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An HLA-DRB1-Derived Peptide Associated with Protection Against Rheumatoid Arthritis Is Naturally Processed by Human APCs

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Predisposition to rheumatoid arthritis (RA) is thought to be associated with HLA-DR1, -DR4, and -DR10. However, epidemiological observations are better explained by a model in which the DQ alleles that are linked to these DR alleles, i.e., DQ5, DQ7, and DQ8, predispose to RA, while certain DR alleles have a dominant protective effect. All protective DRB1 alleles, e.g., *0402, *1301, and *1302, encode a unique motif, 70DERAA74. The protection may be explained by the presentation of DRB1-derived peptides by DQ to immunoregulatory T cells, because it was demonstrated in various autoimmune disease models that T cell responses to certain self-Ags can be involved in disease suppression. The aim of this study was to analyze whether peptides carrying the DERAA motif are naturally processed by human APC and presented in the context of the RA-predisposing DQ. Using a synthetic peptide carrying the DRBI*0402-derived sequence KDILEDERRAVDTYC, we generated DERAA peptide-specific cell responses to certain self-Ags can be involved in disease suppression. The aim of this study was to analyze whether peptides carrying the DERAA motif are naturally processed by human APC and presented in the context of the RA-predisposing DQ. Using a synthetic peptide carrying the DRBI*0402-derived sequence KDILEDERRAVDTYC, we generated DERAA peptide-specific cell responses to certain self-Ags can be involved in disease suppression.


These DR-DQ linkage disequilibria and the observation that in collagen-induced arthritis, a mouse model for RA, the predisposing MHC class II molecule is H2-A^* (equivalent of HLA-DQ), whereas certain H2-E (equivalent of HLA-DR) alleles can protect, formed the basis for a new hypothesis (6). In this model, termed RA protection, certain DQ alleles predispose to RA, whereas DR alleles can modulate the effect of DQ by either enhancing or dominantly protecting against the DQ-associated predisposition. The predisposing DQ alleles are DQ5 and the DQA1*03-chain-containing alleles, DQ4, DQ7, DQ8, and DQ9 (Table I), collectively referred to as DQ3 hereafter. The enhancing DRB1 alleles are DRB1*0401 and *0404, whereas DRB1 alleles such as DRB1*0402, *1301, and *1302, which all encode the amino acid sequence motif 70DERAA74 in the HV3 region of the protein, are dominantly protective (6).

Consistent with this model, DQ8 transgenic mice, but not DQ6 (DQB1*0601/DQA1*0103) transgenic mice, develop collagen-induced arthritis (7). The DQ3-DR4 haplotypes have a stronger association with RA and predispose to a more severe disease than the DQ5-DR1 and DQ5-DR10 haplotypes (8, 9). Particularly, individuals with two doses of RA-predisposing DQ3 are significantly more predisposed to RA than individuals with two doses of shared epitope (9). Shared epitope-negative DQ9-DR9 homozygous individuals are predisposed to RA (10), and DQ9-DR9 increases the risk of developing disease in DQ3- and DQ5-heterozygous individuals. Noticeably DRB1*0402 with the motif DERAA is the only DR4 allele clearly not associated with RA (11). Moreover, DRB1*1301 and *1302 have a dominant protective effect on disease predisposition over DQ5 (9) and on disease severity over both DQ3 and DQ5 (12).
The mechanism of the DERAA-related protection may involve the presentation of DRB1-derived peptides by DQ leading either to the deletion of potential autoreactive T cells in the thymus or to the generation of DRB1-specific, DQ-restricted immunoregulatory CD4+ T cells. In various murine autoimmune disease models, CD4+ T cell responses to self-MHC Ags have been related to suppression of the disease (13, 14). In addition, MHC-derived peptides constitute a large fraction of the natural ligands eluted from MHC class II molecules (15). Whether the immunoregulatory CD4+ T cells arise directly in the thymus or acquire their regulatory capacity later in the periphery is still controversial (reviewed in Ref. 16). Their existence and their mode of action, i.e., by cytokines such as TGF-β, IL-4, and IL-10 or by cell-cell contact with the APC, are nonetheless becoming increasingly clear (17–20). The regulatory T cells may have the same or a different specificity as the autoaggressive T lymphocytes, but they will need to home to the autoimmune-targeted site to fulfill their suppressive role (21). CD4+ T cells specific for an epitope of an MHC class II molecule seem well suited for a protective role in autoimmune diseases involving inflammation, because MHC class II molecules are always expressed at sites of inflammation.

