Endogenous Processing and Presentation of T-cell Epitopes from *Chlamydia trachomatis* with Relevance in HLA-B27-associated Reactive Arthritis*

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*Chlamydia trachomatis* triggers reactive arthritis, a spondyloarthropathy linked to the human major histocompatibility complex molecule HLA-B27, through an unknown mechanism that might involve molecular mimicry between chlamydial and self-derived HLA-B27 ligands. *Chlamydia*-specific CD8 T-cells are found in reactive arthritis patients, but the immunogenic epitopes are unknown. A previous screening of the chlamydial genome for putative HLA-B27 ligands predicted multiple peptides that were recognized in vitro by CD8 T-lymphocytes from patients. Here stable transfectants expressing bacterial fusion proteins in human cells were generated to investigate the endogenous processing and presentation by HLA-B27 of two such epitopes through comparative immunoproteomics of HLA-B27-bound peptide repertoires. A predicted T-cell epitope, from the CT610 gene product, was presented by HLA-B27. This is, to our knowledge, the first endogenously processed epitope involved in HLA-B27-restricted responses against *C. trachomatis* in reactive arthritis. A second predicted epitope, from the CT63 gene product, was not detected. Instead a non-predicted nonamer from the same protein was identified. Both bacterial peptides showed very high homology with human sequences containing the HLA-B27 binding motif. Thus, expression and intracellular processing of chlamydial proteins into human cells allowed us to identify two bacterial HLA-B27 ligands, including the first endogenous T-cell epitope from *C. trachomatis* involved in spondyloarthropathy. That human proteins contain sequences mimicking chlamydial T-cell epitopes suggests a basis for an autoimmune component of *Chlamydia*-induced HLA-B27-associated disease. *Molecular & Cellular Proteomics* 8:1850–1859, 2009.

*Chlamydia trachomatis* is an obligate intracellular parasite that infects the urogenital epithelium. It is a very common pathogen and one frequently inducing reactive arthritis (ReA)\(^1\) (1). Multiple strategies, including down-regulation of major histocompatibility complex (MHC) class I and class II expression (2–4) and persistence, have been developed by the bacteria to evade the immune system. Yet both CD4\(^+\) and CD8\(^+\) T-cell responses are activated upon infection (5). In particular, HLA-B27-restricted CD8\(^+\) T-lymphocytes are found in patients with *Chlamydia*-induced ReA (6, 7). The role of these cells in the pathogenesis and evolution of ReA to chronic disease is probably mediated by IFN-γ. Secretion of this cytokine is critical for the protective immunity against *Chlamydia* (8) because it inhibits the bacterial growth (9). However, this is often insufficient to promote complete clearance of *C. trachomatis*, and actually IFN-γ-induced depletion of the tryptophan pool induces the differentiation of the metabolically active reticular bodies to persistent forms (10), which sustain chronic infection and ReA. The high prevalence of HLA-B27 among patients with *Chlamydia*-induced ReA (11), especially in its chronic form, suggests a pathogenetic mechanism based on interactive effects of the bacteria and HLA-B27 that seems unrelated to the capacity of *C. trachomatis* to infect or replicate into HLA-B27-positive cells (12). One such mechanism could be T-cell-mediated autoimmunity elicited by molecular/anti- genic mimicry between chlamydial and self-derived HLA-B27 ligands. Antigenic mimicry between chlamydial and homologous α-myosin-derived peptides is crucial to inducing autoimmune myocarditis in mice (13). Breakdown of cytotoxic T-lymphocyte (CTL) tolerance to HLA-B27 was observed in transgenic rats upon exposure to *C. trachomatis* (14). Cross-reactivity between HLA-B27-restricted *Chlamydia*-specific CTL with self-derived HLA-B27 epitopes has not been reported. However, a biochemical basis for it was provided by the finding of an endogenously processed and presented peptide from the DNA primase of

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\(^{1}\) The abbreviations used are: ReA, reactive arthritis; MHC, major histocompatibility complex; CTL, cytotoxic T-lymphocyte; GFP, green fluorescent protein; NQRA, Na\(^+\)-translocating NADH-quinone reductase subunit A; PqqC, pyrroloquinoline-quinone synthase-like protein; htMDM, high throughput mass data manager; s/n, signal-to-noise ratio; IFN, interferon; fr., fraction; E3, ubiquitin-protein isopeptide ligase; TCR, T-cell antigen receptor.
C. trachomatis with high homology to a self-derived HLA-B27 ligand (15, 16).

