

HLA-B*0702 Antibody Epitopes Are Affected Indirectly by Distant Antigen Residues

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ABSTRACT: We examine the effect of mutations in the HLA-B*0702 α_1 domain on the binding of several well-characterized monoclonal antibodies. BB7.1 recognizes the α -helix, with a special requirement for residue 67. Combined with an established requirement for the α_2 α -helix, BB7.1 appears to span the B*0702 peptide-binding groove. Alternatively, BB7.1 epitope conformation may be altered by distant B*0702 sites. ME1 and B27M1 recognize connecting loop residues 41 and 43 and α_1 α -helical residues 67–71. Instead of contacting residue 67–71 side chains directly, however, ME1 appears to recognize a B*0702 configuration that depends upon the proper interaction of these and other HLA residues. In

addition to solvent-accessible residues 41 and 43, the B27M1 epitope depends on solvent-inaccessible residue 32 at the bottom of the peptide-binding groove. MB40.2, known to require residues 169–182 near the α_2 – α_3 junction, also requires the proper combination of distant residues in the α_1 β -strand and α -helix. The effect of mutations near the peptide-binding groove suggests that bound peptides may directly or indirectly affect HLA epitopes. These results illustrate that HLA epitope conformation is very sensitive to changes at distant HLA sites and forecast that epitope models based on sequential amino acid residues will often fail to predict HLA epitopes. *Human Immunology* 36, 69–75 (1993)

ABBREVIATIONS

Å angstrom
HLA human leukocyte antigen

mAb monoclonal antibody
MHC major histocompatibility complex

INTRODUCTION

Class I human leukocyte antigen (HLA) molecules consist of a three-domain transmembrane heavy chain and a single-domain light-chain, β_2 -microglobulin [1]. The polymorphic heavy-chain α_1 and α_2 domains form a peptide-binding groove of α -helical walls and a β -strand floor [1–3]. The peptide-binding groove is supported by the relatively nonpolymorphic α_3 domain and the invariant β_2 -microglobulin domain. Most major histocompatibility complex (MHC) class I polymorphic residues are found in the α -helical and β -strand residues

lining the peptide-binding groove [3, 4]. Allogeneic class I MHC molecules induce vigorous antibody responses. Antibodies to human class I MHC molecules (HLA-A,B,C) are clinically important, mediating hyperacute graft rejection [5], chronic graft rejection [6], and the destruction of transfused platelets [7]. Anti-HLA antibodies also may participate in autoimmune disease [8, 9].

Antibody epitopes on globular proteins typically are formed by solvent-accessible antigen residues (reviewed by Benjamin [10]). Nonetheless, solvent-inaccessible antigen residues can alter antibody binding [11, 12]. Furthermore, antigen residues distant from the antibody-binding site clearly influence antibody binding [13, 14]. Thus, while antibody epitopes are formed by solvent accessible residues, other antigen residues may also affect antibody binding.

Most variable amino acid residues of typical globular proteins reside on the protein surface and are accessible

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to antibodies. In contrast, most polymorphic HLA residues are concentrated in the peptide-binding groove where they are largely buried by bound peptide [3, 4]. Therefore, it is worth testing whether HLA solvent-inaccessible residues and other distant sites affect antibody epitopes. We address this question using well-characterized antibodies reactive with HLA-B*0702. Previous reciprocal blocking studies indicate that BB7.1 and MB40.2 bind one B*0702 site, whereas ME1 and B27M1 bind a second nonoverlapping B*0702 site [15]. The MB40.2 epitope has been mapped to the carboxy terminus of the α_2 domain [16, 17], including residues 169, 176/178, 180, and 182 [18]. BB7.1 blocks MB40.2 binding [15], probably by binding α_2 residues 166 and 169 [18]. Because BB7.1 binding also requires B*0702 α_1 domain residues [16], the BB7.1 epitope apparently straddles the top of the peptide-binding groove; α_1 residues also are required for ME1 and B27M1 binding [19–21]. In this study we further map the BB7.1, ME1, and B27M1 epitopes. We also show that the MB40.2 and B27M1 epitopes are affected indirectly by B*0702 distant residues.

MATERIALS AND METHODS

Cell lines. Supernatant fluids were collected as described [18] from the following rodent hybridomas: MA2.1, GAP A3, R17.217, CR11-351, L368, BBM1, MB40.5, W6/32, BB7.7, BB7.1, MB40.2, ME1, B27M1, MB40.3, SFR8-B6, and BB7.6. 4E and F4/326 were donated as ascites by Dr. S.Y. Yang. HLA-A,B,C null 721.221 cells were donated by R. DeMars. P. Ivanyi donated BPor cells (HLA-A1,2;B*0703,8 [22, 24] [D.F. Epperson, personal communication]).

