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Molecular mimicry mediated by MHC class Ib molecules after infection with Gram-negative pathogens

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The development of many autoimmune diseases has been etiologically linked to exposure to infectious agents[1]. For example, a subset of patients with a history of *Salmonella* infection develop reactive arthritis[2–6]. The persistence of bacterial antigen in arthritic tissue and the isolation of *Salmonella* or *Yersinia* reactive CD8⁺ T cells from the joints of patients with reactive arthritis support the etiological link between Gram-negative bacterial infection and autoimmune disease[7,8]. Models proposed to account for the link between infection and autoimmunity include inflammation-induced presentation of cryptic self-epitopes, antigen persistence and molecular mimicry[1]. Several studies support molecular mimicry as a mechanism for the involvement of class II epitopes in infectious disease-induced self-reactivity[9–12]. Here, we have identified an immunodominant epitope derived from the *S. typhimurium* GroEL molecule. This epitope is presented by the mouse H2-T23-encoded class Ib molecule Qa-1 and was recognized by CD8⁺ cytotoxic T lymphocytes induced after natural infection. *S. typhimurium*-stimulated cytotoxic T lymphocytes recognizing the GroEL epitope cross-reacted with a peptide derived from mouse heat shock protein 60 and recognized stressed macrophages. Our results indicate involvement of MHC class Ib molecules in infection-induced autoimmune recognition and indicate a mechanism for the etiological link between Gram-negative bacterial infection and autoimmunity.

CD8⁺ T cells contribute to the protective immune response to infection with *Salmonella typhimurium*[13]. Characterization of this *S. typhimurium*-specific CTL response showed that most CD8⁺ T cells recognized bacterial epitopes presented on the class Ib molecule Qa-1^b. We generated a panel of Qa-1^b-restricted, *Salmonella*-specific CTL clones; clone SalTc1.69 recognized a bacterial epitope presented by Qa-1^b in a proteasome-sensitive and TAP (transporter associated with antigen processing)-dependent manner. This clone also recognizes cells infected with other Gram-negative pathogens, indicating the recognition of a conserved bacterial epitope. To identify the epitope, we isolated peptides bound to Qa-1^b in *Salmonella*-infected cells, separated them by reverse-phase high-performance liquid chromatography and tested fractions for the presence of the epitope by assaying for the ability to sensitize RMA-S cells for recognition by SalTc1.69 (ref. 14). We identified bioactive fractions and used MALDI (matrix-assisted laser desorption/ionization) mass spectroscopy to identify a complex mixture of peptide species (data not shown). We re-chromatographed the bioactive fractions using the ion-pairing agent heptafluorobutyric acid and identified a bioactive fraction (Fig. 1*a*, fraction 46). MALDI mass spectroscopy of fraction 46 identified a unique protonated molecular ion (MH+) of 1087.2 (Fig. 1*a*, inset) with a mass/charge ratio of 1087.2 (Fig. 1*a*, inset). Partial digestion with aminopeptidase M (Fig. 1*b*) or carboxypeptidase P (Fig. 1*c*) yielded amino-acid ladders from which we deduced the sequences GM(K/Q)FD and GY(L/I), respectively. We deduced the final sequence GM(K/Q)FDRGY(L/I) by insertion of the residue R from the molecular weight data. A database search identified a peptide of 1,086.2 Da, GMQFDRGYL, derived from residues 192–200 of the *Salmonella* GroEL protein (SalGroEL), and an analog peptide of similar molecular weight, GMKFDRGYI, from residues 216–224 of the mouse heat shock protein 60 (mhsp60). To distinguish between these two sequences, we digested samples *in situ* with trypsin and analyzed them by MALDI time-of-flight. Trypsin digestion yielded fragments with MH+ mass/charge ratios of 352.4 (GYL) and 753.9 (GMQFDR), a result consistent with *Salmonella* GroEL being the parental protein of the isolated peptide (Fig. 1*d*). The bacterial GroEL peptide has a methionine at the second position and a leucine at the ninth position, conforming to the Qa-1^b peptide-binding motif[15].