Because of the likely involvement of HLA-B27 in the pathogenesis of chronically evolving ReA, the role of CD8+ T-cell responses in the protective immunity against C. trachomatis and the presence of HLA-B27-restricted T-cells in patients with Chlamydia-induced ReA, the identification of relevant chlamydial epitopes becomes crucial to establish the pathogenetic mechanism of this disease. Unfortunately a direct analysis of chlamydial HLA-B27 ligands expressed on infected cells is exceedingly difficult because of their extremely low amounts, which challenge even the most sensitive techniques of MS. In the case of Chlamydia, the situation is further complicated by the down-regulation of MHC class I expression shortly after infection (3, 4). To our knowledge, only one MHC class I ligand was recently identified, in the mouse system, from Chlamydia muridarum-infected cells using state-of-the-art MS techniques (17). Due in part to this difficulty, alternative approaches, such as expression cloning and synthetic peptide epitope mapping (18, 19) or MHC class I tetramer arrays (20), have been used to identify MHC class I-restricted chlamydial T-cell epitopes in mice. In a previous study (6) predictive algorithms were used to screen the whole genome of C. trachomatis for nonamer peptide sequences containing the HLA-B*2705 binding motif and a high probability of being generated by proteasomal cleavage. This led to identifying multiple sequences that, when used as synthetic peptides in vitro, stimulated CD8+ T-cells from patients with Chlamydia-induced ReA. Such cells could also be detected in the synovial fluid of these patients using HLA-B27 tetramers complexed to some of these peptides (7).

Although these strategies identify chlamydial sequences that are recognized by CD8+ T-cells they do not prove that these peptides are the endogenously processed epitopes that activated the natural T-cell responses to the bacteria in vivo. Because of the intrinsic cross-reactivity of T-cells (21, 22), it is conceivable that synthetic peptides recognized in vitro may be different from the natural epitopes generated by endogenous processing of the chlamydial proteins that elicit the HLA-B27-restricted T-cell responses in ReA patients. To investigate this issue we focused on two predicted epitopes (6).

Isolation of HLA-B27-bound Peptides—B*2705-bound peptides were isolated from 1–2 × 10^6 C1R-B*2705 cells and C1R-B*2705 transfectants expressing chlamydial proteins as described previously (24). Briefly cells were lysed in 1% Igepal CA-630 (Sigma), 20 mM Tris/HCl buffer, 150 mM NaCl, pH 7.5 in the presence of a mixture of protease inhibitors. HLA-B27-peptide complexes were isolated by affinity chromatography of the soluble fraction with the W6/32 monoclonal antibody (IgG2a, specific for a monomorphic HLA-A,B,C determinant) (25). HLA-B27-bound peptides were eluted at room temperature with 0.1% aqueous TFA, concentrated with Centricon 3 (Amicon, Beverly, MA), and fractionated by HPLC as described previously (26).

Molecular & Cellular Proteomics 8.8

Automated Comparative Analysis of MALDI-TOF MS Data—A systematic comparison of the MALDI-TOF MS spectra of the HPLC fractions from HLA-B27-bound peptide pools was carried out with an algorithm programmed in Visual Basic 6.0 and implemented as a macro within Microsoft® Excel, designated as high throughput mass data manager (htMMD). This tool recognizes the raw numeric data list from each individual mass spectrum from the MALDI-TOF/TOF mass spectrometer software. First, for comparison of distinct peptide pools, the ion peaks with a signal-to-noise ratio (s/n) smaller than 3-fold, relative to the average s/n of the MS spectrum, were removed. Second, ion peaks with a s/n below 5% of the maximal s/n observed in the MS spectrum were also ignored. Third, an exclusion list of matrix-related ion peaks was generated from direct MS analysis of the matrix alone. The ion peaks whose m/z was identical (±0.4) to those in this list were excluded. For comparison of MS spectra corresponding to a correlation (±1) HPLC fractions from two peptide pools, ion peaks were considered to reflect identical peptides when their observed m/z were within a user-defined range, which depends on the
**RESULTS**

Identification of Endogenously Processed HLA-B27-restricted T-cell Epitopes from *C. trachomatis in Human Cells*—The following strategy (Fig. 1) was originally developed in a previous study from our laboratory (16) and was here adapted to investigate the endogenous processing and presentation of HLA-B27 ligands from *C. trachomatis* recognized by CD8$^+$ T-cells from ReA patients (6). 1) B*2705-C1R cells were used as recipients to generate stable transfectants expressing bacterial fusion proteins. 2) To achieve efficient expression of chlamydial proteins in human cells, fusion constructs were generated in which the GFP coding sequence was placed at the 5'end of the corresponding chlamydial gene. 3) B*2705-bound peptide repertoires were isolated only in the bacterial protein transfectant (5). The amino acid sequence of the differentially expressed peptides was determined by MS/MS fragmentation analysis (6). The corresponding peptide was chemically synthesized, and the MS/MS spectrum of the synthetic peptide was compared with that of the endogenous ligand (7).

