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

Koen Vos,* † Geziena M. T. Schreuder,*, Ferdinand C. Breedveld, † Rene´ R. P. de Vries,* and Eric H. Zanelli*

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 69KDILEDERAAVD79, we generated DERAA peptide-specific T cell clones (TCC) from a DQ8 homozygous individual carrying DERAA-negative DR4 alleles. By analyzing the proliferation of these TCC, we demonstrated natural processing and presentation of the DERA sequence by the APC of all the individuals (n = 12) carrying a DERAA-positive DRB1 allele and either DQ8 or the DQ8-related DQ7. Using a panel of truncated synthetic peptides, we identified the sequence 67(I)LDEDERAADV(TY)78 as the minimal determinant for binding to DQ8 and for recognition by the TCC. These findings support a model in which self-MHC-derived peptide can modulate predisposition to autoimmune disease in humans. The Journal of Immunology, 2001, 166: 4987–4993.
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 to the autoimmune 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 DERAA-specific T cell clones (TCC) generated from a DQ8 homozygous individual with DR4 alleles lacking the DERAA motif, we found natural processing and presentation of the DERAA sequence by APC of all individuals carrying a DERAA-positive DRB1 and a DQ8 allele, i.e., DQ7a or DQ8. Our results clearly demonstrate that DRB1 molecules not only act as restriction elements, but can also act as 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 KDILEDERAAYDVTYC (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, Multisynetych, 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 spectrometry 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 and proved to be at least 70% (UV, 214 nm). The integrity of the peptides was determined by mass spectrometry as previously described (22).

cDNA production and nucleotide sequence determination

RNA from the DRB1*0402 homozygous B lymphocyte cell line (BLCL) YAR [IH99092] (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'-CTGTTTGCCTCCTTG-3' (starting 18 nucleotides upstream from the ATG start codon) and 5'-TGGAGGAATGAGAGC-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 pmol of Texas Red 5'-labeled sequencing primers (Isogen Bioscience, Maarssen, The Netherlands). The primer sequences were 5'-GTCCTCCTG TCTCCACAGCAT-3' (F1), 5'-CCGGCATCTATCTTGCT-3' (F2), 5'-AGAA TGAGACCAAGCG-3' (R1), and 5'-TGGCTGGATGAAGACCAGC-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 2% 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 XL1-blue (Stratagene, La Jolla, CA) as host strain, DRB1*0402 protein expression was induced by isopropyl-β-d-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 1. HLA-DQ and DR alleles relevant for this study

<table>
<thead>
<tr>
<th>Short Name of the DQ Molecule</th>
<th>DQB1/DQA1</th>
<th>Linked to</th>
<th>In RA Predisposition</th>
<th>In shared epitope model</th>
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</thead>
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<tr>
<td>DQ2</td>
<td>*0201/*0501</td>
<td>DR3</td>
<td>+a</td>
<td>+b</td>
</tr>
<tr>
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<td>*04/*03</td>
<td>DR4</td>
<td>+a</td>
<td>+b</td>
</tr>
<tr>
<td>DQ5</td>
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<td>+b</td>
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<td>*06/*01</td>
<td>DR13, DR15</td>
<td>+a</td>
<td>+b</td>
</tr>
<tr>
<td>DQ7a/b</td>
<td>*0301/*03</td>
<td>DR4</td>
<td>+a</td>
<td>+b</td>
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<td>+a</td>
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<tr>
<td>DQ7e/f</td>
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<td>+b</td>
</tr>
<tr>
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<td>*0302/*03</td>
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<td>+a</td>
<td>+b</td>
</tr>
<tr>
<td>DQ8a/b</td>
<td>*0303/*03</td>
<td>DR9</td>
<td>+a</td>
<td>+b</td>
</tr>
</tbody>
</table>

a Only in the absence of the dominantly protective, DERAA motif-containing DRB1 alleles, i.e., DRB1*0103, *0402, *1102, *1103, *1301, and *1302.
b Only for the shared epitope-containing DRB1 alleles, i.e., DRB1*0101, *0401, *0404, *0405, and *1001.
c DQB1*06 was *0602 when present with DR15, *0603 with DRB1*1301, and *0604 with DRB1*1302.
d Because the name DQ7, defined by serology, represents three different DQ molecules we designated them DQ7a, DQ7b, and DQ7c throughout this study.

*1302.

Table I. HLA-DQ and DR alleles relevent for this study

*1302.

Table I. HLA-DQ and DR alleles relevent for this study

*1302.
Generation of DERAA-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 $\mu$g/ml peptide KDILEDERAAVDVTYC ($>99\%$ 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 ($2 \times 10^6$ cells in 1 ml of culture medium) were restimulated with autologous monocyte-derived DC ($5 \times 10^7$/well) preincubated with 10 $\mu$g/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 ($5 \times 10^7$/well) preincubated with the peptide (second restimulation). After 2 days 10% IL-2-containing medium (Lymphocult-T, Biostest, 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 cell lines 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-DQ-matched PBMC as APC. After the third round of restimulation, peptide-specific T cell lines were frozen. After 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 $\mu$g/ml PHA, and 20$\mu$m 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. Viability of T cells ($10^7$/well) were cultured in 200 $\mu$l of culture medium in the presence of irradiated (3000 rad) PBMC from HLA-DR/DQ-typed individuals ($5 \times 10^6$/well) and synthetic peptide. Cells were cultured for 3 days at 37°C in 5% CO$_2$ and subsequently for another 16–20 h with $[^3]$H]thymidine (0.5 $\mu$Ci/ml). $[^3]$H 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 DQ8 molecules BLCL BSM [IHW9032] (DQB1*0302/DQA1*0301/DRB1*0401) homozygous for DQ8 was used. Cells were cultured in RPMI 1640 supplemented with 2 $\mu$mL-glutamine, 100 U penicillin/100 $\mu$m streptomycin solution, and 10% heat-inactivated FCS (all from Life Technologies, Paisley, U.K.). DQ8 molecules were purified from cell lysates by affinity 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 fluorescein-labeled standard peptide in the presence of protease inhibitors. The standard peptide in this study was fluorescent-labeled Epp0–103 (LPKPPKPVSKMLMATPILLMQALFM). 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 ($A-B/A=\text{inhibition factor}$). 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*0402 differs from nonprotective DRB1*04 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/HLA database submission no. HWS10007777) 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 $^{67}$ILEDERAA$^{74}$ is the only candidate region that can explain the protective effect of DRB1*0402 in RA.

