

# Functional Interaction between Human Histocompatibility Leukocyte Antigen (HLA) Class II and Mouse CD4 Molecule in Antigen Recognition by T Cells in HLA-DR and DQ Transgenic Mice

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## Summary

Studies in vitro have suggested that a species barrier exists in functional interaction between human histocompatibility leukocyte antigen (HLA) class II and mouse CD4 molecules. However, whether mouse CD4<sup>+</sup> T cells restricted by HLA class II molecules are generated in HLA class II transgenic mice and respond to peptide antigens across this barrier has remained unclear. In an analysis of T cell responses to synthetic peptides in mice transgenic for HLA-DR51 and -DQ6, we found that DR51 and DQ6 transgenic mice acquired significant T cell response to influenza hemagglutinin-derived peptide 307-319 (HA 307) and *Streptococcus pyogenes* M12 protein-derived peptide 347-397 (M6C2), respectively. Inhibition studies with several monoclonal antibodies showed that transgenic HLA class II molecules presented these peptides to mouse CD4<sup>+</sup> T cells. Furthermore, T cell lines specific for HA 307 or M6C2 obtained from the transgenic mice could respond to the peptide in the context of relevant HLA class II molecules expressed on mouse L cell transfectants that lack the expression of mouse MHC class II. These findings indicate that interaction between HLA class II and mouse CD4 molecules is sufficient for provoking peptide-specific HLA class II-restricted T cell responses in HLA class II transgenic mice.

Interaction of the CD4 coreceptor with class II products of the MHC has a critical role in intrathymic selection (1, 2) and peripheral immune response to foreign antigens of CD4<sup>+</sup> T cells (3). The CD4 molecule binds to the  $\beta$  domain of the MHC class II molecule (4, 5) and provokes T cell responses by increasing avidity of T cell-APC interaction and/or by transmitting an intracellular activation signal (6, 7). Although human (HLA) and mouse (H-2) MHC have evolved from common ancestral genes (8), several studies suggest that a species barrier exists in functional interaction between mouse CD4 and human class II molecules in vitro (9-11). Lamarre et al. (9) considered the possibility that mouse CD4 probably did not interact with the HLA-DP molecule by analyzing the response of CD4<sup>-</sup>CD8<sup>-</sup> T cell hybridoma. Vignali et al. (10) showed that the responses of mouse CD4<sup>+</sup> T cells to peptide antigens were reduced by replacing the  $\beta$ 2 domain of the I-A molecule on APC with that of the DR molecule. Furthermore, T cells from human CD4 transgenic mice have been shown to respond to human class II molecules in a xenogeneic MLR stronger than that from

normal mice (11). However, it has remained unclear whether mouse CD4<sup>+</sup> T cells restricted by human class II molecules are generated in HLA class II transgenic mice and respond to foreign peptide antigen across this barrier. It is, therefore, essential to clarify this issue if we want to employ the HLA class II transgenic mice for investigating the role of HLA class II in regulating immune response and disease susceptibility.

We generated transgenic C57BL/6 (B6) mice carrying genes for both HLA-DQ6A (DQA1\*0103) and -DQ6B (DQB1\*0601) encoding  $\alpha$  and  $\beta$  chains of the HLA-DQ6 molecule, respectively (DQ6-B6) (12), that for HLA-DRA (DRA\*0101) encoding the monomorphic  $\alpha$  chain of the HLA-DR molecule (DRA-B6) (13), and that for HLA-DR51B (DRB5\*0102) encoding the  $\beta$  chain of HLA-DR51 molecule (DR51B-B6). Here we show that DQ6-B6 and DR51-B6 obtained by crossing DR51B-B6 with DRA-B6 acquired peptide-specific, HLA-restricted T cell responses, and that functional interaction between HLA class II and mouse CD4 molecules was involved in these responses.

