Evidence of Widespread Binding of HLA Class I Molecules to Peptides

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Summary

We have tested the binding of HLA class I proteins to peptides using a solid-phase binding assay.
We tested 102 peptides, mostly derived from the HIV *gag* and HIV *pol* sequences. Most peptides
did not bind to any class I protein tested. The pattern of binding among the three class I proteins
tested, HLA-A2, -B27, and -B8, was appropriately 85% concordant. Further, all five of the known
HIV-1 *gag* T cell epitopes detected by human CTL bound at least one class I protein. Binding
of class I to the peptides could be detected either by directly iodinated class I proteins, or indirectly
using monoclonal antibodies specific for class I. The binding to the plates could be blocked with
MA2.1, which binds in the α1 region of A2, but not by W6/32, which binds elsewhere. The
data presented here show that binding of class I to peptides is specific, but that many peptides
bind to more than a single class I protein.

The MHC plays an important role in regulation of the immune response. The roles of both class I and class II molecules have been extensively investigated (1). It is now clear that class I molecules play a crucial role in recognition of virus infected cells by cytotoxic T cells (2). Class II molecules regulate afferent immune responses by their presentation of peptide fragments, primarily to helper T cells. Both Grey and colleagues and Unanue and colleagues have directly shown that fragments of peptides from immunogenic proteins bind directly to MHC class II molecules (3–6). Further they showed the binding patterns of peptides to class II molecules correlated fairly well with the ability of presenting cells to functionally display those same peptides to T cells (5). There is ample indirect evidence that similar peptide fragments of proteins can bind to MHC class I molecules as well. This binding converts cells expressing appropriate class I proteins into targets for antigen-specific CTL (7, 8). Competition experiments showed that most peptides that were epitopes presented by that molecule could compete for the ability to sensitize targets for T cell killing (9, 10), although in some cases competition was unidirectional (11).

It has been much more difficult to demonstrate the direct binding of class I proteins to peptides than the binding of class II proteins to peptides. Two recent preliminary reports have appeared, suggesting that binding of class I proteins to peptides can occur in vitro (12, 13). The results which we present here expand on those previous results and demonstrate substantial specificity of binding of class I to peptides.

Surprisingly the data here show that a peptide which binds to a class I molecule is likely to bind to other class I proteins.

Materials and Methods

Peptides. The HIV-1 (SF-2) *gag* peptides used in these studies were supplied by the MRC AIDS directed research program, and were synthesized by Cambridge Research Biochemicals. The *pol* peptides were a gift of R. Young, Whitehead Institute, Cambridge, MA. Influenza A matrix protein peptide 57–68 with lysine substituted at position 65 (K65), and influenza nucleoprotein peptide 380–393 were kindly supplied by J. Rothbard, Immulogic Corp. All peptides used were judged >80% pure by HPLC. The sequences of all the peptides used in these studies are shown in Fig. 1.

Purification of Class I Molecules. HLA class I molecules were purified essentially as described by Bouillot (13). In brief, 2 × 10⁹ lymphoblastoid cell lines (9004 [(HLA-A2; B27) from the ASHI Serum Bank and Cell Repository] or B cell line JM [A2;B51,62], and PF 04015 [A1;B8]) were extracted in 100 ml of TSB (10 mM Tris-HCl, 0.15 M NaCl, pH 7.5) with protease inhibitors and 1% NP-40 for 60 min. Extracts were centrifuged at 8,000 rpm for 25 min at 4°C. Supernatants were passed at 5 ml/h at 4°C over a mock protein-coupled CNBr-activated Sepharose column, and next over a sequence of three columns coupled with irrelevant IgG1; mAb MA2.1 (HLA-A2 specific); and mAb ME.1 (HLA-B27 specific), respectively (14–16). The A1/B8 molecules were eluted from a W6/32 column. All mAbs were purified from tissue culture supernatant by affinity chromatography using Sepharose–protein A columns. The columns coupled with MA2.1 and ME.1 were washed separately with 100 ml of TSB, 1% NP-40; TSB, 1% NP-40, 0.5 M NaCl; and TSB, 0.1% NP-40. HLA-A2 and HLA-B27

