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Possible association of non-binding of HSP70 to HLA-DRB1 peptide sequences and protection from rheumatoid arthritis

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Abstract The β-chains of HLA-DR molecules associated with susceptibility to rheumatoid arthritis (RA) share a common amino acid sequence in their third hypervariable region at position 70–74. This shared epitope could either contribute to preferential binding of a given disease-associated peptide, be involved in disease-induction by molecular mimicry or, by binding to heat shock proteins, influence antigen presentation. It is known that the *Escherichia coli* M_r 70,000 heat shock protein DnaK can bind peptides from the shared epitope. Using a highly sensitive method, we show that peptides covering the third hypervariable region of associated, but also most of the non-associated *HLA-DR* alleles, bind to DnaK. Similar binding specificities could be found for the constitutively expressed mammalian M_r 70,000 heat shock protein Hsc73 and the inducible mammalian Hsp72. However, peptides containing the amino acid sequence DERAA, found in *HLA-DR* alleles and strongly associated with protection from RA, did not bind any HSP70. Thus, our results suggest a possible association of non-binding of HSP70 to HLA-DR molecules or its 70–74 fragments and protection from RA.

Keywords Heat shock protein · Shared epitope · HLA-DR · Rheumatoid arthritis · Protection

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Introduction

Heat shock proteins (HSPs) are ubiquitously distributed proteins with an exceptional degree of evolutionary conservation. They are subdivided in different families according to their molecular weight. Members of the HSP70 family occur in all known species and show the highest degree of interspecies conservation among all HSPs. HSPs are found in different cellular compartments, are highly immunogenic, play a major role in protecting cells against damage (Ang et al. 1991; Lindquist 1986), serve as chaperones for other proteins and polypeptides, and participate in antigen processing and presentation (Gething 1996; Panjwani et al. 1999; Pepin et al. 1996; Schild et al. 1999; Spiess et al. 1999; Williams and Watts 1995). An important function for HSPs not only in innate but also adaptive immunity and autoimmunity has been demonstrated (Gaston 1991; Multhoff et al. 1998; Singh-Jasuja et al. 2000; Srivastava and Udono 1994; Wells and Malkovsky 2000).

A link of HSPs with arthritis was first implied by van Eden and co-workers (1985), when a T-cell clone capable of transferring adjuvant arthritis in Lewis rats was shown to recognize the mycobacterial HSP60. Much of the following discussion on HSP and arthritis has included the idea of molecular mimicry (Cohen and Young 1991; Gaston 1997; Quayle et al. 1992). Although the disease-associated epitope in adjuvant arthritis was found not to be conserved between mycobacterial and rat HSP60 (van Eden et al. 1988), preimmunization using either mycobacterial HSP, HSP-derived peptides or naked DNA encoding for HSP has been shown to protect against induction of adjuvant arthritis (Bloemendal et al. 1997; Kingston et al. 1996; Ragno et al. 1997; Wendling et al. 2000). In humans, T cells from patients with rheumatoid arthritis (RA) showed responses to human but not mycobacterial HSP60 (van Roon et al. 1997). Recognition of the human HSP (overexpressed in arthritic joints) triggered regulatory T cells resulting in a less severe course of RA (Macht et al. 2000; van Roon et al. 1997).

Predisposition to RA is associated with the expression of particular *HLA-DR* alleles sharing five very similar amino acid residues (Q/R K/R RAA) in their third hypervariable (*HV3*) region (aa 70–74) of their β1 chain. This finding has led to the shared epitope (SE) hypothesis (Gregersen et al. 1987). Auger and co-workers (1996) demonstrated that peptides containing the SE are able to bind the HSP70 of *Escherichia coli*, DnaK. The co-chaperone of DnaK, DnaJ contains a QKRAA motif itself and binding of DnaJ to DnaK can be inhibited by HLA-DR-derived peptides containing Q K/R RAA (Auger and Roudier 1997). Furthermore, these authors found co-precipitation of the constitutively expressed mammalian HSP70 (Hsc73) and HLA-DRB1 molecules containing the SE motif in immunoprecipitation experiments, suggesting a binding of DnaK to complete HLA-DR molecules containing the SE motif. This view, however, has been challenged by Rich and co-workers (1998), who failed to confirm these data by showing a non-specific co-precipitation of Hsc73 with HLA class I and HLA class II DR molecules.

