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CD8$^+$ T cells specific for a potential HLA-A*0201 epitope from *Chlamydophila pneumoniae* are present in the PBMCs from infected patients

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Abstract

Infection with the common pathogen *Chlamydophila pneumoniae* (Cpn, previously *Chlamydia pneumoniae*) has a high prevalence in patients suffering from arteriosclerosis and may trigger or contribute to heart disease. In mice, CD8-positive T cells are critical for the eradication of the infection and the development of immune memory against Cpn. Although several H2-class I epitopes have been described, no HLA-class I-associated peptides from Cpn are known. In order to define HLA-A*0201 epitopes from Cpn, we focused on the bacterial heat shock proteins (HSP) 60 and 70 which are known to be recognized by the immune system. Using epitope prediction, peptide binding studies and peptide-specific CTLs from HLA-A2 transgenic mice, we could define a potential HSP-70-derived epitope. The study of PBMCs from Cpn-infected individuals using fluorescent MHC tetramers revealed that some patients have CD8$^+$ T cells capable of recognizing the Cpn HSP-70 HLA-A*0201 epitope. Our studies pave the way to the immunomonitoring of the anti-Cpn CTL immune response present in patients suffering from different diseases potentially linked to Cpn or anti-Cpn immunity.

Introduction

Chlamydophila pneumoniae (Cpn, formerly *Chlamydia pneumoniae*) is a common intracellular pathogen that is estimated to infect nearly every human being several times during lifetime (1). Cpn infections and re-infections are in the vast majority of cases not associated with clinical signs. Yet, in rare cases, Cpn is the cause of community-acquired pneumoniae, bronchitis, pharyngitis and sinusitis. Importantly, Cpn infection can be persistent (2) and may be associated with chronic diseases such as multiple sclerosis (3), asthma (4) and coronary heart diseases (review by Taylor-Robinson and Thomas) (5). Seroepidemiological studies (6), animal experiments (7) and detection of Cpn within arteriosclerotic plaque tissues (8) suggest that Cpn infections may participate in the development of arteriosclerosis. Consequently, a prophylactic or therapeutic vaccine that would be efficient in the prevention or clearance of a persistent Cpn infection may be of great interest. In mice, the eradication of Cpn as well as the anti-Cpn memory immunity require CD8-positive CTL (9). Accordingly, vaccine formulations able to trigger CTLs such as DNA-based vaccines can partially protect against Cpn infection (10, 11). Among all proteins expressed by Cpn, surface molecules (especially OMP2) and heat shock proteins (HSP) seem to be relevant antigens, shown to be naturally recognized during infection (12, 13). Wizel *et al.* reported the identification of mouse H2-class I epitopes from these Cpn proteins among others. Because HSP-70 (12, 14, 15) and HSP-60 (12, 15) are naturally recognized during Cpn infection and because of the link between immune recognition of Cpn HSP and Cpn-associated pathologies (16, 17), we focused on these chaperones to discover human MHC class I-associated epitopes derived from Cpn. Since HLA-A*0201 is one of the most prevalent human class I molecules, we predicted

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potential HLA-A*0201-associated epitopes from Cpn HSP-60 and HSP-70. Using CTLs derived from HLA transgenic mice and fluorescent MHC class I tetramers, we found that one HSP-70-derived epitope presented by HLA-A*0201 is recognized by human CTLs. Our discovery opens the door to larger studies where the presence of such CTLs could be compared with the level of Cpn infection and the onset of diseases such as arteriosclerosis, multiple sclerosis, Alzheimer disease, asthma, etc.

Methods

Tumor cell lines

HeLa cells were transfected with a plasmid coding for a chimeric HLA-A*0201 molecule containing the α3, transmembrane and cytoplasmic domains of H-2D^b to give HeLa/A2A2D^b transfectants (18). Cells were grown in RPMI 1640 enriched with 10% FCS, 2 mM L-glutamine and 1% penicillin/streptomycin (Life Technologies, Inc.) supplemented at 1 mg ml^{-1} G418 (Life Technologies, Inc.).

Animals

Six- to twelve-week old HHDII mice were bred in our animal facility. The local animal ethics committee of Tübingen gave its approbation for peptide vaccination of mice (number IM/98).

