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THYMIC DELETION OF Vβ11⁺, Vβ5⁺ T CELLS IN H-2E NEGATIVE, HLA-DQβ⁺ SINGLE TRANSGENIC MICE[1]

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DQw6b transgenic mice have been generated by microinjecting a linearized cosmid clone containing 34-kb DQb genomic DNA, isolated from HLA-homozygous B cell line AKIBA (DR2, Dw12, DQw6), into embryos of (CBA × B10.M)F_2 or (SWR × SJL)F_2. Among 85 mice screened, eight mice were transgene-positive. The transgene in seven of eight founders was germline-transmitted. FACS analysis and immunohistochemical studies with DQβ-specific mAb demonstrated that DQβ molecules in association with mouse Aα^f molecules are expressed on peripheral mononuclear cells, spleen cells, and in thymic medulla. More interestingly, Vβ11-, Vβ5.1-, and Vβ5.2-bearing T cells, but not Vβ8.2-bearing T cells, were clonally deleted in the H-2E-negative but DQβ⁺ progeny of two selected founders (260-23 and 258-10). The deletion was found to take place intrathymically during the transition stage from immature to mature thymocyte development. We postulate that although human DQ genes are more homologous to mouse H-2A genes, Aα^f/DQβ hybrid molecules may possess the same self-peptide- (or superantigen)-presenting epitope as Eα/Eβ molecules critical for deletion of Vβ11-, Vβ5.1-, and Vβ5.2-bearing T cells in thymus. Our results also confirm the previous findings that accessory molecules on thymocytes such as CD4 may be involved in thymic selection, and further suggest that an interaction of mouse CD4 and mouse Aα chain is required for the clonal deletion.

The peripheral TCR repertoire is shaped through positive and negative selection by self-peptide associated with MHC molecules (1, 2, 3). Studies with antigen-specific TCR transgenic mice (4, 5) and mAb specific for individual TCR Vβ molecules (6, 7, 8, 9) demonstrated that both selections take place in the thymus during T cell ontogeny. For example, Vβ11-, Vβ5.1-, Vβ5.2-, and Vβ17a-bearing T cells were found to be clonally deleted in H-2E (EαEβ)-positive mouse strains (10, 11, 12). Partial deletion of Vβ11⁺ cells was detected in recombinant and transgenic mice expressing EαAβ hybrid molecules, indicating that Eα chain is the primary site for "self-peptide" presentation for Vβ11 (13). The degree of clonal deletion was correlated with the quantitative level of Eα chains expressed on the cell surface. Transgenic mice expressing human DRα/mouse Eβ dimers could also clonally delete Vβ11⁺ T cells (26). This was not surprising, since there is more than 75% homology between Ea and DRa genes.

In this report, clonal deletion of Vβ11-, Vβ5.1-, and Vβ5.2-bearing T cells was observed in our H-2E-negative, HLA-DQβ-positive single transgenic mice. This was very surprising, since DQ genes are more homologous to mouse H-2A genes, which do not delete Vβ11⁺ and Vβ5⁺ T cells. Thus, human DQβ chain may possess the same self-peptide- (or superantigen)-presenting epitope as Eα chains critical for the deletion of Vβ11-, Vβ5.1-, and Vβ5.2-bearing T cells. Furthermore, these results are clearly different from those recently reported by Nishimura et al. (14) in DQw6α/DQw6β double transgenic mice in which no clonal deletion of Vβ TCR-bearing T cells was found. Since both transgenic mice have the same allelic form of DQβ (DQw6β), interaction of mouse CD4 and mouse Aα is probably required for the negative selection of T cells.

MATERIALS AND METHODS

Mice. DQb transgenic mice were generated by microinjecting the linearized 40-kb cosmid clone containing 34-kb DQb genomic DNA isolated from HLA-homozygous human B cell line AKIBA (HLA-DR2, Dw12, DQw6), purified by the PEG methods (15), into pronuclei of (SWR × SJL)F_2 or (CBA × B10.M)F_2. The inbred strains RIII, B10.M, B10.K, and SWR were bred and maintained in our animal facility.

