

A function for the QKRAA amino acid motif: mediating binding of DnaJ to DnaK. Implications for the association of rheumatoid arthritis with HLA-DR4.

I Auger, J Roudier

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Research Article

The amino acid motif QKRAA, when expressed on HLA-DRB1, carries susceptibility to develop rheumatoid arthritis. This motif is the basis of strong B and T cell epitopes. Furthermore, it is highly overrepresented in protein databases, suggesting that it carries a function of its own. To identify this function, we used QKRAA peptide affinity columns to screen total protein extracts from *Escherichia coli*. We found that DnaK, the *E. coli* 70-kD heat shock protein, binds QKRAA. Of interest, DnaK has a natural ligand, DnaJ, that contains a QKRAA motif. We found that QKRAA-containing peptides inhibit the binding of DnaK to DnaJ. Furthermore, rabbit antibody to the QKRAA motif can inhibit binding of DnaJ to DnaK. These data suggest that QKRAA mediates the binding of *E. coli* chaperone DnaJ to its partner chaperone DnaK.

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A Function for the QKRAA Amino Acid Motif: Mediating Binding of DnaJ to DnaK

Implications for the Association of Rheumatoid Arthritis with HLA-DR4

Isabelle Auger and Jean Roudier

Laboratoire d'Immuno Rhumatologie, Faculté de Médecine de Marseille, 13005 Marseille, France

Abstract

The amino acid motif QKRAA, when expressed on HLA-DRB1, carries susceptibility to develop rheumatoid arthritis. This motif is the basis of strong B and T cell epitopes. Furthermore, it is highly overrepresented in protein databases, suggesting that it carries a function of its own.

To identify this function, we used QKRAA peptide affinity columns to screen total protein extracts from *Escherichia coli*. We found that DnaK, the *E. coli* 70-kD heat shock protein, binds QKRAA. Of interest, DnaK has a natural ligand, DnaJ, that contains a QKRAA motif. We found that QKRAA-containing peptides inhibit the binding of DnaK to DnaJ. Furthermore, rabbit antibody to the QKRAA motif can inhibit binding of DnaJ to DnaK.

These data suggest that QKRAA mediates the binding of *E. coli* chaperone DnaJ to its partner chaperone DnaK. (*J. Clin. Invest.* 1997; 99:1818-1822.) Key words: QKRAA motif • DnaJ • DnaK • rheumatoid arthritis

Introduction

Short amino acid motifs may carry important biological properties, independently from the protein that contains these motifs. This is well documented, for example, for motifs like RGDS, which mediates the binding of the central domain of fibronectin by integrins (1), or KFERQ, which allows the targeting of proteins to lysosomes (2).

We have been interested in identifying the biological function carried by the amino acid motif QKRAA. Indeed, this 5 amino acid motif, when expressed in the third hypervariable region of HLA-DRB1, is known to carry susceptibility to develop rheumatoid arthritis, a chronic inflammatory joint disease (3). We recently studied the representation of QKRAA in protein databases and observed that this motif was overrepresented, suggesting that it carries an original property (4).

Address correspondence to Jean Roudier, Laboratoire d'Immuno Rhumatologie, Faculté de Médecine de Marseille, 13005, Marseille, France. Phone: 33 4 91 83 44 97; FAX: 33 4 91 83 09 26; E-mail: roudier@aix.pacwan.net

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In this report, we show that QKRAA, expressed in the NH₂-terminal region of most bacterial 40-kD chaperones (DnaJ proteins), mediates binding of DnaJ to its partner chaperone DnaK. Indeed, affinity columns made with peptides that contain a QKRAA motif allow the purification of DnaK from total protein extracts from *Escherichia coli*. Furthermore, QKRAA-containing peptides inhibit the binding of DnaK to DnaJ, and rabbit antibody to the QKRAA motif can inhibit binding of DnaJ to DnaK.

Methods

Synthetic peptides. Peptides were synthesized by the solid phase method of Merrifield, and then purified by reverse phase HPLC to a purity higher than 80% (Neosystem, Strasbourg, France). Amino acid sequences of the peptides are: DRB1*0401 p: KDLLEQKRAAVD-TYC, 401 short p: QKRAA, 401 mutated p: QKKAA, 401²p: EQK-RRAAEQKRAA, EBVgp110p: QKRAAQRAA, *E. coli* DnaJp: VLTDSQKRAAYDQYG, DRB1*0101p: KDLLEQRRAAVD-TYC, DRB1*0402p: KDLLEDERAAVDTYC, DRB1*0403p: KDLLEQR-RAEVDTYC, DRB1*0801p: KDFLEDRRALVDTYC, DRB1*1001p: KDLLERRRAAVDTYC, Pigeon cytochrome p: KAERA-DLIALYLKQATAK, RNase B p: KESAAAKFERQHMDS.