## Materials and Methods

**Mice.** DRA-B6 and DQ6-B6 were generated and characterized as previously described (12, 13). DR51B-B6 were produced by injection of B6 oocytes with a 5.6-kb *Apal*/*EcoRI* fragment containing the HLA-DR51B (DRB\*0102) cDNA (14). This transgene includes a 4.0-kb *Apal*/*SacI* fragment containing the 5' upstream region of DR51B gene and connected to the SV40 poly A signal sequence at the 3' side. Mice carrying the transgene were identified by Southern blot analysis using DR51B cDNA as a probe. DR51-B6 were obtained by crossing DRA-B6 with DR51B-B6.

**Reverse Transcriptase-PCR Analysis.** Total cellular RNA was extracted from homogenized tissues or single cell suspensions. cDNA were made from RNA extracts digested by DNase I to avoid contaminating transgenic DNA and were then subjected to PCR to amplify the DR51B for  $A\beta^b$  gene. The primers used were (5'-CACTGATGGTGCTGAGCTCCCCAC-3') and (5'-CCAAGG-AAGAGCAGGCCAGCAC-3') for DR51B, and (5'-CGAAAG-GCATTTTCGTGTACCAG-3') and (5'-ATGCTAGACTCTGAG-TCGGAG-3') for  $A\beta^b$ . PCR products were electrophoresed in a 1.2% agarose gel, transferred onto a nylon membrane, and hybridized with end-labeled oligonucleotide probes for DR51B (5'-GAG-TGTCATTTCTTCAACG-3') and  $A\beta^b$  (5'-CGGCGGCTTGAA-CAGCCCA-3') as described (15).

**Flow Cytometry.** Cell surface expressions of HLA-DR51 molecules on B cells, dendritic cells, and macrophages were examined by flow cytometry (FCM)<sup>1</sup> as follows. Spleen cells from each strain of mice were cultured with IL-4 (50 U/ml) for 48 h and then doubly stained with biotinylated goat anti-mouse IgG plus IgM (Tago, Inc., Burlingame, CA) followed by streptavidin-coupled PE, and TAL16.1, specific for HLA-DR51 (16), followed by FITC-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA). IgG plus IgM positive cells were gated to be analyzed as B cells. Splenic dendritic cells were prepared as described (17) and stained with TAL16.1. About 70–80% of cells prepared were positive for splenic dendritic cell marker 33D1 (18). Dish adherent spleen cells were prepared and cultured with IFN- $\gamma$  (250 U/ml) for 48 h, and then doubly stained with M1/70HL, specific for MAC-1 antigen expressed on macrophages (Hybritech, Inc., San Diego, CA) and TAL16.1. MAC-1 positive cells were gated to be analyzed as splenic macrophages. All the cells expressed a comparable level of the I-A<sup>b</sup> molecule. For analysis of the expression of DR $\alpha$ E $\beta^b$  molecules in DRA-B6 and DR51-B6, spleen cells were stained with biotinylated 17-3-3, mAb specific for E $\beta^b$  (19), followed by streptavidin-coupled PE. FCM was done by FACScan<sup>®</sup> (Becton Dickinson & Co., Mountain View, CA).

**T Cell Proliferation Assays and Blocking Experiments.** Mice were immunized by giving 0.2 ml of soluble HA 307 peptide (20  $\mu$ g, PKYVKQNTLKLAT) or M6C2 peptide (20  $\mu$ g, AKKQVEKDLANLTAELDKVKE) emulsified with CFA (Difco, Detroit, MI) into the hind footpads and at the base of the tail. These peptides were synthesized by a peptide synthesizer (model 9050 Plus; Millipore Corp., Bedford, MA) and purified by reversed-phase HPLC. Popliteal and inguinal lymph nodes were removed 9–12 d later and single cell suspensions were prepared in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD) supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100  $\mu$ g/ml streptomycin, 2 mM L-glutamine, 50  $\mu$ M 2-ME, and 20 mM Hepes (complete medium). To purify lymph node cells to T cells, I-A<sup>b</sup> positive cells were eliminated by using sheep anti-mouse IgG coated magnetic beads (Dyna-