Mutagenesis. Variants were generated by site-directed mutagenesis as described [18]. Except for the last nucleotide of exon 1, all mature protein-encoding nucleotides subjected to in vitro mutagenesis were sequenced. The B7/8 recombinant molecule was generated by subcloning a genomic B*0801 *Bgl*II-*Kpn*I fragment [23], containing the last third of exon 2 and all of exon 3, into a genomic B*0702 clone. The B8/7 recombinant molecule was generated by subcloning a genomic B*0801 *Xba*I-*Bgl*II fragment [23], containing all of exon 1 and the first two-thirds of exon 2, into a genomic B*0702 clone.

Transfection. HLA genes were electroporated into HLA-721.221 human B-lymphoblasts and maintained in Hygromycin B (Calbiochem) as previously described [18], and 721.221 cells transfected with B*0801 in the pHPTe shuttle vector [23] were selected in a mixture

of hypoxanthine (5×10^{-3} M), aminopterin (2×10^{-5} M), and thymidine (8×10^{-4} M) (Sigma).

Flow cytometry. Flow cytometry was performed and analyzed as previously described [18]. Corrected median or mean fluorescence intensity on a logarithmic scale was calculated by subtracting the goat-anti-mouse antibody only control value (always similar to the isotype-matched negative control value). Values were normalized to the signal produced by the pan-HLA monoclonal antibody (mAb), W6/32 (corrected experimental value $\div$ corrected W6/32 value). For each variant, the corrected fluorescence intensity for each antibody was compared with a *wild-type* B*0702 transfectant in the same experiment; each variant was tested in at least two separate experiments.

RESULTS

Mutations in the α_1 α -helix abrogate BB7.1 binding. Parham et al. [15] mapped the BB7.1 epitope to the α_1 domain by showing that BB7.1 binds B*0702 and B*4201, but not other HLA-B alleles tested. Using recombinant B7/B27 molecules, Toubert et al. [16] showed that the BB7.1 epitope requires at least one of the B*0702 α_1 -domain residues 63, 67, or 70. To map the BB7.1 epitope further, we tested three natural and experimental recombinants of B*0702 and B*0801 (Fig. 1). B*0703 matches B*0702 except at α -helical residues 69–71 [22] (D.F. Epperson, personal communication). B7/8 matches B*4201 except at α -helical positions 67, 69, 70, and 71, whereas B8/7 matches B*0702 except at β -strand positions 9, 11, and 12 (Fig. 1). We find that BB7.1 binds B*0703 and B8/7, but not B*0801 or B7/8 (Table 1 and Fig. 1). As B*0703 and B7/8 differ in the α_1 domain only at position 67, this residue appears to play a critical role in BB7.1 binding.

BB7.1 is not affected by α_1 -domain mutations Q32E, A41E, and P43R (Table 1). β -Strand residue 32 lies in the peptide-binding groove; the residue 32 side chain points down to make close contact with β_2 -microglobulin [3]. Residues 41 and 43 are on a connecting loop that packs against the outer face of the α_1 α -helix [3]. Combined, these data concur with previous results mapping of the BB7.1 epitope to the B*0702 α -helices [16, 21, 18] and emphasize the importance of residue 67.

Mutations in the α_1 domain influence MB40.2 binding to B*0702. We and others [16–18] have mapped the MB40.2 epitope to B*0702 residues 169–182 near the α_2 – α_3 -domain junction (Fig. 2A). MB40.2 also binds B*4201 [15], but does not bind B*0801 (Fig. 1). This is unexpected because B*4201 matches the α_2 domain of