Affinity column binding studies. Cyanogen bromide-activated Sepharose 4B (Sigma Chemical Co., St. Quentin, France) was washed with 1 mM HCl solution and incubated with peptide in 0.1 M NaHCO₃, 0.5 M NaCl, pH 8, buffer overnight at 4°C. 5 mg of peptide were used per milliliter of Sepharose. Free Sepharose groups were then blocked with 0.2 M glycine, pH 8, buffer for 2 h at room temperature. Columns were washed at 4°C with the following buffers: 0.1 M NaHCO₃, 0.5 M NaCl, pH 8, buffer, and then 0.5 M CH₃COONa, pH 4, buffer, and finally phosphate-buffered saline, pH 7.5.

E. coli were grown overnight at 37°C in Luria broth medium. Cells were collected by centrifugation and stored as frozen pellets at -20°C. Cell pellets were lysed in 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 MgCl₂. Protein extracts from 2 × 10⁸ bacteria were added to 1 ml of each affinity column and incubated overnight at 4°C. After washing with 25 mM Tris-HCl, 0.5 mM NaCl, 0.5% Triton X-100, pH 7.5, bound proteins were eluted at room temperature with 10⁻³ M ATP, 10 mM Mg²⁺. Eluted proteins were separated on 8% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked for 1 h in TBS, pH 7.5, 5% powdered milk and incubated with anti-DnaK monoclonal antibody 8E2 (StressGen Biotechnologies Corp., Victoria, Canada), followed by peroxidase-conjugated antibody. Blots were revealed by chemiluminescence (Boehringer Mannheim, Mannheim, Germany).

ELISA for DnaJ/DnaK binding. DnaJ was obtained from StressGen Biotechnologies Corp. and was > 90% pure. DnaK was obtained from Boehringer Mannheim and was > 95% pure. Binding of DnaJ to DnaK was assayed as described previously (5). Briefly, ELISA plates were coated with *E. coli* DnaJ (0.5 µg/well) for 1 h at room temperature. Plates were blocked with PBS, 0.2% BSA, and

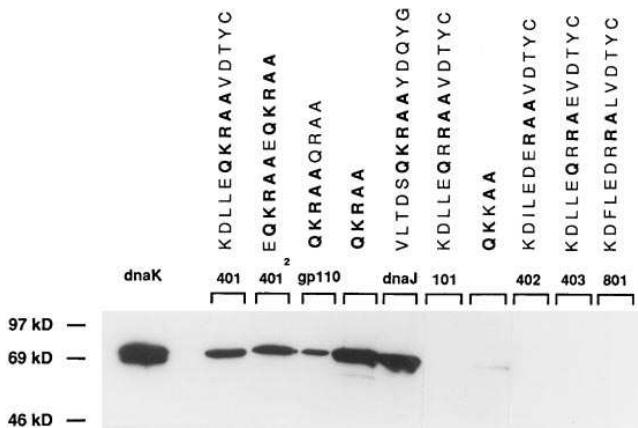


Figure 1. QKRAA-containing peptides bind DnaK in *E. coli* protein extracts. Protein extracts from *E. coli* were loaded on peptide affinity columns. Bound proteins were eluted in ATP buffer. A 70-kD protein that bound QKRAA-containing peptides was identified by Western blotting with anti-DnaK monoclonal antibody 8E2 (StressGen Biotechnologies Corp.). The name and amino acid sequence of the peptide used for each affinity column is indicated above each lane.

washed with 25 mM Hepes, 150 mM KCl, 25 mM NaCl, 5 mM MgCl₂, 0.1 mM EDTA, 1 mM DTT, and 0.1% Triton X-100, pH 7.6.

For peptide inhibition studies, *E. coli* DnaK (0.5 µg/well) was preincubated for 1 h with peptide (10 µg/well). For antibody inhibition studies, *E. coli* DnaK was added to the wells in presence of either rabbit antibody specific for the QKRAA motif (6) or rabbit anti-rat IgG antibody at 15 µg/well. After washing, plates were incubated in 0.1% glutaraldehyde and bound DnaK was detected by mouse anti-DnaK monoclonal antibody 8E2 diluted 1/1,000, followed by peroxidase-conjugated anti-mouse IgG antibody (Boehringer Mannheim) diluted 1/1,000. OD was read at 405 nm. ELISA were performed in duplicates.