The SE hypothesis has been challenged by a proposed RA protection model (Zanelli et al. 1995). This model is based on experiments with collagen-induced arthritis (CIA) in mice (Gonzalez-Gay et al. 1996; Zanelli et al. 1996, 1997) and on human epidemiological studies (Reviron et al. 2001; Seidl et al. 2001; van der Horst-Bruinsma et al. 1999; Vos et al. 2001; Zanelli et al. 1998). The RA protection model proposes that certain *HLA-DQ* alleles predispose to RA, whereas *HLA-DR* are able to modulate this predisposition by either enhancing or mediating strong protection against this predisposition. Accordingly, in the mouse, CIA-predisposing MHC class II molecules are H2-A^q (equivalent of HLA-DQ) whereas H2-E molecules (equivalent of HLA-DR) can mediate protection (Zanelli et al. 1995). In this model, the RA-predisposing HLA-DQ molecules have been shown to be DQB1*03/DQA1*03 (DQ7, DQ8 and DQ9, collectively referred as DQ3) and DQB1*0501/DQA1*01 (DQ5) (Seidl et al. 2001; Zanelli et al. 1998, 2000). The *HLA-DR* alleles mediating strong protection from the predisposition are *DRB1*0402*, *DRB1*1301* and *DRB1*1302* (Reviron et al. 2001; Zanelli et al. 2000), all encoding the unique amino acid sequence DERAA in their *HV3* region.

The aim of the present study was to investigate the binding of three different HSP70s to peptides derived from the *HV3* region of HLA-DRB1 molecules using a highly sensitive method (Kalbacher and Beck 1999). The HSP70s used were *E. coli* HSP70 DnaK, the constitutively expressed mammalian M_r 70,000 heat shock cognate protein Hsc73 and the inducible mammalian Hsp72. Our results demonstrate that not only do the RA-associated *HLA-DR* sequences bind to all examined HSP70s, but also peptides originating from other HLA-DR molecules. However, there is one important exception: peptides containing DERAA, comprising the aa 70–74 of RA-protective *HLA-DR* alleles (e.g., *DRB1*0402*), did not bind to any of our tested HSP70s. This strong correlation suggests a role in the protection from RA for those *HLA-DR* alleles not able to interact with bacterial and mammalian HSP70 molecules.

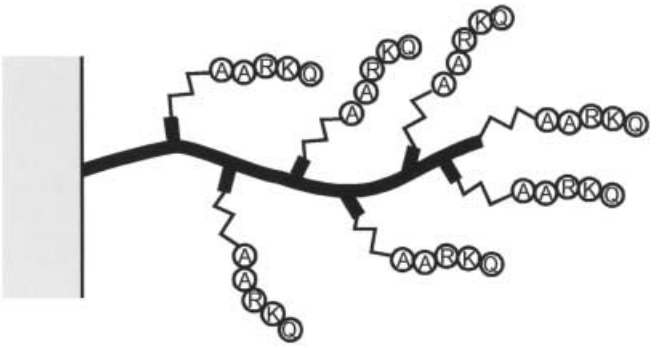


Fig. 1 Structure of the Fractogel polyacrylamide tentacle-type resin loaded with QKRAA peptide

Materials and methods

Introduction of an ε-aminohexanoic-acid spacer on Fractogel EMD amino

Fractogel EMD amino was a kind gift of Merck (Darmstadt, Germany). Fractogel EMD chromatography phases are organic matrices with a particle size of 20–40 µm and a hydrophilic surface, on which linear polyelectrolyte-chains (tentacles) were introduced by copolymerization (Fig. 1).

This tentacle-based resin has several advantageous properties for binding experiments (Kalbacher and Beck 1999). First, peptides can be synthesized directly on the resin. Second, the peptide-bound resin can be used as an affinity matrix allowing direct binding of proteins in an aqueous system. Due to the controlled orientation of the peptide on the resin and the flexibility mediated by the glycidylmethacrylate matrix and an additional ε-aminohexanoic spacer, the peptide has an excellent accessibility to proteins allowing an optimal interaction with peptides in an hydrophilic environment. As the peptide is immobilized specifically and no further coupling procedure to a matrix is needed, nonspecific coupling by side chains of trifunctional amino acids, such as in procedures using free peptides and CNBr-activated matrices, is excluded. The gel was activated with Fmoc-ε-aminocaproic-acid, using Fmoc/TBTU [TBTU: 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium-tetrafluororborate] chemistry (Fields and Noble 1990). Quantification of Fmoc revealed a coupling efficiency of about 0.3 mmol/g.

