Immune response of HLA-DQ8 transgenic mice to peptides from the third hypervariable region of HLA-DRB1 correlates with predisposition to rheumatoid arthritis

ERIC ZANELLI[†], CHRISTOPHER J. KRCO[†], JEANINE M. BAISCH[‡], SHEN CHENG[§], AND CHELLA S. DAVID[¶]

Department of Immunology, Mayo Clinic and Medical School, Rochester, MN 55905

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ABSTRACT The major histocompatibility complex class II genes play an important role in the genetic predisposition to many autoimmune diseases. In the case of rheumatoid arthritis (RA), the human leukocyte antigen (HLA)-DRB1 locus has been implicated in the disease predisposition. The “shared epitope” hypothesis predicts that similar motifs within the third hypervariable (HV3) regions of some HLA-DRB1 alleles are responsible for the class II-associated predisposition to RA. Using a line of transgenic mice expressing the DQB1*0302/DQA1*0301 (DQ8) genes in the absence of endogenous mouse class II molecules, we have analyzed the antigenicity of peptides covering the HV3 regions of RA-associated and nonassociated DRB1 molecules. Our results show that a correlation exists between proliferative response to peptides derived from the HV3 regions of DRB1 chains and nonassociation of the corresponding alleles with RA predisposition. While HV3 peptides derived from nonassociated DRB1 molecules are highly immunogenic in DQ8 transgenic mice, all the HV3 peptides derived from RA-associated DRB1 alleles fail to induce a DQ8-restricted T-cell response. These data suggest that the role of the “shared epitope” in RA predisposition may be through the shaping of the T-cell repertoire.

Predisposition to rheumatoid arthritis (RA) has been linked to the major histocompatibility complex (MHC) class II HLA-DRB1 locus (1–4). Within the human leukocyte antigen (HLA)-DR4 specificity, DRB1*0401 (Dw4), DRB1*0404/0408 (Dw14), and DRB1*0405 (Dw15) alleles confer genetic predisposition to RA, while DRB1*0402 (Dw10) does not (Fig. 1) (2–4). Because DR4 molecules mainly differ in their third hypervariable (HV3) regions (5), Gregersen and colleagues proposed, in their “shared epitope” hypothesis, that the amino acid motif Leu, Gln, Arg or Lys, Ala (L/Q/R or K/A) at positions 67, 70, 71, and 74 of the HV3 regions of RA-associated DRB1 alleles is the molecular basis for RA predisposition (refs. 6 and 7 and Fig. 1). In addition, the association of the DRB1*1402 allele (Dw16) with RA susceptibility in American Indians of the Yakima Nation further supports the “shared epitope” hypothesis, since this allele has the same L/Q/R/A motif (Fig. 1) (8). However, the association of RA with the DRB1*0301, B1*0901, and B1*1001 alleles in other populations is inconsistent with the shared epitope hypothesis (refs. 7 and 9–11 and Fig. 1).

Collagen-induced arthritis (CIA) is an experimental model of autoimmune polyarthritis which has numerous similarities with RA—in particular, a strong association between disease susceptibility and genes within the MHC class II region (12). In mice, CIA susceptibility is associated with the H2Ab locus (HLA-DQB1 equivalent) of the MHC. Using H2Eb (HLA-DRB1 equivalent) transgenic mice, we reported that the ex-

pression of a functional H2E molecule effectively protected susceptible H2A^q mice against CIA (13). This protective effect on CIA is dictated by polymorphisms within the second exon of the H2Eb gene, and a strict correlation exists between the ability of the H2A^q molecule to present H2E_β^d and H2E_β^s HV3 region-derived peptides and CIA protection of the corresponding H2E_β molecules (14). These data suggested that a possible mechanism for the H2E-mediated protection in CIA may be by the presentation of H2E_β HV3 peptides in the context of H2A^q molecule.