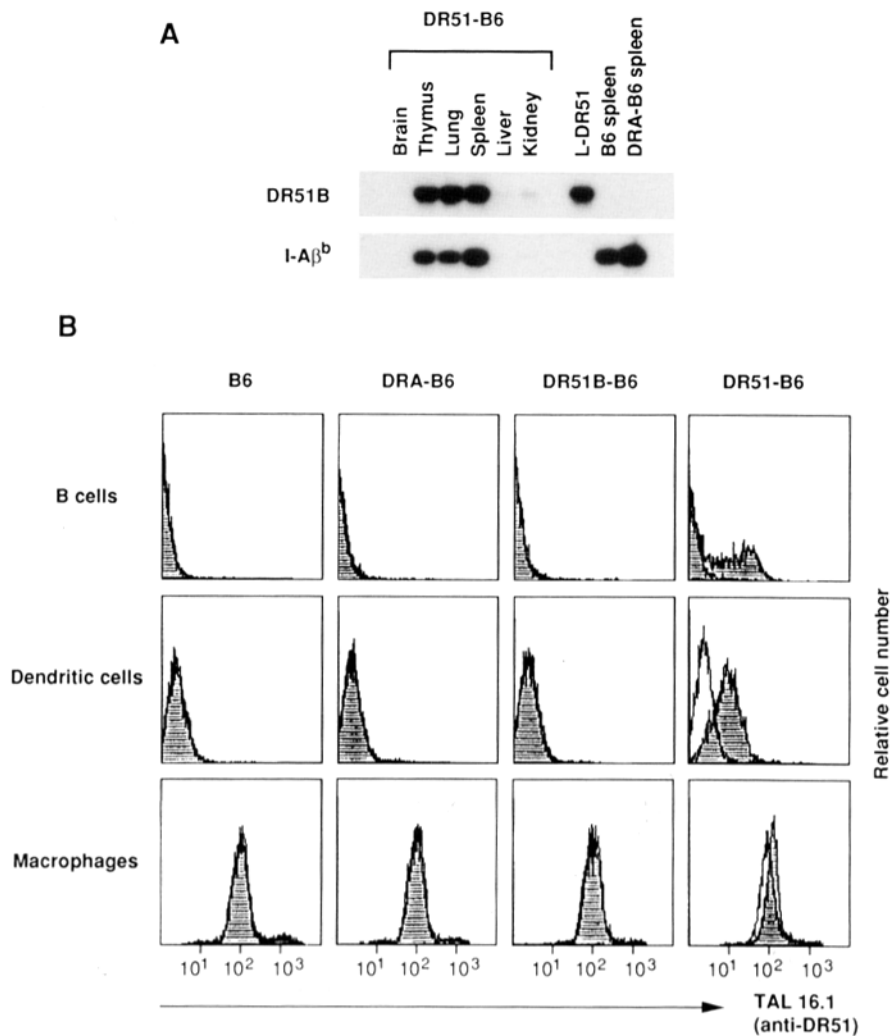
Oslo, Norway) and anti-I-A<sup>b</sup> mAb (Meiji, Odawara, Japan) as follows. Magnetic beads were added to the cell suspension and the preparation was centrifuged for 10 min at 1,500 rpm followed by resuspension of the cells and removal of the beads/IgG positive B cells. This step was repeated twice and cell suspensions were subsequently incubated with anti-I-A<sup>b</sup> mAb at a saturating concentration for 30 min at 4°C. The cells were then washed three times with complete medium and further enriched to T cells using magnetic beads. The enriched T cells ( $4 \times 10^5$ ) were cultured in 0.2 ml of complete medium in triplicate in 96-well flat-bottom tissue culture plates, containing  $8 \times 10^5$  irradiated (3,000 rad) syngeneic spleen cells as APCs in the presence or absence of antigen peptide at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> for 48 h. 1  $\mu$ Ci of [<sup>3</sup>H]thymidine was added during the final 12 h and then incorporated radioactivity was measured. In blocking experiments on the T cell responses with mAbs, a 200-fold diluted ascites form of anti-Lyt2.2 (anti-CD8; Meiji), 3JP (anti-A $\alpha^b$ ) (20), HU11 (anti-DQ) (21), or a fourfold diluted culture supernatant GK1.5 (anti-CD4) (22), or 1.3  $\mu$ g/ml purified L243 (anti-DR) (23), or 10  $\mu$ g/ml purified 17-3-3 (anti-E $\beta^b$ ), 17/227 (anti-A $\beta^b$ ) (24), 20-8-4S (anti-K<sup>b</sup>D<sup>b</sup>) (25) was added to the culture.