The existence of protective, DERAA-specific, DQ-restricted T cells in RA requires the natural processing and presentation of the DERAA sequence. The aim of the present study was to test whether the DERAA-positive DRB1 proteins are processed by human APCs, resulting in the presentation of DRB1-derived peptides carrying the DERAA motif in the context of the RA-predisposing DQ molecules. By analyzing the proliferation of DQ-restricted T cell clones (TCC) generated from a DQ8 homozygous individual with DR4 alleles lacking the DERAA motif, we found natural T cell responses to self-MHC Ags that are processed and generate DQ-restricted epitopes associated with protection against RA.

### Materials and Methods

#### Peptides and mAbs

The 15-mer DERAA peptide with the sequence KDILEDERAAVDITYC (boldface intended to emphasize the presence of the DERAA motif in the sequence) was purchased from NeoSystem (Strasbourg, France). Its purity was >99%. All other peptides used in this study were synthesized on an automated multiple peptide synthesizer (Syroll, Multisyntech, Germany) and isolated as previously described (22). The purity of the peptides was determined by analytical reverse phase HPLC as previously described (22) and proved to be at least 70% (UV, 214 nm). The integrity of the peptides was determined by mass spectroscopy as previously described (22).

mAbs B8.11.2 specific for HLA-DR (23) and SPVL3 specific for HLA-DQ (24) were provided by Arend Mulder (Leiden University Medical Center, Leiden, The Netherlands). Fluorescent-labeled mAbs used for FACS analysis were purchased from Becton Dickinson (CD4, CD8, CD28, FACS analysis were purchased from Becton Dickinson (CD4, CD8, CD28, CD29, HLA-DR) or from Leuco (St. Louis, MO; HLA-DQ).

#### cDNA production and nucleotide sequence determination

RNA from the DRB1*0402 homozygous B lymphocyte cell line (BLCL) YAR ([HWW9026] (50 × 10^6 cells) was isolated using RNAzol (Cinna/ Biotec Laboratories, Houston, TX) and reverse transcribed using oligo(dT) and AmpliTaq from Novagen (Madison, WI). Using the DRB1*04-specific primer pair 5'-GGCACTGCGTTCGCC-3' (starting 18 nucleotides upstream from the ATG start codon) and 5'-TGGAAGAATGAGAGCCAAGCG-3' (starting 85 nucleotides downstream from the TGA stop codon), we amplified a 904-bp cDNA fragment containing the complete coding region of DRB1*0402. PCR products were purified using MicroSpin S-400 HR columns (Amersham Pharmacia Biotech, Roosendaal, The Netherlands).

Sequencing reactions were set up using the Thermo Sequenase core sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech), with 2 μmol of Texas Red 5'-labeled sequencing primers (Isogen Bioscience, Maarssen, The Netherlands). The primer sequences were 5'-GCCGAGTCTTCGAGCAT-3' (F1), 5'-GGCCGAGTCTTCGAGCAT-3' (F2), 5'-AGAA TGAGAGCCAAGCG-3' (R1), and 5'-TGCTTGAGCATTACACACCT-3' (R2). Cycle sequencing was performed on a Peltier Thermal Cycler (PTC- 200, MJ Research, Waltham, MA), and subsequent electrophoresis on a Vista DNA Sequencer 725 (Amersham Pharmacia Biotech) for 6 h using a 6% Rapidgel-XL (U.S. Biochemical, Cleveland, OH). We determined the nucleotide sequences of two independently amplified PCR products and obtained identical results.

#### Production and purification of recombinant DRB1*0402 protein

The full-length DRB1*0402 cDNA fragment was inserted into the expression vector pET28b (Novagen). Using Escherichia coli XLI-blue (Stratagene, La Jolla, CA) as host strain, DRB1*0402 protein expression was induced by isopropyl-1-thiogalactopyranoside. After overnight culture the cells were harvested and lysed. The inclusion bodies were purified by repeated washing/centrifugation cycles and resuspended in phosphate-Tris-buffered 8 M urea, pH 8, to a final protein concentration of 3 mg/ml. Purity, estimated by Coomassie-stained SDS-PAGE, was about 90%.