We propose that an extended DQ/DR haplotype is responsible for RA predisposition and that the polymorphism of HLA-DRB1 alleles dictates the degree of protection (15). The molecular basis of this protection is through the presentation of DRB1 HV3 peptides by RA-associated DQ molecules (15). Indeed, HLA class II-derived peptides constitute a major fraction of naturally processed peptides bound to HLA class II molecules (16, 17). HLA-DQB1*0301 and -DQB1*0302 alleles are in linkage disequilibrium with RA-associated DR4 haplotypes. According to our model, the negative association of the DQB1*0302/DQA1*0301/DRB1*0402 haplotype with RA should be reflected in the capacity of the DQ8 molecule to present the HV3 peptide from the DRB1*0402 chain and induce T-cell proliferation. Conversely, the lack of DQ8-restricted immunogenicity of HV3 peptides derived from DRB1*0401, DRB1*0404, or DRB1*1402 alleles should correlate with the RA association of the corresponding Dw4, Dw14, and Dw16 subtypes. To analyze this hypothesis, we took advantage of the DQ8.Ab⁰ transgenic mice recently generated in our laboratory, which express a functional DQB1*0302/A1*0301 dimeric molecule in the absence of endogenous mouse class II molecule (18). The DQ8.Ab⁰ mice were immunized with a series of DRB1 HV3 peptides corresponding to most HLA-DRB1 alleles, and the *in vitro* T-cell responses against the immunizing peptides were assessed. Our results are consistent with our hypothesis and show that the presentation of HV3 peptides derived from the DRB1 alleles by DQ8 molecules correlate with their nonassociations with RA. These data suggest that the HV3 region of DRB1 molecules may play an important role in shaping the T-cell repertoire and thereby determining predisposition to RA.

MATERIALS AND METHODS

Generation of DQ8 (DQB1*0302/DQA1*0301).Ab⁰ Transgenic Mice. DQ8.Ab⁰ mice were generated using cosmid

Abbreviations: Ab⁰, class II-deficient; CIA, collagen-induced arthritis; HV3, third hypervariable; mAb, monoclonal antibody; MHC, major histocompatibility complex; PBL, peripheral blood lymphocyte; RA, rheumatoid arthritis.

[†]E.Z. and C.J.K. made equivalent contributions to this study.

[‡]Present address: Gene Screen, Inc., 2600 Stemmons Freeway, Suite 133, Dallas, TX 75207.

[§]Present address: DeKalb Poultry Research, Inc., 3100 Sycamore Road, DeKalb, IL 60115.

[¶]To whom reprint requests should be addressed at: Department of Immunology, Guggenheim Building, Mayo Clinic, Rochester, MN 55905.

Standard Peptide: KD L L E Q R R A A V D T Y C								
DRB1 Alleles	HV3 region							RA Association
	67	70	71	73	74	77	78	
0101/0102/0404/0405/0408/0410/1402	L	Q	R	A	A	T	Y	Yes
1501/1502/1503	I	Q	A	A	A	T	Y	No
1601/1101/1104/1305/1202	F	D	R	A	A	T	Y	No
1602	L	D	R	A	A	T	Y	No
0301/0302	L	Q	K	G	R	N	Y	Yes
0401/0409	L	Q	K	A	A	T	Y	Yes
0403/0406/0407/0411	L	Q	R	A	E	T	Y	Yes?
0103/0402/1102/1301/1302/1304	I	D	E	A	A	T	Y	No
1103	F	D	E	A	A	T	Y	No
1401/1404/1405	L	R	R	A	E	T	Y	No
1303	I	D	K	A	A	T	Y	No
1403	L	D	R	A	L	T	Y	No
0701	I	D	R	G	Q	T	V	No
0801/0802/0804	F	D	R	A	L	T	Y	No
0803	I	D	R	A	L	T	Y	No
0901	F	R	R	A	E	T	V	Yes
1001	L	R	R	A	A	T	Y	Yes

FIG. 1. Distribution of polymorphic residues in the region 65–79 among DRB1 alleles and their association with RA. The same HV3 sequence is shared by several independent DRB1 alleles. Polymorphic residues at positions 67, 70, 71, 73, 74, 77, and 78 are listed with the standard one-letter amino acid code. The region 65–79, covering this polymorphic region, constitutes the 17 DRB1 HV3 peptides used in the present study. They will be referred to in the text and the figures using the DRB1 alleles written in bold.

H11A and X10A, generously provided by J. Strominger, as previously described (18–20).