P17-1 CMGARASVLSGGELDK
-2 GELDKWEKIRLRPGG
-3 CLRPGGKKKYKLKHIV
-4 CLKHIVWASRELERFA
-5 CLERFAVNPGLLETSE
-6 LETSEGCRQILGQLQ
-7 CLGQLQPSLQTGSEEL
-8 CGSEELRSLYNTVATL
-9 TVATLYCVHQRIDVK
-10 CRIDVKDTKEALEKIE
-11 CLEKIEEEQNKSKKKA
-12 CSKKKAQQAAAAAGTG
-13 CAAGTGNGSQVSQNY

P24-1 PIVQNIQGQMVHQAIC
-2 VHQAISPRTLNAWVKC
-3 NAWVKVVEEKAFPEVC
-4 AFPPEVIPMFSALSEC
-5 SALSEGATPQDLNTMC
-7 GHQAAMQMLKETINEC
-8 ETINEEAAEWDRVHPC
-9 DRVHPVHAGPIAPGQC
-10 IAPGQMREPRGSDIAC
-13 NPPIPVGEIYKRWIIC
-14 KRWIILGLNKIVRMYC
-16 LDIRQGPKEPFRDYVC
-17 FRDYVDRFYKTLRAEC
-20 VQNANPDCKTILKALY

-21 ILKALGPAATLEEMMC
-22 LEEMMTACQGVGGPGY
-23 CVGGPGHKARVL
P15-14 CAEAMSQVTNPANIMM
-15 CANIMMQRGNFRNQRK
-16 RNQRKTVKCFNGKE
-17 NCGKEGHIAKNCPAPN
-18 NCRAPRKKGCWRCGR
-19 WRCGREGHQMKDCWE
-20 KDCWERQANFLGKIW
-21 CLGKIWPSYKGRPGNF
-22 CRPGNFLQSRPEPTAPPE
-23 CEPTAPPEESFRFGEE
-24 CRFGEEKTTPSQKQEPI
-25 CPQKQEPIDKELYPLTS
-26 CYPLTSLRSLFGNDPSSQ

HIV *pol* Peptides

36. MSLPGRWKPKMIGGIGGFIKVRQYD
37. FIKVRQYDQILIEICGHKAIGTVLV
38. KAIGTVLVGPTPVNIIGRNLLTQIG
39. RNLLTQIGCTLNFPISPIETVPVKL
40. IETVPVKLKPGMDGPKVKQWPLTEE
41. KQWPLTEEKIKALVEICTEMEKEGK
42. TEMEKEGKISKIGPENPYNTPVFAI
43. YNTPVFAIKKKDSTKWRKLVDFREL
44. KLVDFRELNKRTQDFWEVQLGIPHP

45. VQLGIPHPAGLKKKKSVTVLDVGDA
46. TVLDVGDAYFSVPLDEDFRKYTAFT
47. FRKYTAFTIPSINNETPGIRYQYNV
48. GIRYQYNVLPQGWKGSPAIFQSSMT
49. AIFQSSMTKILEPFRKQNPDIVITQ
50. NPDIVITQYMDDLYVGSDLEIGQHR
51. DLEIGQHRTKIEELRQHLLRWGLTT
52. LLRWGLTTPDKKHQKEPPFLWMGYE
53. PFLWMGYELHPDKWTVQPIVLPEKD
54. PIVLPEKDSWTVNIDQKLVGKLNWA
55. LVGKLNWASQIYPGIKVRQLCKLLR
56. RQLCKLLRGTKALTEVIPLTEEAEL
57. PLTEEAELELAENREILKEPVHGVY
58. KEPVHGVYYDPSKDLIAEIQKQGQG
59. EIQKQGQGQWTYQIYQEPFKNLKTG
60. PFKNLKTGKYARMRGAHTNDVKQLT
61. TNDVKQLTEAVQKITTESIVIWGKT
62. SIVIWGKTPKFKLPIQKETWETWWT
63. ETWETWWTEYWQATWIPEWEFVNTP
64. EWEFVNTPPLVKLWYQLEKEPIVGA
65. EKEPIVGAETFYVDGAANRETRLGK
66. NRETRLGKAGLYTNKGRQKVVPLTN
67. QKVVPLTNTTNQKTELQAIYLALQD
68. AIYLALQDSGLEVNIVTDSQYALGI
69. DSQYALGIIQAQPDQSESELVNQII
70. SELVNQIIEQLIKKQKVYLAWVPAH
71. YLAWVPAHKGIGGNEQVDKLVSAGI
72. DKLVSAGIRKILFLDGIDKAQDEHE
73. DKAQDEHEKYHSNWRAMASDFNLPP