#### Human cells and HLA typing

All cells used in this study were isolated from heparinized blood (PBMC) from either healthy volunteers or patients visiting the out-patient clinic of the Department of Rheumatology of Leiden University Medical Center. All donors gave their informed consent. HLA typing was performed in Leiden as described previously (9).

<table>
<thead>
<tr>
<th>Short Name of the DQ Molecule</th>
<th>DQB1/DQA1 Linked to</th>
<th>In RAP model</th>
<th>In shared epitope model</th>
</tr>
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<tr>
<td>DQ9</td>
<td>*0303/*03</td>
<td>DR9</td>
<td>+−</td>
</tr>
</tbody>
</table>

* Only in the absence of the dominantly protective, DERAA motif-containing DRB1 alleles, i.e., DRB1*0103, *0402, *1102, *1103, *1301, and *1302.
* Only for the shared epitope-containing DRB1 alleles, i.e., DRB1*0101, *0401, *0404, *0405, and *1001.
* DQB1*06 was *0602 when present with DR15, *0603 with DRB1*1301, and *0604 with DRB1*1302.
* Because the name DQ7, defined by serology, represents three different DQ molecules we designated them DQ7a, DQ7b, and DQ7c throughout this study.
* Although DQ9 can represent different DQ molecules, in this study DQ9 was only the DR9-linked DQ9 (DQB1*0303/DQA1*03).
**Generation of DERRA-specific T cell lines and clones**

PBMC from an HLA-DQ8 homozygous donor were cultured at 10^6 cells/ml in 5 ml of culture medium (IMDM supplemented with 10% human serum) in the presence of 10 μg/ml peptide KDILDERAAVDTYC (>99% pure). In parallel, monocytes from the same donor were isolated by adherence, and DC were generated using GM-CSF and IL-4, as previously described (25). After 8 days, the surviving T cells (2 × 10^5/well in 1 ml of culture medium) were restimulated with autologous monocyte-derived DC (5 × 10^5/well) preincubated with 10 μg/ml of the same peptide. After 11 days of culture the T cells in individual wells were restimulated separately with irradiated (2000 rad), autologous PBMC (5 × 10^5/well) preincubated with the peptide (second restimulation). After 2 days 10% IL-2-containing medium (Lymphocult-T, Biostat, Serum Institute, Frankfurt, Germany) was added and refreshed every 3–5 days. The resulting T cell lines were harvested 11 days after the last restimulation. Some of the T cells were restimulated with irradiated, peptide-pulsed, autologous PBMC and supplied with IL-2-containing medium as before, and some were tested for their peptide specificity by proliferation assay using irradiated (3000 rad), HLA-DQ8-matched PBMC as APC. After the third round of restimulation, peptide-specific T cell lines were frozen. On thawing, the T cells were cloned nonspecifically by limiting dilution (0.3 cells/well) using 10^3 irradiated (3000 rad) allogeneic feeder cells (pooled PBMC from five donors), 1 μg/ml PHA, and 20U/ml human rIL-2. After in vitro expansion with feeder cells, PHA, and rIL-2, the resulting TCC were frozen and stored in liquid nitrogen.

**In vitro lymphocyte proliferation assay**

Proliferation of T cells was measured in flat-bottom 96-well plates (Corning Costar) in triplicate cultures. Viable T cells (10^4/well) were cultured in 1 ml of culture medium in the presence of irradiated (3000 rad) PBMC from an HLA-DR/DQ-typed individuals (5 × 10^5/well) and synthetic peptide. Cells were cultured for 3 days at 37°C in 5% CO2 and subsequently for another 16–20 h with [3H]thymidine (0.5 μCi/ml). [3H]incorporation was measured by liquid scintillation counting. Results are expressed as the mean of triplicate cultures. The SD was <20% of the mean value in all results of this study.

**Purification of DQ molecules and DQ-peptide binding assay**

As a source of DQ molecules BLCL BSM [HW9032] (DQB1*0302/ DQA1*0301 DRB1*0401) homogenous for DQ8 was used. Cells were cultured in RPMI 1640 supplemented with 2 mM l-glutamine, 100 U penicillin/100 μg/ml streptomycin solution, and 10% heat-inactivated FCS (all from Life Technologies, Paisley, U.K.). DQ8 molecules were purified from cell lysates by heparin chromatography essentially as described previously for DR (26). The only modification was the exchange of the last chromatography column from one specific for DR to one carrying mAb SPVL-3 that is specific for DQ.

