

HLA-DQB1 Codon 57 Is Critical for Peptide Binding and Recognition

By W.W. Kwok,* M.E. Domeier,* M.L. Johnson,† G.T. Nepom,*‡ and D.M. Koelle†

*From the *Virginia Mason Research Center and the †University of Washington School of Medicine, Seattle, Washington 98101*

Summary

The association of specific HLA-DQ alleles with autoimmunity is correlated with discrete polymorphisms in the HLA-DQ sequence that are localized within sites suitable for peptide recognition. The polymorphism at residue 57 of the DQB1 polypeptide is of particular interest since it may play a major structural role in the formation of a salt bridge structure at one end of the peptide-binding cleft of the DQ molecules. This polymorphism at residue 57 is a recurrent feature of HLA-DQ evolution, occurring in multiple distinct allelic families, which implies a functional selection for maintaining variation at this position in the class II molecule. We directly tested the amino acid polymorphism at this site as a determinant for peptide binding and for antigen-specific T cell stimulation. We found that a single Ala→Asp amino acid 57 substitution in an HLA-DQ3.2 molecule regulated binding of an HSV-2 VP-16-derived peptide. A complementary single-residue substitution in the peptide abolished its binding to DQ3.2 and converted it to a peptide that can bind to DQ3.1 and DQ3.3 Asp-57-positive MHC molecules. These binding studies were paralleled by specific T cell recognition of the class II-peptide complex, in which the substituted peptide abolished T cell reactivity, which was directed to the DQ3.2-peptide complex, whereas the same T cell clone recognized the substituted peptide presented by DQ3.3, a class II restriction element differing from DQ3.2 only at residue 57. This structural and functional complementarity for residue 57 and a specific peptide residue identifies this interaction as a key controlling determinant of restricted recognition in HLA-DQ-specific immune responses.

MHC class II proteins act as peptide-binding molecules for presenting antigenic peptides to T cells. Both peptide sequence-specific and peptide sequence-nonspecific interactions with the MHC molecules contribute to the stabilization of the MHC-peptide complex (1-3). Interactions of peptide main-chain carbonyl and amide groups with MHC residues by hydrogen bond and Van der Waals force provide the free energy for sequence-nonspecific binding. Interactions of peptide side chains with polymorphic residues within the binding pockets of the MHC impose peptide-binding specificity, which varies for different MHC molecules. X-ray crystallography of one of these MHC molecules, HLA-DR1, indicated there are four major binding pockets in the DR1 molecules, termed pockets 1, 4, 6, and 9 (3, 4), which accommodate specific anchor residues from the peptide. Much of the sequence polymorphism among HLA-DR alleles clusters in these pockets, providing the structural basis by which different molecules bind and present different antigens. Preferential interactions between polymorphic pockets and specific anchor residues create peptide "motifs" in which the pattern of residues within a peptide sequence dictates its preferential MHC binding partner (5-7).

In contrast to HLA-DR, binding motifs for HLA-DQ molecules remain largely unknown (8-10), although polymorphisms in binding pockets that distinguish among DQ molecules are associated with several autoimmune diseases, including type 1 diabetes mellitus (11, 12). In particular, the HLA-DQ3.2 molecule, highly associated with disease, is highly homologous to two naturally occurring alleles, HLA-DQ3.1 and HLA-DQ3.3. These three DQ molecules have identical DQ α chains (DQA1*0301) and DQ β chains with extremely high amino acid sequence similarity; the DQ3.2 and DQ3.3 β alleles (DQB1*0302 and DQB1*0303, respectively) differ only in an Ala→Asp polymorphism at codon 57, whereas the DQ3.1 β allele (DQB1*0301) encodes Asp 57 and three additional polymorphisms at positions 13, 26, and 45. The aspartic residue in DQ3.1 and DQ3.3 likely interacts with an arginine at residue 79 of the DQA1 chain to form a salt bridge by analogy with a similar structure in HLA-DR1 (3), whereas DQ3.2 lacks this interaction. An influence of this potential salt bridge in DQ on peptide binding has been suggested (13) but not yet directly tested. We have analyzed these three different DQ alleles to examine peptide-binding specificity to DQ molecules in-

fluenced by the codon 57 polymorphism. A peptide-specific, DQ3.2-restricted T cell clone was also used to assess the functional consequences of modulating peptide binding on TCR interaction with the DQ-peptide complex.