Flow Cytometry. Expression of CD4, CD8, HLA-DQ, T-cell receptor V_{β} chains, and mouse H-2A and H-2E molecules on peripheral blood lymphocytes (PBLs) was analyzed by flow cytometry using monoclonal antibodies (mAbs) GK1.5 (anti-CD4), 53-6-72 (anti-CD8), IVD12 (anti-HLA-DQ), MR9-8 (anti- V_{β} 5.1), KJ-16 (anti- V_{β} 8.1.2), MR10-2 (anti- V_{β} 9), 25-5-16 (anti-H-2A^b), 4D5 and 7-16.17 (anti-H-2A^b), and Y-17 (anti-H-2E^b) as described (13).

Peptide Synthesis. The 17 DRB1-(65–79)-peptides of sequence KDXLEXXRXXVDXXC, where amino acids X at positions 67, 70, 71, 73, 74, 77 and 78 are listed in Fig. 1, were synthesized by the Peptide Core Facility at the Mayo Foundation using an automated 430A peptide synthesizer (Applied Biosystems) and were purified by high-pressure liquid chromatography. Amino acid compositions were confirmed by N-terminal sequencing using Edman's method.

T-Cell Proliferation Assay. Mice were immunized with 100 μ g of peptide emulsified in a saline solution and complete Freund's adjuvant, and T-cell proliferation assays were performed (21). For inhibition experiments, 20 μ l ($\approx$ 5 μ g of antibody) of culture supernatants containing mAb GK1.5 (anti-CD4), 53-6-72 (anti-CD8), IVD12 (anti-HLA-DQ), 25-5-16 (anti-H-2A^b), 4D5 (anti-H-2A^b), 7-16.17 (anti-H-2A^b), Y-17 (anti-H-2E^b) or MB-40 (anti-HLA-A,B,C) was added to the cells challenged *in vitro* with peptide DRB1*0402 at 100 μ g/ml. Results are representative of at least three independent experiments. For each experiment, two mice were used per peptide.

RESULTS

Expression of HLA-DQ8, CD4, and T-Cell Receptor V_{β} Molecules in DQ8.Ab⁰ Mice. The MHC class II chains H2A^b and H2E^b synthesized in the Ab⁰ mice do not pair with each other (18). Thus, no class II molecules reach the cell surface in these mice. This results in the dramatic reduction in the percentage of CD4⁺ T cells among PBLs ($\approx$ 0.1%) (Table 1). The expression of the *DQB1*0302* and *DQA1*0301* genes in DQ8.Ab⁰ animals ($31.5 \pm 5.6\%$ of PBLs) restored the CD4 expression in PBLs to a substantial level. The percentage of CD4⁺ T cells in 6-week-old DQ8.Ab⁰ mice was between 8% and 11%, compared with 18% in B10 mice (Table 1). Although the relative percentages of specific V_{β} -bearing CD4⁺ T cells in DQ8.Ab⁰ mice are distinct from those in B10 mice, they show that the T-cell repertoire education in these mice is normal (Table 1). The percentage of CD8⁺ T cells was similar in all the strains of mice (data not shown). The possibilities of potential interisotypic H2E β /DQA1*0301 and H2A α /DQB1*0302 dimers were ruled out by showing the absence of staining on flow cytometry using mAbs 4D5 and 7-16.17 (anti-H2A^b).

Table 1. Expression of HLA-DQ, CD4, and T-cell receptor V_{β} molecules in PBLs

Mice	% HLA-DQ ⁺	% of CD4 ⁺ T cells			
		Total	V_{β} 5.1	V_{β} 8.1.2	V_{β} 9
Ab ⁰	0	0.1	—	—	—
B10	0	18–20	4.5–6	17.5–18.5	2–3
DQ8.Ab ⁰	25–36	8–11	11–13.5	14–18	7.5–8

specific) (data not shown). Additionally, the DQB1*0302 chain is not expressed on the cell surface in the absence of the DQA1*0301 molecule in singly transgenic mice (data not shown). Therefore, in the absence of mouse class II molecule, the HLA-DQ8 molecule can positively select CD4⁺ T cells.