Peptide binding to DQ8 was determined as described previously (27). In short, purified DQ8 molecules were incubated with fluorochrome-labeled standard peptide in the presence of protease inhibitors. The standard peptide in this study was fluorescent-labeled LPKPPKPVSKM RMATPLLMLQALPM. Peptides for which the DQ binding capacity was to be determined were added to DQ molecules simultaneously with the standard peptide. The DQ-peptide complexes were separated from free peptide by gel filtration, and the fluorescent emission of the complexes was measured as previously described (27). From the amount of MHC-bound fluorescence obtained in the presence of the labeled standard peptide only (A) and the amount of fluorescence obtained in the additional presence of the unlabeled peptide (B), the ability of each peptide to inhibit the binding of the labeled peptide was calculated (A - B)/A = inhibition factor). The binding capacity of each test peptide to DQ was expressed as the ratio of the inhibition factor of that peptide and the inhibition factor obtained with the unlabeled standard peptide.

**Results**

**DRB1*0404 differs from nonprotective DRB1*0401 alleles in the HV3 region only**

The DQ3-DRB1*0401 haplotype is associated with the most severe form of RA, probably because the DRB1*0401 allele enhances the DQ3-associated disease. In contrast, the closely related allele DRB1*0402, which is also in linkage disequilibrium with DQ3, is dominantly protective. At the start of this study, DRB1*0402 was known to differ from DRB1*0401 in the HV3 region and therefore the HV3 region was thought to be associated with protection. However, a complete sequence comparison between DRB1*0401 and *0402, showing all regions potentially associated with protection, was impossible because the region of DRB1*0402 encoding amino acid residues 92–237 had never been sequenced. Therefore, we determined the nucleotide sequence of the complete coding region of DRB1*0402. For the sequence analysis we used cDNA amplified from the DRB1*0402 homozygous BLCL YAR- and DRB1*04-specific primers.

The resulting DRB1*0402 sequence (EMBL nucleotide sequence databank accession no. AJ245881, IMTG/HILA database submission no. HWS10000777) differed from the known DRB1*0401 sequence only at the three known polymorphic positions, which are in the HV3 region, i.e., in codon 67, L→I, 70, Q→D, and 71, K→E. This result implies that 67ILEDERAA24 is the only candidate region that can explain the protective effect of DRB1*0402 in RA.

**Generation of DERRA-specific, DQ-restricted TCC**

As tools for the analysis of the natural processing of the DERAA peptide, we generated DERRA peptide-specific, DQ-restricted TCC. We started by subjecting PBMC from a DQ8-homozygous individual carrying DR4 molecules without a DERRA motif to repeated stimulation with a synthetic DERRA peptide with the sequence KDILDERAAVDTYC6, which is present in all but one of the DERRA-positive DRB1 alleles (DRB1*1103 carries F instead of I at position 67). Despite the fact that it is generally assumed impossible to generate T cell responses in vitro from an unprimed individual, we obtained two DERRA peptide-specific T cell lines. From one of these lines (Tp7.7) a panel of 28 TCC was generated that were DERRA-specific because their proliferation in the presence of DERRA-negative, DQ8 APC was fully dependent on the addition of exogenous DERRA peptide. The results of a representative TCC are shown in Fig. 1. Peptide-specific proliferation was completely inhibited by the DQ-blocking mAb SPVL-3.