Materials and Methods

Cell Lines. EBV-transformed B cell lines were obtained from the X International Histocompatibility Workshop. The VP16-restricted T cell clone 2A.334 was derived from the HSV-2 lesion of a patient (HLA DQA1*0301/DQB1*0302, DQA1*0103/DQB1*0603) with herpetic vesicles. Briefly, cells from herpetic vesicles were centrifuged through Ficoll-Hypaque density gradient; 10^5 cells were stimulated in bulk with 1.5×10^5 allogeneic irradiated PBMC in RPMI 1640 supplemented with 10% heat-inactivated pooled human serum, 1% penicillin-streptomycin, 2 mM L-glutamine, PHA (0.4 $\mu\text{g}/\text{ml}$), and human IL-2 (50 U/ml; Schiparelli Biosystems, Fairfield, NJ) (14). After 16 d of growth, 5×10^5 cells were restimulated with HSV-2 antigen and 5×10^5 autologous irradiated PBMC in 1-ml cultures, and 10–12 d later they were cloned at 1 cell/well using PHA, IL-2, and allogeneic feeder cells (15). Clones judged to have HSV-2 type-specific responses (stimulation index with HSV-2 antigen >4.0 and no specific stimulation with HSV-1 antigen) were screened for reactivity with HSV-1 \times HSV-2 intertypic recombinant virus RP-2. HSV-2 type-specific T cell clones reactive with RP-2 are specific for VP16 (16).

Peptide-binding Assays. 10^6 EBV-transformed human B cells (PRIESS, DQ3.2; PF, DQ3.1; HID, DQ3.3) were incubated with 0.5% paraformaldehyde for 10 min and washed three times in HBSS. Cells were then resuspended with 10 μM biotinylated peptide in 150 mM citrate-phosphate buffer, pH 4.4, 1 mM PMSF, in a 100 μl reaction volume for 24 h at 37°C. Cells were washed twice in HBSS before lysis in 100 μl buffer containing 0.5% NP-40, 0.15 M NaCl, 50 mM Tris, pH 8.0, and protease inhibitors. The lysates were cleared by centrifugation, and then transferred to 96-well plates precoated with 10 $\mu\text{g}/\text{ml}$ mAb SPVL3 (specific for HLA-DQ) and blocked with 5% FCS in PBS. The lysate was neutralized with 100 μl of 50 mM Tris, pH 8.0, containing 0.02% *n*-dodecyl β -D-maltoside and incubated overnight at 4°C. The wells were then washed with PBS containing 0.02% Tween 20. Europium-labeled streptavidin (Wallac, Gaithersburg, MD) was added in the presence of 200 μl DELFIA assay buffer (Wallac) and incubated at 4°C for 4 h. After washing, 200 μl of enhancement buffer (Wallac) was added, and the resultant fluorescence was measured using a DELFIA 1232 fluorometer. Peptides were provided by Drs. R. Burke and M. Tigges (Chiron Corp., Emeryville, CA) or synthesized as previously described (17).

Cytotoxic T Cell Assays. 10^6 target cells were incubated with 125 μCi ^{51}Cr and 0.005–5 $\mu\text{g}/\text{ml}$ of peptide for 90 min at 37°C. Cells were washed and plated at 3×10^3 cells/wells into 96-well U-bottom plates. Cloned T cells were added at an E/T ratio of 20:1, with a final volume of 200 $\mu\text{l}/\text{well}$ with or without 10 $\mu\text{g}/\text{ml}$ mAb SPV L3 or L243 (specific for HLA-DR). After 4 h at 37°C, 30 μl of supernatant was counted using a scintillation counter (Lumaplates and Topcount; Packard, Meriden, CT). Results are reported as percentage of specific lysis, defined as [mean experimental cpm – mean spontaneous cpm]/(mean maximum cpm – mean spontaneous cpm) $\times 100$. Assays were performed in triplicate, and spontaneous release was usually $<30\%$ of total release.

Proliferation Assays. T cells ($10^4/\text{well}$) and autologous irradiated PBMC ($10^5/\text{well}$) as APC were added to a final volume of

200 μl of T cell medium in 96-well U-bottom plates. Peptide was used at a final concentration of 5 $\mu\text{g}/\text{ml}$. After 3 d, [^3H]thymidine (1 $\mu\text{Ci}/\text{well}$) was added for 18 h; cells were harvested with a semiautomatic harvester and counted by liquid scintillation. Stimulation index is defined as mean cpm of [^3H]thymidine incorporation for antigen/mean cpm of [^3H]thymidine incorporation for control antigen.