**Fig. 1. Summary of the strategy used to identify endogenously processed HLA-B27 ligands from *C. trachomatis*.** The B*2705-bound peptide repertoires were isolated in parallel experiments from about 1–2 × 10$^{10}$ C1R transfectant cells expressing (2) or not expressing (1) a GFP bacterial fusion protein by immunopurification of HLA-B27 and acid extraction (3). The peptide pools were fractionated by HPLC under identical conditions and in consecutive runs (4). Each individual HPLC fraction was analyzed by MALDI-TOF MS, and the corresponding MS spectra of correlative fractions were systematically compared to identify peptides found only in the bacterial protein transfectant (5). The amino acid sequence of the differentially expressed peptides was determined by MS/MS fragmentation analysis (6). The corresponding peptide was chemically synthesized, and the MS/MS spectrum of the synthetic peptide was compared with that of the endogenous ligand (7).

The htMDM tool also allows searching for specific ion peaks, such as candidate peptide epitopes or peptides of a particular size. A user-defined inclusion list with the m/z values of interest can be stored and used to automatically screen all the MS spectra for the presence of ion peaks with identical (±0.4) m/z for their quick identification. The htMDM output consists of a listing of filtered m/z values with the corresponding retention time or HPLC fraction number. Matches with the exclusion and inclusion directories are marked as well as shared and specific m/z signals from each of the peptide repertoires compared. To verify the performance of the htMDM tool, both automatic and manual comparisons were carried out.

**Peptide Sequencing—MALDI-TOF-TOF** MS/MS fragmentation spectra were acquired with Data Explorer, version 4.9, at 1 kV using collision-induced dissociation with atmospheric air and a precursor mass window of ±2.5 Da. A s/n of 10 was used for processing data. Interpretation of the MS/MS spectra was done manually but was assisted by various tools as follows. Manual inspection of the spectrum usually allowed us to derive a tentative sequence. This was used to screen the chlamydial protein sequences NQRA and PqqC (UniProtKB/Swiss-Prot accession numbers O84639 and O84616, respectively) for a possible match using the Mascot software, version 2.2 (Matrix Science Inc, Boston, MA). The MS-product tool (version 5.1.8) (University of California San Francisco), which generates a list of theoretical fragment ions from a given peptide sequence, was then used to match the candidate sequences from the chlamydial proteins to our experimental MS/MS spectra. The assigned sequences were formally confirmed by comparing the experimental MS/MS spectra with those of the corresponding synthetic peptides.

**Homology Searches—** The sequence homology between chlamydial peptides and human proteins was analyzed in the non-redundant protein database using the Blastp software (National Center for Biotechnology Information, Bethesda, MD). The most recent versions of the database (Release 33, January 22, 2009) and search algorithm available were used.
expected retention time of the peptide epitope and in neighbor fractions using a narrow gate corresponding to the m/z of the peptide.

Expression of Chlamydial Fusion Proteins in B*2705-C1R Cells—We focused on the CT634 and CT610 genes, coding for the NQRA and PqqC proteins, respectively (27), which contain sequences recognized in vitro as exogenously added synthetic peptides by CD8+ T-cells from ReA patients (6). In addition, PqqC stimulated IFN-γ secretion by peripheral blood mononuclear cells from Chlamydia-infected patients with urogenital infection (28). NQRA is a polypeptide of 465 residues involved in Na+ transport from the cytoplasm to the periplasm. PqqC is a polypeptide of 231 residues that is expressed in the native state as a homodimer. It is a Chlamydia-specific toxin that is secreted into the host cytoplasm and induces apoptosis in mammalian cell lines (29, 30). Both proteins are natural components of elementary bodies (31). GFP-chlamydial gene constructs (Fig. 2A) were transfected into B*2705-C1R cells. The stable expression of the fusion proteins was established by flow cytometry (Fig. 2B) and Western blot analyses (Fig. 2C).