Solid-phase peptide synthesis

Peptide synthesis was performed on a modified SyRo multiple peptide synthesizer (MultiSynTech, Witten, Germany) in a 0.02 mmol scale, using Fmoc/TBTU chemistry (Fields and Noble 1990). Protecting groups were removed by incubation with 90% TFA, 2.5% 1,2-ethandithiol, 2.5% H_2O, 2.5% phenol for 2.5 h, with the peptide still remaining on the resin. After extensive washing with CH_2Cl_2 and isopropanol, the gel was dried under vacuum. The gel was then resuspended in PBS and used for affinity chromatography.

Synthesis control was performed for each multiple peptide synthesis, either by quantitative amino acid analysis or for selected peptides by microsequencing. Some of the sequences were synthesized on Fractogel EMD amino derivatized with a cleavable Rink-amide linker, yielding free peptide amides. The deprotected and acid-cleaved peptides were controlled for homogeneity and identity by RP-HPLC and ESI-MS, revealing a purity of >90%.

Peptides, used for coupling to CNBr-activated Sepharose (cyanogen bromide-activated Sepharose 4B) (Sigma, Germany) were synthesized by classical Fmoc/But chemistry (Fields and Noble 1990) using a 4-HMP-polystyrene-resin (HMP: hydroxymethylphenoxy).

Bacterial protein extraction

E. coli bacteria were grown in LB AGAR (Sigma, Germany) overnight at 37°C. Approximately 3×10^8 bacteria were lysed in 0.5 ml lysis buffer (50 mM Tris HCl (pH 8), 50 mM glucose, 0.1 mM phenylmethylsulfonyl fluoride, 25 mg/ml lysozyme, 1% Triton X-100, 0.04 mg/ml DNase and 10 mM $MgCl_2$) (Auger et al. 1996). The lysis mixture was incubated for 30 min at 44°C, followed by ultrasound treatment. The lysate was centrifuged at 14,000 *g* for 15 min and the supernatant was either used immediately or stored at –80°C.

Monoclonal antibodies and HSPs

The monoclonal antibodies 8E2 (SPA-880, specific for DnaK), DnaK (SPP-630), Hsc73 (SPP-751) and Hsp72 (SPP-755) were purchased from Stressgen (Victoria, BC, Canada).

Affinity column binding studies

Binding experiments with CNBr-activated Sepharose were done as described by Auger and co-workers (1996). For Fractogel binding studies, the gel was washed once with 2 ml 0.1 M $NaHCO_3$, 0.5 M NaCl (pH 8) buffer after synthesis of peptides. The Fractogel was then incubated with 15 ml 0.2 M glycine (pH 8) overnight at 4°C. Columns were consecutively washed with each of the following buffers: (1) 0.1 M $NaHCO_3$, 0.5 M NaCl (pH 8) buffer, (2) 25 mM Tris-HCl, 0.5 mM NaCl, 0.5% Triton X-100 (pH 7.5), (3) 0.5 M CH_3COONa (pH 4), (4) 0.1 M $NaHCO_3$, 0.5 M NaCl (pH 8), and finally resuspended in 0.5 ml phosphate-buffered saline (PBS). To each column HSP was added and incubated overnight at 4°C. Bound HSP was eluted, after washing with 25 mM Tris-HCl, 0.5 mM NaCl, 0.5% Triton X-100 (pH 7.5) at 4°C, with 0.5 ml glycine buffer 0.2 M (pH 2) at room temperature.

Electrophoresis and immunoblotting

Eluted proteins were lyophilized, dissolved in 80 µl sample buffer (0.13 M Tris, 4% SDS, 0.078 M DTT, 20% glycerin, 0.02% bromophenol blue) and neutralized with 2 N NaOH. Separation was done on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), controlled by Rainbow colored protein molecular weight marker (Amersham, England), followed by staining with a silver-staining kit (Bio-Rad, Germany). For a few experiments, eluted proteins were transferred to nitrocellulose membranes (Millipore, Germany). Nitrocellulose was blocked with Rotiblock-solution (Bio-Rad, Germany) overnight at 4°C and incubated with anti-DnaK antibody (1/8000) 1 h at room temperature. Blots were revealed by chemiluminescence (Boehringer Mannheim, Germany).