ELISA for DnaK/peptide binding. ELISA plates were coated for 1 h at room temperature with 10 µg peptide/well. For peptide inhibition studies, we added DRB1*0401 peptide (10 µg/well) to *E. coli* DnaK (0.5 µg/well). Bound DnaK was detected by mouse anti-DnaK monoclonal antibody 8E2 diluted 1/1,000 followed by peroxidase-

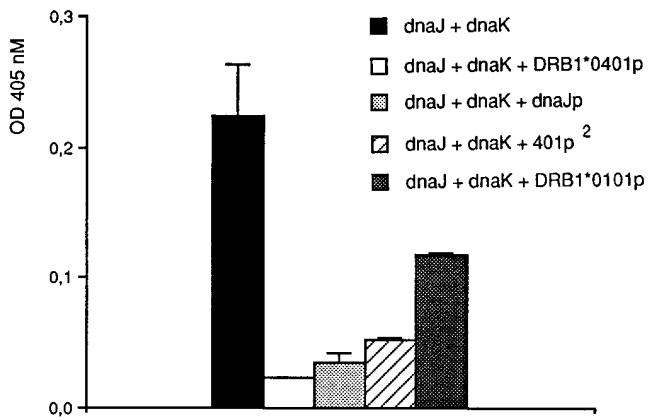


Figure 2. QKRAA-containing peptides inhibit binding of DnaK to DnaJ. ELISA plates were coated with *E. coli* DnaJ (0.5 µg/well) for 1 h at room temperature. For peptide inhibition studies, *E. coli* DnaK (0.5 µg/well) was preincubated for 1 h with peptide (10 µg/well). Bound DnaK was detected by mouse anti-DnaK monoclonal antibody 8E2 diluted 1/1,000, followed by peroxidase-conjugated anti-mouse IgG antibody (Boehringer Mannheim) diluted 1/1,000. OD was read at 405 nm. The background OD, obtained for wells where DnaK was added without prior coating with DnaJ was 0.06. It was subtracted from all the data. Peptides used for competition studies were: DRB1*0401 p: KDLLEQKRAAVDTYC, *E. coli* DnaJp: VLTDSQKRAAYDQYG, 401²p: EQKRAAEQKRAA, DRB1*0101p: KDLLEQRRRAEVDTYC. DRB1*0101p, a peptide that does not allow the purifying of DnaK from total protein extracts from *E. coli* is still capable of inhibiting partially the interaction between DnaJ and DnaK. We consider it a weak DnaK binder.

conjugated anti-mouse IgG antibody (Boehringer Mannheim) diluted 1/1,000. OD was read at 405 nm.

Results

QKRAA-containing peptides bind DnaK in *E. coli* protein extracts. Protein extracts from *E. coli* were loaded on peptide affinity columns. Bound proteins were eluted in ATP buffer, and