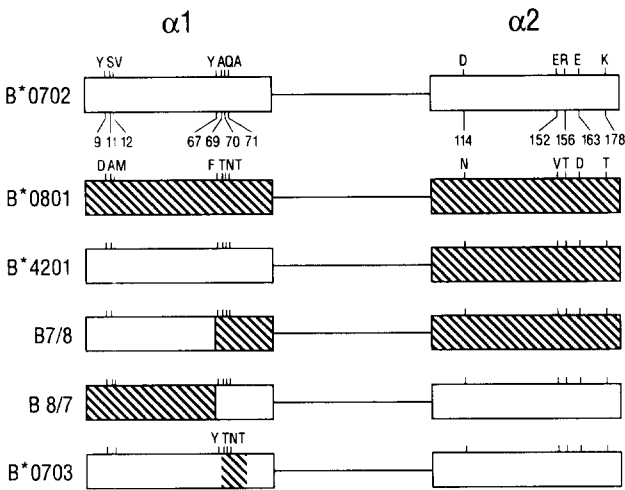


FIGURE 1 Recombinant HLA molecules affect antibody binding. Shown schematically are the α_1 and α_2 domains of B*0702, B*0801, and various natural and laboratory-generated recombinant HLA molecules. Amino acid differences between B*0702 and B*0801 are indicated. Regions matching B*0801 are *batched*, whereas regions matching B*0702 are shown *without batching*. All molecules are identical in the α_3 domain [25, 22] (D.F. Epperson, personal communication). Approximate binding values of selected antibodies are shown; for quantitative results, see Table 1. As B*4201 was not studied here, strong binding reported by Parham et al. [15], is denoted *yes*.

B*0801 and the α_1 domain of B*0702 (Fig. 1). Thus, in addition to the α_2 domain, the B*0702 α_1 domain influences MB40.2 binding. To dissect the α_1 contribution, we analyzed B*0703, B7/8, and B8/7 (Fig. 1). MB40.2 binds all three recombinant molecules, with only slightly reduced binding to B7/8 (Table 1). This demonstrates that separately B*0801 residues in neither the α_1 α -helix (B*0703, B7/8) nor the α_1 β -strand (B8/7) abrogate MB40.2 binding. Therefore, the interaction of B*0801 α_1 residues abrogates MB40.2 binding near the α_2 - α_3 -domain junction (Fig. 2A and B). As expected, MB40.2 binding is not affected by mutations at residue 32, 41, or 43 (Table 1).

Mutations in the α_1 domain abrogate B27M1 binding. B27M1 binds B*0702 and B8/7, but not B*0703, B*0801, or B7/8 (Fig. 1 and Table 1). These data suggest a role for one or more of the residues 67, 69, 70, and 71 for B27M1 binding to B*0702. Additional mutations in the α_1 domain were tested to delineate further the B27M1 epitope. Mutations A41E and P43R abrogate B27M1 binding (Table 1). Connecting loop residues 41 and 43 are solvent accessible [4] and could contact B27M1 (Fig. 2C).

The Q32E mutation also completely abrogates B27M1 binding. However, residue 32 is not accessible

to solvent, lying in the peptide-binding groove (Fig. 2C) with side chain pointing down toward β_2 -microglobulin [3]. This implies that residue 32 does not directly contact B27M1; instead this mutation must affect the B27M1 epitope from a distance.

ME1 has a potential contact site in the α_1 domain. ME1 blocks B27M1 binding to B*0702 [15], suggesting overlapping epitopes. Like B27M1, ME1 shows little or no binding to A41E, P43R, or B*0703 molecules (Table 1), consistent with contact of both the HLA α -helix and the connecting loop. These data suggest that B27M1 and ME1 could block each other's binding through competition for connecting loop residues 41 and 43, and possibly α -helical residues. However, the B27M1 and ME1 epi-

TABLE 1 α_1 Mutations affect mAb binding

Antibody	HLA variant ^a						
	Q32E	A41E	P43R	B*0801	B7/8	B8/7	B*0703
BB7.1	1.27 ^b	1.23	0.91	0.01	0.08	1.01	0.78
MB40.2	1.54	1.48	1.32	0.09	0.71	1.31	1.00
ME1	1.49	0.05	0.00	0.43	0.36	0.94	0.08
B27M1	0.02	0.05	0.00	0.00	0.00	0.93	0.04

^a 721.221 cells transfected with B*0702, B*0801, or recombinant genes, as described in *Materials and Methods*. B*0703 is expressed by BPot cells [22]. mAb BBM1, MB40.5, 4-10, BB7.7, W6/32, 4E, and F4/326, BB7.6, and SFR8-B6 bound well to all of the HLA molecules shown, whereas MB40.3 bound well to all except B*0801 and B7/8 (data not shown).

^b Change in mean fluorescence intensity for each antibody, normalized on pan-anti-HLA mAb, W6/32. On 721.221 transfectants, W6/32 staining reflects transfected HLA gene expression, whereas, on BPot cells, W6/32 staining reflects B*0703 and other HLA-A,B,C gene expression. Values showing near total abrogation in mAb binding are shown in bold, whereas values markedly different from control but still clearly positive are underlined.