Results

DnaK binds to 15 amino acid-long peptides contained in the *HV3* region

Using the same experimental conditions as Auger and co-workers (1996), we started our binding studies with 15 aa residue peptides of *HLA-DRB1*0401* (KDLLEQKRAAVDTYC) or *HLA-DRB1*0101* (KDLLEQRRAAVDTYC) bound to CNBr-activated Sepharose. DnaK did bind to both peptides. However, in order to detect the bound HSP, we either had to silver stain the gel (Fig. 2) or to blot it to a nitrocellulose membrane for chemiluminescence. Protein staining by Coomassie Brilliant Blue R was not sufficient to detect any bound HSP, probably due to a low protein concentration.

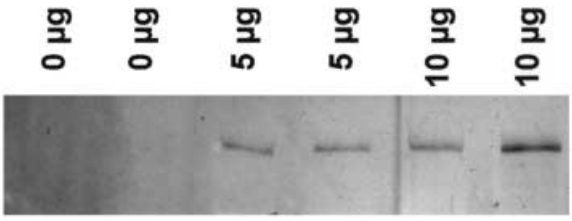
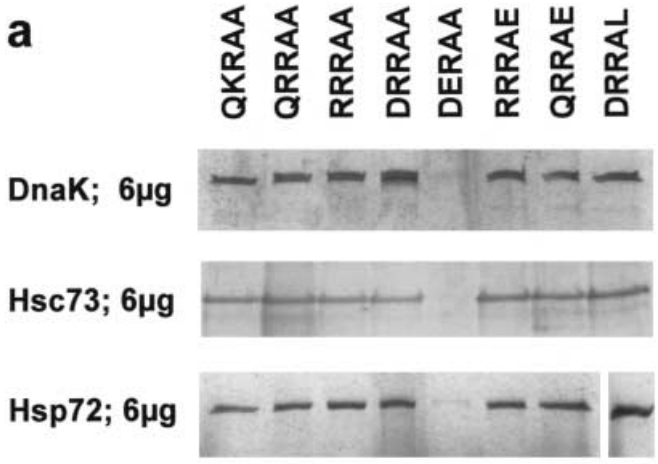


Fig. 2 DnaK binds to the peptide KDLLEQKRAAVDTYC on Sepharose columns. The peptide KDLLEQKRAAVDTYC was bound to CNBr-activated Sepharose and used for affinity columns. The columns were incubated with 5 or 10 µg DnaK (HSP70 of *E. coli*, Stressgen) overnight at 4°C. Bound HSP was eluted with 02 M glycine pH 2 at room temperature. The eluates (two independent eluates for each concentration of DnaK) were separated on SDS-PAGE and silver stained

HSP70s do not bind to DERAA, contained in the *HV3* region of RA protective alleles

In order to optimize binding conditions, we used Fractogel columns for further experiments (Kalbacher and Beck 1999). Peptides fixed onto this matrix are unfolded, selectively bound, and, due to a spacer between the peptides and the resin, are easily accessible for binding HSPs (Fig. 1). Using Fractogel-peptide columns and silver-staining or immunoblotting for protein detection, we analyzed distinct peptides, each peptide comprising aa 70–74 from either RA-protective, RA-neutral or RA-associated *HLA-DRB1* alleles (Crilly et al. 1999; Fugger and Svejgaard 1997; Marsh 1998; Sherritt et al. 1996). We tested binding of all sequences found in the *HV3* region of human *HLA-DRB1* alleles to three different HSP70s: DnaK, Hsc73 and Hsp72. As shown in Fig. 3, almost all peptides did bind DnaK, Hsp72 and Hsc73, with the important exception of DERAA (e.g., *HLA-DRB1*0402*, *1301*, and *1302*). Repeated experiments using newly synthesized Fractogel-peptide columns and silver-staining after SDS-PAGE did not reveal any binding of HSP70s to DERAA. There was a weak binding of DnaK and no binding of Hsc73 or Hsp72 to DRAAA, a peptide originating from *HLA-DRB1*1603*, an allele expressed with low frequency in the population, but no other non-binding peptide could be observed. To exclude the possibility of non-specific ion-ion interactions, an experiment was repeated with high salt washing buffers (1 M), yielding the same results for DERAA, DRRAL and QKRAA (results not shown).