We tested synthetic peptides for their ability to sensitize Qa-1^b-transfected target cells for CTL lysis (Fig. 2*a*). The *Salmonella* GroEL peptide, but not a related *Mycobacteria* GroEL peptide nor the class I leader peptide Qdm, can sensitize L cells expressing Qa-1^b cells (L-Qa-1^b) to lysis by SalTc1.69. Target cells lacking Qa-1^b did not elicit target cell lysis (Fig. 2*e*). The *Salmonella*-specific CTL clone SalTc1.69 also recognized the mouse hsp60 peptide (Fig. 2*a* and *e*). Thus, clone SalTc1.69, in addition to recognizing a *Salmonella*-derived peptide epitope presented by Qa-1^b cells, also cross-recognizes a peptide epitope derived from a self-protein. This recognition pattern was not unique to clone SalTc1.69, as other independently isolated, Qa-1^b-restricted, *Salmonella*-specific CTL clones have this recognition pattern (Fig. 2*d*). Moreover, a *Salmonella*-specific CTL line (SalCTL3) and CTLs in splenocytes from *Salmonella*-infected mice recognized cells pulsed with SalGroEL and mhsp60 peptides (Fig. 2*b* and *c*). These data indicate that this peptide recognition pattern is a general feature of CTLs evoked after infection with *S. typhimurium*.

We addressed whether T cells recognizing GroEL/mhsp60 peptides presented by Qa-1^b were a dominant or minor population

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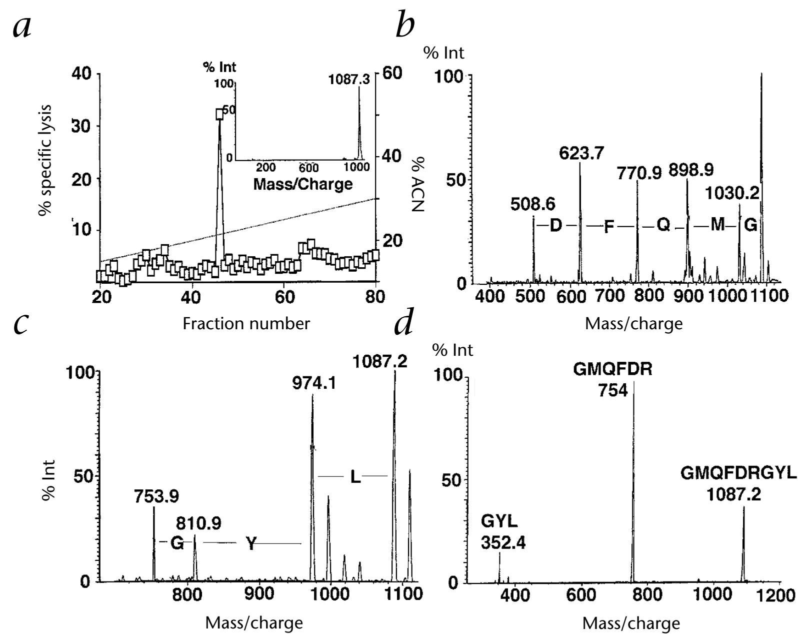
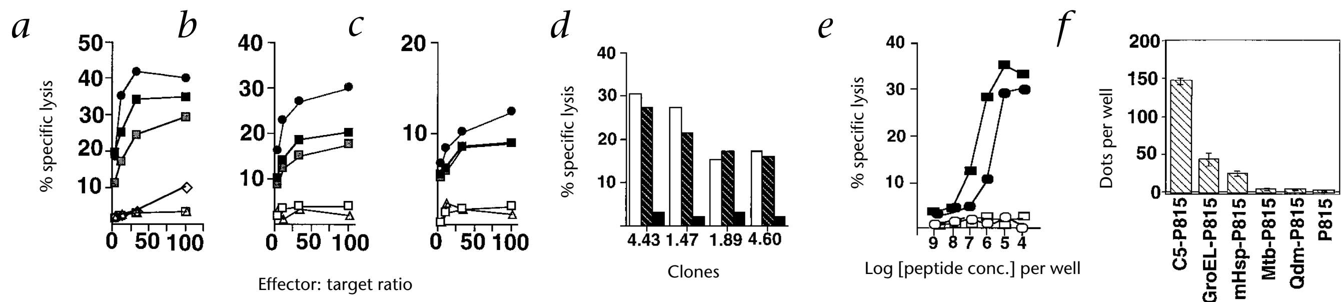