Patients

The study was made after acceptation of the project by the ethic commission of Tübingen: 'Untersuchung der T-Zell-vermittelten Immunantwort gegen synthetische Peptide aus Chlamydia pneumoniae (Cpn) Antigenen bei infizierten Patienten mit koronarer Herzerkrankung (KHK)' (study number 125/2002V). The patients were admitted to the Department of Medicine III (Cardiology) of the University Hospital Tübingen for the diagnosis and treatment of coronary heart disease. Upon their agreement (documented by their signature of a letter describing the goals of the research), 55 ml of blood was drawn from the patients: 50 ml in heparinized syringes and 5 ml in syringes without heparin. The heparinized blood was overlaid on a Ficoll solution for the preparation of PBMCs. PBMCs were frost in FCS 10% dimethyl sulfoxide (DMSO) at 10 million cells per aliquot and stored in liquid nitrogen. The serum was recovered from non-heparinized blood and kept at −80°C. Each patient was typed for HLA-A*0201 using FACS analysis of 0.5 million fresh PBMCs stained with the mAb BB7.2 (American Type Culture Collection, Manassas, VA, USA). The detection of anti-Cpn antibodies in sera was performed using the ELISA kit from Medac GmbH, according to the manufacturer's instructions.

Epitope prediction

Prediction of potential HLA-A*0201 ligands was carried out as described (24). Briefly, proteins were screened against a matrix pattern, which evaluates every amino acid within nonamer or decamer peptides fitting the HLA-A*0201 motif. Anchor residues are given values of 1; other residues, 0–1, reflect amino acid preferences for certain positions within the peptide. Such motif predictions are available using the database SYFPEITHI (http://www.uni-tuebingen.de/uni/kxi/).

Peptides

Peptides were synthesized in an automated peptide synthesizer 432A (Applied Biosystems, Foster City, CA, USA) following the 9-fluorenylmethyloxycarbonyl/*tert*-butyl strategy. After removal from the resin by treatment with trifluoroacetic acid : phenol : ethanedithiol : thioanisole : water (90 : 3.75 : 1.25 : 2.5 : 2.5 by volume) for 1 or 3 h (arginine-containing peptides), peptides were precipitated from methyl-*tert*-butyl ether, washed once with methyl-*tert*-butyl ether and twice with diethyl ether and re-suspended in water before lyophilization. Synthesis products were analyzed by HPLC (System Gold; Beckman Instruments, Fullerton, CA, USA) and matrix-assisted laser desorption/ionization time of flight mass spectrometry (G2025A; Hewlett-Packard, Palo Alto, CA, USA). Peptides of <80% purity were purified by preparative HPLC.

Peptide binding assay

T2 cells were used for binding studies. A 1 mM peptide stock solution in PBS 10% DMSO was made, and cells were incubated with the peptide at a final concentration of 10 μM, 1 μM or 0.1 μM in RPMI 1640 overnight at 37°C. HLA surface expression was monitored after staining with the primary antibody BB7.2 and a FITC-coupled goat anti-mouse (GAM) IgG (Dianova, Hamburg, Germany) on a FACSCalibur cytometer (Becton-Dickinson, San Jose, CA, USA). Figure 1 presents the results of these studies: – means no binding detectable at any peptide concentration, + means binding detectable at only the highest peptide concentration (10 μM), ++ means binding detectable starting at the 1 μM peptide concentration and +++ means binding detectable starting at the 0.1 μM peptide concentration.

Generation of mouse CTLs

HHD mice (18) were injected subcutaneously in the neck with 25 μg of synthetic peptides mixed with 25 μg of the Hepatitis B virus core$_{128–140}$ helper epitope (25) and 50 μl of Titermax (Sigma). Ten days later, spleens were removed, and splenocytes were put in culture in 10 ml of Iscove's modified Dulbecco's medium supplemented with 10% FCS, 2 mM L-glutamine, 1% penicillin/streptomycin (Life Technologies, Inc.), 50 μg ml^{-1} gentamicin (Life Technologies, Inc.), 5×10^{-5} M β-mercaptoethanol and 1 mM sodium pyruvate (Sigma). Peptide was added at a final concentration of 1 μM. Three days later, 25 units of recombinant IL-2 (Proleukin; Chiron) were added. At day 6, the cytotoxicity of the cultures was tested on 5000 unloaded or peptide-loaded ^{51}Cr-labeled HLA-A2-positive target cells. CTL cultures exhibiting detectable peptide-specific cytotoxicity were re-stimulated weekly using 2×10^7 million peptide-loaded (1 μM peptide for 1 h), freshly irradiated (200 Gy) HHD splenocytes. Long-term cultures were used for intracellular cytokine staining.