Nucleotide analysis. For the Southern analysis, 20 μg of mouse tail DNA was digested with the restriction endonuclease Bam H1 and loaded on 0.8% agarose gel. The DNA were then transferred onto nitrocellulose membrane (Hybond-N, Amersham) and hybridized with a 1.2-kb full length DQw6b cDNA probe (DC101) by the combined procedures described by Maniatis et al. (15). For the Northern analysis, mice were sacrificed and fresh tissues were removed and immediately frozen in liquid nitrogen. Total RNA of spleens, thymus, kidneys, lungs, liver, and heart from transgenic mice and the negative littermate was extracted by the GIT-CsCl method (15). Twenty micrograms of total RNA from each tissue as well as from AKIBA cell line was loaded on 1.0% agarose gel. The RNA was then transferred and blotted as described in the Southern analysis.

FACS analysis. For analyzing DQb transgene expression, peripheral blood mononuclear cells were isolated by Ficoll gradient and incubated with FITC-conjugated Leu10 (Becton Dickinson) for 45 min on ice. Cells were then washed twice with FACS buffer (PBS containing 1% BSA and 0.02% NaN_3) and resuspended in 0.5 ml of FACS buffer with 1% formaldehyde. Cytofluorography was performed on FACS IV (Becton Dickinson, Mountain View, Ca).

For the two color analysis of Vβ5.1⁺, Vβ5.2⁺, Vβ8.2, or Vβ11⁺ CD4 or CD8 T cells, peripheral blood mononuclear cells or thymocytes were isolated by Ficoll gradient and stained with mAb MR9-4 (anti-Vβ5.1 and 5.2), MR9-8 (anti-Vβ5.1) (12), F23.2 (anti-Vβ8.2) (1), or

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RR3-15 (anti-Vβ11) (10). Cells were then stained with FITC-conjugated mouse-anti-rat IgG or FITC-conjugated goat-anti-mouse IgG Fc (Accurate Chem. Westbury, N.Y.). After washing with FACS buffer, the cells were then labeled with biotin-conjugated anti-CD4 GK1.5 or anti-CD8 53-6.72 (ATCC, Rockville MD). Cells were then labeled with streptavidin-conjugated phycoerythrin (TAGO Inc.). All the washing conditions and FACS analysis are as described above.

For analysis of Vβ5 and Vβ11 TCR expression in immature and mature thymocytes, thymocytes were treated in two different ways before fluorescence staining. In the first method, thymocytes were cultured for 4 h at 37°C to increase the density of TCR on immature thymocytes before being stained with mAb MR9-4 and RR3-15 (16). In the second protocol, 4 mg of hydroxycortisone acetate (Sigma Chemical Co. St. Louis, MO) per mouse was administered intraperitoneally to destroy the cortisone-sensitive immature thymocytes and indirectly enriched for mature thymocytes (17). After 48 h, thymocytes of these cortisone-treated mice were isolated and stained with the same mAb as described above.

Thymic staining of DQb transgene product. Frozen thymic sections from DQb transgenics and the negative littermate were stained as previously described (18). Briefly, the sections were incubated with biotin-conjugated anti-DQβ mAb 61.11.1 (mouse IgG2a) and stained with the standard vectastain ABC peroxidase reagents (Vectastain, Burlingame, CA) according to the procedure suggested by the manufacturer.

RESULTS

Identification of DQb transgenic mice. As shown in Figure 1, the transgene construct contains 20 kb 5′ upstream and 4 kb 3′ downstream sequences, which are sufficient for proper expression of this gene (19, 20). Among 85 mice screened by Southern analysis, eight mice were transgene-positive (Fig. 2), and the transgene in seven of eight founders was stably germline transmitted. Figure 3 shows the result of Northern blot analysis of the progeny from one selected founder (260-23). The high level of DQβ transcripts was found in thymus and spleen, which agrees with the presence of B cells, macrophages, thymic epithelia, and dendritic cells in these tissues. A fair amount of DQβ transcripts was also found in the lung and kidney, but not in the liver and heart.