Fig. 1 Identification of *Salmonella*-derived epitopes presented by Qa-1^b. C5-infected L-Qa-1^b cells were lysed and peptides were extracted from anti-Qa-1^b immunoprecipitates and separated by reverse-phase high-performance liquid chromatography. ***a***, RMA-S cells were labeled with ^{51}Cr and pulsed with solubilized peptides, then SalTc1.69 was added (effector:target ratio,10:1) and a cytotoxic T cell assay was done. Fraction 33 was positive (data not shown) and contained peptide mixtures (as determined by MALDI time-of-flight), and was subjected to rechromatography using heptafluorobutyric acid as the ion-pairing agent[14]. Fraction 46 was then identified as positive. Dashed line, acetonitrile (ACN) gradient. Inset, mass/charge ratio of 1087.2 of a peptide from the bioactive fraction 46, identified by MALDI time-of-flight. ***b*** and ***c***, Peptide ladders generated from partial digestion of the peptide substrate in fraction 46 with aminopeptidase M (*b*) and carboxypeptidase P (*c*). ***d***, To differentiate between K and Q for the third position, near-complete *in situ* trypsin digestion was followed by mass spectrometry. %int (vertical axes), relative signal intensity; largest peak nomalized to 100%.

by determining the relative frequencies of *Salmonella*-specific and GroEL peptide-specific T cells in the spleens of infected mice. We measured the frequencies of antigen-specific, gamma interferon (IFN-γ)-secreting T cells by enzyme-linked immunospot (ELISPOT) assays of Qa-1^b-expressing P815 cells; these were 146.75/10^5 for *Salmonella typhimurium* strain C5 (C5)-infected cells; 43/10^5 for GroEL-pulsed cells and 24.5/10^5 for mhsp60-pulsed cells (Fig. 2*f*). We used limiting dilution analysis to estimate antigen-specific CTL frequencies; these were 15.6/10^5 (1/6,415) CD8$^+$ for C5-infected target cells, 10.8/10^5 (1/9,267) CD8$^+$ for GroEL-pulsed target cells and 9.3/10^5 (1/10,698) CD8$^+$ for mhsp60-pulsed target cells (Fig. 2*g*). Thus, after infection with *Salmonella*, Qa-1^b-restricted, GroEL/mhsp60 peptide-reactive CTLs are a substantial component of the host response to infection. The finding that Qa-1^b can present a peptide derived from GroEL to CD8$^+$ T cells is consistent with studies indicating