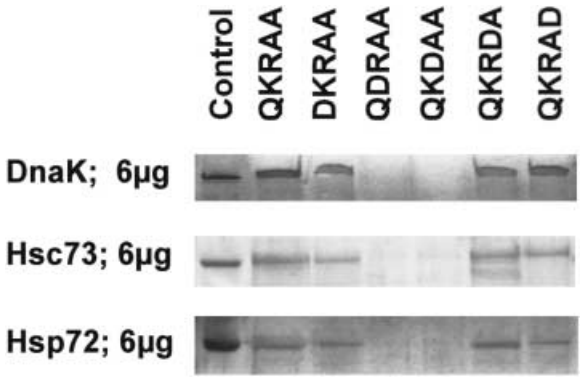


Fig. 4 Binding of peptides not contained in the *HV3* region of the *HLA-DRB1* alleles to DnaK, Hsc73 and Hsp72. Influence of an acidic aa (D) at position 1 to 5 on the binding to DnaK, Hsc73 and Hsp72 (aspartic acid scan). Experimental conditions were the same as in Fig. 3

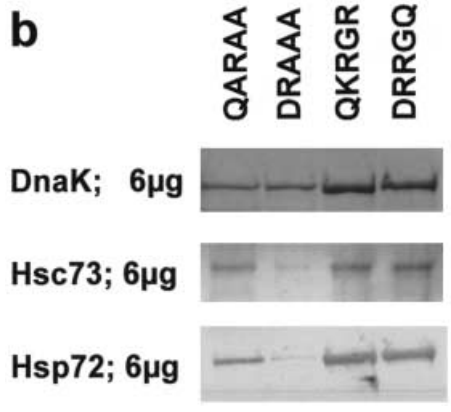


Fig. 3 Binding of peptides representing position 70–74 of human HLA-DR *HV3* regions to DnaK, Hsc73 and Hsp72 using Fractogel columns. The 5 aa peptides are (Marsh 1998): (**a**) QKRAA (*DRB1*0401*), QRRAA (*DRB1*0101*), RRRAA (*DRB1*1001*), DRRAA (*DRB1*1601*), DERAA (*DRB1*0402*), RRRAE (*DRB1*0901*), QRRAE (*DRB1*0403*), DRRAL (*DRB1*0801*). (**b**) QARAA (*DRB1*1501*), DRAAA (*DRB1*1603*), QKRGR (*DRB1*0422*), DRRGQ (*DRB1*0701*). The Fractogel columns were incubated with 6 µg of DnaK, Hsc73 or Hsp72 overnight at 4°C in PBS. After washing, bound HSP was eluted with 0.2 M glycine pH 2 at room temperature. The eluate was lyophilized, separated on 10% SDS-PAGE and silver stained. Experiments were repeated at least twice; one representative experiment is shown

Fig. 5 Binding of 14 aa peptides to DnaK, Hsc73 and Hsp72. The peptides KDLLEQKRAAVDTYC and KDILEDERAAVDTYC were synthesized on Fractogel and incubated with 7 µg of HSP. Elution of bound HSP, separation and identification were done as described above (for details, see legend to Fig. 3)

Negatively charged side chains prevent binding of HSP70s

To analyze the pattern of amino acids preventing binding to HSP70s, we performed an aspartic acid (D) scan for each position of the five amino acids and constructed peptides not contained in the *HV3* region of *HLA-DRB1* alleles (Fig. 4). The peptides QDRAA and QKDAA did not bind to DnaK, Hsc73 or Hsp72, suggesting that aa with a negatively charged side chain in positions 2 and 3 of the peptides (position 71 and 72 of the HLA-DR β1-chain) prevent the binding of HSP70s.

One could argue that binding of HSPs to peptides only five amino acids long is different from binding to longer peptides used in previous work (Auger et al. 1996; Gragerov et al. 1994; Jordan and McMacken 1995). We therefore constructed Fractogel columns with peptides 15 aa long (corresponding to aa position 65–79 of the HLA-DR β1-chain). Again, the peptide KDLLEQKRAAVDTYC (*HLA-DRB1*0401*) did bind DnaK, Hsc73 and Hsp72, whereas the peptide KDILEDERAAVDTYC (*HLA-DRB1*0402*) did not bind to any of the HSPs (Fig. 5).