![FIGURE 1](http://www.jimmunol.org/) The DERRA peptide is naturally processed and presented in the context of DQ8. TCC Tp7.7–13, a representative sample from a panel of TCC, was incubated with irradiated PBMC from different DQ8 individuals in medium without additions, medium with 10 μg/ml of DERRA peptide (KDILDERAAVDTYC6), or medium with DERRA peptide and the DQ-specific mAb SPVL-3 as indicated. Proliferation was measured by [3H]thymidine incorporation. The relevant DQ/DR typing of the PBMC (APC) is indicated, and the DERRA-positive DRB1 alleles are underlined. When APC from different individuals with the same DR/DQ typing were tested (two individuals with DRB1*1301 and three with *1302), similar proliferation levels were obtained. The full DR allele designation of the control APC was DRB1*0401/0404, and those for the other APC were, respectively, DRB1*0402/0405, DRB1*0301/0402, DRB1*0401/1301 or *0404/*1301, and DRB1*0401/1302. Four other DERRA-specific TCC were tested, yielding similar results.
(Fig. 1), but was unaffected by the DR-blocking mAb B8.11.2 (data not shown), showing that the 28 DERAA-specific TCC were DQ restricted. In agreement with their MHC class II restriction and their resting state, the TCC expressed high levels of CD4, no CD8, and very little CD28, as determined by Ab staining and flow cytometry (data not shown). As usual for human TCC, they also expressed high levels of HLA-DR and intermediate levels of HLA-DQ. In most of the experiments described below five DERAA-specific TCC were tested, but because these TCC yielded essentially the same results only those for TCC Tp7.7–13 are shown.

Natural processing of DERAA peptide by human APC

Proliferation of the DERAA-specific, DQ-restricted TCC in the presence of APC from individuals carrying DQ8 and a DERAA-positive DRB1 allele showed that all three DRB1 molecules tested (DRB1*0402, *1301, and *1302) were processed by human APC and generated a DERAA peptide that was presented in the context of DQ8 (Fig. 1). The addition of exogenous DERAA peptide enhanced the proliferation only slightly, indicating that the natural processing of the endogenous DERAA-positive DRB1 proteins was efficient. Proliferation was blocked by DQ-specific mAb SPVL-3, showing that the response of the TCC to the naturally processed peptide was also restricted to DQ. After testing APC from six different DQ8-heterozygous, DERAA-positive individuals, we found no evidence for differences in processing among DQ8-heterozygous individuals (Fig. 1), as reported for MHC class II heterozygous mice (28).

Presentation of DERAA peptide restricted to DQ8 and DQ7a

To test which DQ molecules could act as restriction elements, we tested five selected DERAA-specific TCC, among which were Tp7.7–13, against a whole panel of APC from DERAA-negative individuals carrying different DQ alleles with synthetic DERAA peptide or recombinant DRB1*0402 protein as the Ags. Of the six different DQ molecules tested (Fig. 2A), only the two DQA1*03-chain-containing molecules, DQ8 and DQ7a (Table I), could act as restriction elements for the DERAA-specific TCC. These DQ molecules presented both the synthetic and the naturally processed DERAA peptide (derived from recombinant DRB1*0402 protein) to the TCC (Fig. 2A). Surprisingly, the recombinant protein acted as a much more potent Ag on a molecular basis than the peptide. In our opinion there are three possible explanations for this observation. 1) Complete inclusion bodies of the recombinant protein were added to the cultures, and these are large enough to sink to the bottom of the well, as we could demonstrate using the Bradford assay to determine protein concentration. Thus, the effective concentration of the recombinant protein was increased. 2) Complete inclusion bodies may be taken up by the APC much more efficiently than peptides. 3) In a complete protein or in inclusion bodies the epitope is probably more protected from proteolytic degradation than in a small peptide.

A panel of APC from five different individuals carrying DQ7a and a DERAA-positive DRB1 allele (Fig. 2B) was analyzed using proliferation of the DERAA-specific TCC, Tp7.7–13, as a read-out. Again, all APC processed their endogenous DERAA-positive DRB1 molecule and presented the DERAA peptide in the context of DQ, showing on that occasion that a fourth DERAA-positive DRB1 allele, DRB1*1103, which differs from the other DERAA-positive DRB1 alleles at position 67 (I=F), also yielded naturally processed DERAA peptide.