74. ASDFNLPPVVAKEIVASCDKCQLKG
75. CDKCQLKGEAMHGQVDCSPGIWQLD
76. SPGIWQLDCTHLEGKVILVAVHVAS
77. LVAVHVASGYIEAEVIPAETGQETA
78. AETGQETAYFLLKLAGRWPVKTIHT
79. WPVKTIHTDNGSNFTSATVKAACWW
80. TVKAACWWAGIKQEFGIPYNPQSQG
81. PYNPQSQGVVESMNKELKKIIGQVR
82. KKIIGQVRDQAEHLKTAVQMAVFIH
83. VQMAVFIHNFKRKGGIGGYSAGERI
84. GYSAGERIVDIIATDIQTKELQKQI
85. TKELQKQITKIQNFRVYYRDSRNPL
86. YRDSRNPLWKGPSKLLWKGEGAVVI
87. KGEGAVVIQDNSDIKVVPRRKAKII
88. PRRKAKIIRDYGKQMAGDDCVASRQ
89. DDCVASRQDED
90. LTQIGCTLNF
91. PISPIETVPV
92. LVSAGIRKIL
93. FLDGIDKAQD

Other Peptides

K65 KGILGFVFKLTV
FMP182-
195 LASTTAKAMEQMAGS

Figure 1. Sequences of peptides used in these studies. Sequences are shown in the one-letter amino acid code.

were eluted with diethylamine (0.05 M, pH 11.5) + 0.1% NP-40. 1-ml fractions were collected in tubes containing 0.8 ml of 0.1 M phosphate buffer, pH 6.7. Protein-containing fractions as determined with the BCA reagent (Pierce Chemical Co., Rockland, IL) were combined and dialyzed for 16 h against PBS + 0.1% NP-40 at 4°C. Purity was assessed by SDS-PAGE and staining with Coomassie Blue. Purified preparations gave two bands, one at 43 kD and one at 12 kD, as expected for class I heavy chain and β_2 (data not shown). Special care was taken with all preparations to ensure each was free of actin contamination. Isoelectric focusing was also performed on the samples following iodination, as previously described (17). No A2 was detected in the B27 preparations, and no B27 was detectable in the A2 preparations (data not shown). Aliquots were stored at −70°C until used.

Iodination of MHC Class I Proteins. 100 µl of purified material was incubated for 60 s at room temperature with 0.5 mCi of ^{125}I and 10 µl of chloramine T (5 mg/ml). The reaction was terminated by the addition of 30 µl of sodium metabisulfite and 2.5 ml PATB buffer (PBS, 1% BSA, 0.05% tween 20, 0.02% sodium azide). The iodinated material was freed of unbound iodine by passage over Sephadex G-25 columns, PMSF (0.1 mM) and trypsin inhibitor (10 µg/ml) were added, and it was stored at 4°C. All preparations were used within 14 d.

Binding Assays. The direct binding assay of class I to peptide was carried out essentially as described by Bouillot et al. (13). Flat- or U-bottomed flexible polyvinyl chloride plates (No. 3912, Falcon Labware, Oxnard, CA) were pretreated for 2 h with 2.5% glutaraldehyde (in water). Peptides were dissolved in 50% DMSO at 1-5 mg/ml, (or in some experiments testing the *pol* peptides, 10% DMSO in RPMI 1640) and diluted in PBS to 50-100 µg/ml. The *pol* peptides were coated on untreated plates. 100 µl was added per well and incubated overnight at 4°C. Next the plates were blocked with PATB (2 h RT). For direct binding assays, 50 µl of ^{125}I A2 or B27 was added per well at 2 × 10^6 cpm/ml. After overnight incubation at 4°C, the plates were washed three times with PATB buffer, and individual wells were counted.

Alternatively, unlabeled HLA was incubated in peptide coated plates overnight at 4°C. Binding of unlabeled HLA was detected using reactivity with class I–specific mAbs (MA2.1 for A2; ME.1 for B27; or W6/32 for others), either directly iodinated or with a sandwich assay, using unlabeled antibodies either ^{125}I (Amersham) or alkaline phosphatase (Sigma Chemical Co., St. Louis, MO) labeled goat anti–mouse antibody. Alkaline phosphatase was detected using *p*-nitrophenyl phosphate (Sigma) and reading the plates directly on a plate reader at 405 and 690 nM.