Results and Discussion

The T cell clone 2A.334, reactive with VP16, was identified by screening HSV-2 type-specific clones with a panel of HSV-1 and HSV-2 intertypic recombinant viruses (16). The specificity of CD4⁺ T cell clone 2A.334 was defined using a set of overlapping peptides corresponding to amino acids 410–469 of VP16 of HSV-2, the region of maximum sequence divergence between HSV-2 and HSV-1. Initial screening by proliferation assay using overlapping 15-mer peptides identified reactivity to VP16 430–444 (Fig. 1 A). Subsequent analysis of cytolytic activity using 13-mers derived from this sequence demonstrated reactivity with 433–445, but not 429–441, defining an epitope included within the sequence DMTPADALDDFDL (Fig. 1 B). Fig. 1 C summarizes the restriction pattern of this clone, which recognized the VP16 433–445 peptide of HSV-2 presented by DQA1*0301/DQB1*0302; the cytolytic activity of the clone was inhibited by the anti-DQ mAb SPVL3 but not by the anti-DR mAb L243. Since neither DQB1*0301 nor DQB1*0303 presented the HSV-2 peptide to clone 2A.334, we tested these alleles in peptide-binding assays with peptide 433–445. Fig. 2 A shows that the peptide bound DQB1*0302, but not DQB1*0301 nor *0303. Since the DQ3.3 molecule differs from DQ3.2 only in an Ala \rightarrow Asp 57 polymorphism, this directly correlates variation at this residue with altered peptide binding. To determine which residues on the HSV-2 peptide likely interact with the codon 57 residue, we synthesized modified HSV-2 peptides that reconstituted binding to the DQ3.1 and DQ3.3 alleles. These peptide analogues were based on two assumptions: (a) that peptide-binding pockets in DQ and DR molecules should be fairly homologous, and (b) that the HSV-2 peptide is held in an extended conformation, similar to the extended conformation of the HA307–319 peptide in DR1 (3). In this scenario, an anchor residue in position *n* of the peptide should fit into pocket one, and residues *n* + 3, *n* + 5, and *n* + 8 should interact with pockets 4, 6, and 9 of the DQ molecule, respectively. In this model, Asp57 residues may influence pocket 9 of the DQ3.1 and DQ3.3 alleles by forming a salt bridge with Arg79 of the DQ α chain. The DQ3.2 molecule, however, has an alanine at codon 57; there are both steric and charge implications of this polymorphism, including the likelihood that the positively charged Arg79 may interact with negatively charged residues from bound peptide in the absence of the Asp57 salt bridge.

The sequence of the HSV-2 433–445 peptide is consistent with this model: methionine at position 434 may act as an anchor residue for pocket 1, and aspartic acid at position