T-Cell Response of DQ8.Ab⁰ Mice to a Series of Peptides Derived from the HV3 Region of DRB1 Molecules. DQ8.Ab⁰ mice were immunized with the 17 peptides covering the sequence 65–79 of the HV3 regions of most HLA-DRB1 alleles. As shown in Fig. 1, these 15-aa peptides differ from each other at seven residues, mostly at positions 67, 70, 71, and 74. For each peptide, a representative result of three to five independent T-cell proliferation assays is shown in Fig. 2. The DRB1 HV3 peptides showed dramatically different abilities to induce T-cell responses in DQ8.Ab⁰ mice. Of the 17 peptides, peptides DRB1*0402 and DRB1*1103 induced by far the strongest response, followed, in decreasing order, by peptides B1*0801, B1*1501, B1*1601, B1*1303, B1*0803, B1*1602, and B1*1403. No significant proliferation was observed for the 8 remaining peptides (Fig. 2). Transgene-negative littermates showed no response to any of the 17 peptides (data not shown).

Role of Residues 67, 70, 71, and 74 of DRB1 HV3 Peptides in DQ8-Restricted T-Cell Response. As shown in Table 2, the 17 DRB1 HV3 peptides mostly differ at positions 67, 70, 71, and 74. Table 2 also summarizes the DQ8-restricted T-cell response to each peptide and the predisposition of the corresponding DRB1 allele to RA. Position 67 is probably not critical for MHC class II binding, since polymorphism at this position affects only the intensity of the T-cell response (peptide DRB1*0402 versus peptide B1*1103, B1*1601 versus B1*1602, and B1*1403 versus B1*0801 and B1*0803). It is likely that the amino acid at position 67 interacts with the T-cell receptor. Conversely, the presence of an amino acid with a short lateral chain seems to be required at position 74, as peptides B1*0301, B1*0403, B1*1401, B1*0701, and B1*0901—with R, E, or Q residues at position 74—failed to induce T-cell responses. Amino acids at positions 70 and 71 have the most effect on the T-cell response against DRB1 HV3 peptides. Any of the residues A, E, K, and R is acceptable at position 71, but the combination QR, QK, or RR at positions 70 and 71 prevents T-cell response. Conversely, with the exception of peptide B1*0701, every peptide having a D residue at position 70 induces a DQ8-restricted T-cell response

Table 2. Effect of polymorphisms at positions 67, 70, 71, and 74 on DQ8-restricted DRB1 T-cell response and RA association of the corresponding haplotypes

DRB1 peptide	Amino acid				T-cell response	RA association
	67	70	71	74		
0101	L	Q	R	A	No	Yes
0401	L	Q	K	A	No	Yes
0301	L	Q	K	R	No	Yes
0403	L	Q	R	E	No	Yes
1001	L	R	R	A	No	Yes
0901	F	R	R	E	No	Yes
1401	L	R	R	E	No	No*
0701	I	D	R	Q	No	No*
1501	I	Q	A	A	Yes	No
0803	I	D	R	L	Yes	No
1403	L	D	R	L	Yes	No
0801	F	D	R	L	Yes	No
1601	F	D	R	A	Yes	No
1602	L	D	R	A	Yes	No
0402	I	D	E	A	Yes	No
1103	F	D	E	A	Yes	No
1303	I	D	K	A	Yes	No

*Not linked to RA-associated DQ5, DQ7, or DQ8 alleles.

(Table 2). Even peptide B1*1303, with the combination DK at positions 70 and 71, induces a significant proliferative response. The presence of a Q residue at position 74 is very likely responsible for the absence of proliferation against peptide B1*0701. Thus, unlike position 67, amino acid differences at positions 70 and 71 dictate the ability of DQ8.Ab⁰ mice to respond against DRB1 HV3 peptides (Table 2; peptide B1*1303 versus peptide B1*0401, B1*1602 versus B1*0101, and B1*1501 versus B1*0101).

