Jpn J Human Genet 42, 225-232, 1997

IDENTIFICATION OF AN HLA-DQ6-DERIVED PEPTIDE RECOGNIZED BY MOUSE MHC CLASS I H-2D^b-RESTRICTED CD8$^+$ T CELLS IN HLA-DQ6 TRANSGENIC MICE

Tsutao TAKESHITA,[1] Yoshinori FUKUI,[1] Ken YAMAMOTO,[1] Kazuaki YAMANE,[1] Takeshi INAMITSU,[2] Nobuhiro KAMIKAWAJI,[1] and Takehiko SASAZUKI[1,*]

[1] *Department of Genetics, Medical Institute of Bioregulation and* [2] *Department of Pediatrics, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812, Japan*

Summary CD8$^+$ T cells from C57BL/6(B6) mice show cytotoxicity to B cell blasts prepared from syngeneic transgenic mice expressing HLA-DQ6 molecules in a mouse MHC class I H-2D^b restricted manner. Although these results suggest that CD8$^+$ T cells recognize peptides derived from DQ6 molecule bound to H-2D^b on target cells, no direct evidence so far has been obtained. To clarify this, we synthesized 23 peptides corresponding to DQ6α or β chain and carrying the motifs of D^b-binding peptides, and examined their capacity to induce cytotoxicity in the CD8$^+$ T cell line. We show here that DQA1-2, one of these peptides, induced cytotoxicity of the CD8$^+$ T cells when this peptide was pulsed to H-2D^b expressing target cells, as efficiently as HLA-DQ6 expressing target cells did. Thus, our results suggest that DQA1-2 can be naturally processed from DQ6 molecules and recognized by the CD8$^+$ T cells in the context of H-2D^b molecules. These results suggest that allogeneic HLA class II molecules are involved in the rejection not only as the ligand for T cell receptor of alloreactive CD4$^+$ T cells but also as self-peptides bound to HLA class I molecules recognized by CD8$^+$ T cells.

Key Words HLA-DQ6, transgenic mice, CTL, binding motifs, peptide

INTRODUCTION

The HLA multigene family consists of HLA class I and class II genes with extremely high polymorphism, and plays a central role in immune response through the presentation of antigenic peptides to CD8$^+$ or CD4$^+$ T cells, respectively. So far many studies have revealed that certain HLA-haplotypes are associat-

Received December 9, 1996; Accepted December 27, 1996.

* To whom correspondence should be addressed.

ed with immune response to natural antigens (Sasazuki *et al.*, 1983; Sasazuki, 1990) and predisposition to autoimmune disease (Todd *et al.*, 1988). It is also well established that HLA matching have a profound effect in the success of the organ transplantation (Thomas *et al.*, 1975; Beatty *et al.*, 1985). The strong linkage disequilibria between HLA alleles, however, precludes analyzing the role of individual HLA molecule in immune regulation and immune related disorder. To overcome this problem, we developed several lines of transgenic mice expressing either HLA-DR or DQ molecules (Nishimura *et al.*, 1990; Fukui *et al.*, 1993a, b; Yamamoto *et al.*, 1994).

We have previously reported that HLA-DQ6 molecules expressed in transgenic mice function as appropriate MHC class II to mount immune response to the natural antigen and the antigenic peptide (Nishimura *et al.*, 1990; Esaki *et al.*, 1994; Yamamoto *et al.*, 1994). However, we found that $CD8^+$ T cells with cytotoxicity to DQ6-expressing B cell blasts were generated when syngeneic non-transgenic T cells were cultured with the transgenic spleen cells *in vitro*. Since this cytotoxicity is blocked by the monoclonal antibody (mAb) specific for mouse MHC class I H-$2D^b$ molecules, it was suggested that $CD8^+$ T cells recognized DQ6-derived peptide in the context of H-$2D^b$ molecules (Inamitsu *et al.*, 1992). To clarify this possibility, we have tried the identification of DQ6-derived peptide which elicit cytotoxicity of the $CD8^+$ T cells in the present study.

MATERIALS AND METHODS

Mice. The establishment of HLA-DQ6 transgenic mice have been previously reported (Nishimura *et al.*, 1990). Briefly, transgenic mice were obtained by introducing genomic *HLA-DQ6 A* and *B* genes into fertilized eggs from C57BL/6(B6).

Peptides. All peptides were synthesized using the peptide synthesizer (Millipore Peptide Synthesizer 9050 Plus) and purified by HPLC.