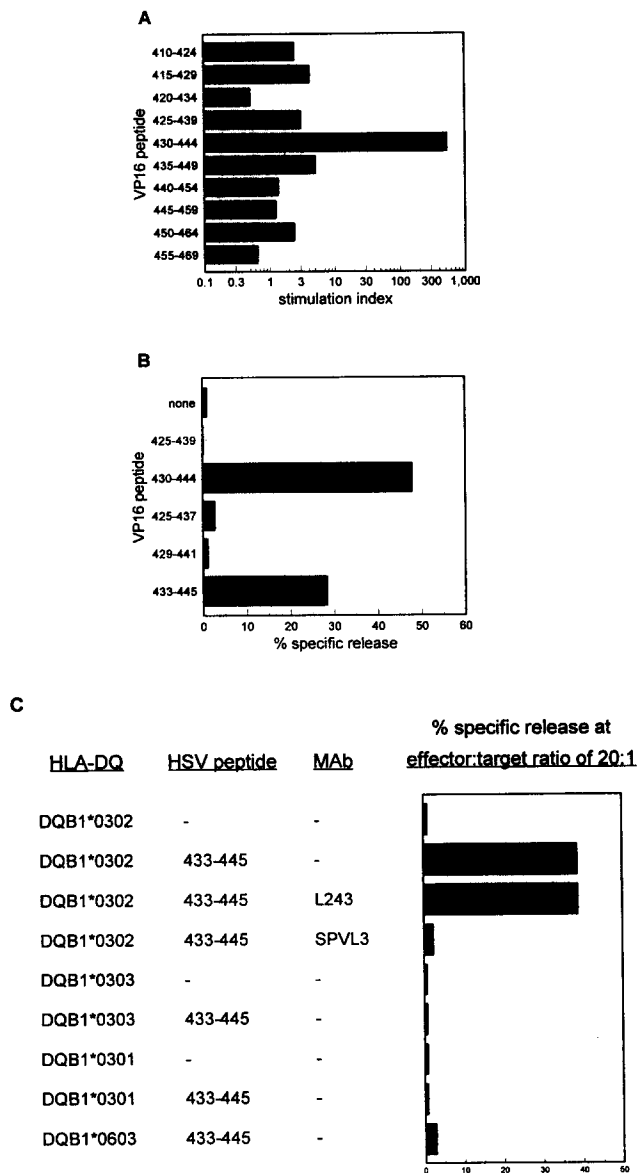


Figure 1. Peptide specificity and restricted recognition of clone 2A.334. Proliferative response of clone 2A.334 with autologous PBMC as APC (A) and cytotoxic activity of clone 2A.334 at an E/T ratio of 20:1 with EBV-transformed autologous B-LCL as target cells (B) or homozygous EBV-transformed B-LCL with different DQ alleles as target cells (C). Peptides were used at 5 μ g/ml for both proliferative (A) and 51 Cr release assays (B and C).

442, corresponding to the $n + 8$ residue, may interact with pocket 9. To test this hypothesis, the HSV-2 10A peptide was synthesized with D \rightarrow A substitution at position 442 ($n + 8$) and tested for the ability to bind to the DQ3.1, DQ3.2 and DQ3.3 molecules. As shown in Fig. 2 B, the HSV-2 10A peptide bound to both DQ3.1 and DQ3.3 but did not bind to DQ3.2, giving a pattern reciprocal to the wild-type HSV-2 433-445 peptide (Fig. 2 A). This identifies residue 442 as a key anchor for binding to the three DQ alleles, complementary to the effect of the polymorphism at codon 57. To further validate the role of M at position 434 and D

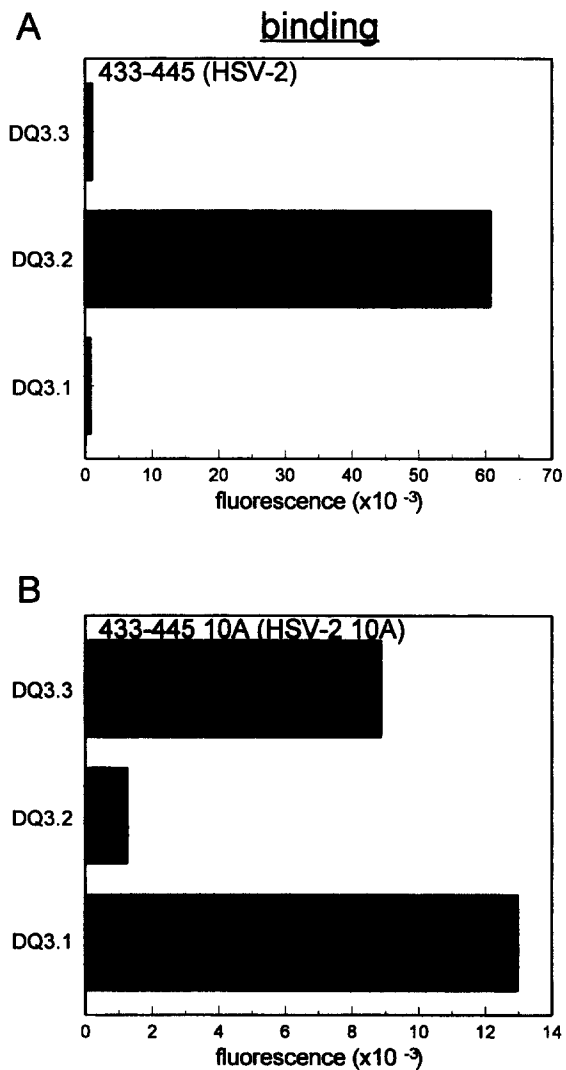
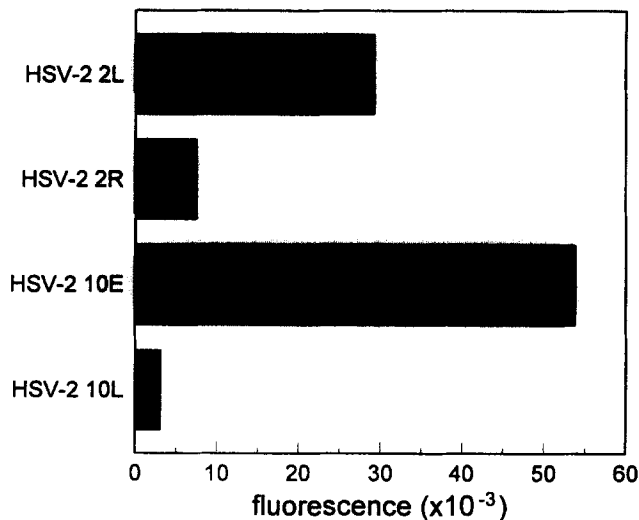