For competition assays, after blocking and washing, 50 µl PATB buffer with different concentrations of competing peptide and 50 µl ^{125}I-labeled HLA (2-4 × 10^6 cpm/ml) were mixed, incubated for 2 h at RT, and added to coated plates. Incubation, washing, and harvesting were as described for the direct binding experiments.

Results

HLA Class I Molecules Bind to Peptide-coated Plates. To examine the binding of purified MHC class I molecules to peptides, class I was iodinated and added to glutaraldehyde-treated polyvinylchloride plates coated with synthetic peptides. Two peptides derived from the influenza A matrix protein were used in these studies: K65, an analogue of influenza matrix protein (FMP) 57–68 with a lysine substitution for threonine at position 65, which will block sensitization of A2 target cells with FMP57–68 (and therefore must bind to A2), and FMP 182–195, which does not sensitize targets and therefore presumably does not bind (8, 9). Both peptides are soluble under out experimental conditions. Fig. 2 shows a titration curve of iodinated HLA-A2 binding to plates. As easily seen, the binding is fivefold greater than the binding to FMP 182–195–coated wells. It is dependent on the amount of radiolabeled A2 added. In this experiment the observed binding represents 10% of the labeled protein added. The fraction of bound class I counts varied among experiments. In most experiments between 10 and 25% of the added counts bound to the plate.

Despite the purity of the class I preparations established by SDS-PAGE and IEF, we still wished to exclude the possibility that the observed binding was due to some contaminating protein. To exclude this, we modified the binding assay to detect the binding of A2 to peptide-coated plates using mAbs to MHC proteins. Both direct and indirect assays have been used, which give essentially identical results. The direct binding assay uses a directly iodinated mAb (e.g., MA2.1), the indirect assay uses iodinated or alkaline phosphatase–coupled goat anti–mouse antibody second antibody. Shown in Fig. 3 is a titration of a preparation of unlabeled HLA A2 binding to K65-coated plates, detected with [125]I-

labeled MA2.1 Fab fragments. Similar results were seen using the monomorphic class I–specific antibody W6/32. A typical experiment using W6/32 and goat anti–mouse antibody coupled to alkaline phosphatase is shown in Fig. 4. Here we see no binding to peptide P24-4, but good binding to both K65 and P24-2. These results demonstrate that the material binding in the direct assay must contain intact class I protein, and not some contaminating material. This must be so since both MA2.1 and W6/32 require β_2 microglobulin association for antibody binding.

Binding Assay Is Peptide Specific. To examine the specificity of binding of class I proteins to peptide-coated plates, we tested a series of 102 peptides from the HIV *gag* and HIV *pol* genes. All were tested at least three times in the binding assay. The *gag* peptides were tested both using the direct binding by iodinated A2 and detection of binding using W6/32 and goat anti–mouse antibody coupled to alkaline phosphatase. The peptides that produced binding in the binding assay >10 SD above the binding to the negative control (BSA coated wells) are designated ***, those >5 SD more than the negative controls are indicated by **; those that bound >3 SD are indicated by *; those that bound <3 SD are considered negative and are designated –. Known cytotoxic T cell epitopes are boxed. The binding of the *gag* peptides is shown in Table 1. The *pol* peptides that bound HLA-A2 were: 37, 39, 55, 62, 75, 77, 78, 85, 86, 87, and 88. The remainder of the *pol* peptides did not bind A2. Thus, most of the peptides (*gag*, *pol*, and influenza), 74 of 102 tested, do not bind HLA-A2. We confirm the pattern of A2 binding to the three *gag* peptides previously reported (13). We conclude from these experiments that most peptides do not have a structure that is compatible with binding to these purified class I molecules.
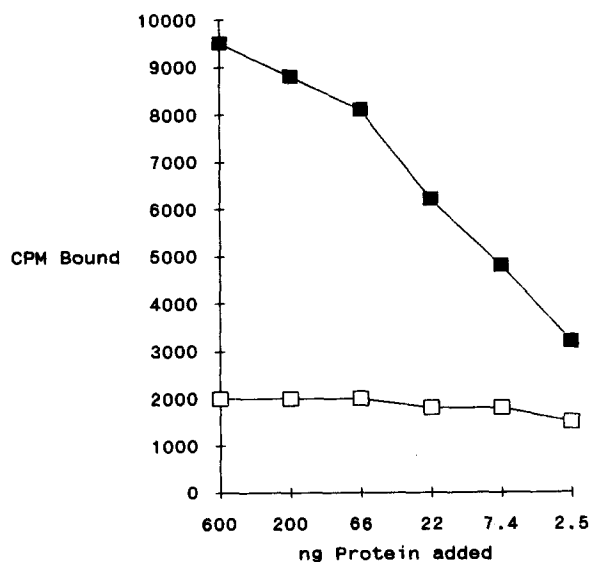