Antibodies. Anti-HLA-DQ(4+5+6) mAb, HU-11, was a gift of Dr. A. Wakisaka, Hokkaido University, Sapporo, Japan. Anti-mouse CD4 mAb GK1.5 was obtained from American Type Culture Collection, Rockville, MD. Anti-I-$A^{b,k}$mAb (Meiji, Odawara, Japan), anti-mouse IgG coating magnetic beads, anti-rat IgG coating magnetic beads (Dynal A.S, Oslo, Norway) were used for establishment of DQ6-specific $CD8^+$ T cell line. Fluorescein isothiocyanate-conjugated anti-HLA-DQ mAb, phycoerythrin-conjugated anti-CD4 mAb and fluorescein isothiocyanate-conjugated anti-CD8 mAb (Becton Dickinson, Mountain View, CA) were used for flow cytometric analysis.

Generation of the DQ6-specific $CD8^+$ T cell line. B6 mice were immunized with irradiated DQ6 transgenic mice spleen cells (2×10^7) into the tail base and foot pads. Two weeks later, immunized mice were boosted with irradiated DQ6 spleen cells. Seven days later, lymph nodes were prepared and treated with

anti-I-A^b mAb and anti-mouse IgG coating magnetic beads to remove B cells. Purified T cells were stimulated several times with irradiated DQ spleen cells, and DQ6-specific $CD8^+$ T cell lines (>95% $CD8^+$ purified) were established by removing $CD4^+$ T cells with rat anti-mouse CD4 mAb (GK1.5) and goat anti-rat IgG coating magnetic beads.

CTL assays. Spleen cells from B6 and DQ6-transgenic mice were treated by lipopolysaccharides (LPS) and used as target cells. ^{51}Cr-pulsed target cells (1×10^4) were cultured with a various number of effector cells in a final volume of 200 μl of medium with or without peptides in 96-well round-bottom plate, and incubated for 4 hr at 37°C in 5% CO_2. In some experiments, mAbs specific to mouse MHC class I were added to the culture. Plate was centrifuged and radioactivity in 100 μl of supernatant were counted using a gamma counter. Percent specific lysis was calculated as follows: $(E-S)/(M-S) \times 100$ where E, S, M indicate the ^{51}Cr release in the presence of cytolytic T lymphocytes (CTL), ^{51}Cr release in the absence of CTL, ^{51}Cr release in the presence of 0.1% NP40, respectively.

RESULTS

Selection of DQ6-derived peptides with binding motifs to H-2D^b molecules

The peptide motif binding to mouse class I molecules have been reported (Falk *et al.*, 1991). According to this, H-2D^b molecule specifically binds 9 to 11 amino acid length peptides which preferentially encode asparagin at position 5 and methionine at position 9. To identify peptide recognized by the DQ6-specific $CD8^+$ T cell line in the context of D^b molecules, we synthesized 23 candidate peptides corresponding to amino acid sequence of DQ6 α or β chains (*DQA1-0103*, *DQB1-0601*) which have asparagin at position 5 and/or methionine at position 9. The amino acid sequence of these 23 peptides are shown in Table 1.

CTL assay using DQ6-derived peptides

B cell blasts prepared from B6 were pulsed with 23 synthesized peptides and the cytotoxicity of $CD8^+$ T cells was examined (Fig. 1). When the peptide DQA1-2 (SCGVNLYQF) was pulsed, definite cytotoxic activity of the $CD8^+$ T cells was observed. This cytotoxicity did not result from the peptide toxicity to cells, because the DQA1-2 did not induce any cytotoxicity in the absence of DQ6-specific $CD8^+$ T cell (Fig. 2). Since this peptide induce the cytotoxicity of another DQ6 specific $CD8^+$ T cell line (im-5) and is only the peptide to elicit the cytotoxicity (Fig. 3), we conclude that "SCGVNLYQF" is the peptide recognized by DQ6-specific $CD8^+$ T cells.

The cytotoxic activity induced by DQA1-2 was inhibited by D^b-specific mAb, but not by K^b-specific mAb and DQ-specific mAb (Table 2). Thus, these results indicate that DQ6-specific T cells recognized DQA1-2 in the context of D^b molecule.

Table 1. DQ6-derived peptides with binding motifs to H-2D^b molecules.