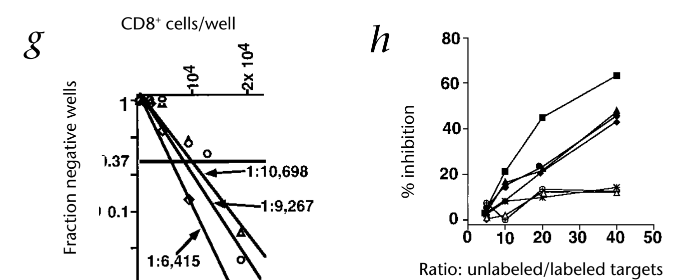


Fig. 2 Recognition of bacterial GroEL$_{192-200}$ and self homolog hsp60$_{216-224}$ by Qa-1^b-restricted CTLs. ***a–c***, ^{51}Cr-labeled L-Qa-1^b cells were infected with C5 (ò), left untreated (□) or pulsed with the peptides GroEL$_{192-200}$ (GMQFDRGYL; ■), mhsp60$_{216-224}$ (GMKFDRGYI; shaded squares), *M. tuberculosis* hsp65 peptide (GMRFDKGYI; ◇) or Qdm (AMAPRTLLL; △), then SalTc1.69 (*a*), the *Salmonella*-specific CTL line SalCTL3 (*b*) or *in vitro* re-stimulated splenocytes from C5-infected BALB/c mice (*c*) were added (effector:target ratios, horizontal axes). *c*, Data represent five analyses using spleen cells from independently infected mice. ***d***, Qa-1^b-restricted, *Salmonella*-specific CD8$^+$ CTL clones 4.43, 1.47, 1.89 and 4.60 were analyzed for their ability to lyse Qa-1^b expressing L cells either unpulsed (■) or pulsed with the GroEL (open bars) or mhsp60 (striped bars); effector:target ratio, 5:1. ***e***, L-Qa-1^b cells (filled symbols) or untransfected control L cells (open symbols) were pulsed with GroEL$_{192-200}$ (■) or mhsp60$_{216-224}$ (ovals) and tested for recognition by SalTc1.69 (effector:target ratio, 10:1). ***f***, Dots/well assay for measuring antigen-specific IFN-γ–secreting T cell frequencies. Immune splenocytes from infected BALB/c mice were co-cultivated with C5-infected P815 or P815 cells pulsed with or without peptide, and captured IFN-γ was detected. Data represent the averaged data from four independently infected mice. ***g***, Measurement of *Salmonella*- and peptide-specific CTL frequencies by limiting dilution analysis in immune BALB/c splenocytes against C5-infected (◇), GroLE peptide-pulsed (○) or mhsp60 peptide-pulsed (△) L-Qa-1^b cells as described[13]. The total number of CD8$^+$ T cells containing one antigen-specific CTL was extrapolated, where 37% (dotted line) of the wells of a given dilution yielded negative CTL activity. ***h***, Cold target inhibition of ^{51}Cr-labeled C5-infected P815 cells with unlabeled C5-infected P815 cells (■), uninfected (△) or C5-infected (◆) L-Qa1^b, uninfected (⊕) or C5-infected (*) Qa1^b-negative LV cells and GroEL (●) or mhsp (▲) peptide-pulsed L-Qa1^b. The long-term *Salmonella*-specific CTL line SalCTL3 was used at an effector:target ratio of 5:1.

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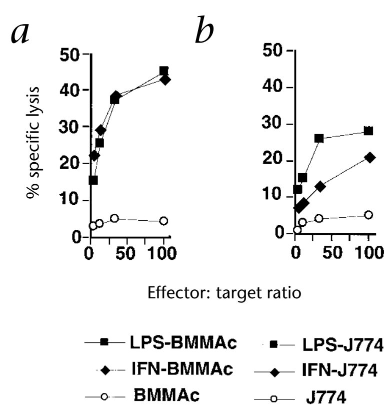

Fig. 3 Qa-1^b-restricted CTLs recognize uninfected stressed macrophages. Monolayers of bone marrow-derived macrophages from BDF1 mice (H-2$^{b/d}$; ***a***) and the J774 macrophage cell line (H-2^d; ***b***) were preincubated with IFN-γ or LPS, labeled and used as targets for recognition by SalTc1.69.

that Qa-1 can bind heat shock protein-derived peptides[16]. All the CD8$^+$ CTL clones selected for bacterial reactivity that recognized *Salmonella* GroEL also cross-recognized mhsp60 (Fig. 2*d* and data not shown). Furthermore, cold target-inhibition studies showed that Qa-1^b-expressing cold target cells pulsed with GroEL or mhsp60 peptides were equally effective in blocking recognition of labeled C5-infected target cells (Fig. 2*h*). These data, as well as the similar frequencies of GroEL- and mhsp60-reactive T cells, indicate that most GroEL/Qa-1^b-reactive CTLs evoked after *Salmonella* infection also cross-recognize mhsp60 and are thus self-reactive.