Generation of DERAA-specific, DQ-restricted TCC

As tools for the analysis of the natural processing of the DERAA peptide, we generated DERA-specific, DQ-restricted TCC. We started by subjecting PBMC from a DQ8-homozygous individual carrying DR4 molecules without a DERA motif to repeated stimulation with a synthetic DERA peptide with the sequence $^{46}$KDILEDERAAVDTYC$^{53}$, which is present in all but one of the DERA-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 unpurified individual, we obtained two DERA peptide-specific T cell lines. From one of these lines (Tp7.7) a panel of 28 TCC was generated that were DERA-specific because their proliferation in the presence of DERA-negative, DQ8 APC was fully dependent on the addition of exogenous DERA 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.
Natural processing of DERAA peptide by human APC

To test which DQ molecules could act as restriction elements, we tested five selected DERRA-specific TCC, among which were Tp7.7–13, against a whole panel of APC from DERRA-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 DERRA-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 DERRA-positive DRB1 allele (Fig. 2B) was analyzed using proliferation of the DERRA-specific TCC, Tp7.7–13, as a readout. Again, all APC processed their endogenous DERRA-positive DRB1 molecule and presented the DERRA peptide in the context of DQ, showing on that occasion that a fourth DERRA-positive DRB1 allele, DRB1*1103, which differs from the other DERRA-positive DRB1 alleles at position 67 (I→F), also yielded naturally processed DERRA peptide.

Reduced efficacy of DQ7a compared with DQ8 in presenting the DERAA peptide to DERRA-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 DQα chain (DQA1*03). However, in the β-chain DQ7a (DQB1*0301) differs from DQ8 (DQB1*0302) at positions 13, 26, 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 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 68LEDERAAVDTY76 is the core epitope for binding to the DQ8 molecule, but that the flanking residues K65, D66, I67, 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 LEDERAAVDTY 76 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.

**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 DQ7a7a DR4/4 (DRB1*0401) in medium supplied with increasing concentrations of the DERAA peptide (KDILEDERAAVDTYC). Proliferation was measured by [3H]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.

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

<table>
<thead>
<tr>
<th>DQ</th>
<th>DQB1</th>
<th>Polymorphic Amino Acid Residues</th>
<th>Proliferation of TCC Tp7-7-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQ7a</td>
<td>0301</td>
<td>A Y E D</td>
<td>+</td>
</tr>
<tr>
<td>DQ8</td>
<td>0302</td>
<td>G L G A</td>
<td>11</td>
</tr>
<tr>
<td>DQ9</td>
<td>0303</td>
<td>G L G D</td>
<td>1</td>
</tr>
<tr>
<td>DQ7b</td>
<td>0304</td>
<td>A Y E A</td>
<td>11</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.*
In this study we analyzed whether peptides carrying the amino acid sequence DERAA encoded by HLA-DRB1 alleles that are associated with protection against RA are generated by natural processing and presented in the context of DQ. This analysis is an essential step in determining the mechanisms on which the protective effect of these DRB1 alleles in RA is based. In agreement with our hypothesis that the DERAA-associated protection could be mediated by regulatory T cells recognizing the DERAA sequence in the context of DQ or by deletion of potential autoaggressive T cells in the thymus, we found that DERAA is naturally processed from all four DERAA-positive DRB1 alleles tested (Figs. 1 and 2). This result also indicated that DRB1 polymorphisms in the regions flanking the DERAA sequence had no influence on the processing. Even DRB1*1103 yielded correctly processed DERAA peptide despite a polymorphism at position 67. In contrast to the study by Moudgil et al. (28) in mice suggesting differences in processing among heterozygous individuals, we found no differences among heterozygous individuals in processing of the DERAA sequence, even though APC from a total of 16 individuals (5 DERAA-negative and 11 DERAA-positive) were tested.

In agreement with the binding motif of DQ8 defined by Kwok et al. (29), the DERAA core epitope identified in this study carried a negatively charged residue (D76) at position 9. However, their motif predicts that a negatively charged residue at position 4 as we found (E25) will result in a 10-fold reduction of the binding to DQ8 (29), suggesting an intermediate affinity of the DERAA peptide for DQ8. Nevertheless, in a previous study (27), the DRB1*0402-derived DERAA peptide (65–79) showed a similar affinity for DQ8 as the type I diabetes-associated glutamic acid dehydrogenase (GAD65 peptide 250–273) that carries a core epitope for DQ8 binding with a noncharged residue (E258) at position 4 and a negatively charged residue (E261) at position 9 (29).

In support of our 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 ILEDERAAVDTY, 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.
Acknowledgments
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References