Figure 2. Direct binding of ^{125}I-labeled HLA-A2 to peptides. (■) Wells coated with K65; (□) wells coated with FMP 182–195.
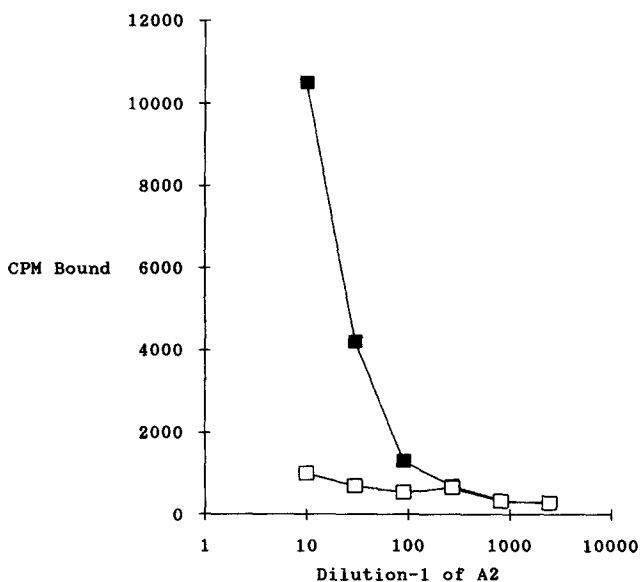

Figure 3. Detection of native HLA-A2 binding to wells coated with K65 (■) or BSA alone (□). A2 is detected by binding of ^{125}I-labeled MA2.1 Fab fragments.
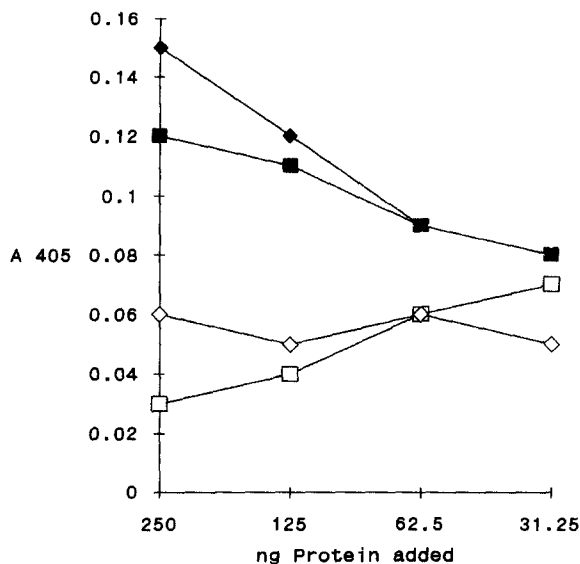
Figure 4. Binding of HLA-A2 binding using an indirect ELISA assay. Wells were coated with K65 (■); P24-2 (◆); P24-4 (◇); or BSA alone (□). W6/32 and alkaline phosphatase–conjugated goat anti-mouse IgG were used for detection.

Determination of Binding Constants of Purified Class I Protein for Peptide. We could inhibit the binding of HLA class I molecules to the peptides bound on plates by preincubation of the class I proteins with soluble peptides. This is a crucial result since it demonstrates that the binding of class I proteins is saturable. Peptides that do not bind in the solid-phase assay are not able to inhibit the binding to peptides fixed on plates. These results allow us to calculate the affinity of A2 for any given peptide in solution. By using the direct binding assay, we can measure the amount of bindable A2 in any preparation since we know the specific activity of the A2. By using a competition in solution assay we can estimate the concentration of unbound A2 and knowing the specific activity can calculate the total amount of A2, and therefore solve at every point all the relevant information to perform a Scatchard analysis of binding of class I to peptides. The result of this analysis of binding of HLA-A2 to K65 is shown in Fig. 5. A single line drawn through these points allows the K_a to be extrapolated to be $\sim$1.5 × 10^3, and the K_d is $\sim$6 × 10^{-4}. A similar experiment was performed with B27 which gave a similar binding constant when tested with the FMP matrix analogue K65. It is possible that more than a single affinity can be inferred from this plot. If two lines are drawn the high affinity K_a is increased only by a factor of 2–3 to $\sim$5 × 10^3.