**Antigen-specific T Cell Line.** T cells were purified from lymph node cells of DR51-B6 or DQ6-B6 preimmunized with 20  $\mu$ g of peptide by eliminating I-A<sup>b</sup> positive cells as described above. Purified T cells ( $6 \times 10^6$ ) were cultured with  $25 \times 10^6$  irradiated (3,000 rad) syngeneic spleen cells with HA 307 or M6C2 peptide at 10  $\mu$ g/ml in complete medium for 5 d. Viable cells were collected by density gradient centrifugation with Lympholyte-M (Cedarlane Laboratories, Hornby, ON, Canada) and further cultured without stimulator cells and antigen for 5 d. The stimulation and resting cycle was repeated twice in medium supplemented with rat Con A supernatant (Becton Dickinson & Co.). A T cell line ( $5 \times 10^4$ ) was cultured with  $3 \times 10^4$  indicated mouse L cell transfectants pretreated with mitomycin C in the presence or absence of peptide for 36 h. 1  $\mu$ Ci of [<sup>3</sup>H]thymidine was added during the final 6 h and then incorporated radioactivity was measured.

## Results and Discussion

We previously reported that HLA-DQ6 molecules were expressed in the thymus, lungs, lymph nodes, and spleen in a tissue-specific manner as was MHC class II in DQ6-B6 (12). In DR51-B6 obtained by crossing DR51B-B6 with DRA-B6, the DR51B transgene was expressed in the thymus, spleen, and lung (Fig. 1A). This suggested that HLA-DR51 molecules were expressed on various immunocompetent cells such as thymic epithelial cells, B cells, macrophages, and dendritic cells in DR51-B6 because DRA-B6 expressed DR $\alpha$  chain as DR $\alpha$ E $\beta^b$  molecule on these cells (13). Cell surface expressions of DR51 molecules on these immunocompetent cells were confirmed by FCM analysis (Fig. 1B). It should be noted that DR51-B6 also expresses a xenogeneic mixed isotype molecule, DR $\alpha$ E $\beta^b$ , as in DRA-B6 (Fig. 2). Expression of the DR $\alpha$ A $\beta^b$  molecule in DR $\alpha$  single transgenic mice was not demonstrated by immunoprecipitation (26) nor was the A $\alpha^b$ DR51 $\beta$  molecule in DR51B-B6 demonstrated by FCM using anti-DR $\beta$  polyclonal antibody (data not shown). Thus, DR51-B6 apparently expresses three different MHC class II molecules, DR51, DR $\alpha$ E $\beta^b$ , and I-A<sup>b</sup>. In DQ6-B6, mixed

<sup>1</sup> Abbreviation used in this paper: FCM, flow cytometry.



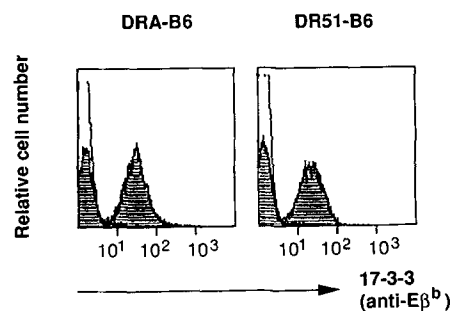
**Figure 1.** Expression of HLA-DR51B transgene in transgenic HLA-DR51 C57BL/6 mice (*DR51-B6*). (A) Reverse transcriptase-PCR analysis of DR51B and I-A $\beta^b$  gene transcripts in various organs in DR51-B6 or in cell lines. (B) Cell surface expression of the DR51 molecule on B cells, splenic dendritic cells, and splenic macrophages of B6, DRA-B6, DR51B-B6, and DR51-B6 (*shaded*). The background stainings for each sample is presented in the same panel (*unshaded*).

isotype molecules were not detected by immunoprecipitation (12).