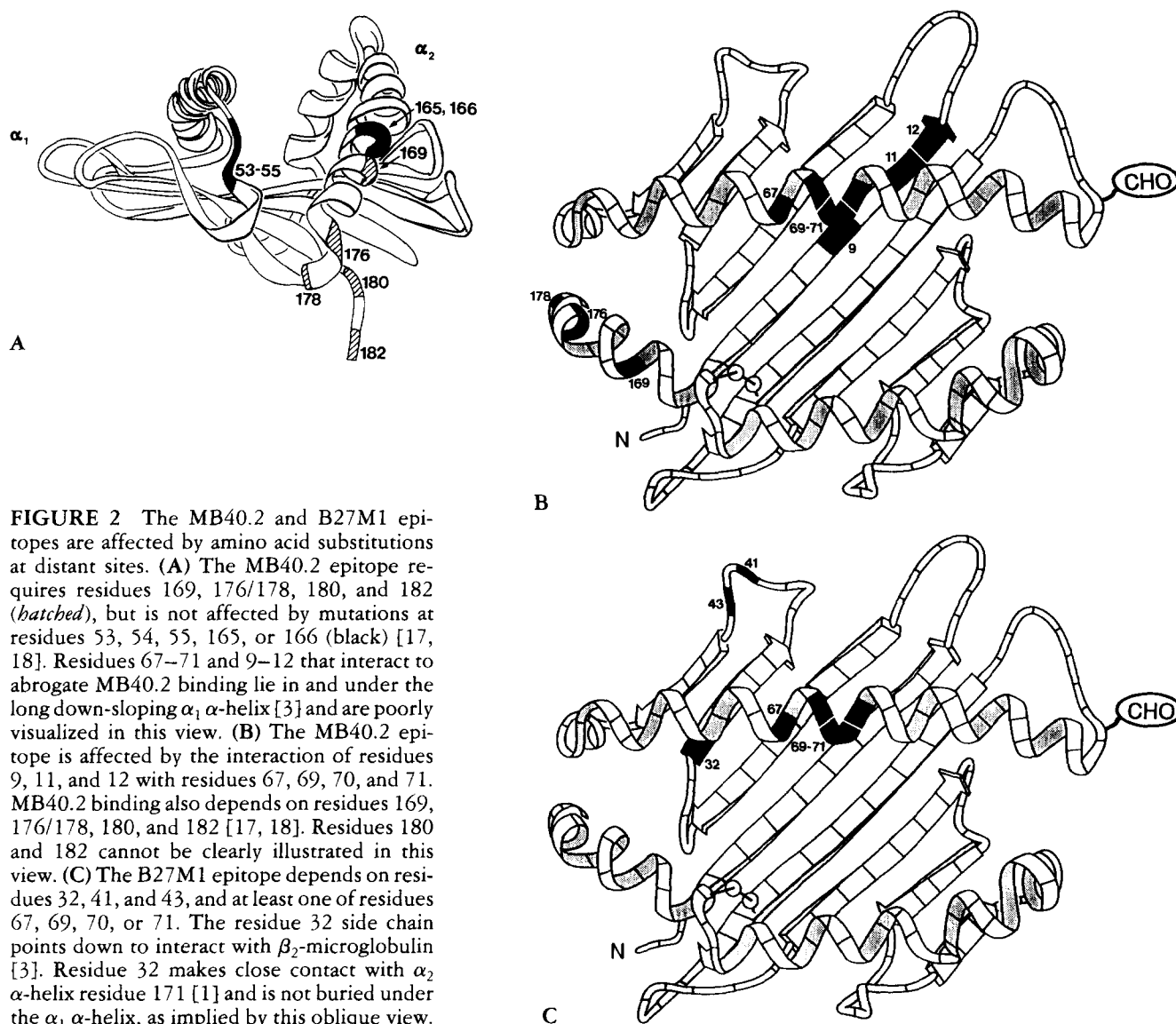


FIGURE 2 The MB40.2 and B27M1 epitopes are affected by amino acid substitutions at distant sites. (A) The MB40.2 epitope requires residues 169, 176/178, 180, and 182 (*hatched*), but is not affected by mutations at residues 53, 54, 55, 165, or 166 (black) [17, 18]. Residues 67–71 and 9–12 that interact to abrogate MB40.2 binding lie in and under the long down-sloping α_1 α -helix [3] and are poorly visualized in this view. (B) The MB40.2 epitope is affected by the interaction of residues 9, 11, and 12 with residues 67, 69, 70, and 71. MB40.2 binding also depends on residues 169, 176/178, 180, and 182 [17, 18]. Residues 180 and 182 cannot be clearly illustrated in this view. (C) The B27M1 epitope depends on residues 32, 41, and 43, and at least one of residues 67, 69, 70, or 71. The residue 32 side chain points down to interact with β_2 -microglobulin [3]. Residue 32 makes close contact with α_2 α -helix residue 171 [1] and is not buried under the α_1 α -helix, as implied by this oblique view.