Infection of cells with Cpn

Cells were infected at 1 infectious unit per cell as described (26). The efficiency of infection was controlled 24 h after infection

	Cpn Sequence *Human sequence*	SYFPEITHI score	Experimental binding	Immunogenicity in HHD mice
HSP60	KLQERLAKL *--N------*	3.667	-	NT
	KLSGGVAVI *---D----L*	3.444	-	NT
	ALVRCIPTL *--L----A-*	3.444	++	Yes
	KIHKGVKTL *LMLQ--DL-*	3.111	-	NT
	GIKDFLPVL *S-QSIV-A-*	3.000	-	NT
	ILPGGGTAL *-VL---C--*	3.000	-	NT
HSP70	ALAYGIDKV *-I---L--G* *005527*	3.667	+	Yes
	YLGETVTEA *F--HP--N-*	3.111	+++	No
	EIGDGVFEV *T-D--I---*	3.111	++	No
	VLGGEVKDV *I-M-DKSEK*	3.111	+	No

Fig. 1. Predicted HLA-A*0201 epitopes from Cpn HSP-60 and HSP-70. Using the SYFPEITHI algorithm potential HLA-A*0201 epitopes from Cpn HSP-60 and HSP-70 were predicted. Only potential epitopes with a score ⩾3.000 are listed. Under each peptide sequence, the sequence in italic corresponds to the ortholog peptides in the human HSP proteins. The Cpn peptides presented were synthesized and tested in a T2 binding assay. Column 'Experimental binding' indicates the affinity of the peptides: High affinity (+++: stabilization of HLA-A*0201 molecules at a concentration of 0.1 μM), intermediate affinity (++: stabilization of HLA-A*0201 molecules at a minimal concentration of 1 μM) and low affinity (+: stabilization of HLA-A*0201 molecules at a minimal concentration of 10 μM) for HLA-A2 and peptide showing no binding capacity in this test (−: no accumulation of HLA-A2 molecules at the surface of T2 cells after overnight culture with up to 10 μM peptides). Each peptide showing binding capacity to HLA-A*0201 was injected in HHDII mice. Immunogenic peptides (as detected after *in vitro* stimulation of splenocytes with peptides and cytotoxic assays, data not shown) are indicated with 'yes' in the column 'Immunogenicity in HHD mice'. 'NT' means that the peptides were not injected in mice (showing no binding to HLA-A2 in the T2 assay).

using the following method. The cells were fixed on a cover slip for 10 min in PFA 2% in PBS. After washing, the extracellular bacteria were stained using a mouse monoclonal anti-Cpn and a secondary GAM-FITC antibody. After washing, cells were permeabilized with Triton 2% in PBS for 4 min at room temperature. Then, cells were incubated again with the anti-Cpn antibody and a secondary GAM-Cy3 antibody. Cover slips were analyzed by confocal microscopy. Inclusion bodies appeared blue, whereas free bacteria outside the cells appeared green.

Intracellular cytokine staining

Mouse CTL lines were co-incubated overnight with infected or non-infected target cells (24–36 h after infection) in a medium supplemented with GolgiStop (Pharmingen, San Diego, CA, USA). Then, cells were permeabilized and stained using the Cytofix/Cytoperm Plus kit and CD4 FITC, anti-IFN-γ PE, and CD8 CyC antibodies, according to the manufacturer's recommendations (BD-Pharmingen, Heidelberg, Germany). FACS analysis was performed using a FACSCalibur cytometer (Becton-Dickinson). Data in Fig. 2 show the IFN-γ and CD8 expression in gated CD4-negative lymphocytes.

Staining of PBMCs with HLA–peptide tetrameric complexes

PBMCs were defrosted and kept at 5 million cells ml^{-1} for more than 1 week in X-Vivo15 medium (Bio Whittaker, Belgium) supplemented with 10 μM Cpn HSP-70 ALAYGIDKV peptide and 5 mM IL-7 (R&D Systems). At day 4, 10 U ml^{-1} rIL-2 (Proleukin; Chiron) were added. MHC class I–peptide tetrameric complexes were produced as previously described (27). In brief, the heavy chain was modified by deletion of the transmembrane domain and COOH-terminal addition of a sequence containing the BirA enzymatic biotinylation site. The HLA-A2 heavy chains and β2-microglobulins were produced using a prokaryotic expression system (pET/HLA-A2 plasmid and bacteria provided by Paul Travers), purified and re-folded *in vitro* by limiting dilution with the HLA-A*0201-binding peptide derived from Cpn HSP-70 ALAYGIDKV. The re-folded complexes were purified by gel filtration (Superdex 75, Pharmacia) using fast protein liquid chromatography, biotinylated by BirA (Avidity, Denver, CO, USA) in the presence of biotin (Sigma Chemical), ATP (Sigma Chemical) and Mg^{2+} (Sigma Chemical). The biotinylated product was separated from free biotin by gel filtration using fast protein liquid chromatography. Tetramers