The Binding Assay Is not Allele Specific. It is interesting to note from the data in Table 1 that there is little difference in the pattern of binding among A2, B27, and the A1/B8 mixture. Of the 34 *gag* peptides where both A2 and B27 were tested, 32 are concordant. 11 bind both A2 and B27, and 2 bind only A2. The concordance between A2 and the A1/B8 sample is similar, where 12 of 15 peptides are concordant. It is interesting to note that the peptides that bind only

Table 1. *Direct Binding of Purified HLA Class I Molecules to HIV gag peptides*

Peptide	A2	A1/B8	B27
P24-1	–	NT	NT
P24-2	**	NT	**
P24-3	–	NT	–
P24-4	–	NT	NT
P24-5	–	NT	NT
P24-7	–	NT	–
P24-8	–	NT	NT
P24-10	–	NT	–
P24-13	–	*	NT
P24-14	*	NT	*
P24-16	–	NT	NT
P24-17	–	NT	NT
P24-20	–	*	–
P24-21	–	NT	–
P24-22	–	NT	–
P24-23	*	**	–
P17-1	–	NT	–
P17-2	*	–	–
P17-3	**	**	**
P17-4	*/–	NT	–
P17-5	–	NT	–
P17-6	–	NT	–
P17-7	***	**	***
P17-8	**	NT	*

continued

a single class I protein were relatively weak binders. However, two of these weakly binding peptides are functional CTL epitopes recognized by CTL. Inspection of the raw data suggests that even some of these peptides bound B27 above background as well, but the number of counts bound did not reach statistical significance. Thus, even the small number of discrepancies that we see here may over represent the number of peptides that bind specifically to a single class I protein.

Antibodies to α1α2 Block Peptide Binding. To examine the site of binding of the class I molecules, we used mAbs W6/32 and MA2.1 to block the binding of A2 to the plate. MA2.1 binds to residues in α1, while W6/32 binds elsewhere, and would not be expected to alter binding (18). As shown in Fig. 6, addition of MA2.1 at the same time as A2 to the plate, caused a 50% inhibition of binding. In contrast, addition of W6/32 had no effect. As a control, we tested MA2.1 on B27 binding to K65. In this experiment, we saw no inhibition of B27 binding. Thus the inhibition is specific. These experiments suggest that the binding of class I to peptide is mediated by the α1α2 peptide binding domain.

Table 1. *(continued)*

Peptide	A2	A1/B8	B27
P17-9	**	NT	**
P17-10	–	NT	–
P17-11	–	–	–
P17-12	–	–	–
P17-13	–	NT	–
P15-14	*	NT	*
P15-15	***	**	***
P15-16	*	**	**
P15-17	–	NT	–
P15-18	**	**	**
P15-19	–	–	–
P15-20	–	–	–
P15-21	**	NT	NT
P15-22	–	NT	–
P15-23	**	NT	**
P15-24	–	–	NT
P15-25	–	NT	–
P15-26	–	NT	NT
K65	**	**	**

Results of direct binding of ^{125}I-labeled class I molecules to peptides. All samples were tested in sextuplicate and the standard deviation of the cpm bound determined. Binding is indicated relative to BSA-blocked wells. (–) Binding < 3 SD greater than the BSA-blocked wells; (*) binding 3–5 SD above the blocked wells; (**) binding 5–10 SD above the blocked wells; (***) binding >10 SD above the blocked wells. (NT) Not tested. Peptides that are known to be recognized by *gag*-specific CTL are boxed. Peptides P24-13, P24-20, and P17-3 are recognized by B8-restricted CTL; peptide P24-14 is recognized by B27-restricted CTL; peptide P17.8 is recognized by A2-restricted CTL.