Correlation Between T-Cell Response to DRB1 HV3 Peptides and RA Predisposition. The combinations QR, QK and RR are found on all the HV3 peptides from RA-associated DRB1 alleles (i.e., peptides B1*0101, B1*0301, B1*0401, B1*0901, and B1*1001) (Fig. 1 and Table 2). Therefore, the different DRB1 alleles of the DR4 specificity can be distinguished by the ability of their DRB1 HV3 peptides to induce a DQ8-restricted T-cell response. While the RA-associated DRB1 HV3 peptides B1*0101 and B1*0401 fail to induce T-cell response, the non-RA-associated peptide B1*0402 induces the strongest proliferation (Fig. 2). The absence of T-cell proliferation in response to HV3 peptide from B1*0403 can be explained by its motif Q/R/E at positions 70, 71, and 74. On the basis of our hypothesis, this allele should predispose to RA. However, DR4 alleles DRB1*0403, DRB1*0406, DRB1*0407, and DRB1*0411 (Dw13 and KT2 specificities) have been described as non-RA-associated (3, 4, 7). Unlike DRB1*0402, both DQ7 and DQ8 are in linkage disequilibrium with these DR4 subtypes. We propose that only Dw13 individuals carrying DQ7 are not predisposed to RA. Indeed, DRB1*0403 has been described as a protective allele in Tlingit American Indians, where DQB1*0301/DQA1*0501/DRB1*1402 is the RA-predisposing haplotype (22). Conversely, in Polynesians, where DQ8 is predominant (23), the frequencies of DRB1*0403 allele in RA patients and healthy subjects are similar, and the relative risks for developing RA in DRB1*0403 and DRB1*0401 individuals are nearly identical (24).

T-Cell Response to DRB1 HV3 Peptides in DQ8.Ab⁰ Mice is DQ8-Restricted and Driven by CD4⁺ T Cells. Although no mouse H2A_α or H2E_β chains or mouse/human hybrid molecules are expressed on the cell surface of peripheral cells in these animals (data not shown), we wanted to rule out any role of the mouse class II chains on the T-cell response against the DRB1 HV3 peptides. DQ8.Ab⁰ mice and negative full sibs

FIG. 2. T-cell proliferation assay using cells from DQ8.Ab⁰ mice immunized with the 17 DRB1 HV3 peptides listed in Fig. 1.

were immunized with the peptide B1*0402, which induces the strongest response, and challenged *in vitro* with various concentrations of the same peptide (Fig. 3A) or in the presence of mAb specific for HLA-DQ, H2A, H2A α , H2E β , HLA-A,B,C, or mouse CD8 or CD4 (Fig. 3B). A dose-dependent T-cell response was observed when lymph node cells were stimulated *in vitro* with peptide B1*0402 (Fig. 3A). Of the mAbs used in blocking experiments, only anti-HLA-DQ and anti-CD4 mAbs strongly inhibited the response against the peptide B1*0402 (Fig. 3B). No proliferation was observed in transgene-negative littermates (data not shown). Therefore, the DRB1 HV3 peptide-specific T-cell proliferations in the DQ8.Ab⁰ mice is HLA-DQ-restricted and driven by CD4⁺ T cells. No interisotypic DQB1*0302/H2A α or DQA1*0301/H2E β molecules are used as restriction elements in the T-cell response to DRB1 HV3 peptides in these mice, since anti-H2A α and anti-H2E β antibodies can detect hybrid and interisotypic molecules.

DISCUSSION

We report two important findings in this paper. First, we show that, in the absence of endogenous mouse class II molecule, a human class II molecule can be used as a restrictive element by mouse T cells *in vivo*. Moreover, we show that the DQ8-restricted response to an antigenic peptide in these transgenic mice is identical to the normal syngeneic situation, where substitution of only one or two residues on the antigenic peptide dramatically affects its ability to induce T-cell recognition (Figs. 1 and 2). Second, and most important, the present

results are consistent with the model of RA predisposition that we have recently proposed (15).