Salmonella CTL recognition of a peptide derived from a self stress protein indicates that such CTLs may also cross-recognize uninfected stressed target cells. To address this, we pretreated mouse bone marrow-derived macrophages and the J774 macrophage cell line with recombinant INF-γ or bacterial lipopolysaccharide (LPS) to induce stress protein expression[17]. SalTc1.69 recognized LPS- or IFN-γ-activated but not unactivated macrophages (Fig. 3). These results show the self-reactive nature of the hsp60-specific SalTc1.69 in stress conditions.

The mouse Qa-1 and human HLA-E molecules are functional counterparts, based on their ability to bind class I leader sequence-derived peptides and serve as a ligand for the CD94–NKG2A receptor complex[18,19]. Thus Qa-1 and HLA-E are essential in natural killer cell recognition of class I-deficient target cells. Our studies indicate that Qa-1 can also function in the binding and presentation of an immunodominant peptide epitope recognized by *Salmonella*-specific CD8$^+$ CTLs. A databank search showed that the GroEL peptide sequence GMQFDRGYL is highly conserved, being present in the GroEL molecules of several Gram-negative pathogens, including *S. typhimurium*, *S. typhi*, *Escherichia coli*, *Yersinia enterocolitica*, *Klebsiella pneumoniae* and *Helicobacter pylori*. Given that HLA-E and Qa-1 have identical peptide binding motifs, our observations using a mouse system may be applicable to understanding the human immune response to *Salmonella* and other related intracellular pathogens. Therefore, our studies expand the current understanding of class Ib molecule function to include involvement in the presentation of pathogen-derived epitopes and as a target for self-reactive T cells.

Members of the heat shock protein family are highly conserved among prokaryotes and eukaryotes. T cells recognizing bacterial heat shock proteins may cross-react with self heat shock proteins, and this response may be involved in the development of autoimmune disease. CD8$^+$ T cells primed *in vitro* with *Mycobacterium* hsp65 also recognize stressed macrophages[17]. *Mycobacteria* hsp65-reactive CTLs are found in BCG-infected mice[20]. These studies with *Salmonella* demonstrate that, during a natural infection with a virulent pathogen, a host antibacterial CTL response is generated that has the capability to be self-reactive towards cells that express endogenous hsp60. Such heat shock protein-specific, self-reactive, class Ib-restricted T cells may contribute to the etiological link between infection with Gram-negative pathogens and autoimmune diseases. Because Qa-1 has limited polymorphism, the development of these self-reactive CD8$^+$ T cells may be a common feature of immune responses in most hosts infected with *Salmonella* or other bacterial pathogens. Additional genetic and/or environmental factors likely influence the development of autoimmune disease in susceptible individuals. Our hypothesis is supported, in part, by the identification of MHC-unrestricted CD8$^+$ CTLs from the synovial fluid of a patient with *Yersinia*-induced reactive arthritis that recognize target cells infected with a variety of Gram-negative bacteria[21]. Also, T cells with specificity for heat shock proteins have been isolated from arthritic patients[22]. Given that *Salmonella* vectors have been used as vaccine carriers for a variety of exogenous epitopes[23], our findings should be considered in determining the overall safety of such a vaccine approach.