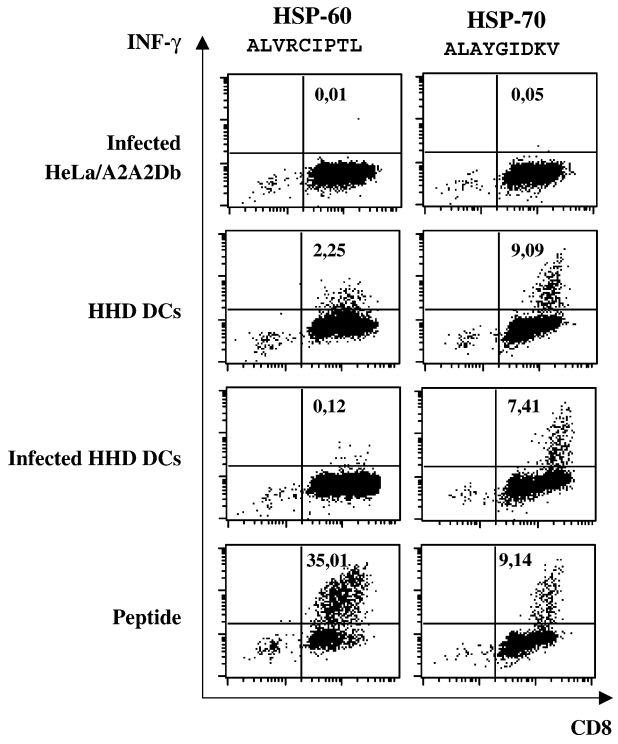

Fig. 2. Response of peptide-specific CTLs to Cpn-infected cells. HHD mouse CTL lines specific for HSP-60 ALVRCIPTL or HSP-70 ALAYGIDKV peptides were co-incubated overnight with Cpn-infected HeLa/A2A2D^b cells, infected or non-infected BM-DCs from HHDII mice and peptide-pulsed HeLa/A2A2D^b cells. After permeabilization, cells were stained for CD4, CD8 and INF-γ. The numbers indicate the percentage of CD8-positive, INF-γ-positive cells among gated CD4-negative cells.

were made by mixing biotinylated protein complexes with streptavidin–PE (Molecular Probes) at a molecular ratio of 4 : 1. At days 6–10 of culture, cells were stained with MHC class I–HLA-A*0201 tetramers: a mixture of two tetramers comprising the PE-labeled Cpn HSP-70 tetramer and an antigen presenting cell (APC)-labeled HLA-A*0201-tetramer containing the dominant HLA-A*0201 epitope from CMV pp65 (28). Both tetramers were incubated with 2 million PBMCs at a final concentration of 5 μg ml^{-1} in a final volume of 100 μl of PBS 50% FCS and 2 mM EDTA for 30 min at 4°C. Then, CD19 FITC, CD4 FITC, CD8 PerCp antibodies (all from BD-Pharmingen) were added in 100 μl PBS 50% FCS and 2 mM EDTA. The cells were incubated at 4°C for 30 min and washed twice with PFEA (PBS with 2% FCS, 2 mM EDTA) before fixation with PBS containing 1% formaldehyde. Stained PBMCs were analyzed by four-color flow cytometry (FACSCalibur, Becton-Dickinson). Data presented in Fig. 3 are gated on CD8-positive, CD19-negative and CD4-negative cells.