Discussion

The evidence for MHC class II proteins binding to peptides seems unequivocal. Both functional studies, which showed that peptides added to fixed APCs could stimulate T cell proliferation, and the direct binding experiments using purified class II molecules have given largely consistent results (3–6). Further, there has been a good correlation of the ability of peptides that bind to a given allelic form of a class I molecule to stimulate a T cell response restricted by that same molecule (5). Thus, there is a strong correlation of the biochemical binding data and the antigen presentation function of the class II proteins tested. Further, the results of Roy et al. have suggested that every peptide is likely to bind some class II molecule (19). When they tested the peptides derived from λ repressor protein, almost all of the peptides could stimulate a T cell proliferative response in one or another mouse strain. The data suggest that all these peptides could bind to at least one class II protein.

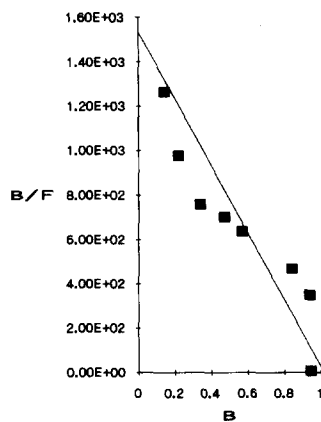In contrast to the biochemical work on class II, the data

Figure 5. Scatchard plot of the binding of HLA-A2 to K65.

reported for class I by Chen and Parham (12), Bouillot et al. (13), and Chen et al. (20) and those reported here are consistent with each other and are different from the class II binding data (5). We note that A2 and B27 bind largely, but not exactly, the same peptides from the set derived from the HIV *gag* sequence. The A2-restricted *gag* epitope P17-8 (Nixon, D., and F. Gotch, personal communication), and the B27-restricted epitope P24-14 (21) are both bound by both A2 and B27. This is consistent with the other results reported here that peptides that bind either A2 or B27 usually bind both proteins. The other known *gag* CTL epitopes (P24-13, P24-20, and P17-3) (F. Gotch, personal communication), all of which are restricted by B8, bind at least one class I protein.

The apparent differences between class I binding and class II binding are not as great as they might first appear. We have examined only three human class I proteins, derived from different loci, HLA-A and -B. In contrast, much of the direct binding studies performed in the mouse used class II proteins and peptides that had already been shown to be under *Ir* gene control. That means that rather than scanning a large protein sequence as we have with *gag* and *pol*, they tested a small series of peptides, which had already been shown to be differently recognized by mice expressing different class
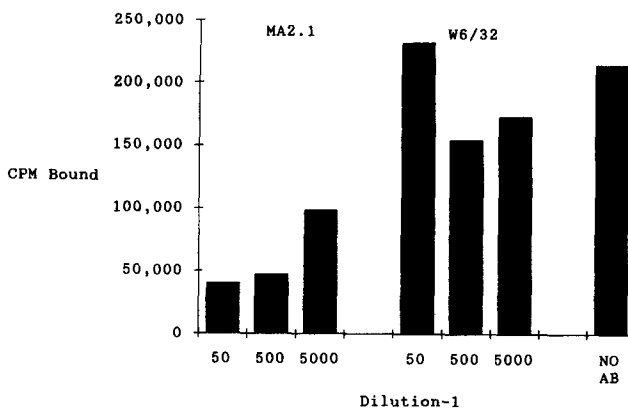

Figure 6. MA2.1 but not W6/32 inhibits the binding of HLA-A2 to K65. Dilutions of each of the mAbs were added to the assay as described in Materials and Methods.

II proteins. Thus, a direct comparison of the data is not appropriate. However, some of the peptides are not bound by all class I molecules and so would be good candidates for peptides under Ir-like gene control.

There is likely to be a fundamental difference between the mechanism of peptide association with class I and class II. It appears that for class II binding to peptides in vivo, much of the association occurs after class II synthesis and expression via protein recirculation in the endosomes. In contrast, it appears that peptide association with class I in vivo may be a critical event in the initial assembly of class I molecules for export to the cell surface, and relatively little recycling occurs (22). The binding we describe must reflect the binding of already assembled proteins to the appropriate peptides. Judging from the relatively small number of peptides (compared with all possible peptides) we have tested it appears the correlation with known CTL epitopes is excellent.

Recent studies using competition for sensitization of target cells with a known CTL peptide epitope have suggested that while a large number of peptides bind to class I proteins, the binding is allele specific (23). Our results here are in apparent conflict with this conclusion. Although 25–42% of a set of peptides tested were noted to inhibit CTL in an allele specific manner, this result cannot be extrapolated to all class I proteins since over 100 different class I proteins are known. Further, another important difference is the peptides we tested are an ordered set derived from a single protein sequence rather than "random" peptides.