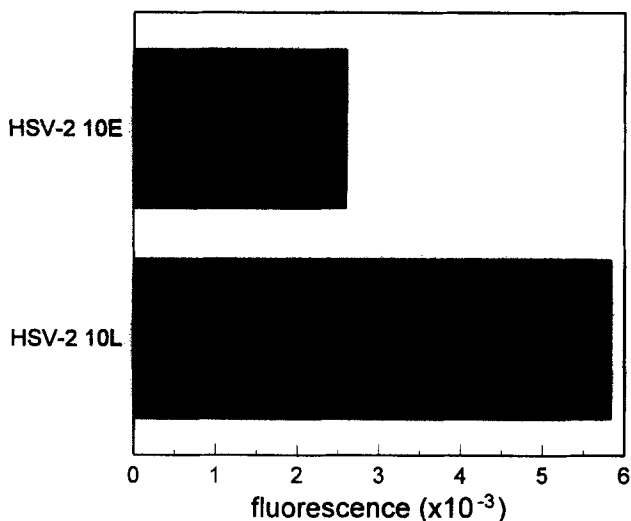
Figure 2. Modulation of HLA-DQ binding by HSV-2 433-445 10D \rightarrow 10A substitution. Binding of 433-445 (A) and 433-445 10A (B) to B-LCL that expressed various DQw3 alleles.

at position 442 as anchoring residues for pocket 1 and pocket 9, respectively, additional HSV peptide analogues were synthesized. HSV-2 2L and HSV-2 2R contain an L and an R substitution at position 434, respectively, whereas HSV-2 10E and HSV-2 10L have an E and an L substitution at position 442, respectively. These HSV-2 peptide analogues were tested for binding to DQ3.2, DQ3.3, and DQ3.1 B-LCLs, as shown in Fig. 3. The conservative M \rightarrow L substitution at position 434 (HSV-2 2L) had little influence on binding to DQ3.2 compared with the native HSV-2 433-445 peptide, whereas there was a substantial decrease in binding with the more drastic M \rightarrow R substitution in the HSV-2 2R peptide (Fig. 3 A). Similarly, a conservative E substitution at position 442 (HSV-2 10E) bound to DQ3.2 much better than HSV-2 10L. As with the HSV-2 10A peptide, which failed to bind to DQ3.2 but was capable of binding to DQ3.1 and DQ3.3 (Fig. 2 B), the HSV-2 10L peptide also bound to