Results and Discussion

*Some predicted HLA-A*0201 epitopes from Cpn HSP-60 and HSP-70 bind HLA-A*0201*

Using the algorithm SYFPEITHI (http://www.uni-tuebingen.de/uni/kxi/), we predicted HLA-A*0201 epitopes from Cpn HSP-60 and HSP-70 (Fig. 1). Peptides with a score ⩾3.000 were synthesized: six potential epitopes for HSP-60 and four potential epitopes for HSP-70. All these peptides are different from their ortholog sequences in the human HSP-60 or HSP-70 (see sequence in italics in Fig. 1). Three of the HSP-60-predicted epitopes have only one or two residues different from the human sequence. These epitopes could be candidates to support the postulated pathogenic cross-recognition of human-derived peptides by immune cells directed against Cpn (16). We used a T2 binding assay to measure the actual binding of the predicted epitopes to HLA-A2. In this test, the accumulation of HLA-A*0201 molecules at the cell surface (recorded using indirect antibody staining and FACS analysis) reflects the capacity of exogenous peptides to bind and stabilize this class I molecule. While all four predicted epitopes from HSP-70 were found to bind to HLA-A*0201, only one of the six potential HSP-60 epitopes showed detectable binding to HLA-A*0201. These five HLA-A*0201-binding peptides were injected into HLA-A*0201 transgenic mice [H2 class I deficient: HHDII mice (18)]. Two of the peptides (HSP-70 ALVRCIPTL and HSP-60 ALAYGIDKV) were immunogenic and allowed the derivation of CTL lines capable to specifically kill target cells expressing HLA-A2 and loaded with the respective peptide (data not shown).

Mouse CTLs directed against Cpn HSP-predicted epitopes do not specifically recognize Cpn-infected cells

In order to test whether the two candidate epitopes were presented at the cell surface of Cpn-infected cells, we used the transgenic human tumor cell line HeLa/A2A2D^b (HeLa transfected with the gene coding the chimeric A2A2D^b protein) (18). Such cells could be infected by Cpn as confirmed by intracellular antibody staining and confocal studies (data not shown). The infected or non-infected target cells were co-incubated overnight with the two peptide-specific CTLs derived from HHDII mice. The activation of the CTLs was recorded using intracellular IFN-γ staining and FACS analysis. As shown in Fig. 2, the two CTL lines reacted to the peptides loaded on HLA-A2-positive cells (lower two dot plots 'peptide'): 35.01 and 9.14% CD8-positive, IFN-γ-positive cells in the CTL lines specific for (Cpn HSP-60) ALVRCIPTL and (Cpn HSP-70) ALAYGIDKV peptides, respectively. However, none of the CTL line was activated in the presence of infected HeLa/A2A2D^b cells (upper dot plot, infected HeLa/A2A2D^b). Apparently, in these cells, the two Cpn-derived peptides could not be processed and presented by HLA-A*0201. Since Cpn develops in vesicules similar to endosomes, we hypothesized that in tumor cells the proteins produced from Cpn do not have access to the cytosol and consequently cannot be processed by the MHC class I presentation machinery (proteasome, transporter associated with antigen processing-TAP-pumps). The optimal cell types as far as transfer of antigens from endosomes to the cytosol is concerned are dendritic cells (19). Thus, we tested the presentation of the two predicted HLA-A*0201 epitopes at the surface of infected mouse dendritic cells. Bone marrow-derived dendritic cells (BM-DCs) were prepared from HHDII mouse hind legs. Since Cpn infection is known to mature dendritic cells (20), we used LPS-matured BM-DCs as