Because the RA- and non-RA-associated DR4 haplotypes can be distinguished on the basis of their DRB1 allele polymorphism, it has been proposed that a similar motif in the DRB1 HV3 region is responsible for the RA association of both DR4 and DR1 haplotypes (3, 4, 7). More recently, it has been suggested that residues at positions 70, 71, and 74 of the DRB1 chain strongly influence T-cell recognition (25). Therefore, RA-associated DR1 and DR4 alleles might bind similar "arthritogenic" peptide(s) because of these similarities. However, this model ignores the fact that DR1 and DR4 molecules differ at three and six positions in their first and second hypervariable regions, respectively, which also strongly influence T-cell recognition. Moreover, the "shared epitope" hypothesis does not explain the differences in disease severity between DR1 and DR4 RA patients (26, 27), the absence of RA association of DQ7DR4 haplotype in Indian populations (28), or the increased severity of RA manifestations in patients "homozygous" for the shared epitope (29–31). The shared epitope also needs to be extended to explain the RA association of DR9 and DR10 haplotypes (3, 7, 9, 10).

Rather than the limited similarities between DRB1 alleles, the close identity between DQ7, DQ8, and DQ9 alleles of RA-associated haplotypes is impressive (Table 3). For instance, Dw16-positive American Indians of the Yakima and Tlingit Nations have the DQB1*0301 (DQ7) allele found in the RA-associated Dw4 subtype, and a DQA1*0501 allele, very similar to DQA1*0301 at critical residues of the antigen-

FIG. 3. The response of DQ8.Ab⁰ mice to peptide DRB1*0402 is dose-dependent (A) and is HLA-DQ-restricted and driven by CD4⁺ CD8⁻ T cells (B). The values are expressed in Δcpm (mean cpm in experimental wells – mean cpm in control wells without peptide). The value of Δcpm for Ab⁰ mice challenged with peptide DRB1*0402 was 556 ± 24. The background counts were 6557 ± 394 cpm for cells from DQ8.Ab⁰ mice immunized with peptide DRB1*0402 and cultured with medium alone.

Table 3. Distribution of DQB1, DQA1, and DRB1 alleles among HLA class II haplotypes and RA association

Haplotype	DQB1	DQA1	DRB1	RA association
DQ5Dw1	0501	0101	0101	Yes
DQ5Dw20	0501	0101	0102	Yes
DQ5DwBON	0501	0101	0103	No
DQ7Dw4	0301	0301	0401	Yes
DQ8Dw4	0302	0301	0401	Yes
DQ8Dw10	0302	0301	0402	No
DQ7Dw13	0301	0301	0403	No
DQ8DwKT2	0302	0301	0403	Yes?
DQ8Dw14	0302	0301	0404	Yes
DQ7Dw16	0301	0501	1402	Yes
DQ9Dw23	0303	0301	0901	Yes
DQ5DR10	0501	0104	1001	Yes

binding groove (8, 22). Similarly, DR9 RA patients in Chili carry the DQ9 (DQB1*0303/DQA1*0301) molecule, which differs from DQ8 only at position 57 of the β chain (10). The increased frequency of the DQw3 specificity (DQ7 and DQ8), but not DR4, in Arab RA populations is another factor in favor of our hypothesis (11). Further, DR1 and DR10 haplotypes associated in some populations such as Israeli and Spaniards with a less severe arthritis, characterized by the absence of rheumatoid factor, share an identical DQB1*0501/A1*01 molecule (4, 6, 7, 9, 26, 27). Thus, the HLA class II contribution to RA predisposition may be the result of the interaction between HLA-DQ predisposing and HLA-DR nonprotective alleles on both haplotypes carried by an individual. We have recently found in our laboratory that the DQ8.Ab⁰ mice are susceptible to CIA whereas HLA-DQ6 transgenic mice are resistant (18). We have also found that the *DRB1*1502* gene, linked to resistance to RA in Japanese populations, protects

mice from arthritis (M. A. Gonzalez-Gay, unpublished work in our laboratory).