Expression of DQb gene in transgenic mice. FACS analysis of the progeny from the same founder shows that a low level of DQβ molecules can be detected by a monoclonal antibody Leu-10 on the surface of 3–16% of peripheral mononuclear cells (Fig. 4). DQβ molecules were also found on 20–30% of splenic cells and lymph node cells (data not shown). The level of DQβ expression can be dramatically increased by in vitro stimulation with mouse rIL-4 and LPS as well as by in vivo injection of CFA (data not shown). Thymic staining showed that the DQβ molecules are mainly detected in the medulla (Fig. 5). H-2 typing of the same founder (260-23) and its progeny (backcrossed to B10.M) showed that all are H-2^f haplotype and no E molecules have been detected by mAb specific for Eα (14-4-4s), as well as for Eβ (17-3-3s) (21). The level of Aβ^f expression in the transgenics and their negative littermates was similar as determined by mAb MKS4 (22) (data not shown). The levels of DQβ expression on the basis of fluorescent density are one-fourth to one-fifth of the endogeneous H-2A expression, indicating that the chain pairing between the mixed heterodimers of Aα^f/DQβ is much less efficient than endogeneous A_a^f/A_b^f pairing. Similarly, low levels of Aα^f/DQβ molecules were observed by thymic staining.

Clonal deletion of T cells in DQβ transgenic mice. Experiments were carried out to determine the role of human class II molecules in thymic selection of mouse TCR using the transgenic mice. Several Vβ specific mAb as well as mAb against CD4 and CD8 were used to determine the frequency of specific Vβ-positive cells in each subset of peripheral T cells from DQβ transgenic mice and their negative littermates in the progeny of two selected founders (260-23 and 258-10) (Table 1). RIII strain, which has a massive deletion of Vβ TCR genes, was used to indicate background staining (23). B10.M was used to indicate normal Vβ TCR level in H-2E negative strain. Percentages of Vβ11-, Vβ5.1-, and Vβ5.2-bearing T cells in CD4 and/or CD8 subsets of the DQβ negative littermates from the progeny of both selected founders were quite similar to those of the parental B10.M strain. However, in the DQβ⁺ transgenic mice from the progeny of both founders, the Vβ11-bearing T cells were absent in the CD4⁺ subset, and a large percentage of Vβ11-bearing T cells was also deleted in CD8⁺ subset, similar to an H-2E positive mouse. A large percentage of Vβ5.1- and Vβ5.2-bearing T cells in CD8 subset were also deleted. The level of Vβ5.2-bearing T cells in CD8⁺ subset was quite similar to the results in H-2E positive strain reported by Woodland et al. (11). The clonal deletion seems specific, since no deletion has been found in the Vβ8.2-bearing T cells in both CD4⁺ and CD8⁺ subsets determined by mAb F23.2 (1). In DQβ transgenics, 22% of CD4⁺ and 16% of CD8⁺ cells are Vβ8.2⁺, and in the negative littermate, 20% of CD4⁺ and 14% of CD8⁺ cells are Vβ8.2⁺.

Clonal deletion occurs in mature thymocytes. Roehm et al. (15) have shown that immature thymocytes express low levels of TCR (TCR dull), whereas mature thymocytes express high levels of TCR (TCR bright). Studies were carried out with anti-Vβ5 and anti-Vβ11 mAb to compare Vβ5 and Vβ11 expression in immature and mature thymocytes between DQβ transgenics and their negative littermates. Figure 6 shows the fluorescence histograms of adult thymocytes stained with anti-Vβ5 antibody (MR9-4). SWR, which has a deletion of Vβ TCR gene, including Vβ5, was used for background staining (24); B10.M and B10.K were chosen to provide the level of Vβ5 expression in I-E negative and I-E positive strains, respectively. As expected, virtually no Vβ5⁺ thymocytes have been detected from strain SWR. About 1% of high