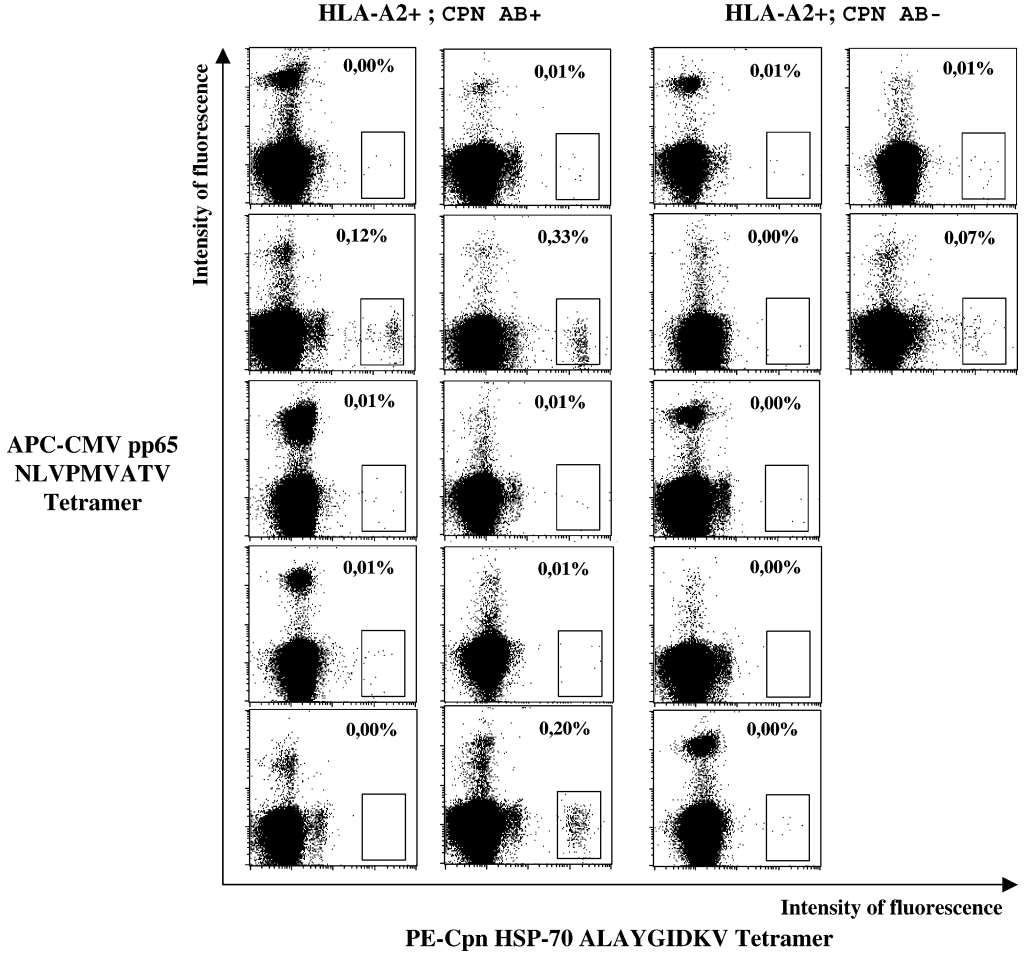

Fig. 3. MHC class I tetramer staining of *in vitro* cultured human PBMCs. PBMCs from 10 HLA-A2-positive, anti-Cpn antibody-positive (HLA-A2+ Cpn AB^+) and 7 HLA-A2-positive and anti-Cpn antibody-negative (HLA-A2+ Cpn AB^-) cells were cultured for 1 week in the presence of the Cpn HSP-70 ALAYGIDKV peptide and stained with two fluorescent HLA-A*0201 tetramers: one folded around the Cpn HSP-70 ALAYGIDKV peptide (PE labeled) and one folded around the CMV pp65 NLVPMVATV peptide (APC labeled). The numbers indicate the percentage of CD8-positive, Cpn HSP-70 ALAYGIDKV tetramer-positive cells among gated CD4-negative, CD19-negative cells. Those cells are not stained by the control APC tetramer, indicating that the recognition by the PE tetramer is specific.

negative controls in the assay. Infection of BM-DCs was confirmed by intracellular detection of Cpn (data not shown). As shown in Fig. 2, the HSP-60-derived peptide-specific CTL lines were not activated by Cpn-infected autologous BM-DCs ('infected HHD DCs'). Looking for H2-class I epitopes, neither our group (13) nor Wizel *et al.* (15) could identify HSP-60-derived CTL epitopes. This family of proteins is highly conserved between species and immune tolerance may prevent the development of CTLs against HSP-60. The HSP-70-derived peptide-specific CTL line was activated by infected and also by non-infected BM-DCs. Thus, a peptide that can be recognized by this CTL line is expressed at the surface of BM-DCs. In this situation, the actual presentation of the Cpn HSP-70 potential epitope could not be addressed using our *in vitro* system. Nevertheless, this experiment indicates that CTLs specific for a Cpn-peptide can recognize non-infected cells. Since a large number of evidences suggest that some pathologies associated with Cpn infection, particularly in arteriosclerosis, are due to cross-recognition by immune cells of self and Cpn antigens (16, 17, 21, 22), we decided to further investigate the presence of CTLs specific for the (Cpn HSP-70) ALAYGIDKV potential epitope in the blood of HLA-A2-positive heart disease patients.