Peptide name	Corresponding amino acid residues	Sequence	Peptide name	Corresponding amino acid residues	Sequence
DQA1			DQB1		
-1	(68-76)	VAKHNLNIM	-1	(15-23)	CYFTNGTER
-2	(10-18)	SCGVNLYQF	-2	(29-37)	RYIYNREED
-3	(61-69)	GALRNMAVA	-3	(58-66)	AEYWNSQKD
-4	(70-78)	KHNLNIMIK	-4	(78-86)	VCRHNYEVA
-5	(83-91)	TAATNEVPE	-5	(106-114)	TEALNHHNL
-6	(102-110)	LGQPNTLIC	-6	(109-117)	LNHHNLLVC
-7	(110-118)	CLVDNIFPP(V)	-7	(146-154)	PLIRNGDWT
-8	(117-125)	PPVVNITWL	-8	(193-201)	ESAQNKMLS
-9	(123-131)	TWLSNGHAV	-9	(6-14)	DFVLQFKAM
-10	(58-66)	DPQGALRNM	-10	(152-160)	DWTFQILVM
-11	(181-189)	WEPEIPAPM	-11	(155-163)	FQILVMLEM
			-12	(191-199)	QSESAQNKM

Amino acid sequences of the peptides corresponding to DQ6α (*DQA1-0103*) or β (*DQB1-0601*) chains with H-2D^b binding motifs. H-2D^b molecule bind 9 to 11 amino acid length peptide, which preferentially carrys asparagin at position 5 and methionine at position 9. Putative binding motifs are indicated by underlines.

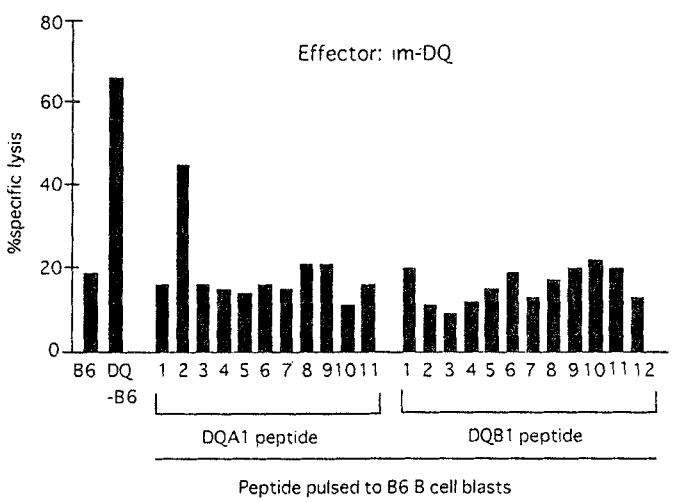

Fig. 1. The cytotoxicity of the CD8$^+$ T cell line (im-DQ) in the presence of DQ6-derived peptides. The middle parts of figure shows the cytotoxicity of the CD8$^+$ T cells (im-DQ) when B6 target cells were pulsed with peptides DQA1- (1 to 11) and DQB1- (1 to 12) respectively at final concentration of 10 μg/ml. The left part of the figure shows the cytotoxicity when B cell blasts from B6 or HLA-DQ6 transgenic mice (DQ-B6) were used as target cells without peptides.

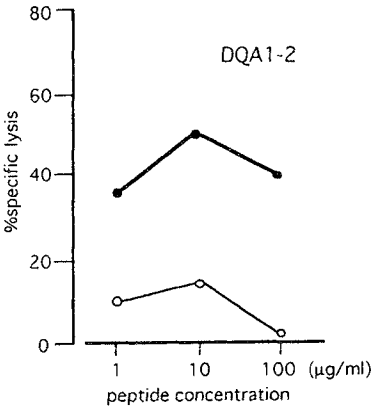

Fig. 2. The cytotoxic activity of CD8+ T cell line (im-DQ) to B6 B cell blasts pulsed with various concentration of the DQ6α-derived peptide. The cytotoxicity in the presence of DQA1-2 peptide is shown. The figure shows the cytotoxicity in the presence (●), or absence (○) of the DQ6-specific CD8+ T cells (im-DQ).