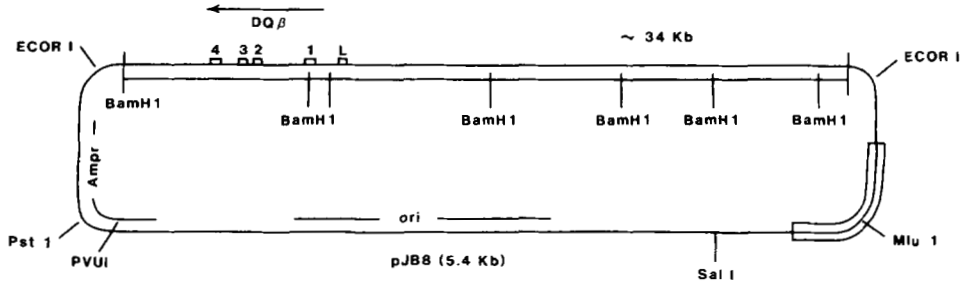

Figure 1. DQβ transgene construct. The DQβ transgene construct was derived from a cosmid clone containing DQβ genomic DNA isolated from EB virus-transformed human B cell line AKIBA (DR2, Dw12, DQw6). Briefly, the 40-kb cosmid clone was isolated with the PEG method and linearized at the PVU I site, as indicated by the *arrow*. To generate transgenics, 2 pl of the linearized DNA at the concentration of 2 μg/ml was microinjected into each fertilized egg.

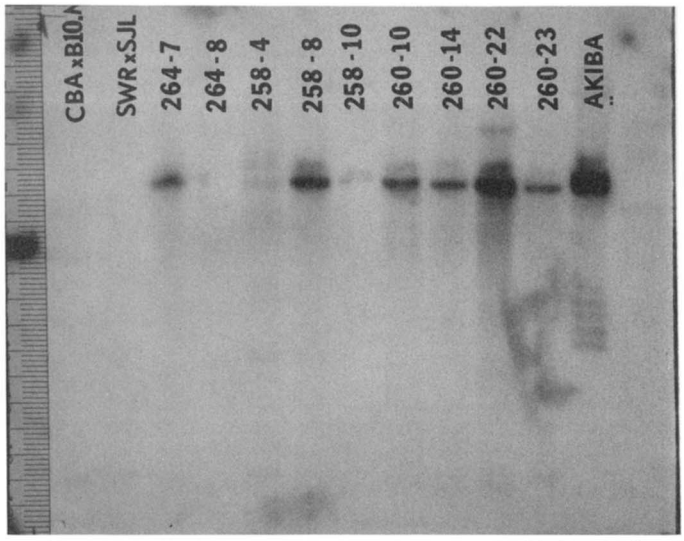

Figure 2. Southern blot analysis shows a unique 9.6-kb Bam H1 band of transgene in eight DQβ transgenic founders, as well as in the positive control AKIBA, but not in the negative control (CBA × B10.M)F_1 and (SWR × SJL)F_1.

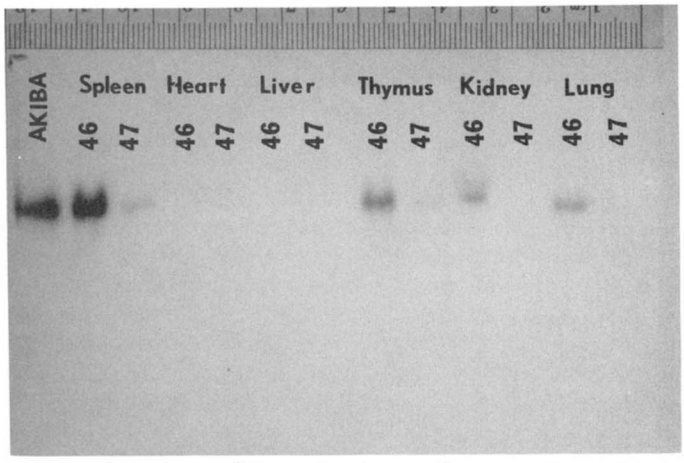

Figure 3. Detection of tissue distribution of transgene transcripts in the progeny of one selected founder (260-23) by Northern blot analysis. A unique 1.2-kb band of DQβ transcripts was detected in spleen, thymus, kidney, and lung from DQβ transgenics (*#46*), as well as in the positive control AKIBA, but not in heart and liver of DQβ transgenics or in the tissues from a negative littermate (*#47*).