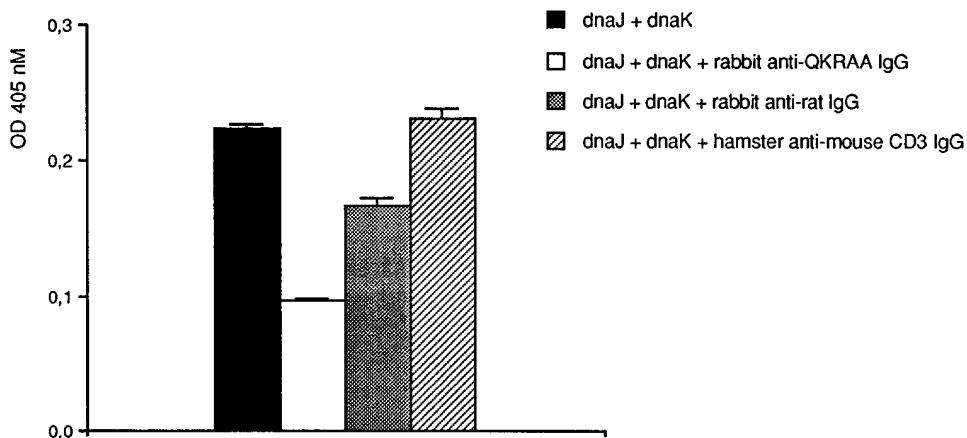


Figure 3. Antibody to the QKRAA motif inhibits binding of DnaK to DnaJ. ELISA plates were coated with *E. coli* DnaJ (0.5 µg/well) for 1 h at room temperature. *E. coli* DnaK was added to the wells in the presence of either rabbit antibody specific for the QKRAA motif (8), rabbit anti-rat IgG antibody, or hamster anti-mouse CD3 antibody at 15 µg/well. Bound DnaK was detected by mouse anti-DnaK monoclonal antibody 8E2 diluted 1/1,000, followed by peroxidase-conjugated anti-mouse IgG antibody (Boehringer Mannheim) diluted 1/1,000. OD was read at 405 nm. Background OD obtained by adding the competing anti-body to a well containing neither DnaJ nor DnaK, was 0.07 for the three antibodies used in this assay. It was subtracted from all the data.

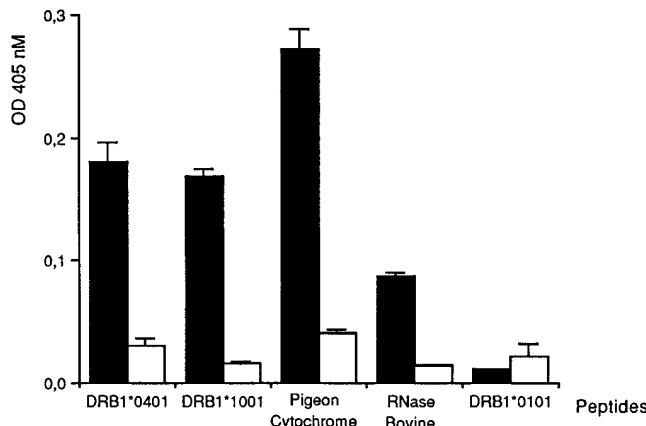


Figure 4. QKRAA-containing peptide inhibits binding of DnaK to DnaK binding peptides. ELISA plates were coated for 1 h at room temperature with 10 μ g/well of peptide. Peptides used for coating were: DRB1*0401 p: KDLLE**QKRAA**VDTYC, DRB1*1001p: KDLLE**RRRAA**VDTYC, Pigeon cytochrome p: KAERADLIY-LKQATAK, RNase B p: KESAAAKFERQHMD, DRB1*0101p: KDLLE**QRRAA**VDTYC (weak binder). For peptide inhibition studies, we added DRB1*0401 peptide (10 μ g/well) to *E. coli* DnaK (0.5 μ g/well). Bound DnaK was detected by mouse anti-DnaK monoclonal antibody 8E2 diluted 1/1,000, followed by peroxidase-conjugated anti-mouse IgG antibody (Boehringer Mannheim) diluted 1/1,000. OD was read at 405 nm. The background signal obtained by adding DnaK to a well with no coating peptide, and then detecting bound DnaK with anti-DnaK antibody, was 0.07 OD. We subtracted it from the data presented on the graph. ■, peptides + Dnak; □, peptides + Dnak + DRB1*0401p.

then analyzed on 8% SDS-PAGE gels. A 70-kD protein that bound QKRAA-containing peptides was identified by Western blotting as DnaK (Fig. 1). Peptide QKRAA was capable of binding DnaK, but peptide QKAA or peptides containing the QRRAA, QRRAE, DERA, and DRRAL motifs were not.

QKRAA-containing peptides inhibit binding of DnaK to DnaJ. Binding of DnaK to DnaJ can be assayed by ELISA (5). Incubation of DnaK with three peptides containing the QKRAA motif, DRB1*0401p, DnaJp (a peptide from the J region of DnaJ), and 0401p completely inhibited binding of DnaK to DnaJ. Incubation of DnaK with DRB1*0101p that contains a QRRAA motif instead of QKRAA resulted in very incomplete inhibition of DnaJ/DnaK binding (Fig. 2). Incubation of DnaK with DRB1*0403p that contains a QRRAE motif did not inhibit at all the interaction between DnaJ and DnaK (data not shown). Thus, minor changes in the QKRAA motif seem to diminish its ability to compete with DnaJ for binding to DnaK.

Antibody to the QKRAA motif inhibits binding of DnaK to DnaJ. Binding of the QKRAA motif of DnaJ to DnaK was confirmed as a rabbit antipeptide antibody specific for the QKRAA motif (6) inhibited DnaK binding to DnaJ (Fig. 3).