The binding of peptides that are not epitopes is expected. The immune response in any individual is the result of both the innate T cell repertoire and selection. We would be surprised if all of the synthetic peptides that bound to class I were actually recognized. A fraction might never be produced from the antigenic protein in the cell by natural cellular processing. Some would be unreactive because the appropriate TCR was removed from the repertoire by tolerance, and others because no appropriate TCR was encoded in the germline. Given these constraints, together with the necessity to make a CTL response to a wide array of viral proteins, it is advantageous for class I proteins to bind peptides permissively.

It is possible that during purification and iodination that some of the class I becomes denatured and the denatured proteins are the ones that participate in the binding assay in vitro, where, as they bind peptide, they renature. This would be consistent with the large fraction of class I protein molecules that are detected in the direct binding by iodinated class I compared with the fraction of molecules detected in the class II binding assays. These results are also in general consistent with the results of Townsend and colleagues on peptide stabilized chain association (22).

The binding of class I is specific, as neither A2 nor B27 binds nonspecifically to the BSA blocking the plate, or to numerous other peptides. Similarly, the labeled MA2.1 and W6/32 did not bind nonspecifically to peptide or plates. We know that the class I molecule is binding rather than some other molecule present in the iodinated class I preparations. Two lines of evidence support this contention. First, the preparations are almost pure. When the iodinated peptide is immunoprecipitated, and run on isoelectric focusing gels, only the A2 or B27 heavy chain bands are detected (along with β_2 microglobulin). Actin contamination was minimal, as was contamination with other class I proteins. The second line of evidence is the detection of class I binding to the plates using mAbs to class I. mAbs directed at both monomorphic and polymorphic epitopes on class I molecules could be used to detect specific binding of class I proteins to the plates.

The binding constants we have measured are lower than those reported for class II binding to peptide. This cannot be the result of a solid-phase assay vs. a solution assay, since we have measured the inhibition of binding by peptide to HLA in solution, and only used the solid-phase assay to measure the binding of the unbound fraction of the class I. There are two sources of error in the estimation of the binding constants. First is the estimation of the total concentration of class I introduced into the assay. This error is likely to be less than a factor of two, since A2 was measured by an immunoassay. The other likely source of error is the estimation of the unbound class I fraction. This might be underestimated because some of the molecules would be inactivated during iodination, and hence contribute to the total number of counts, but would of necessity remain unbound. We have corrected for this error in the calculation, since we only determine the binding constant using the total number of bindable counts, rather than the total added. If there is significant exchange of peptides by the class I protein at 4°C during the solid-phase assay, we would have underestimated the bound fraction and therefore underestimated the K_a. Thus, while there are sources of potential error in the measurement of affinity of assembled class I for peptide they are unlikely to alter the results by several orders of magnitude. The calculated affinities are $\sim$10-fold less than those reported for class II. The ability of this assay to detect such low affinity binding could result, at least in part, in the apparent differences we see in the binding of class I and class II to peptides.

The central issue raised by the data presented here is the promiscuous binding. The question we must address is whether the binding measured has any relationship to the binding detected by the CTL. Several points can be made to encourage us that this binding is relevant. First, three of five of the peptide epitopes that have been identified in HIV *gag* bind A2, although only one A2-restricted epitope is known to be present in this set of peptides. When the other two peptides were tested with the B8 (their defined restriction element) and A1 mixture, those peptides were shown to bind as well. Thus, all of the identified peptide epitopes bind to class I in the binding assay. Indeed, when we examined a subset of the peptides reported here, for their ability to sensitize A2 and B27 target cells, we noted that the two peptides that consistently blocked, K65 and P15-5, also bound class I (Wain, E., J.A. Frelinger, and A.J. McMichael, unpublished data).

Thus, while the binding is permissive, it is not without meaning. The biology of the immune response is consistent with permissive binding. Nearly every virus can induce a

specific CTL response, in nearly all individuals who must have T cell repertoires selected by different MHCs. Thus the variation in peptides recognized which correlates with MHC type might not be the result of altered binding of peptides, but rather the result of altered thymic selection. This is consistent with the result that mutations in class I proteins in the $\alpha 1$ domain result in loss of recognition by only some T cells clones, rather than the all or none pattern that would be expected from altered peptide binding (24).

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