level (bright) as well as 6% of low level (dull) of Vβ5 expression was observed in thymocytes from the DQβ negative littermates and H-2E negative strain B10.M (see Fig. 6A). However, in the DQβ⁺ transgenics and H-2E positive strain B10.K, 6% of dull Vβ5⁺ cells were detected, but no bright Vβ5⁺ expression could be detected. Thus, the results clearly demonstrate that in thymus, only mature thymocytes, and not immature thymocytes, were clonally deleted. Similar results were observed in the cortisone-treated thymus enriched for mature thymocytes (see Fig. 6B). Similar results were also obtained with anti-Vβ11 mAb RR3-15 (data not shown). These results demonstrate that the intrathymic clonal deletion of Vβ11-, Vβ5.1-, and Vβ5.2-bearing T cells takes place in the H-2E negative, DQβ⁺ transgenic mice as in H-2E positive strains; presumably the $A\alpha^f$/DQβ hybrid molecules in the DQβ transgenics can act like H-2E molecules to clonally delete these Vβ-bearing T cells in the transition stage from immature to mature thymocytes.

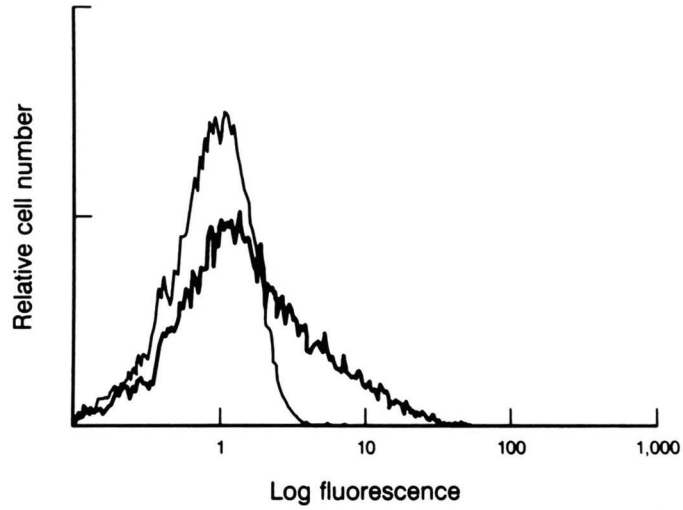

Figure 4. Detection of surface expression of DQβ transgene. DQβ transgene product is detected with anti-DQβ mAb Leu10 on the surface of peripheral mononuclear cells from DQβ transgenics (*#66*), but not on the negative littermate (*#62*).

DISCUSSION

The ability of human DQβ chain to pair with mouse Aα and be expressed on the cell surface is not surprising, since the nucleotide sequence comparison of human and mouse class II genes showed higher homology between DQ and H-2A than H-2E genes, whereas DR genes have higher homology with H-2E genes (25). It has been recently demonstrated that DRα chain can pair with Eβ chain and can function like E molecules in clonal deletion of certain T cell receptor genes and antigen presentation (26). In our DQb transgenics, DQβ chain does not pair with Eα molecules, since introducing Eα gene did not enhance any DQβ expression (data not shown). The low level of DQβ expression in the transgenics could reflect the competition of chain pairing to $A\alpha^f$ between DQβ and the endogenous $A\beta^f$ molecules (27).

However, the ability of $A\alpha^f$/DQβ hybrid molecules in clonally deleting Vβ11-, Vβ5.1-, and Vβ5.2-bearing T cells is very intriguing. Several mechanisms can be invoked to explain this phenomenon. First, the clonal deletion is due to the activation of another unidentified gene product by the integration of DQβ transgene, but not due to the expression of DQβ. The product of this unidentified gene could be involved in negative selection of these Vβ5- and Vβ11-bearing T cells. This is probably not the case, since the same clonal deletion was observed in the progeny from two selected founders (260-23 and 258-10), whose transgenes most likely integrated in different sites of mouse genome. Second, introducing DQb transgene products could have decreased the endogeneous I-A expression, which might be necessary for sustaining Vβ5.1-, Vβ5.2-, and Vβ11-bearing T cell survival. However, FACS analysis with mAb MKS4 showed that this is not true, since identical levels of $A\beta^f$ expression was observed between DQβ transgenics and their negative littermates. Third, although the thymic staining and FACS analysis with mAb Leu10 and 61.11.1 indicated that the intact DQβ molecules are expressed on the cell surface, the results cannot rule out the remote possibility that a small amount of DQβ transgene products was fragmented and served as a "self-peptide" presented by the endogeneous A molecules, which leads to the deletion of Vβ11-,