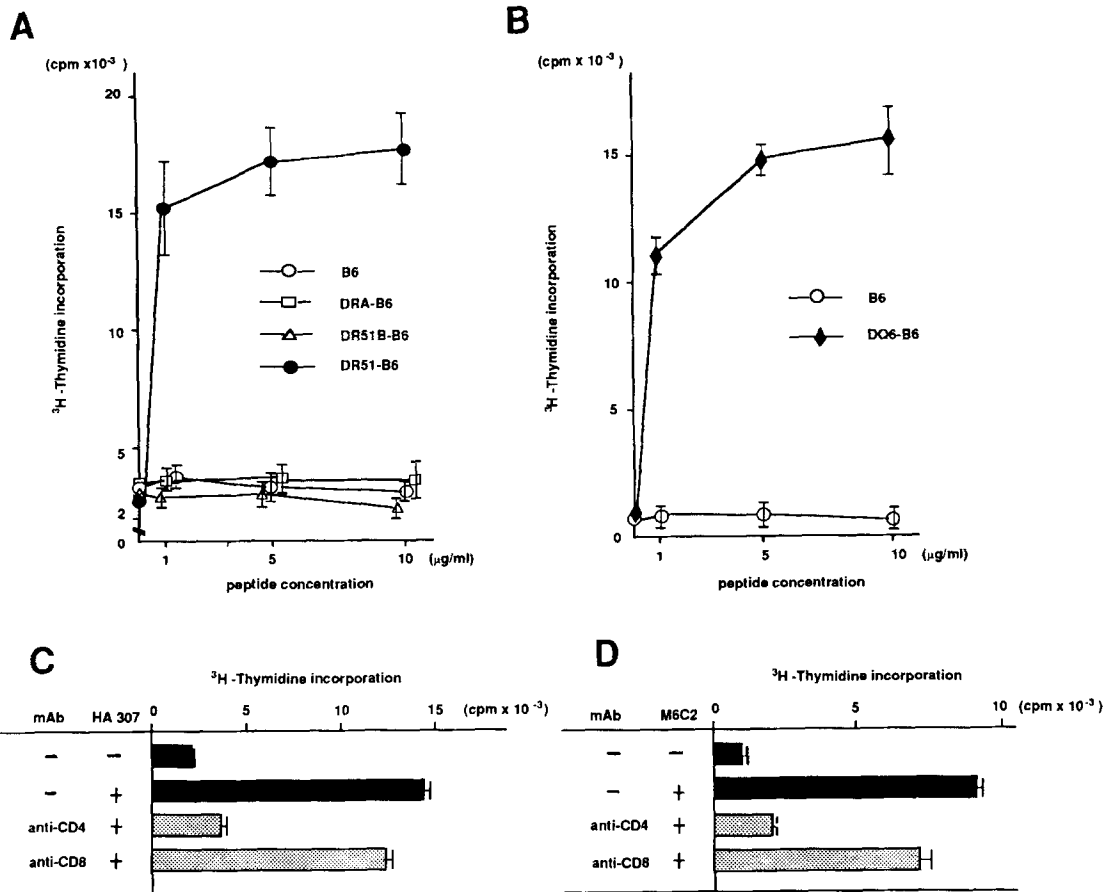
In a previous study (12), it was shown that DQ6-B6 did not produce antibodies specific for the DQ6 molecule, and that loss of MLR against the DQ6 molecule was observed in DQ6-B6, suggesting that DQ6-B6 acquired a tolerance

to HLA-DQ6 molecule at the B and T cell levels. To investigate whether DR51-B6 acquired a tolerance to the HLA-DR51 molecule, mice were immunized with the EBV-transformed human B cell line, TOK, which expresses HLA-DR51 molecules, and the production of antibodies specific for DR51 molecule was examined. As expected, DR51-B6 did not produce antibodies against DR51 molecule, whereas B6, DRA-B6, and DR51B-B6 produced the antibodies (data not shown). In addition, a primary MLR using DR51-B6 spleen cells as stimulator showed that the transgenic DR51 molecule could stimulate T cells from B6, DRA-B6, and DR51B-B6, but not DR51-B6 (data not shown). These findings indicate that DR51-B6 acquired a tolerance to transgene products as had DQ6-B6, suggesting that transgenic HLA class II molecules contributed to eliminate self-reactive T cells as had MHC class II molecules.