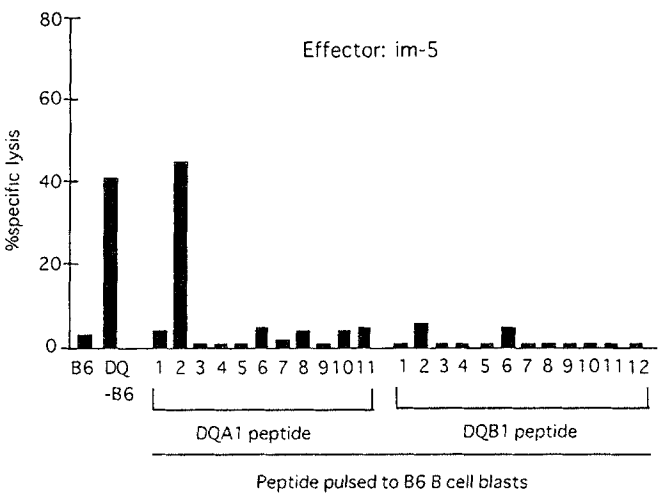

Fig. 3. Cytotoxic activity of the CD8+ T cell line (im-5) to B6 B cell blasts pulsed with DQ6-derived peptides.

DISCUSSION

Clinical studies have revealed that the matching of HLA class II alleles affects the success of organ transplantation as class I do (Beatty *et al.*, 1985; Festenstein *et al.*, 1986). Murine allograft studies have also shown that difference in class II haplotype induce graft rejection (de Waal *et al.*, 1983; Rosenberg *et al.*, 1986).

Table 2. Identification of the restricting molecules using mAb specific to either mouse MHC class I or HLA-DQ6 molecules.

mAb	% Inhibition of cytotoxicity	
	DQ-B6 target	B6 target+DQA1-2
None	0.0	0.0
Anti-K^b	21.2	12.6
Anti-D^b	87.4	70.0
Anti-DQ	9.8	14.4

The cytotoxicity to indicated target cells of the $CD8^+$ T cells was examined with or without 200 fold diluted ascites form of K^b-specific mAb, D^b-specific mAb or DQ-specific mAb (Hu-11).

% Inhibition was calculated by the formula; % Inhibition = [1 − (% Specific lysis with mAb/ % Specific lysis without mAb)] × 100.

Regarding the effect of MHC class II molecules on the rejection, two possibilities may be raised. First, $CD4^+$ or $CD8^+$ T cells might directly recognize allogeneic class II molecules, resulting in the graft rejection (Swain, 1983; Golding *et al.*, 1987). Second, cytotoxic $CD8^+$ T cells which recognize class II-derived peptide bound to class I molecules might play a crucial role in the graft rejection as was suggested by Shinohara *et al.* (1986).

It is well established that exogenous antigens are processed into peptides in antigen presenting cells and mainly presented by class II molecules, whereas endogenous antigen are processed into peptides and mainly presented by class I molecules. We have previously shown that HLA-DQ6 molecules are recognized by mouse $CD8^+$ T cells with the restriction of MHC class I, H-$2D^b$ molecules (Inamitsu *et al.*, 1992), suggesting that DQ6-derived peptide bound to H-$2D^b$ molecules are recognized by the $CD8^+$ T cells. Supporting this, elution studies of MHC class I binding peptides have suggested that many peptides derived from MHC class I and class II molecules are bound to MHC class I molecules (Falk *et al.*, 1990, 1991). In the present study, we have identified the DQα chain-derived peptide (SCGVNLYQF) which elicits the cytotoxicity of the $CD8^+$ T cells by testing 23 peptides corresponding to DQα or DQβ chains with H-$2D^b$ binding motifs. Our results strongly suggest that "SCGVNLYQF" is processed in antigen presenting cells and is recognized by $CD8^+$ T cells in the context of H-$2D^b$ molecules.

Our results thus imply that the difference in HLA class II alleles between donors and recipients, in association with the particular HLA-class I molecules, have a profound negative effect on the success of the organ transplantation in humans *via* induction of cytotoxic $CD8^+$ T cells which recognize HLA class II-derived peptide bound to HLA class I molecules. It is possible that HLA class I molecules and class II molecules encoded for by the genes on different chromosome of the donor generate the particular HLA class II-derived peptide bound to HLA class I molecules and are involved in the cytotoxic $CD8^+$ T cell induction. In this point of view, further experiments should be done in a greater detail for the

organ transplantation with the disparate HLA class II but the shared HLA class I molecules.

Acknowledgments We thank Dr. Ronald N. Germain of The National Institutes of Health, Dr. Ralph T. Kubo of National Jewish Center, and Dr. Masao Kimoto of Saga Medical School, Japan, for pertinent advice and for the gift of the monoclonal antibodies. We also thank Dr. M. Hirata and Dr. T. Maeda for advice on techniques. This work was supported by grants from The Science and Technology Agency, Japan.