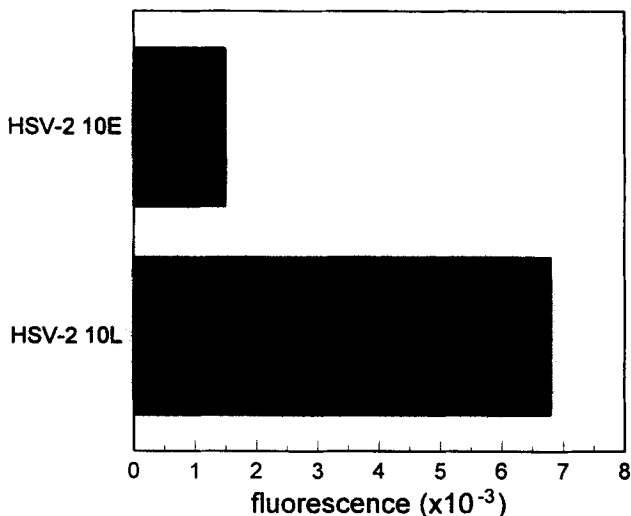
A DQ3.2



B DQ3.3



C DQ3.1



DQ3.3 and DQ3.1 (Fig. 3, B and C). These results substantiate the assumption that in this peptide, residue 434 is the pocket 1 anchor and that a negatively charged residue at the $n + 8$ position is critical for allele-specific binding to DQ3.2. Presumably, in the absence of the Asp57 salt bridge, the negatively charged residue at position $n + 8$ of the peptide can interact with the accessible Arg79 of the DQ α chain, and, conversely, in the presence of the salt bridge, a negatively charged residue at the $n + 8$ position appears to be unfavorable for peptide binding.

These HSV-2 peptide analogues were then tested for recognition by T cell clone 2A.334. As anticipated from the binding studies, HSV-2 2L and native 433–445 peptide stimulated clone 2A.334 with DQ3.2 B-LCL as APC (Fig. 4, A and B). The HSV-2 2R peptide, however, was presented only at much higher peptide concentration (Fig. 4 C), in agreement with the observation that this peptide bound less efficiently to DQ3.2. Peptide analogues with substitutions at position 442 were also analyzed. Peptide HSV-2 10E (Fig. 4 D) was presented by DQ3.2, like the native HSV-2 433–445 peptide. The HSV-2 10A and HSV-2 10L analogues, however, showed no cytolytic recognition by clone 2A.334 on DQ3.2 target cells (Fig. 4, E and F). These results correlated with the lack of binding of these peptides to DQ3.2 B-LCL. However, clone 2A.334 was able to recognize the HSV-2 10A and HSV-2 10L peptides when they were presented by DQ3.3 (Fig. 4, E and F). Thus, both HSV-2 peptides with a negatively charged residue at position 442 complexed with DQ3.2 and also HSV-2 peptides with an aliphatic side chain residue at position 442 complexed with DQ3.3 were recognized by the clone 2A.334, indicating the generation of similar MHC-peptide complexes by the structural complementation of residue 442 and codon 57. Interestingly, though the HSV-2 10A and 10L peptides bound to DQ3.1, these DQ-HSV-2 complexes were not recognized by the 2A.334 clone. This indicates modulation of T cell recognition by the additional polymorphic sites, which are unique to DQ3.1, and two alternative explanations are feasible. Polymorphic residues 13 and 26 of the DQ3.1 molecule are likely to lie in the floor of the peptide-binding groove, such that the HSV-2 peptides could be presented by the DQ3.1 molecule in a different conformation because of these changes. Similar observations have been reported by others for DR4-restricted clones (18). Alternatively, the DQ3.1 molecule has a bulky residue at position 45, that, though located outside the peptide-binding groove and the α helix, may interfere directly with interaction with the TCR. This alternative is consistent with previous observations that residue 45 is an immunodominant mAb-binding epitope on the DQ3.1 molecule (19, 20).

In sum, we have demonstrated a critical role for the DQ β polymorphism at codon 57 as a structural and functional

Figure 3. Binding of HSV 433–445 position 2 and 10 analogues to (A) DQ3.2, (B) DQ3.3, and (C) DQ3.1.

