, 49). Although the motif of HLA-B*2702 demonstrates a dominant W at P9 (11) and this is also a preferred residue in the motif of HLA-B*2703 (50, 51), few peptides or T cell epitopes identified to date contain this C-terminal residue (12, 52). Further studies are necessary to elucidate a possible role in disease pathogenesis for peptides containing a C-terminal W.

The role of the free C67 in the B27 B pocket in disease mechanism has been under investigation. Studies suggest that the free C67 can be modified due to its chemical reactivity (53), and homocysteine-modified B27 can be recognized by homocysteine-specific CTL in vivo.

### Table VII. Capacity of Gogo-B*0101-positive cell lines to bind a panel of peptides

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Origin</th>
<th>Sequence</th>
<th>IC₅₀ (nM)</th>
<th>721.221-Gogo-B*0101</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>153.07</td>
<td>SIV (B27-like)</td>
<td>IRYPKTFGW</td>
<td>34</td>
<td>54</td>
<td>60</td>
</tr>
<tr>
<td>155.01</td>
<td>SIV (B27-like)</td>
<td>IRFKKTGW</td>
<td>12</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>330-1</td>
<td>Human Fau protein (B27)</td>
<td>RRFVNNVYPTF</td>
<td>64</td>
<td>196</td>
<td>11</td>
</tr>
<tr>
<td>330-3</td>
<td>HBNPBβ-subunit 35–43 (B27)</td>
<td>SRDKTIIMW</td>
<td>31</td>
<td>128</td>
<td>11</td>
</tr>
<tr>
<td>405.1</td>
<td>Human actin (B27)</td>
<td>KRGLILKLY</td>
<td>316</td>
<td>668</td>
<td>11</td>
</tr>
<tr>
<td>405.2</td>
<td>Unknown (B27)</td>
<td>GRFKLIVLY</td>
<td>1572</td>
<td>3091</td>
<td>11</td>
</tr>
<tr>
<td>405.5</td>
<td>HIV env 584–592 (B27)</td>
<td>ERYLKDQQL</td>
<td>7972</td>
<td>14579</td>
<td>61</td>
</tr>
<tr>
<td>405.6</td>
<td>Flu Histone H3.3 (B27)</td>
<td>RRYQKSTEL</td>
<td>26</td>
<td>53</td>
<td>10</td>
</tr>
<tr>
<td>5423.3</td>
<td>HFPS 191–199 (B27)</td>
<td>KRYKSVKCY</td>
<td>36</td>
<td>100</td>
<td>11</td>
</tr>
<tr>
<td>5423.4</td>
<td>Human cytochrome C oxidase (B27)</td>
<td>KRKKAYADF</td>
<td>3267</td>
<td>6115</td>
<td>11</td>
</tr>
<tr>
<td>155.01</td>
<td>SIV (B27-like)</td>
<td>IRFPKTFGW</td>
<td>12</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>Chiron 50</td>
<td>SIV with substitution</td>
<td>IFFPKTFGW</td>
<td>1836⁶</td>
<td>3274</td>
<td>⁶</td>
</tr>
<tr>
<td>Chiron 51</td>
<td>SIV with substitution</td>
<td>ILFPKTFGW</td>
<td>423</td>
<td>998</td>
<td></td>
</tr>
<tr>
<td>Chiron 52</td>
<td>SIV with substitution</td>
<td>IFFPKTFGW</td>
<td>2108</td>
<td>3174</td>
<td></td>
</tr>
<tr>
<td>Chiron 53</td>
<td>SIV with substitution</td>
<td>INFPKTFGW</td>
<td>17064</td>
<td>23443</td>
<td></td>
</tr>
<tr>
<td>Chiron 54</td>
<td>SIV with substitution</td>
<td>IDFPKTFGW</td>
<td>65727</td>
<td>107383</td>
<td></td>
</tr>
</tbody>
</table>

*Experiments performed with radiolabelled 153.07 IRYPKTFGW.

⁶Numbers in bold indicate three times or greater decrease in binding capacity.

### FIGURE 3. Molecular model of Gogo-B*0101 compared with that of HLA-B*27. In A–C, the top view of the PBR is shown with negative charge in red, positive charge in blue. In C, substitutions that result in a difference in charge or size are shown in pink, changes that remain similar in charge and size are shown in light blue. In D and E, the B pocket environment is shown with the peptide and it’s P2 side chain in pink.
the role in disease susceptibility of this molecule and the peptides associated with disease susceptibility in gorillas. However, given the humans. It is unclear from this study whether Gogo-B01 is asso-
cussions and critical review of initial versions of this manuscript.

-associated SpAs in

In conclusion, here we present data showing the remarkable
as indicated by DNA-DNA hybridization.

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