Methods

Peptide epitope identification by MALDI time-of-flight mass spectroscopy. C5-infected L-Qa-1^b cells (1×10^{10}) were lysed in 20 ml 1% Triton X-100 lysis buffer for 15 min at 4 °C and centrifuged at 10,000*g* for 15 min. After the supernatant was precleared with mouse immunoglobulin coupled to sepharose beads, Qa-1 immunoprecipitates were obtained by incubating samples for 2 h at 4 °C with rabbit antisera directed against the carboxyl terminus of Qa-1^b and coupled to sepharose beads[24]. Anti-Qa-1^b beads were washed sequentially in PBS and water and were acid-extracted by incubation for 15 min at 4 °C in 10% trifluoroacetic acid (Pierce, Rockford, Illinois). Extracts were size-fractionated using Amicon Centriprep-10 membrane filters. The low-molecular-weight fractions were 'pooled', lyophilized, resuspended in 1 ml 0.1% trifluoroacetic acid and 20% acetonitrile (J.T. Baker, Phillipsburg, New Jersey) in water and subjected to reverse-phase HPLC using a C18 (Vydac, Hesperia, California) column integrated into a HPLC (Waters, Milford, Massachusetts) system. Peptides were chromatographed, and bioactive fractions were identified as described[14]. RMA-S cells were cultured in RPMI supplemented with 10% fetal bovine serum and incubated at room temperature for 15 h, then labeled with 0.2 mCi ^{51}Cr. Of each fraction recovered from HPLC, 25 µl were used to pulse ^{51}Cr-labeled RMA-S cells (5,000 cells/well) for 60–90 min at room temperature. At the end of the incubation, SalT1.69 was added to each well at the effector:target ratio of 10:1. The plates were incubated for 4 h at 37 °C and the percent specific lysis was determined using standard techniques.

For experiments using target cells pulsed with synthetic peptides, 5,000 ^{51}Cr-labelled target cells were pulsed for 60–90 minutes at 37 °C with the sythetic peptides (final concentration of 10 µg/ml) in 96 well plates. At the end of the incubation, CTL effector populations were added to the wells. Mass spectral analysis used a Kratos (Manchester, England) Kompact MALDI/III mass spectrometer. An aliquot of the bioactive HPLC fraction was lyophilized and resuspended in 5 µl 0.1% trifluoroacetic acid and 20% acetonitrile. Then, 0.3 µl of the sample solution was deposited on a sample site and mixed with 0.3 µl saturated ammonium sulfate and 0.3 µl matrix ultraviolet chromophore (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% ethanol). The mixture was air-dried in a dark chamber at room temperature, and the sample was inserted into the mass spectrometer. These ions were then mass-analyzed using a time-of-flight mass spectrometer[14]. For ladder sequencing of peptides, samples were microdigested as follows: For aminopeptidase M, 0.3 µl peptide substrate was deposited on one of the sample sites, and 0.3 µl aminopeptidase M (2 µg/ml) was then added and the mixture incubated at room temperature for 180 s. For carboxypeptidase P, 0.3 µl substrate was deposited on the sample site, and then 0.3 µl ammonium citrate buffer (25 mM, pH7.5) and 0.3 µl enzyme (0.05 µg/µl) were added and incubated for 15 s. For trypsin, near-complete *in situ*

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trypsin digestion was accomplished by mixing 0.5 µl sample with 0.5 µl trypsin (0.05 µg/µl) and 2 µl ammonium bicarbonate buffer. An aliquot (0.3 µl) of the reaction mixture was deposited onto the sample grid. For all digestions, the reaction was stopped by the addition of 0.3 µl of the matrix solution and the sample was dried and mass-analyzed.

Generation and analysis of *Salmonella*-specific CTLs. *Salmonella*-specific CTLs were generated *in vivo* by vaccination of BALB/c mice with the avirulent *S. typhimurium* strain SL3235 followed by challenge with virulent *S. typhimurium* strain C5. At 7 d after C5 infection, spleen cells were isolated, re-stimulated *in vitro* with C5-infected J774 macrophages and assayed for CTL activity against C5-infected or peptide-pulsed target cells. Conditions for mouse infection, *in vitro* re-stimulation and the preparation of *Salmonella*-infected target cells have been described[13]. The isolation and characterization of the *Salmonella*-specific long-term line SalCTL3 and CTL clones have been described[13].