A Single HLA-B27 Chlamydial Ligand Differentially Detected in NQRA Transfectants Is Unrelated to the Predicted T-cell Epitope—HLA-B27-bound peptide pools were isolated from B*2705-C1R cells, B*2705-C1R transfected with GFP alone, and B*2705-C1R expressing the NQRA fusion protein. A total of 4895 ion peaks from the NQRA transfectant were compared with 4958 and 4526 ion peaks from untransfected B*2705-C1R and GFP transfectants, respectively. Only one ion peak from the NQRA transfectant, with m/z 1054.7 in HPLC fr. 208, was absent from B*2705-C1R cells and from the transfectant expressing GFP alone (not shown). The amino acid sequence of this peptide was determined by MALDI-TOF/TOF MS/MS and assigned as a nonamer, KRALLEIVI, spanning residues 86–94 of the chlamydial NQRA protein. The assignment was confirmed by comparison of the MS/MS spectrum with that of the corresponding synthetic peptide (Fig. 3B). The NQRA-(86–94) peptide was unrelated to the predicted T-cell epitope from this protein spanning residues 330–338 (MRDHTITLL) (6). The endogenously processed peptide was not predicted in that report.
The possibility that low amounts of NQRA-(330–338) might have escaped detection in our comparison was addressed by determining the elution position of the synthetic peptide in the same chromatographic conditions used for fractionation of the HLA-B27-bound peptide pools and looking for the corresponding ion peak in the MALDI-TOF MS spectra of that and neighbor (H11006) HPLC fractions from the bacterial transfectant. No evidence for an ion peak consistent with NQRA-(330–338) was found within the detection limits of the instrument used (data not shown). Of note, this peptide was very inefficient in inducing HLA-B27 refolding in vitro (7), suggesting that it is a low affinity ligand.

The Predicted T-cell Epitope from the Chlamydial PqqC Protein Is Endogenously Processed and Presented by HLA-B27—The same strategy was used for determining the endogenous processing of HLA-B27 ligands from PqqC. Upon systematic comparison of the MALDI-TOF MS spectra from the HPLC-fractionated HLA-B27-bound peptide pools involving 4905 ion peaks from the PqqC transfectant, two ion peaks not found in the corresponding HPLC fractions from B*2705-C1R cells (Figs. 4A and 5A) or B*2705-C1R expressing GFP alone (data not shown) were detected. One of these peaks, with \( m/z \) 1054.7 in HPLC fr. 184 from the PqqC transfectant (Fig. 4A), had the same molecular mass and chromatographic retention time as the predicted T-cell epitope from this protein spanning residues 70–78, ARKLLLDNL (6). The amino acid sequence of the differentially expressed B*2705 ligand was determined by MALDI-TOF/TOF MS/MS, and its assignment as the chlamydial T-cell epitope was formally confirmed by comparison of the MS/MS spectrum with that of the corresponding synthetic peptide (Fig. 4B).
The second differential ion peak, with \( m/z 1183.5 \), was found in HPLC fr. 182 from the PqqC transfectant (Fig. 5A). The amino acid sequence of this peptide was determined to be TRFSYAEYF (Fig. 5B). It was unrelated to the chlamydial PqqC protein but matched exactly the N-terminal region of the human ankyrin repeat and SOCS box protein 2 (ASB2; UniProtKB/Swiss-Prot accession number Q96Q27). This is a likely substrate recognition component of E3 ubiquitin-protein ligase complexes (32).

NQRA-(86–94) and PqqC-(70–78) Are Highly Homologous to Human Protein Sequences with the HLA-B27 Binding Motif—The chlamydial B*2705 ligand NQRA-(86–94) was screened for homologous sequences against the human proteome database. This search identified a highly homologous sequence (Table I) from the DnaJ homolog DJB14 (UniProtKB/Swiss-Prot accession number Q96Q27). This is a likely substrate recognition component of E3 ubiquitin-protein ligase complexes (32).

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Fig. 4. A single endogenous HLA-B27 ligand identified from the chlamydial PqqC protein shows identity with the predicted T-cell epitope. A, MALDI-TOF MS spectra of fr. 184 from the HLA-B27-bound peptide pools isolated from the PqqC transfectant (above) and untransfected B*2705-C1R cells (below) showing a single detected ion peak, at \( m/z 1055.7 \), differentially present in the bacterial transfectant. Only the relevant sections of the spectra are shown. This ion peak was also not detected in the adjacent fractions 183 and 185 of the untransfected cells (not shown). The ion peak at \( m/z 1053.6 \), labeled with an asterisk, was a shared ligand found in the adjacent fr. 185 from B*2705-C1R. B, MALDI-TOF/TOF MS/MS spectrum of the ion peak at \( m/z 1055.7 \) from the bacterial PqqC transfectant showing the assigned sequence (above) and MS/MS spectrum of the corresponding synthetic peptide (below). The periodically spaced ion peaks in the upper MS/MS spectrum are related to each other by 44 \( m/z \) units with a related series differing by 16 \( m/z \) units. They arise from traces of the Igepal CA-630 detergent (octylphenyl polyethylene glycol, \( (C_2H_4O)nC_14H_{22}O \)) used for cell lysis.