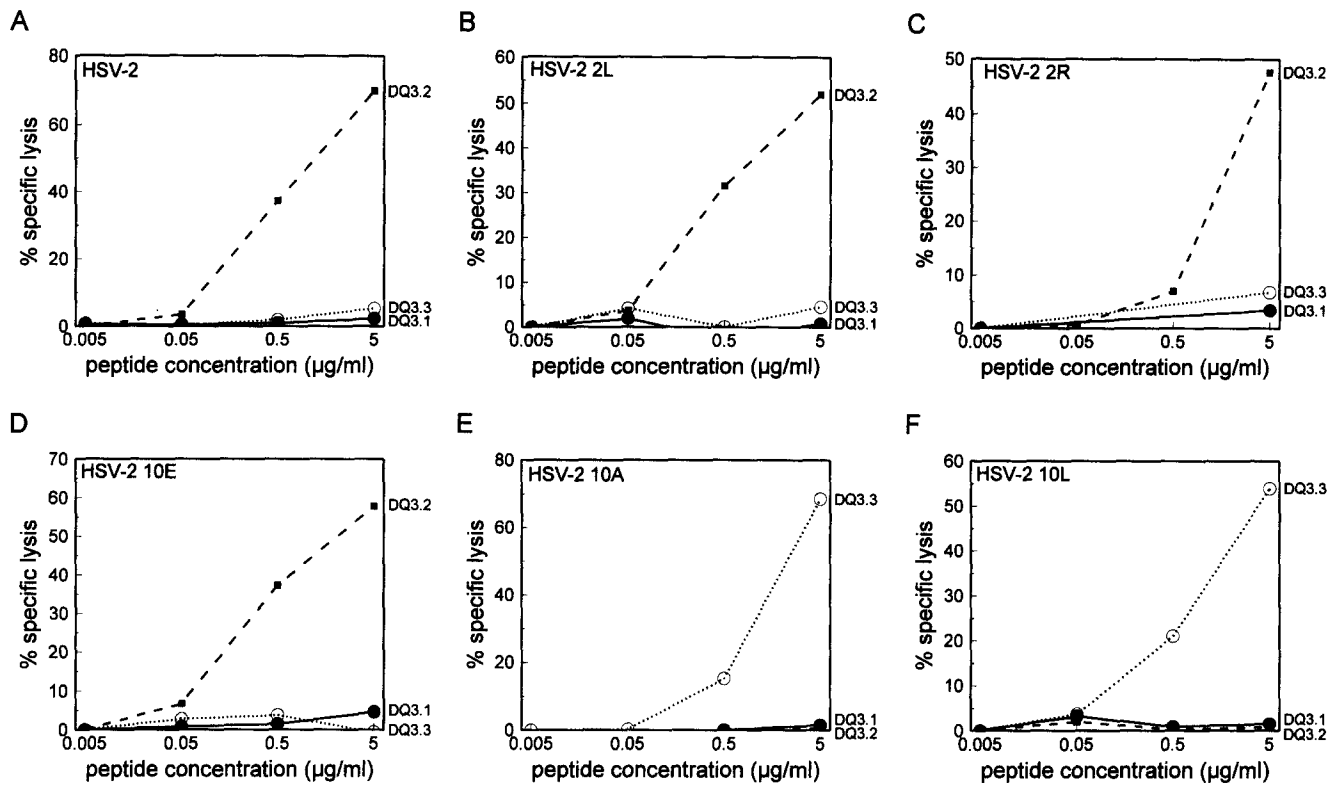


Figure 4. Modulation of T cell recognition by HSV 433-445 analogues. ^{51}Cr release assays with clone 2A.334 on B-LCL that expressed different DQ3 alleles and loaded with (A) HSV-2 433-445, (B) HSV-2 2L, (C) HSV-2 2R, (D) HSV-2 2E, (E) HSV-2 10A, or (F) HSV-2 10L were used as targets.

determinant of peptide-specific recognition. This polymorphism is likely to be a major contributor to allelic specificity, with preferential binding of Asp-negative alleles to

peptides with negatively charged anchor residues interacting with the class II pocket.

We thank Drs. R. Burke and Dr. M. Tigges for providing peptides, and Holly Chase for preparation of the manuscript.

This work was supported by National Institutes of Health grants DK-02319, DK-40964 (W.W. Kwok) and AI-34616 (D.M. Koelle).

Address correspondence to Dr. William W. Kwok, Virginia Mason Research Center, 1000 Seneca Street, Seattle, WA 98101.

Received for publication 17 July 1995 and in revised form 8 November 1995.

References

- Engelhard, V.H. 1994. Structure of peptides associated with class I and class II MHC molecules. *Annu. Rev. Immunol.* 12: 181-207.
- Madden, D.R. 1995. The three-dimensional structure of peptide-MHC complexes. *Annu. Rev. Immunol.* 13:587-622.
- Stern, L.J., J.H. Brown, T.S. Jardetzky, J.C. Gorga, R.G. Urban, J.L. Strominger, and D.C. Wiley, 1994. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature (Lond.)*. 368:215-221.
- Brown J.H., T.S. Jardetzky, J.C. Gorga, L.J. Stern, R.G. Urban, J.L. Strominger, and D.C. Wiley. 1993. Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature (Lond.)*. 364:33-39.
- Hammer, J., P. Valsasini, K. Tolba, D. Bolin, J. Higelin, B. Takacs, and F. Sinigaglia. 1993. Promiscuous and allele-specific anchors in HLA-DR-binding peptides. *Cell*. 74:197-203.
- Chicz, R.M., R.G. Urban, J.C. Gorga, D.A.A. Vignali, W.S. Lane, and J.L. Strominger. 1993. Specificity and promiscuity