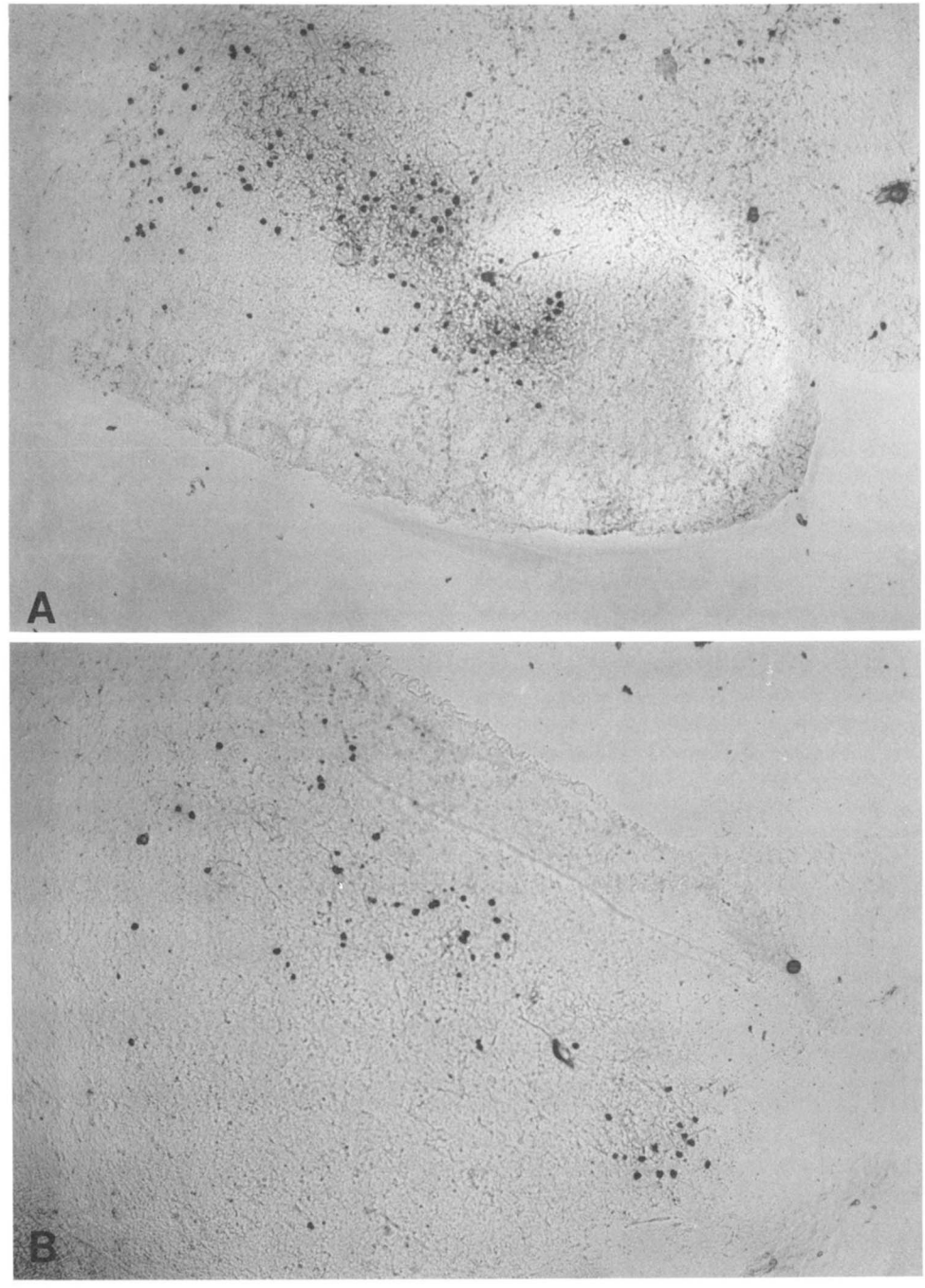

Figure 5. Thymic staining of DQβ transgene expression. Frozen thymic sections from DQβ transgenics (*A*) and the negative littermate (*B*) were stained as described in *Materials and Methods*. The DQβ molecules are mainly detected in the medulla of thymus derived from transgenics. The dark spots in both transgene-positive and transgene-negative mice reflect the background staining of activity of endogenous peroxidase.