topes are not identical. Unlike B27M1, ME1 binds the Q32E B*0702 variant well and binds B*0801 and B7/8 at intermediate levels (Fig. 1 and Table 1). Because ME1 binds B7/8 and B8/7 much better than B*0703, HLA residue 67 appears to interact with residues 69–71 to enable formation of the ME1 epitope.

DISCUSSION

BB7.1 binding requires B*0702 α_2 -helical residues 166 and 169 [18] and α_1 α -helical residues 63, 67, and/or 70 [16]. To map the BB7.1 epitope further, we studied natural and experimental recombinants of B*0702 and B*0801. BB7.1 binds B8/7 and B*0703, but not B7/8. Because BB7.1 also binds B*4201 [15], this maps the BB7.1 epitope to the α_1 α -helix, with a specific requirement for residue 67 (Fig. 1). Because BB7.1 binding

requires both HLA α -helices, we postulate that BB7.1 binds B*0702 from “the top,” across the peptide-binding groove. Alternatively, BB7.1 may recognize an HLA α_2 configuration that depends on the α_1 α -helix or bound peptide. The requirement for both HLA polymorphic α -helices (and possibly bound peptide) can explain why BB7.1 binds a narrow spectrum of HLA-B molecules, B*07 subtypes, and B*4201.

Like BB7.1, ME1 binding requires HLA α_1 residues, although blocking studies show that these mAbs bind nonoverlapping epitopes [15]. The A41E and P43R mutations abrogate binding of ME1 and B27M1, suggesting that these cross-blocking mAbs [15] contact the solvent-accessible connecting loop containing residues 41 and 43. As both mutations change small nonpolar residues (alanine and proline) to larger charged residues (glutamic acid and arginine, respectively), these substitutions

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could abrogate ME1 and B27M1 binding by steric or charge hindrance.

ME1 weakly binds B7/8, but shows little or no binding to B*0703, molecules that differ in the α_1 domain only at residue 67. A role for α -helical residue 67 is not surprising, as the connecting loop containing residues 41 and 43 packs against the outer face of the α_1 α -helix [3]. ME1 strongly binds B*0702, B27 subtypes, Bw22 subtypes, and B*4201 [15]; ME1 weakly binds B*4601, B*1401, B*1501 [15], and B*0801 (this report). Based upon a comparison of ME1-binding alleles, it has been postulated that the ME1 epitope includes alanines at positions 69 and 71 [19]. However, quite different combinations of residues 67 and 69–71 enable ME1 binding, including several weak ME1-binding alleles without alanine at residues 69 and 71 (e.g., C-TNT in B*1401 and B*1402, F-TNT in B*0801, and Y-RQA in B*4601 [25]). Furthermore, ME1 does not bind variant B*2705 molecules with a B*0702 tyrosine at 67 [21], unless it is combined with B*0702 residues N63 and Q70 [20]. We speculate that ME1 does not directly contact the residue 67, 69, 70, or 71 side chains; instead ME1 recognizes an HLA configuration indirectly determined by these side chains.

An alternative hypothesis is suggested by the observation that residue 67 forms part of the peptide-binding B pocket ("45" pocket) in A*0201, A*6801, and B*2705 [2, 3, 26]. ME1 binding might be determined by the complex interaction of HLA residues 67–71 and bound peptides. Indeed, some B*2705 variants with mutations at residue 45 or 67 are not expressed at the cell surface, perhaps because these variants do not bind endogenous peptide [27]. Although all our variants are expressed at the cell surface, some B*0702 substitutions could profoundly influence the spectrum or conformation of bound peptides.