Reduced efficacy of DQ7a compared with DQ8 in presenting the DERAA peptide to DERAA-specific TCC

To analyze whether DQ7a and DQ8 differed in efficacy to present the DERAA peptide to the TCC, we made a dose-response curve of peptide-dependent TCC proliferation for DQ7a and DQ8 carrying APC. We included APC carrying DQ7b or DQ9 (Table I) in the analysis because DQ7b and DQ9 are related variants of DQ8 and DQ7a (Table II). Specifically, these four DQ3 molecules carry the same DQA1 chain (DQA1*03). However, in the β-chain DQ7a (DQB1*0301) differs from DQ8 (DQB1*0302) at positions 13, 26, and 45, and 57; DQ7b (DQB1*0304) at positions 13, 26, and 45; and DQ9 (DQB1*0303) at position 57. The peptide concentration needed for a similar level of TCC proliferation proved similar for
DQ8- and DQ7b-expressing APC and ~10-fold higher for DQ7a-expressing APC (Fig. 3). DQ9-expressing APC gave somewhat unusual results. The APC from all three different DQ9 individuals that we tested (DQ2/9, DQ6/9, and DQ9/9) induced high background proliferation of the TCC, resulting in a low stimulation index (SI) for the DERAA-specific proliferation (SI varied from 3 to 11). However, the high background proliferation could be blocked by addition of the DR-specific mAb B8.11.2 (restoring the SI to ~150–300), whereas the DERAA-specific proliferation was blocked by the DQ-specific mAb SPVL-3 and was unaffected by mAb B8.11.2 (data not shown). The combined proliferation data (Fig. 3) suggest that the impact of the polymorphism at position 57 is greater than that of the polymorphisms at position 13, 26, and 45.

Unfortunately, no DERAA-positive DQ9 APC were available to test natural processing of the DERAA peptide by DQ9 APC. Nevertheless, DQ9 APC were able to process and present the recombinant DRB1*0402 protein to the TCC, as indicated by SI values of 6 for DQ9/9 DR9/9 and 31 for DQ6/9 DR15/9 APC (compared with SI of 4.5 and 11 obtained with the 15-mer DERAA peptide in the same experiment; data not shown).

Minimal epitope for DQ8 binding and for proliferation of DERAA-specific TCC

Using a panel of truncated and mutated peptides in a competition binding assay, we determined the minimal DERAA region involved in binding to DQ8 (Fig. 4). The longest DERAA peptide tested had a slightly higher affinity for DQ8 than the standard peptide (Ii p87–101); all truncated and mutated peptides had lower affinity. The binding results of the truncated peptides indicate that the 9-mer 66LEDRAAVD76 is the core epitope for binding to the DQ8 molecule, but that the flanking residues K65, D66, I77, and T77 increase the DQ8 binding capacity of the peptide. The complete lack of binding by the D76→A substituted peptide shows that the D76 is essential for DQ8 binding and indicates that it occupies the p9 pocket.

To determine whether DQ8, DQ7a, and two other DQA1*03-chain-containing DQ molecules, DQ7b and DQ9, presented the same epitope to the TCC we tested APC carrying these DQ molecules for TCC proliferation with a panel of truncated DERAA peptides (Fig. 5). The shortest peptide that induced significant TCC proliferation (SI, >5) with all four APC was the 12-mer peptide 67 ILEDERAAVDTY 78. The results with the truncated peptides indicated that I67 and Y78 contributed substantially to T cell recognition (Fig. 5), but that 68 LEDERAAVD76 was most likely to occupy the binding groove in all four DQ molecules. In agreement with the results presented in Fig. 3, DQ9 APC gave high background proliferation, hampering clear conclusions, but the proliferation of the TCC with the truncated peptides presented by the three other DQ3 APC decreased in the order of DQ8>DQ7b>DQ7a.

Table II. Only the amino acid difference at position 57 of the DQB1*03 molecules affected DERAA peptide-specific T cell proliferation

<table>
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<tr>
<th>DQ</th>
<th>DQB1</th>
<th>pos13</th>
<th>pos26</th>
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<td>A</td>
<td>++</td>
</tr>
</tbody>
</table>

*Residues 13 and 26 are located inside pocket 4, residue 57 is inside pocket 9, and residue 45 is in a downward loop.

FIGURE 3. Relative efficacy of DQ8, DQ7a, and related DQ3 molecules presenting the DERAA peptide to the DERAA-specific TCC. TCC Tp7-7-13 was incubated with irradiated PBMC from individuals carrying DERAA-negative DR molecules combined with different DQ3 molecules (Table I), i.e., DQ8/8 DR4/4 (DRB1*0401/*0404), DQ6/7b DR4/15 (DRB1*0408/*1500), DQ9/9 DR9/9 (DRB1*0901), or a DQ7a/7a DR4/4 (DRB1*0401) in medium supplied with increasing concentrations of the DERAA peptide (KDILEDERAAVDTYC). Proliferation was measured by [1H]thymidine incorporation.