Next, in order to examine whether HLA class II molecules expressed in mice would provoke T cell responses to foreign peptide antigens, mice were immunized with influenza A hemagglutinin-derived peptide 307-319 (HA 307), known to be a satisfactory DR binding peptide (27), or with *Streptococcus pyogenes* M12 protein-derived peptide 347-367 (M6C2),



**Figure 2.** Cell surface expression of the DR $\alpha$ E $\beta^b$  molecule on spleen cells of DRA-B6 and DR51-B6 (*shaded*). The background stainings for each sample is presented in the same panel (*unshaded*).



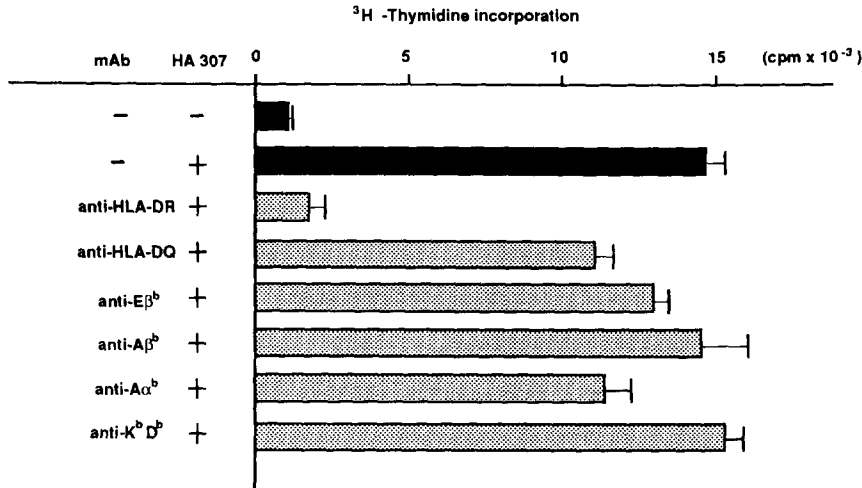
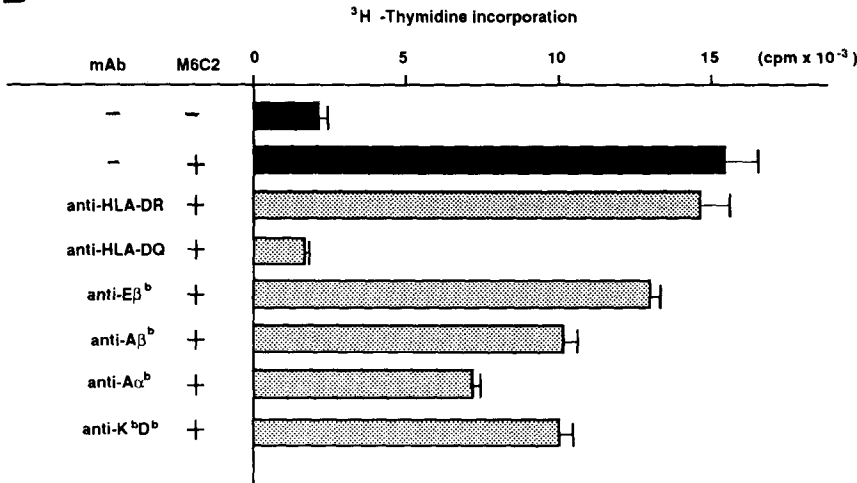
**Figure 3.** Immune responses to peptide antigens in HLA transgenic mice. DR51-B6 and DQ6-B6 acquired the T cell response to HA 307 peptide (A) and M6C2 peptide (B), respectively. Anti-CD4 but not anti-CD8 mAb inhibited these T cell responses (C and D). Data presented are representative of three experiments and are expressed as the mean  $\pm$  SD of triplicate wells.