QKRAA-containing peptide inhibits binding of DnaK to DnaK binding peptides. The previous results suggested that the QKRAA motif on DnaJ binds DnaK. To determine whether QKRAA binds the peptide binding site on DnaK, we performed competition experiments. We coated ELISA plates with different peptides that were known to bind DnaK

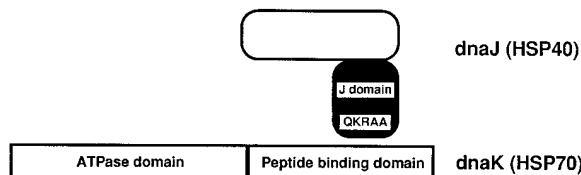


Figure 5. A tentative model of the interaction between DnaJ and DnaK. The QKRAA motif in the J domain of *E. coli* DnaJ binds the peptide binding domain of *E. coli* DnaK.

strongly, like DRB1*0401p, DRB1*1001 peptide, pigeon cytochrome peptide, or RNase B peptide, or very weakly, like DRB1*0101p. DnaK showed strong binding to DRB1*0401p, DRB1*1001 peptide, pigeon cytochrome peptide, weak binding to RNase Bp, and very weak binding to DRB1*0101p.

We then performed competition experiments with a QKRAA-containing peptide, DRB1*0401p. DRB1*0401p could completely inhibit binding of DnaK to every peptide (Fig. 4). This suggests that QKRAA interacts with the peptide binding site of DnaK, with high affinity (Fig. 5).

QKRAA binding to DnaK is not limited to *E. coli*. To test whether the interaction between the QKRAA motif and DnaK applies to other bacteria, we loaded protein extracts from *Brucella ovis*, *Salmonella dublin*, and *Proteus mirabilis* on QKRAA peptide affinity columns. We eluted a 70-kD protein that was identified as DnaK by positive staining with monoclonal antibody 8E2 (specific for *E. coli* DnaK) (Fig. 6).

Discussion

Most patients with rheumatoid arthritis type as HLA-DR4, HLA-DR1, or HLA-DR10 (7, 8). In these alleles, the third hypervariable regions (HV3) of the HLA-DR β 1 chain (the polymorphic chain in the HLA-DR α DR β 1 dimer) contain similar amino acid motifs: QKRAA in HLA-DRB1*0401, QRRAA in HLA-DRB1*0404, *0405, *0408, *0101, *0102, or RRRAA in HLA-DRB1*1001 (8). The role played by the QKRAA, QRRAA, and RRRAA motifs in the development of rheumatoid arthritis is unknown.

While studying the role of the QKRAA motif, expressed on HLA-DRB1*0401 (formerly HLA-DR4Dw4), the allele that is associated with the most severe forms of rheumatoid ar-

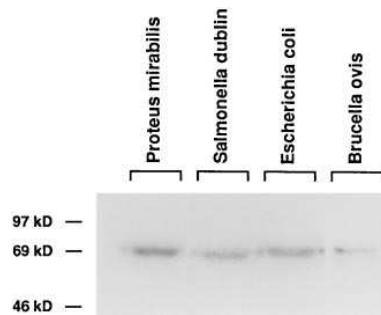


Figure 6. QKRAA peptides bind DnaK in protein extracts from *P. mirabilis*, *S. dublin*, *E. coli*, and *B. ovis*. Protein extracts from *P. mirabilis*, *S. dublin*, *E. coli*, and *B. ovis* were loaded on peptide DRB1*0401p (KDLLE**QKRAA**VDTYC) affinity columns. Bound proteins were eluted in ATP buffer. A 70-kD protein that bound DRB1*0401p was identified by Western blotting with anti-DnaK monoclonal antibody 8E2 (StressGen Biotechnologies Corp.).

Table I. Aligned Amino Acid Sequences of the J Domains of DnaJ Proteins from *E. coli*, *B. ovis*, *C. crescentus*, *L. lactis*, *R. fredii*, *E. rhusiopathiae*, *B. subtilis*, and *H. influenzae*