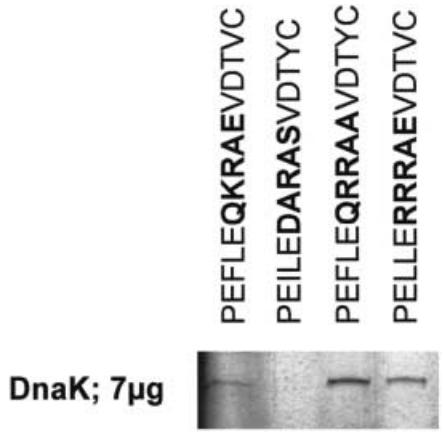


Fig. 6 Non-binding of DARAS to DnaK. In the murine CIA model, the *H2-Eβ*^{d} allele mediates protection. The aa sequence DARAS (*H2- Eβ*^{d}) corresponds to position 70–74 of the human *HLA-DRB1* allele. The peptide sequences are: PEFLEQKRAEVDTVC, H2-Eβ^{b,k}; PEILEDARASVDTYC, H2-Eβ^{d}; PEFLEQRRAAVDTYC, H2-Eβ^{s}; PELLERRRAEVDTVC, H2-Eβ^{p}. For details, see legend to Fig. 3

HSP70s do not bind to DARAS, a peptide mediating strong protection from collagen-induced arthritis in mice

Mouse MHC class II molecules are encoded by *H2-Aα* and *Aβ* for the A molecule, and *Eα* and *Eβ* for the E molecule. All mice do express H2-A, but mice carrying the haplotypes *b*, *s*, *q* and *f* do not express H2-E. Introduction of the transgene *H2-Eβ*^{d} into CIA-susceptible H2-A^{q} mice resulted in functional expression of H2-E molecules and a dramatic reduction in incidence and severity of arthritis. The most efficient reduction of incidence and severity was restricted to H2-Eβ^{d} (Gonzalez-Gay et al. 1994, 1995). *Eβ* genes are homologous to human *DRB1* genes and the H2-Eβ^{d} molecule carries the aa sequence DARAS at the position corresponding to aa 70–74 of the human HLA-DR molecule. We therefore tested binding of DnaK to 15 aa peptides containing sequences of different H2-Eβ molecules (Zanelli et al. 1995). As can be seen in Fig. 6, the peptide containing DARAS (H2-Eβ^{d}, mediating strong protection in CIA) did not bind to DnaK. In contrast, the peptide containing QRRAA (H2-Eβ^{s}, mediating only partial protection) did bind to DnaK.

Discussion

RA is a chronic inflammatory disease of the adult with a prevalence of about 1% (Feldmann et al. 1996). Many pathogenetic details triggering and perpetuating the disease remain poorly defined, but abnormal autoimmune mechanisms are considered to be of central importance. Susceptibility to RA is believed to be associated with the expression of certain *HLA-DR* alleles carrying the SE (Q/R K/R RAA) at positions 70–74 of their β1-chain (Gregersen et al. 1987). Peptides containing the SE motif are able to bind to the HSP70 of *E. coli*, DnaK (Auger et al. 1996). In this study, we analyzed whether peptides originating from the *HV3* region of *HLA-DRB1* alleles bind members of the HSP70 family. We therefore tested binding of *E. coli* DnaK, the constitutive mammalian Hsc73, and the inducible mammalian Hsp72 to all aa sequences found in the *HV3* region of *DRB1* alleles. In contrast to the study of Auger and co-workers (1996) we demonstrate a binding of all three chaperones to most of the aa sequences, with the important exception of peptides containing the sequence DERAA. The differences to the results as reported by Auger and co-workers (1996) may be due to our very sensitive methods using silver staining for detection of bound HSP and highly loaded Fractogel columns. The immunoprecipitation results of Auger and colleagues are also different from the results published by Rich and co-workers (1998), showing a non-specific co-precipitation of Hsc73 with HLA class II DR molecules from cell lines not containing the SE.