which is a DQ6-restricted antigenic peptide in humans (28), and secondary proliferative T cell responses in vitro were measured. Fig. 3 A shows that DR51-B6 acquired the T cell response to HA 307, whereas no proliferative response was observed in B6, DRA-B6, or DR51B-B6. On the other hand, the immune response to M6C2 was observed only in DQ6-B6 (Fig. 3 B). DR51-B6 and DQ6-B6 did not acquire the T cell response to M6C2 and HA 307, respectively (data not shown). Anti-CD4 but not anti-CD8 mAb inhibited these responses (Fig. 3, C and D). These results suggest that the DR51 molecule, but not DR $\alpha$ E $\beta^b$  or I-A<sup>b</sup>, presented HA 307 to mouse CD4<sup>+</sup> T cells in DR51-B6, whereas the DQ6 molecule presented M6C2 to CD4<sup>+</sup> T cells in DQ6-B6. However, it remained to be determined whether functional interaction of mouse CD4 with HLA class II molecules was involved in these responses, because mouse CD4 might interact with the  $\beta$  chain of DR $\alpha$ E $\beta^b$  and/or I-A<sup>b</sup> expressed in addition to DR51 or DQ6 molecules on the same APC (9, 12).

To clarify this point, inhibitory effects by several mAbs specific for mouse MHC class II molecules on the T cell re-

sponses were examined. As shown in Fig. 4, mAbs specific for E $\beta^b$  (17-3-3), which are known to react with DR $\alpha$ E $\beta^b$  (13, 29) or those for A $\beta^b$  (17/227), failed to block the responses as well as had anti-A $\alpha^b$  (3JP). By contrast, the response was completely inhibited by anti-DR (L243) in DR51-B6 and anti-DQ (HU11) in DQ6-B6. These results suggest that the DR $\alpha$ E $\beta^b$  or I-A<sup>b</sup> molecules did not play a major role in these responses.

To examine more closely the interaction of mouse CD4 with HLA class II, CD4<sup>+</sup> T cell lines specific for HA 307 or M6C2 were obtained from DR51-B6 or DQ6-B6, respectively, and their restriction elements were investigated using mouse L cells transfected with various HLA class II as APCs that do not express endogenous mouse MHC class II molecules. Consistent with the blocking experiments, each T cell line specific for either HA 307 or M6C2 responded to the peptide antigen in the context of the relevant HLA class II molecules in the absence of mouse class II (Fig. 5, A and B). These responses were restricted to DR51 or DQ6 molecules because the L cells expressing each of the DR1, DR2, or DR4 molecules, which are known to present HA 307 to