For experiments using target cells pulsed with synthetic peptides, 5,000 target cells were pulsed for 60–90 min with the synthetic peptides (final concentration, 10 µg/ml) before the addition of CTL effector populations. All synthetic peptides were obtained from Macromolecular Resources (Fort Collins, Colorado) with a purity greater than 95%. Cold target inhibition experiments were done as described using 5×10^3 ^{51}Cr-labeled, C5-infected P815 cells mixed thoroughly with 5×10^4 (10:1), 10^5 (20:1), 2×10^5 (40:1) or 4×10^5 (60:1) cold target cells before the addition of effector cells (effector:target ratio, 5:1)[13].

Bone marrow-derived macrophages were generated by culturing marrow mononuclear cells in DMEM supplemented with 20% fetal bovine serum and 30% NIH-3T3 culture supernatants. Cells were cultured for 7 d before being used as targets in CTL assays. Bone marrow derived macrophages or the J774 macrophage cell line were subjected to stress by being cultured in the presence of 1,000 units/ml IFN-γ or 20 µg/ml LPS for 48 h before being used in CTL assays.

Detection of peptide-specific T cells by ELISPOT and limiting dilution analysis. Measurement of antigen-specific T cells secreting recombinant IFN-γ by ELISPOT assays was done as described[25]. Multiscreen-HA plates (Millipore, Bedford, Massachusetts) were pre-coated overnight with monoclonal antibody against IFN-γ (R4-6A2) 10 µg/ml (PharMingen, San Diego, California). Immune splenocytes from infected BALB/c mice were depleted of red blood cells by NH_4Cl and were dispensed into each well of the plates, with the numbers of cells adjusted to 1×10^5 $CD8^+$ T cells per well. C5-infected P815 cells, P815 cells pulsed with 5 µg/well peptides, or P815 cells alone were added, in quadruplicates, at a concentration of 1×10^5 cells per well in the presence of 10% T-STIM culture supplement (Collaborative Biomedical Products, Bedford, Massachusetts) 24 h later. Captured IFN-γ was detected with biotinylated monoclonal antibody against IFN-γ (10 µg/ml XMG1.2; PharMingen, San Diego, California) and developed as described[25]. The numbers of dots per well were counted using the software NIH Image.

For measurement of peptide-specific CTL frequencies by limiting dilution, immune splenocytes from C5-infected BALB/c mice were cultured in 11 graded doses (48 wells per dose) with 6×10^5 irradiated (2,000 rads) syngeneic uninfected splenocytes and 5×10^4 irradiated (20,000 rads), C5-infected J774 cells in the presence of 20% T-STIM culture supplement. A week later, the contents of each well were assayed for cytolytic activity against 5×10^3 ^{51}Cr-labeled, C5-infected or peptide-pulsed (5 µg/ml) L-Qa-1^b cells. Wells were scored positive if the percent specific lysis was three standard deviations of the mean from control wells without responders. The fraction of negative wells for each data point represent a set of 48 wells for a single dose of responders.