1	70	
MAKQDYYEILGVSKTAEEREIRK	AYKR	<i>E. coli</i>
M KIDYYEALGVTRTADDKTL	AAFRKLAMQYHPDRNPDPE	<i>B. ovis</i>
MR DYYEILGVTRTIDEAGL	AERKFKEIGEAYETLKDP	<i>C. crescentus</i>
MNNTEYYERLGVDKNASQDEI	AAGRKFKEINEAYSVLSDS	<i>L. lactis</i>
M KRDLYETLGVARNADEKEL	QKRAA YDRFG	<i>R. fredii</i>
MADKRDFYEILGVSKSATDAEI	KEEG AEEKYKEVQEAYETLSDE	<i>E. rhusiopathiae</i>
MSKRDYYEVLGVSKSASKDEI	AEKSFKEINQAYETLKDP	<i>B. subtilis</i>
MYLFSATNYKITMAKKDYYEVLGLQKGASEDEI	QKRAA YDRFG	<i>H. influenzae</i>
KAYRQLAKKYHPDINKEDG	QKRAA YDRFG	
KAYRQLSKKYHPDINKEAGS	QKRAH YDQFG	
DE KFKEVKEAYETLSDD	QKRAH YDQFG	
RAYKRLASKHHPDKNQGSKE	AEEKFKEINEAYEVLGVQ	
	QKRAA YDQFG	

thritis (9), we observed that it carried unique immunological properties.

Indeed, the QKRAA motif, whether expressed on synthetic peptides, HLA-DR molecules, bacterial, or viral proteins, constitutes a strong epitope for B cells, responsible for serological cross-reactions between QKRAA-containing molecules (6, 10). The QKRAA motif is also the basis for T cell epitopes involved in the positive and negative selection of the T cell repertoire (11, 12). People who express QKRAA on their HLA-DR molecules usually tolerate HLA-DR peptides with the QKRAA motif, but respond to bacterial peptides with the QKRAA motif (11, 12). Finally, the QKRAA motif is overrepresented in protein databases (4). We reasoned that these characteristics might indicate that the QKRAA motif carries a unique biological property that we decided to identify.

When screening total protein extracts from *E. coli* with different QKRAA peptide affinity columns, we isolated a 70-kD protein that we identified as DnaK, the 70-kD bacterial chaperone (reference 13 and Fig. 1). DnaK has a specific partner/ligand, DnaJ, the 40-kD bacterial chaperone, which is precisely a QKRAA protein (as are most bacterial DnaJ proteins), (Table I). Our data suggest that the QKRAA motif itself on DnaJ binds DnaK. Indeed, QKRAA-containing peptides inhibit the binding of DnaK to DnaJ and rabbit antibody to the QKRAA motif inhibits binding of DnaJ to DnaK.

Our data are consistent with what is known on the interaction between DnaJ and DnaK in *E. coli*. DnaJ, a 376 amino acid protein interacts with DnaK, a 638 amino acid protein composed of an NH₂-terminal ATPase domain and a COOH-terminal peptide binding domain, to bind unfolded polypeptides. In this interaction, DnaJ directly binds DnaK. The 108 NH₂-terminal amino acids of *E. coli* DnaJ are sufficient to mediate the DnaJ/DnaK interaction, as demonstrated in vitro by using a mutant of DnaJ that lost its 268 COOH-terminal amino acids (14). It is believed that the highly conserved J domain of DnaJ, which encompasses residues 2 to 72, is the part of DnaJ that binds DnaK (15). The QKRAA motif is precisely located in the J domain of DnaJ (residues 61–65) (Table I). Furthermore, the observation that QKRAA peptides inhibit the binding of DnaK to four different DnaK binding peptides suggests that the QKRAA motif may occupy the peptide binding site on DnaK, a finding that is consistent with the recently described affinity of the peptide binding site of DnaK for arginine and lysine (16). Finally, interaction between QKRAA and DnaK is not limited to *E. coli* as demonstrated by binding of DnaK from *B. ovis* (another bacteria whose DnaJ protein contains a QKRAA motif), *S. dublin*, and *P. mirabilis* to QKRAA peptide affinity columns.

Taken together, our data suggest that the QKRAA motif on DnaJ constitutes a binding motif for bacterial HSP70s (DnaK proteins). Whether the same property is conserved when the QKRAA motif is expressed on HLA-DR is still unknown. Consistent with this hypothesis, we have observed that, in lymphoblastoid cells, human HSP73 can associate with HLA-DRB1 chains that contain a QKRAA motif (13).

It is conceivable that, in case of exposure to enterobacteria in people who express HLA-DRB1*0401, bacterial DnaK proteins may bind the QKRAA motif on HLA-DR, triggering strong T cell responses to HSP70s. This is especially interesting because a dominant epitope on human type II collagen is homologous to HSP70s (17).

Thus, QKRAA is a binding motif for bacterial HSP70s and this may be a clue to the role of bacterial heat shock proteins in the development of RA in people who type as HLA-DRB1*0401.

Acknowledgments

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