B*0702 residues also interact in a complex manner to alter the MB40.2 epitope (Fig. 2A and B). MB40.2 binding requires B*0702 residues 169, 176/178, 180, and 182, near the α_2 – α_3 junction [17, 18]; the clustering of these solvent-exposed residues strongly implicates this region as the MB40.2-binding site. MB40.2 also binds B*0703, B7/8, and B8/7 recombinants that collectively match B*0801 throughout the α_1 domain (Figure 1). However, MB40.2 binds B*4201 [15], but not B*0801 (Table 1), alleles with identical α_2 and α_3 sequences (Fig. 1). Thus, MB40.2 binding is compatible with either B*0801 residue cluster 67–71 or 9–12 separately, but not in combination. Residues 67–71 and 9–12 apparently interact to alter conformationally the MB40.2 epitope over a distance. Residues 67–71 and 9–12 lie in and under the long α_1 α -helix (Fig. 2B) and are well separated from residues 176/178, 180, and 182 on a nearly vertical α_2 segment (Fig. 2A). Thus, it would appear that MB40.2 cannot simultaneously bind both

regions (Fig. 2A and B). Reinforcing this conclusion, mutations at intervening residues 53–55, 165, and 166 do not affect MB40.2 binding [18].

At least two models can explain how distant amino acids alter the MB40.2 epitope. First, the unique combination of B*0801 amino acids D9 and F67 in the peptide-binding B pocket [2, 3, 26] may select a unique B*0801 peptide repertoire. Peptide sequence or conformation might alter the MB40.2 epitope conformation at α_2 α -helical residues 169–182. Second, repulsion between nonpolar F67 and acidic D9 may alter the position of nearby β -strand side chains. One can postulate that subtle side-chain movements may be transmitted along the B*0801 β -strand 1, eventually causing side-chain movement in the overlying α_2 α -helix near residue 169. An altered position of residue 169 may prevent MB40.2 binding. In contrast to B*0801, there may be no repulsion between B7/8 residues Y9 and F67, or B8/7 residues D9 and Y67. Similarly, MB40.2-binding alleles (B*07 subtypes B*4001, B*4101, and B*4201) juxtapose compatible side chains at position 9 (histidine or tyrosine) and position 67 (tyrosine or serine). Regardless of the mechanism involved, these data show that the MB40.2 epitope is influenced by distant α_1 -domain residues.

B27M1 may directly contact solvent-exposed connecting loop residues 41 and 43 and one or more nearby α -helical residues (Fig. 2C). Surprisingly, B27M1 binding also is abrogated by the Q32E mutation. Residue 32 lies at the bottom of the peptide-binding groove (Fig. 2C), and the side chain points down toward β_2 -microglobulin [3], excluding direct contact with B27M1. The Q32E mutation probably does not dramatically change the B*0702 peptide repertoire, as the Q32E variant is recognized by four anti-B*0702 cytolytic T-lymphocyte clones (K.D.S., personal observations). We suggest that a slight conformational shift is transmitted down the β -strand to the B27M1-binding site on the connecting loop or the nearby α -helix. The conformational change caused by the Q32E substitution must be subtle, as it does not affect binding of the cross-blocking ME1 mAb.

Long-range, subtle conformational shifts affect some, but not all, antibody epitopes. Collawn et al. [14] found that chemical modification of horse cytochrome c abrogated binding of two non-cross-blocking mAbs, despite relatively little change in overall cytochrome c structure. In contrast, Jin et al. found that 53 human growth hormone variants did not produce long-range conformational effects on 21 mAb epitopes [11]. We find that MB40.2 and B27M1 are affected by HLA residues distant from their binding sites. In addition, Fu et al. have demonstrated that antibody epitopes on HLA-DR β chains are affected both by a solvent-accessible α -helical residue and by distant solvent-inaccessible β -strand residues [28]. HLA epitopes, potentially influenced by two

HLA chains and bound peptide, might be more prone to long-range conformational effects than epitopes on small soluble proteins.

In summary, BB7.1, ME1, B27M1, and MB40.2 binding requires the complex interaction of HLA amino acid residues, possibly involving bound peptide. It has been proposed that most HLA epitopes can be described adequately by comparing polymorphic 2–7 residue HLA segments [29] or that HLA epitopes can be mimicked by synthetic peptides [30]. Our results predict that these approaches will often fail. For example, B27M1 binding requires residues from at least three discontinuous polypeptide segments, including β -strand residue 32, connecting loop residues 41 and 43, and α -helical residues 67–71. MB40.2 binding requires β -strand residues 9–12, α -helical residues 67–71, and distant residues 169–182. It seems unlikely that these conformational epitopes could be modeled by either approach.

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