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1 Rose, N.R. The role of infection in the pathogenesis of autoimmune disease. *Sem. Immunol.* **10**, 5–13 (1998)
2. Maki-Ikola, O. Reactive arthritis after unusual Salmonella infections. *Lancet* **336**, 1387 (1990).
3. Granfors, K. *et al.* Salmonella lipopolysaccharide in synovial cells from patients with reactive arthritis. *Lancet* **335**, 685–688 (1990).
4. Yu, D. & Inman, R. Infectious agents and other nongenetic immunologic factors in spondyloarthropathies. *Curr. Opin. Rheumatol.* **3**, 581–585 (1991).
5. Taggart, A.J. & Bell, A.L. Reactive arthritis: a further consequence of the increase in salmonella infections *Br. Med. J.* **298**, 674 (1989).
6 Kondowe, G.B., Bell, A.L., Middleton, D. & Taggart, A.J. An outbreak of Salmonella reactive arthritis in Northern Ireland. *Ir. J. Med. Sci.* **158**, 274–277 (1989).
7 Hermann, E. T cells in reactive arthritis. *APMIS* **101**, 177–186 (1993).
8. Sieper, J. & Braun, J. Pathogenesis of spondylarthropathies. Persistent bacterial antigen, autoimmunity, or both? *Arthritis Rheum.* **38**, 1547–1554 (1995).
9. Hemmer, B. *et al.* Identification of high potency microbial and self ligands for a human autoreactive class II-restricted T cell clone. *J. Exp. Med.* **185**, 1651–1659 (1997).
10. Wucherpfennig, K.W. & Strominger, J.L. Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. *Cell* **80**, 695–705 (1995).
11. Gross, D.M., *et al.* Identification of LFA-1 as a candidate autoantigen in treatment-resistant Lyme arthritis. *Science* **281**, 703–706 (1998).
12 Zhao, Z.S., Granucci, F., Yeh, L., Schaffer, P.A. & Cantor, H. Molecular mimicry by herpes simplex virus-type 1: autoimmune disease after viral infection. *Science* **279**, 1344–1347 (1998).
13. Lo, W.-F., Ong, H., Metcalf, E.S. & Soloski, M.J. Cytotoxic T cell responses to Gram-negative intracellular bacterial pathogens: A role for $CD8^+$ T cells in immunity to *Salmonella* infection and involvement of class Ib molecules. *J. Immunol.* **162**, 5398–5406 (1999).
14. Aldrich, C.J. *et al.* Identification of a Tap-dependent leader peptide recognized by alloreactive T Cells specific for a class Ib antigen. *Cell* **79**, 649–658 (1994).
15. Kurepa, Z. & Forman, J. Peptide binding to the class Ib molecule, Qa-1b. *J. Immunol.* **158**, 3244–3251 (1997).
16. Imani, F. & Soloski, M.J. Heat shock proteins can regulate expression of the Tla region-encoded class Ib molecule Qa-1. *Proc. Natl. Acad. Sci. USA* **88**, 10475–10479 (1991).
17. Koga, T. *et al.* T cells against a bacterial heat shock protein recognize stressed macrophages. *Science* **245**, 1112–1115 (1989).
18. Lee, N., Goodlett, D.R., Ishitani, A., Marquardt, H. & Geraghty, D.E. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. *J. Immunol.* **160**, 4951–4960 (1998).
19. Braud, V.M. *et al.* HLA-E binds to natural killer cell receptors CD94/NKG2A, B and C. *Nature* **391**, 795–799 (1998).
20. Zugel, U. & Kaufmann, S.H.E. Activation of CD8 T cells with specificity for mycobacterial heat shock protein 60 in *Mycobacterium Bovis* Bacillus Calmette-Guerin-vaccinated mice. *Infect. Immun.* **65**, 3947–3950 (1997).
21. Hermann, E. *et al.* MHC-unrestricted recognition of bacteria-infected target cells by human $CD8^+$ cytotoxic T lymphocytes. *Cell. Immunol.* **143**, 253–260 (1992).
22. Life, P.F., Bassey, E.O.E. & Gaston, J.S.H. T-cell recognition of bacterial heat shock proteins in inflammatory arthritis. *Immunol. Rev.* **121**, 113–135 (1991).
23. Russmann, H. *et al.* Delivery of epitopes by the Salmonella type III secretion system for vaccine development. *Science* **281**, 565–568 (1998).
24. DeCloux, A., Woods, A.S., Cotter, R.J., Soloski, M.J. & Forman, J.F. Dominance of a single peptide bound to the class Ib molecule Qa-1^b. *J. Immunol.* **158**, 2193–2191 (1997).
25. Coico, R. *Current Protocols in Immunology* (John Wiley and Sons, New York, 1994).

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