TABLE I

Vβ11, Vβ5.1, and Vβ5.2 TCR usage in peripheral T cells derived from B10.M, DQβ transgenics and negative littermate

	Percentage			
	Vβ5.1+, CD8+/CD8+	Vβ5.2+, CD8+/CD8+	Vβ11+, CD4+/CD4+	Vβ11+, CD8+/CD8+
B10.M	3.6 ± 0.3 (3)[a]	3.0 ± 0.5 (3)	3.8 ± 0.2 (3)	6.9 ± 0.5 (3)
DQβ− littermate (260-23)	2.6 ± 0.6 (3)	2.5 ± 0.1 (3)	4.0 ± 0.1 (6)	6.7 ± 0.4 (6)
DQβ+ transgenics (260-23)	0.6 ± 0.1 (3)	0.4 ± 0.1 (3)	0.3 ± 0.1 (6)	2.3 ± 0.1 (6)
DQβ− littermates (258-10)	6.7 ± 0.5 (3)	4.4 ± 0.3 (3)	4.0 ± 0.2 (3)	ND[b]
DQβ+ transgenics (258-10)	0.7 ± 0.1 (3)	0.8 ± 0.2 (3)	1.1 ± 0.2 (3)	ND[b]

[a] Numbers in parentheses represent numbers of mice tested.
[b] ND, not done.
[c] Background staining using RIII stain is less than 0.2%.

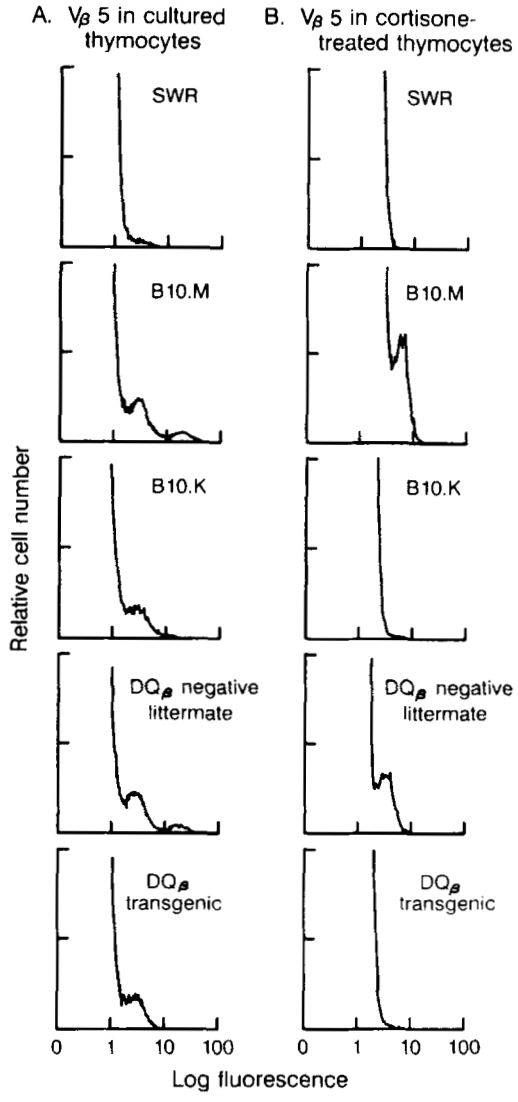

Figure 6. Fluorescence histograms of thymocytes stained with anti-Vβ5.1 and Vβ5.2 mAb (MR9-4). In *panel A*, thymocytes were cultured for 4 h to increase the density of TCR on immature thymocytes and then stained with mAb MR9-4. In *panel B*, mice were administered intraperitoneally with 4 mg of hydroxy-21-cortisone acetate per mouse. 48 h later, the mice were sacrificed and thymocytes were stained with mAb MR9-4. In each sample, 200,000 thymocytes were analyzed.