REFERENCES

Beatty PG, Clift RA, Mickelson EM, Nisperos BB, Flournoy N, Martin PJ, Sanders JE, Stewart P, Buckner CD, Storb R, Thomas ED, Hansen JA (1985): Marrow transplantation from related donors other than HLA-identical siblings. N Engl J Med **313**: 765-771

de Waal RMW, Bogman MJJ, Maass CN, Cornelissen LMH, Tax WJM, Koene RAP (1983): Variable expression of Ia antigens on the vascular endothelium of mouse skin allografts. Nature **303**: 426-429

Esaki Y, Fukui Y, Sudo T, Yamamoto K, Inamitsu T, Nishimura Y, Hirokawa K, Kimura A, Sasazuki T (1994): Role of human major histocompatibility complex DQ molecules in superantigenicity of Streptococcus-derived protein. Infect Immun **62**: 1228-1235

Falk K, Rötzschke O, Rammensee H-G (1990): Cellular peptide composition governed by major compatibility complex class I molecules. Nature **348**: 248-251

Falk K, Rötzschke O, Stevanović S, Jung G, Rammensee HG (1991): Allele specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. Nature **351**: 290-296

Festenstein H, Doyle P, Holmes J (1986): Long term follow-up in London transplant group recipients of cadaver renal allografts. N Engl J Med **314**: 7-14

Fukui Y, Esaki Y, Kimura A, Hirokawa K, Nishimura Y, Sasazuki T (1993a): T-cell repartoire in a strain of transgenic C57/BL6 mice with the HLA-DRA gene on the X-chromosome. Immunogenetics **37**: 204-211

Fukui Y, Yamamoto K, Yokoyama N, Inagawa T, Kurashima C, Esaki Y, Kimura A, Akashi T, Hirokawa K, Sasazuki T (1993b): Restricted expression of transgenic HLA-DRA gene in thymic epithelial cells and its role in acquisition of T cell tolerance to self-superantigens and processed DRα-derived peptide. Eur J Immunol **23**: 1678-1686

Golding H, Mizuochi T, McCarthy SA, Cleveland CA, Singer A (1987): Relationship among function, phenotype, and specificity in primary allospecific T cell populations. J Immunol **138**: 10-17

Inamitsu T, Nishimura Y, Sasazuki T (1992): Different recognition of transgenic HLA-DQw6 molecules by mouse $CD4^+$ and $CD8^+$ T cells. Immunogenetics **35**: 46-50

Nishimura Y, Iwanaga T, Inamitsu T, Yanagawa Y, Yasunami M, Kimura A, Hirokawa K, Sasazuki T (1990): Expression of the human MHC, HLA-DQw6 genes alters the immune response in C57BL/6 mice. J Immunol **145**: 353-360

Rosenberg AS, Mizuochi T, Singer A (1986): Analysis of T-cell subsets in rejection of K^b mutant skin allografts differing at class I MHC. Nature **322**: 829-831

Sasazuki T (1990): HLA-linked immune suppression genes. Jpn J Human Genet **35**: 1-13

Sasazuki T, Nishimura Y, Muto M, Ohta N (1983): HLA-linked genes controlling the immune response and disease susceptibility. Immunol Rev **70**: 51-75

Shinohara N, Bluestone JA, Sachs DH (1986): Cloned cytotoxic T lymphocytes that recognize an I-A region product in the context of class I antigen. J Exp Med **163**: 972-980

Swain SL (1983): T cell subsets and the recognition of MHC class. Immunol Rev **74**: 129-142

Thomas ED, Storb R, Clift RA, Fefer A, Johnson FL, Neiman PE, Lerner KG, Glucksberg H, Buckner CD (1975): Bone marrow transplantation. N Eng J Med **292**: 832-843

Todd JA, Acha-Orbea H, Bell JI, Chao N, Fronek Z, Jacob CO, McDermott M, Sinha AA,

Timmerman L, Steinman L, McDevitt HO (1988): A molecular basis for MHC class II-associated autoimmunity. Science **240**: 1003–1009

Yamamoto K, Fukui Y, Esaki Y, Inamitsu T, Sudo T, Yamane K, Kamikawaji N, Kimura A, Sasazuki T (1994): 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. J Exp Med **180**: 165–171