- among naturally processed peptides bound to HLA-DR alleles. *J. Exp. Med.* 178:27–47.
7. Marshall, K.W., A.F. Liu, J. Canales, B. Perahia, B. Jorgensen, R.D. Gantzios, B. Aguilar, B. Devaux, and J.B. Rothbard. 1994. Role of the polymorphic residues in HLA-DR molecules in allele-specific binding of peptide ligands. *J. Immunol.* 152:4946–4957.
 8. Sidney, J., C. Oseroff, M. del Guercio, S. Southwood, J.I. Krieger, G.Y. Ishioka, K. Sakaguchi, E. Appella, and A. Sette. 1994. Definition of a DQ3.1-specific binding motif. *J. Immunol.* 152:4516–4525.
 9. Verreck, F.A.W., A. van de Poel, A. Termijtelen, R. Amons, J. Drijfhout, and F. Koning. 1994. Identification of an HLA-DQ2 peptide binding motif and HLA-DPw3-bound self-peptide by pool sequencing. *Eur J. Immunol.* 24:375–379.
 10. Chicz, R.M., W.S. Lane, R.A. Robinson, M. Trucco, J.L. Strominger, and J.C. Gorga. 1994. Self-peptides bound to the type I diabetes associated class II MHC molecules HLA-DQ1 and HLA-DQ8. *Int. Immunol.* 6:1639–1649.
 11. Nepom, G.T. 1994. Class II antigens and disease susceptibility. *Annu. Rev. Med.* 46:17–25.
 12. Todd, J.A., J.I. Bell, and H.O. McDevitt. 1987. HLA-DQB gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. *Nature (Lond.)* 329:599–604.
 13. Wucherpfenning, K.W., and J.L. Strominger. 1995. Selective binding of self peptides to disease-associated major histocompatibility complex (MHC) molecules: a mechanism for MHC-linked susceptibility to human autoimmune diseases. *J. Exp. Med.* 181:1597–1601.
 14. Koelle, D.M., H. Abbo, A. Peck, K. Ziegweid, and L. Corey. 1994. Direct recovery of HSV-specific T lymphocyte clones from recurrent HSV-2 lesions. *J. Infect Dis.* 169:956–961.
 15. Moretta, A., G. Pantaleo, L. Moretta, J.-C. Cerottini, and M.C. Mingari. 1983. Direct demonstration of the clonogenic potential of every human peripheral blood T cell. *J. Exp. Med.* 157:743–754.
 16. Koelle, D.M., L. Corey, R.L. Burke, R.J. Eisenberg, G.H. Cohen, R. Pichyangkura, and S.J. Triezenberg. 1994. Antigenic specificity of human CD4⁺ T cell clones recovered from recurrent genital HSV-2 lesions. *J. Virol.* 68:2803–2810.
 17. Kwok, W., G.T. Nepom, and F.C. Raymond. 1995. HLA-DQ polymorphisms are highly selective for peptide binding interactions. *J. Immunol.* 155:2468–2476.
 18. Fu, X., C.P. Bono, S.L. Woulfe, C. Swearingen, N.L. Summers, F. Sinigaglia, A. Sette, B.D. Schwartz, and R.W. Karr. 1995. Pocket 4 of the HLA-DR(α , β 1*0401) molecule is a major determinant of T cell recognition of peptide. *J. Exp. Med.* 181:915–926.
 19. Kwok, W.W., C. Lotshaw, E.C. Milner, N. Knitter-Jack, and G.T. Nepom. 1989. Mutational analysis of the HLA-DQ3.2 IDDM susceptibility gene. *Proc. Natl. Acad. Sci. USA.* 86:1027–1030.
 20. Kwok, W.W., E. Mickelson, S. Masewicz, E.C.B. Milner, J. Hansen, and G.T. Nepom. 1990. Polymorphic DQ α and DQ β interactions dictate HLA class II determinants of allorecognition. *J. Exp. Med.* 171:85–95.