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NQRA-(86–94) and PqqC-(70–78) Are Highly Homologous to Human Protein Sequences with the HLA-B27 Binding Motif—The chlamydial B*2705 ligand NQRA-(86–94) was screened for homologous sequences against the human proteome database. This search identified a highly homologous sequence (Table I) from the DnaJ homolog DJB14 (UniProtKB/Swiss-Prot accession number Q8TBM8). The following features confer special significance to this homology. First, the human counterpart contains the binding motif of HLA-B27, including Arg-2, a nonpolar C-terminal residue (Met-9), and a small N-terminal residue (Ala-1) that is frequent among B*2705 ligands (33). Second, six of nine consecutive residues in the human homolog are identical to the chlamydial peptide. These include Arg-2, the secondary anchor residue Ala-3, and the central region (residues 4–7), which is recognized by the T-cell antigen receptor (TCR) (34). Third, this stretch of identical residues is followed by two conservative differences at positions 8 and 9 involving aliphatic residues.

An analogous search was performed for the chlamydial PqqC-(70–78) epitope. Again this peptide showed a very high homology with residues 172–180 of the exportin 6 (UniProtKB/Swiss-Prot accession number Q96QU8). This human sequence also shows six of nine identical residues, including the...
Arg-2 motif of HLA-B27 ligands, the secondary anchor residue Lys-3, and identity in the whole central region. The two sequences also show chemically similar aliphatic residues at the anchor positions 1 and 9 and polar residues at the accessible position 8 (Table I). Thus, both endogenously processed HLA-B27 ligands from C. trachomatis are highly homologous to human sequences containing the HLA-B27 binding motif and many features that might favor TCR cross-recognition.

**DISCUSSION**

The recognition of HLA-B27-restricted Chlamydia antigens by CTL is potentially important in the pathogenesis of spon-

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**TABLE I**

Homology of naturally processed HLA-B27 ligands from C. trachomatis with human protein sequences

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Identity</th>
<th>Organism</th>
<th>Parental protein (residues)</th>
<th>UniProtKB/Swiss-Prot accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRALLEIVI</td>
<td>C. trachomatis</td>
<td>Na⁺-translocating NADH-quinone reductase subunit A, NQRA-(86–94)</td>
<td>O84639</td>
<td></td>
</tr>
<tr>
<td>ARALLEIIM</td>
<td>Homo sapiens</td>
<td>DnaJ homolog protein, DNAJB14-(43–51)</td>
<td>Q8TBM8</td>
<td></td>
</tr>
<tr>
<td>ARKLLLLNL</td>
<td>C. trachomatis</td>
<td>Pyrroloquinoline-quinone synthase protein, PqqC-(70–78)</td>
<td>O84616</td>
<td></td>
</tr>
<tr>
<td>LRKLLLLQV</td>
<td>H. sapiens</td>
<td>Exportin-6 protein, Exp6-(172–180)</td>
<td>Q96QU8</td>
<td></td>
</tr>
</tbody>
</table>

* Residues in the human sequences identical to the bacterial peptides are underlined.
However, the actual contribution of these pathways to cross-

presentation remains ill defined (41). Thus, the generation of
HLA-B27 epitopes upon processing of endogenously synthe-
sized chlamydial proteins may be similar to antigen process-
ing through the cytosolic pathway as it occurs on antigen-
presenting cells upon natural infection. However, we cannot
rule out that processing of a fusion protein in a B cell line, as
analyzed in this study, might differ from processing of a
natural bacterial protein in dendritic cells. In Chlamydia-in-
fected host cells the most obvious candidates to reach the
cytosolic pathway for MHC class I-mediated antigen presen-
tation are the proteins secreted into the cytoplasm (44) or
those in the vacuolar membrane (18, 19). However, other
chlamydial proteins or protein fragments might reach the cy-
tosol through cross-presentation mechanisms that remain
largely undefined in non-professional antigen-presenting
cells.