Vβ5.1-, and Vβ5.2-bearing T cells in thymus. The fourth possibility, which we favor, is that A_a^f/DQβ hybrid molecules may possess the same self-peptide- (or superantigen)-presenting epitope as E molecules. It has been recently observed that the product of a gene closely linked to Mtv[3]-9 on mouse chromosome 12 or Mtv-9 gene product itself could serve as a cotolerogen in the clonal deletion of H-2E-reactive T cells (11); (Ed Palmer, personal communication). Recently, we have found that breeding DQβ transgene into DBA/1, which is E-negative and Mls-1a⁺, causes the clonal deletion of Vβ6-, Vβ7-, Vβ8.1-, and Vβ9-bearing T cells and indicates that Aα/DQβ hybrid molecules can truly present Mls-1a superantigen (manuscript in preparation). We propose that an ancestral class II gene passed on the Vβ11-, Vβ5.1-, and Vβ5.2-specific self-peptide (or superantigen)-presenting site to mouse Ea, Eb, and human DRa and DQb, but the mouse Aa and Ab lost them during evoluation, duplication, and divergence. It will be interesting to check whether the other human class II genes contain this epitope(s). It could also be a combinatorial epitope(s) generated by the α and β chains.

Recently, Nishimura et al. (14) reported the generation of transgenic mice bearing both HLA-DQw6a and DQw6b transgenes. No clonal deletion of mouse TCR-bearing T cells was found in their double positive transgenics. In the double transgenics, Nishimura et al. could not detect any mixed isotype human/mouse class II molecules, since preferential pairing of DQα and DQβ chains takes place. Several mechanisms can be invoked to explain this discrepancy. First, the different results could be due to the background gene difference. However, since the B6 background in their double transgenics and the B10 background in our single transgenics are quite similar, it is unlikely that the differences are caused by the background genes. Second, the Aα/DQβ hybrid molecules and DQα/DQβ homologous molecule may differ in their self-peptide- (or superantigen)-binding epitope(s). However, most studies reported so far demonstrate that the self-peptide(s) (or superantigen) react outside of conventional peptide-binding pocket (28). We propose that the difference may be due to the requirement of mouse accessory molecules in thymic selection (17, 29). Most likely, mouse CD4 cannot recognize the DQα/DQβ in double transgenics but can recognize the $A\alpha^f$/DQβ hybrid molecules in the DQβ single transgenics (Fig. 7). Thus, besides the trimolecular (TCR, Class II, and self-peptide/superantigen) interaction, CD4 molecules are also involved in the thymic selection. Mouse CD4 can recognize only the CD4 binding site in Aα chain.

The striking functional similarity between $A\alpha^f$/DQβ hybrid molecules and E molecules leads us to search for putative "self-peptide"-presenting epitope in DQβ and Eαβ. We assume that the residue(s) of DQw6β molecule, responsible for Vβ11, Vβ5.1, and Vβ5.2 TCR deletion, should be different from endogenous $A\beta^f$, but similar or identical to Eα/Eβ molecules. In order to determine whether this epitope is conserved in all DQβ alleles, studies will be initiated in our DQw3.2β transgenics. Another important question is whether this epitope(s) is part of the antigen-presenting pocket of the class II molecules or outside the groove similar to staph superantigens (31).

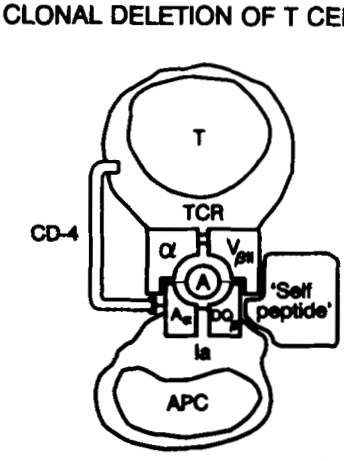

Figure 7. Clonal deletion of T cells—a hypothesis. In the $A\alpha^f$/DQβ transgenic mice, Vβ11⁺ T cells recognize "self-peptide" presented by DQβ chain in conjunction with the interaction between accessory molecules (CD4) and class II Aα, resulting in the clonal deletion of these T cells.

[3] Abbreviation used in this paper: Mtv, mammary tumor virus gene.

Generation of transgenic mice with exon shuffled and mutated class II genes should point out the exact residues involved.

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