According to our model, the incidence and severity of arthritis would depend on the affinity of DRB1 HV3 peptides for RA-predisposing DQ molecules. Residues 70, 71, and 74 of the DRB1 chains that have been involved in RA predisposition are in fact key residues which determine the DQ8-restricted T-cell response to the HV3 peptides. Thus, the intensity of the DQ8-restricted T-cell response against the DRB1 HV3 peptides reflects the capacity of the corresponding DRB1 allele to prevent RA development. Conversely, we did not find such a correlation between the T-cell response to DRB1 HV3 peptides and RA predisposition when using a second line of transgenic mice expressing a functional DQ6 (DQB1*0601/DQA1*0103) molecule which is not associated with RA (data not shown). This observation suggests that the HV3 region of DRB1 molecules is a self-peptide which shapes the T-cell repertoire and influences RA predisposition only in individuals carrying the RA-predisposing DQ alleles. As peptides B1*0402 and B1*1103 induce, by far, the strongest proliferative response, we propose that the corresponding DRB1*0103, DRB1*0402, DRB1*1102, DRB1*1301, DRB1*1302, DRB1*1304 and DRB1*1103 alleles are protective against DQ5-, DQ7-, and DQ8-mediated RA. This model explains the nonassociation of DR4Dw10 and DR1DwBON subtypes with RA, along with the difficulty to find RA patients carrying the genotypes DRB1*0401/DRB1*0402 or DRB1*0404/DRB1*0402, and the decreased frequencies of DR5 and DR6 haplotypes among RA patients. On the other hand, weaker proliferative response against peptides B1*1501, B1*1601, B1*1602, B1*1303, B1*1403, B1*0801, and B1*0803 may correlate with decreased severity of RA, but not incidence. Indeed, the relative risk to develop severe arthritis is much lower in DR2/DR4, DR6/DR4, and DR8/DR4 heterozygous individuals than in individuals carrying the genotype DR1/DR4 or DR4/DR4 (29–31). Finally, the absence of T-cell

FIG. 4. Presentation of DRB1 HV3 peptides by DQ8 molecules modulates RA predisposition: A possible mechanism. During thymic education, the HV3 peptide of DRB1*0402 presented by DQ8 (DQB1*0302/DQA1*0301) induces negative selection of potentially autoreactive T cells. In DRB1*0401 individuals, the same autoreactive T cells are positively selected, because of low affinity of the HV3 peptide of the DRB1*0401 chain for the DQ8 molecule. After activation by a foreign antigen, these CD4⁺ T cells crossreact against components of the cartilage through antigen mimicry, leading to an inflammatory reaction and rheumatoid arthritis.

proliferation against peptides B1*0101 and B1*0401, along with the heterozygosity at the HLA-DQ loci, may account for the predisposition to severe arthritis in DQ5Dw1/DQ8Dw4 or DQ7Dw4/DQ8Dw14 individuals (29–32). Unlike the shared epitope hypothesis, this model agrees with computer simulations using the marker association–segregation χ^2 method, suggesting that the distribution of RA patients is compatible with a biallelic-locus model where a susceptible allele is in linkage disequilibrium with a protective or permissive DRB1 locus (33).

Based on our findings, we propose the following hypothesis on the role of the shared epitope in RA (Fig. 4). As self-antigenic determinants, the HV3 regions of DRB1 molecules shape the peripheral T-cell repertoire. This happens during thymic development when the DRB1 HV3 peptides are presented to T cells by the HLA-DQ molecules. Because of high-affinity interaction with the DRB1 HV3 peptide of DRB1*0103 and DRB1*0402 molecules presented by DQ molecules, some potential autoreactive T cells will be negatively selected. These T cells could be, for instance, the ones recently described in DR1/DR4 RA patients by Walzer-Kuntz *et al.* (34), carrying the amino acid motif GPG in the T-cell receptor $J\beta$ region. On the other hand, the RA-associated DRB1 HV3 motifs will be unable to induce the same negative selection because of low-affinity for the DQ molecule. Thus, in the periphery, the nondeleted, potentially autoreactive CD4⁺ T cells could potentially expand after contact with antigens such as bacterial and viral superantigens or heat shock proteins having a motif similar to the HV3 region of DRB1 molecules, but with a higher affinity (Fig. 4). By molecular mimicry between these foreign antigens and some components of the cartilage (35), the proliferating T cells will crossreact with the self-antigens. Because the microbial agents will also express surface proteins with binding affinity for joint components, the inflammatory response will be preferentially triggered to the joints. For instance, a protein from *Staphylococcus aureus* can specifically bind to type II collagen and trigger arthritis. This model explains the role of the HLA-DQ polymorphism, the shared epitope of DRB1 molecules, the T-cell repertoire, and infectious agents in the predisposition to RA.

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