Another question relates to the sensitivity of MS relative to
T-cell recognition. We grossly estimated that, in our exper-
imental conditions, the detection limit for HLA-B27 ligands
would be around 100–200 copies per cell. This might be 1–2
orders of magnitude below the sensitivity of CTL, which
might recognize only a few epitope copies per cell (39, 40).
Yet it is reasonable to assume that a transfected protein will
yield much more peptide copies presented by HLA-B27
than on infected cells because a much greater protein
amount will reach the cytosolic degradation pathway. Thus,
it is likely that our system can detect peptides that might be
presented upon natural infection in extremely low amounts.
For this reason, a candidate chlamydial peptide not found in
the B27-bound peptide pool in our analysis is unlikely to be
presented on infected cells. Yet this possibility cannot be
ruled out.

With the above considerations in mind, our results demon-
strate both the reach and the limitations of the epitope pre-
diction algorithms used on the chlamydial genome (6). Al-
though they identified at least one peptide, PqqC-(70–78),
they failed to predict another, NQRA-(86–94), and predicted
an epitope that is apparently not processed in vivo, NQRA-
(330–338). Thus, predictive algorithms for putative T-cell
epitopes can only be considered as a first step to orient an
experimental search for naturally processed antigens. To our
knowledge, PqqC-(70–78) is the first HLA-B27-restricted ant-
igen from C. trachomatis recognized by CD8+ T-cells from
ReA patients that has been shown to be endogenously pro-
cessed and presented in vivo. Yet this does not definitely
prove that PqqC-(70–78) is a target of the immune response to
C. trachomatis, which would require showing that the same
peptide is presented on Chlamydia-infected cells or that a
peptide-specific T-cell clone recognizes such cells.

We have previously shown that a peptide derived from the
chlamydial DNA primase with high homology to a natural
self-derived ligand of HLA-B27 is endogenously processed
and presented by this molecule, providing a sound basis for
molecular mimicry between chlamydial and self-derived HLA-
B27 ligands (16). The two chlamydial peptides endogenously processed and presented by HLA-B27 in the present study have closely related human sequences, including the same or similar anchor residues and identical TCR-interacting regions. This level of homology between short bacterial peptides and human proteins may be relatively frequent and not restricted to these examples (45). Whether the human homologs are actually processed as natural HLA-B27 ligands is a question of obvious relevance that was not addressed in this study. In the absence of a complete examination of this issue the significance of these homologies is unknown. However, they show that there are multiple possibilities for the generation of self-derived HLA-B27 ligands with obvious mimicry of naturally processed chlamydial epitopes.

We do not know the reason for the differential expression of a self-derived peptide in the PqqC transfectant. There are three possibilities. First, the peptide could be a shared ligand that was detected by chance only in this transfectant but not in the other cells analyzed. Second, the parental ASB2 protein of this self-derived ligand was up-regulated by chance in the particular PqqC transfectant used in our study. Third, up-regulation of ASB2 might have occurred as a specific consequence of the expression of the GFP-PqqC fusion protein. We consider the first possibility unlikely because the differential ion peak was observed in the same transfectant but not in any of the others in two distinct experiments involving independently obtained peptide pools. We cannot rule out the second possibility as it would require repeating this analysis in a second, independently obtained PqqC transfectant, which was not attempted. The third possibility would be consistent with the speculation that GFP-PqqC expression might up-regulate certain components of the ubiquitin/proteasome pathway of protein degradation to which ASB2 belongs. Because this issue is unresolved to the main point of this study, it was not further pursued.

In conclusion, expression of chlamydial proteins in HLA-B27-positive human cells is a reliable and sensitive way to identify endogenously processed bacterial ligands presented by HLA-B27 in vivo. Using this approach we demonstrated, for the first time to our knowledge, the endogenous processing and presentation of an HLA-B27-restricted epitope recognized by CD8+ T-cells from ReA patients but failed to confirm the endogenous generation of a second T-cell epitope identified by predictive algorithms. In contrast, we identified a non-predicted nonamer from the same parental protein that was endogenously processed and presented by HLA-B27, revealing the limitations of predictive methods for identifying natural MHC class I ligands and T-cell epitopes. The very close similarity of the two bacterial HLA-B27 ligands identified in this study to amino acid sequences containing the HLA-B27 binding motif in the human proteome suggests that this is a possible source of self-derived peptides mimicking chlamydial epitopes. This would be consistent with an autoimmune mechanism of ReA in which T-cell cross-reactivity between Chlamydia-specific and self-derived HLA-B27 ligands may play a crucial role.

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