Detection of CTLs that can recognize the (Cpn HSP-70) ALAYGIDKV potential epitope in the blood of patients with anti-Cpn antibodies

We produced HLA-A*0201 monomers folded around the HSP-70 ALAYGIDKV peptide. The monomers were biotinylated, purified and tetramerized with streptavidin–PE. Using these fluorescent tetramers, we studied the presence of CTLs capable of recognizing the HSP-70 ALAYGIDKV peptide in the blood of patients. Blood samples were collected from 57 heart disease patients. We chose this population of patients since people suffering from heart-related health problems are known to have a high prevalence of Cpn infections (6). Patients were typed for HLA-A2 using antibody staining (BB7.2) of fresh PBMCs and checked for the presence anti-Cpn immunity using sera. Twenty-six patients had detectable anti-Cpn IgG antibodies and among those 13 had detectable

anti-Cpn IgA antibodies. PBMCs from all patients were conserved in liquid nitrogen. Defrosted PBMCs from all HLA-$A2^+$ Cpn AB^+ patients (n = 10) and from HLA-$A2^+$ Cpn AB^- patients (n = 7) were cultivated for 1 week in medium containing the HSP-70 ALAYGIDKV peptide before tetramer staining and FACS analysis (such *in vitro* amplification of CTL precursors allows a more sensitive study of the specific CTLs). The results of these studies are shown in Fig. 3 (dot plots gated on CD8-positive, CD4-negative and CD19-negative cells). We could never detect tetramer-positive CD8-positive cells in the cultured PBMCs from HLA-A*0201-negative patients (whether their sera were positive or negative for anti-Cpn antibodies; data not shown) or in the cultured PBMCs from HLA-A*0201-positive patients having no detectable IgA or IgG against Cpn in their sera (n = 7 patients). On the contrary, three cultures made from the PBMCs of HLA-A2-positive patients having detectable antibodies against Cpn (n = 10 patients) contained detectable $CD8^+$ cells recognized by the fluorescent HLA-A*0201 tetramer folded around the Cpn HSP-70 ALAYGIDKV peptide. The cells recognized by the PE-labeled tetramer folded around the Cpn HSP-70 ALAYGIDKV peptide were not co-stained by the control APC-labeled tetramer (HLA-A*0201 folded around a CMV pp65 peptide). Thus, the PE-positive cells were specific for the Cpn HSP-70 ALAYGIDKV peptide. This analysis shows that CTLs capable of recognizing the Cpn HSP-70 potential HLA-A*0201 epitope are present in the blood of 30% (3 out of 10) of the patients infected with Cpn (having antibodies against Cpn).

Some HLA-A2-positive patients (7 out of 10) having antibodies against Cpn do not have CTL precursors specific for the ALAYGIDKV peptide in frequencies high enough for detection in our screening system. This may be due to the fact that in these patients the frequency of such CTLs is too low to be detected even after *in vitro* amplification. Besides, the phenomenon of immunodominance may inhibit the recognition of the ALAYGIDKV peptide when other immunodominant peptides presented by HLA-class I molecules different from HLA-A*0201 are available (23).

HeLa/A2A2D^b cells infected with Cpn did not activate the ALAYGIDKV-specific T cells. Thus, these tumor cells infected *in vitro* do not process the epitope. *In vivo*, the precise cell types (aortic epithelial cells or smooth muscle cells for example) that are infected by Cpn may have different antigen-processing capacities than HeLa and present the ALAYGIDKV epitope. Besides, a cross-priming presentation whereby secreted Cpn HSP-70 would be captured and processed by APCs may be responsible for the triggering of the ALAYGIDKV-specific T cells. These lymphocytes may recognize some yet non-identified Cpn-infected cells. They may also recognize non-infected cells (see the recognition of BM-DCs by anti-HSP-70 CTLs presented in Fig. 2). Thus, CTLs could participate in the proposed 'antigen mimicry'-mediated autoimmune pathologies associated to bacterial infections (16, 17).

Using our results, the frequency of CTLs in the context of Cpn-associated pathologies such as arteriosclerosis, Alzheimer disease, multiple sclerosis, asthma, etc. (3, 4, 6) can be evaluated and compared with the clinical status of the patients. These studies could result in a better understanding of the role of Cpn infection and anti-Cpn immune response in chronic autoimmune diseases.

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Abbreviations

APC	antigen presenting cell
BM-DC	bone marrow-derived dendritic cell
Cpn	*Chlamydophila pneumoniae*
DMSO	dimethyl sulfoxide
GAM	goat anti-mouse
HSP	heat shock proteins

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