**A****B**

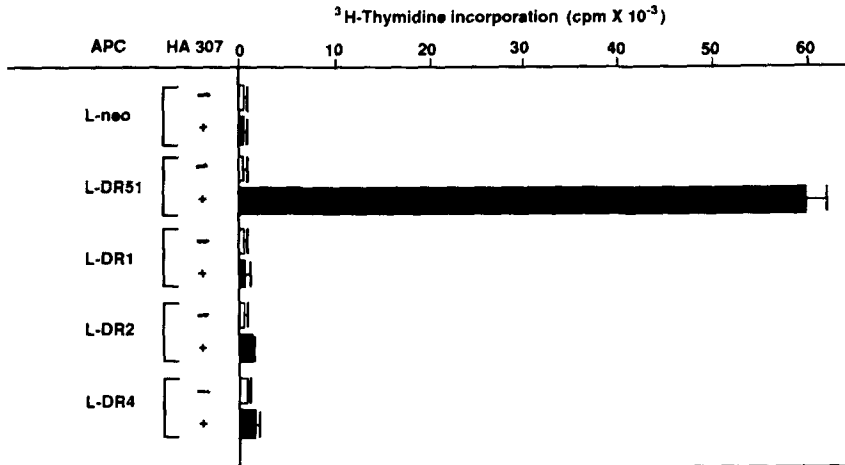
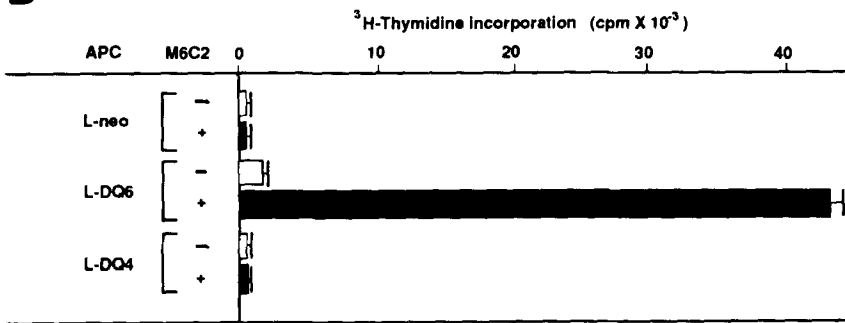
**Figure 4.** Effects of antibodies specific for various MHC molecules on T cell response in HLA transgenic mice. (A) Response to HA 307 in DR51-B6 and (B) that to M6C2 in DQ6-B6. Peptides were used at a concentration of 1  $\mu$ g/ml. Data presented are representative of three experiments and are expressed as the mean  $\pm$  SD of triplicate wells.

human T cells (27), and those expressing DQ4, which binds M6C2 (data not shown), could not present the antigen peptides to these T cell lines.

Spleen cells from bone marrow chimera-B6 and -DRA-B6, which were reconstituted with DR51-B6 BM, could stimulate the HA 307-specific DR51-restricted T cell line, but these bone marrow chimera mice did not acquire the T cell response to HA 307 (data not shown). These observations indicate that the responding T cells observed in the DR51-B6 express TCR antigens that were selected positively in the thymus by the transgene product, but not by I-A<sup>b</sup> or DR $\alpha$ E $\beta$ <sup>b</sup> molecules. Previous study (2) has shown that interaction of CD4 with MHC class II molecules is critical for the generation of MHC class II-restricted mature T cells by analyzing T cell differentiation in CD4-deficient mice. Taken together, we conclude that HLA class II-restricted mouse CD4<sup>+</sup> T cells were generated by significant interac-

tion between HLA class II and mouse CD4 molecules in the thymus of the transgenic mice, and that these cells could recognize antigen peptides in the context of HLA class II molecules.

Our results would contradict the previous observations showing that a species barrier exists in functional interaction between HLA class II and mouse CD4 molecules (9-11). However, it has been also suggested that this barrier is partial and that CD4 can bind to the MHC class II  $\alpha$  chain or a conserved site that itself is not species specific (10, 30). We cannot assess in this study whether T cell responses in the HLA class II transgenic mice will be augmented by introducing human CD4, or whether a HLA class II transgenic mouse expressing chimeric human/mouse MHC class II molecules that contain mouse class II-CD4 interacting region will acquire stronger T cell response than the transgenic mice used in this study. However, one prominent feature of this study was that we established two strains of HLA class II transgenic mice

**A****B**

**Figure 5.** Recognition of HA 307 and M6C2 peptide in the context of the relevant HLA class II molecule expressed on the mouse L cell transfectant by T cell lines established from DR51-B6 (A) and DQ6-B6 (B), respectively. Peptides were used at a concentration of 5  $\mu$ g/ml. 98% of T lymphoblasts were positive for CD4. Data presented are representative of three experiments and are expressed as the mean  $\pm$  SD of triplicate wells.

expressing HLA-DR or -DQ molecules in a tissue-specific manner as MHC class II and T cell responses to peptide antigens were analyzed in an in vivo system. Therefore, our observations indicate that interaction between HLA class II and mouse CD4 molecules is physiologically sufficient for

generating HLA class II-restricted mouse T cell repertoire and for provoking peptide-specific mouse T cell responses. Thus, we propose that HLA class II transgenic mice can serve to investigate the role of HLA class II molecules in regulating immune responses and disease susceptibility in humans.

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