Our results regarding the substrate binding specificities of HSP70 chaperones are in good accordance with published data (Blond-Elguindi et al. 1993; de Crouy-Chanel et al. 1996; Flynn et al. 1989; Gragerov et al. 1994; Rudiger et al. 1997; Takenaka et al. 1995). The peptides DERAA, QDRAA and QKDAA did not bind HSP70s, suggesting that amino acids with a negatively charged side chain (D, aspartic acid or E, glutamic acid) at position two or three of our peptides (corresponding to aa 71 and 72 of the *HLA-DRB* alleles) prevent the binding to DnaK, Hsc73 and Hsp72.

The SE hypothesis is supported by HLA-DR X-ray crystallography, demonstrating an important role for the amino acid residues 70, 71 and 74 for the peptide binding properties of HLA-DR molecules (Brown et al. 1993). The β1-chain of the HLA-DR molecules contains polymorphic residues contributing to five binding pockets. The P4 pocket consists of the residues 70, 71, 74 and 29 of the β1-chain. Peptide-binding studies did suggest that the electrostatic charge of β71 has a strong influence on peptide-binding specificity (Davenport et al. 1997; Hammer et al. 1995). The X-ray crystal structure of DRA*0101, DRB1*0401 complexed with a peptide from human collagen did confirm the importance of position 71 (Dessen et al. 1997). In a recent study, *HLA-DR* alleles with a neutral or negative electric charge in the P4 pocket (e.g., *DRB1*0402*) were found to protect against RA, whereas a positive electric charge in the P4 pocket had no influence on the predisposition (Reviron et al. 2001).

However, the SE hypothesis does not explain the RA association of alleles lacking the SE such as *DRB1*0901* (RRRAE) (Zanelli et al. 2000). An alternative to the SE hypothesis is the RA protection model (Zanelli et al. 1995), proposing that certain *HLA-DQ* alleles predispose to RA whereas *HLA-DR* alleles can modulate this predisposition. In this model the alleles *DRB1*0402*, *DRB1*1301* and *DRB1*1303* (all encoding DERAA in their *HV3* region) mediate strong protection (Zanelli et al. 1995). This protection could, for example, be explained by the presentation of peptides derived from protective DRB1 molecules in associated *DQ* alleles. This

view is supported by data showing that peptides containing DERAA are naturally processed and presented, resulting in DQ8-restricted immunogenicity (Snijders et al. 2001; Zanelli et al. 1997).

Pathogenesis of autoimmune diseases could be influenced by HSPs through different mechanisms. For example, overexpression of Hsc73 in macrophages leads to an increase of antigenic peptide generation (Pepin et al. 1996) and endocytosed antigen interacts with Hsc73 (DeNagel and Pierce 1992; Panjwani et al. 1999). Hsc73 is present in lysosomal compartments and is therefore correctly positioned to participate in antigen processing within the MHC II pathway (Panjwani et al. 1999). In the loading process of MHC II molecules, the CLIP-peptide is removed with the help of H2-M molecules. H2-M can accelerate the association of MHC II molecules with peptides (Denzin et al. 1996), but possesses no peptide binding properties on its own, suggesting a role for HSPs in this process (Panjwani et al. 1999). HSPs could protect peptides from complete proteolysis and transfer them rapidly to vacant MHC II molecules. The interaction between MHC II, Hsc73 and peptide could create a localized concentration of Hsc73-bound antigenic peptide facilitating the formation of MHC II/peptide complexes. In this context, binding or non-binding of HSP70s to the complete HLA-DR molecule or its fragments could prevent or enhance the presentation of peptides involved in the pathogenesis of RA. For example, the presentation of DERAA peptides (non-binding to HSPs) by predisposing DQ-molecules (Snijders et al. 2001) could either lead to deletion of autoreactive T cells in the thymus or to DRB1-specific, DQ-restricted immunoregulatory T cells. Alternatively, antigen presentation by HLA-DR molecules containing DERAA at position 70–74 could be influenced by HSP70s not binding to them. Further studies are required to investigate whether non-binding of HSP70s is restricted to DERAA-containing peptides or extends to complete HLA-DR molecules. Altogether, our results suggest a role for the non-binding of HLA-DR- or H-2E-derived peptides to HSP70s in the protection from RA or CIA and possibly open a new avenue into the understanding of the pathogenesis of these diseases.

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