FIGURE 4. Binding of DERAA peptides to DQ8. Peptides with the indicated amino acid sequences were mixed with the fluorescence-labeled standard peptide and allowed to bind to purified DQ8. From the amount of fluorescence bound in the presence or the absence of unlabeled peptide the capacity of each peptide to bind DQ8 was calculated as described in Materials and Methods. The upper two peptides are the standard peptide and the negative control peptide, respectively. The threshold for binding was arbitrarily set at a binding capacity of 0.4.
In conclusion, our present results provide molecular support for the hypothesis that the roles of the different DQ3 molecules (containing the DQA1*03-chain; Table I) in RA are similar, we found that the DERAA peptide could be presented not only by DQ8 but also by DQ7a, DQ7b, and DQ9. Similarly, others (30) found presentation by DQ8 and DQ9 of two gluten-derived peptides. However, molecular modeling of DQ on the known structure of DR1 (31, 32) indicates that the amino acid sequence differences among DQB1*0301 (DQ7a), DQB1*0302 (DQ8), DQB1*0303 (DQ9), and DQB1*0304 (DQ7b) are located inside pockets 4 and 9 and in a downward loop outside the region that can combine with a TCR (DQ7a differs from DQ8 in pockets 4 and 9, from DQ7b in pocket 4, and from DQ9 in pocket 9). The location of the polymorphic residues (inside the pockets) suggests that the DQ3 molecules have different peptide binding preferences, but are indiscriminate in the interaction with TCR. In agreement with the modeling of DQ, binding studies (29) showed different peptide binding motifs for these DQ molecules. In particular, DQ7a and DQ9 differ from DQ8 in preferring a noncharged residue such as A at position 9. In view of these evident differences in peptide binding preferences among the DQ3 molecules, our TCC proliferation results (Figs. 3 and 5) are best explained by a difference in the affinity of the DERAA peptides for the different DQ3 molecules. Nevertheless, both DQ7a and DQ8 can bind the 15-mer synthetic DERAA sequence and the naturally processed DERAA peptide well enough to cause strong proliferation of the DERAA-specific TCC (Figs. 1 and 2).

There is an extensive literature on the distinct stability and peptide affinity of DQ7a, DQ8, and DQ9 (29, 33–35). These differences have been highlighted to explain the distinct contributions of DQ7, DQ8, and DQ9 to type I diabetes. Indeed, predisposition to type I diabetes is associated with DQ8, but not with DQ7 (36). In contrast with these ideas, our results show that DQ7a, DQ7b, DQ8, and DQ9 are related enough to present common epitopes, such as ILEDERRAVDVTY, which is associated with protection against RA. This finding that all four DQ3 molecules can present the DERAA peptide, albeit with different efficacy, suggests that these molecules may also present the same RA-associated peptides to autoreactive T cells. As a consequence, DQ7a and DQ9 can be expected to make a similar, but weaker, contribution to RA compared with that of DQ8. However, the roles of DQ7a and DQ9 in RA may also be qualitatively different from that of DQ8, as indicated by some epidemiological observations suggesting a primary role for DQ8 in susceptibility to RA and an additive effect of DQ7a and DQ9 when combined with DQ8 (37). Nevertheless, all these observations support some role for DQ7a and DQ9 in susceptibility to RA.

The difference between RA and type I diabetes in the association of DQ7a with disease predisposition may be explained by factors such as the nature of the autoantigens involved and the mechanism of the predisposition. The early age of onset and the very specific nature of the targeted cells, i.e., pancreatic β-cells, suggests a primary role for T cells in the etiology of type I diabetes. Conversely, the rather unspecific reactivity of T and B cells in RA, as underscored by the rheumatoid factor and the indiscriminate destruction of cartilage and bone, suggests a more indirect role for T cells in this disease. Therefore, it is conceivable that the differences among DQ7a, DQ8, and DQ9, which have dramatic consequences for susceptibility to type I diabetes, may be less important in RA.

In conclusion, our present results provide molecular support for a role of DRB1-derived peptide presented by RA-predisposing DQ molecules in disease modulation of RA. Our investigations open new avenues in understanding the role of T cells in the pathogenesis of RA and other autoimmune diseases.
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