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Assessment of major histocompatibility complex class I interaction with Epstein-Barr virus and human immunodeficiency virus peptides by elevation of membrane H-2 and HLA in peptide loading-deficient cells*

Earlier findings indicate that peptides can affect the expression of major histocompatibility complex (MHC) class I molecules on the surface of cells with defective peptide loading mechanism. We have used peptide induced increase of class I antigen expression to assess peptide interaction with MHC class I molecules. A panel of 41 overlapping synthetic peptides derived from the human immunodeficiency virus-1 (HIV-1) gag protein and 33 nonoverlapping peptides from Epstein-Barr virus (EBV) proteins EBNA-1, 2, 3, 4, 5, 6, LMP, BZLF2, BILF2, BSLF2, BALF4 and BcLF1 was assessed for the ability to enhance the expression of HLA-A2.1, H-2D^b, K^b and D^d on the murine RMA-S and human 721.174/T2 (.174/T2) lines by indirect immunofluorescence. Considering doubling of the fluorescence intensity in the peptide-treated samples as positivity, 6 of 39 HIV and 1 of 32 EBV peptides were found to bind to A2.1, 6 of 39 HIV gag and 7 of 16 EBV peptides to D^b, 8 of 39 HIV gag and 5 of 16 EBV peptides to K^b and 2 of 39 HIV gag and 1 of 17 EBV peptides to D^d. The sensitivity of the method is comparable to the *in vitro* class I assembly assay with conformation-dependent monoclonal antibody and is more discriminating than the solid-phase assay. Due to its simplicity this method can also serve for testing large peptide panels for binding capacity to various class I molecules. Moreover, the method provides information about the relevance of *in vitro* tests for class I assembly in living cells.

1 Introduction

Major histocompatibility complex (MHC) class I molecules present endogenously processed peptides to cytotoxic T cells [1, 2]. Using solid-phase binding assay several authors reported indiscriminative binding of peptides to different class I molecules [3–5]. However, both the quantitative class I assembly assay with conformation-dependent mAb [6] and the photoaffinity labeling technique [7] have revealed specific peptide binding to MHC class I molecules. Similarly, distinct subsets of peptides were shown to bind to HLA-A2.1 and HLA-B37, respectively, by the peptide competition assay [8].

Previously our group has developed an MHC class I-deficient variant subline, RMA-S of the Rauscher virus-induced T cell lymphoma, RBL-5 (H-2^b) [9]. Assembly and expression of MHC class I could be enhanced by adding influenza peptides with specific affinity for H-2 D^b and K^b [10, 11]. This suggests that peptide binding is required for the effective assembly or for the stability of class I molecules. The peptide/H-2 allele specific induction of cell surface MHC class I molecules could then be explained in two different ways (a) pinocytosis of peptides into the cells and induction of MHC class I assembly at an intracellular location, leading to an elevated number of transport component molecules [11] or [6] binding of peptides to the few empty MHC class I β2 microglobulin heterodimers that reach the cell surface, which would normally turn over rapidly at 37 °C, but are now stabilized and allowed to accumulate [12]. Both of these mutually not exclusive mechanisms may contribute to specific induction of MHC class I expression in "peptide feeding" experiments. Later, similar findings were made on class I expression-deficient human LCL .174 and its hybrid derivative .174/T2 [13, 14].

We have explored the ability of peptides with affinity to given MHC class I molecules to enhance their surface expression by measuring the elevation of MHC class I on RMA-S and .174/T2 cells after incubation with peptide. We have screened a panel of 41 overlapping peptides derived from the HIV-1 gag protein and 33 nonoverlapping peptides derived from the EBV proteins EBNA-1, 2, 3, 4, 5, 6, LMP, BZLF2, BILF2, BSLF2, BALF4 and BcLF1 for MHC class I induction on RMA-S and .174/T2 cells.

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Abbreviations: EBNA: EBV nuclear antigen complex **LMP:** Latent membrane protein

2 Materials and methods

2.1 Cell lines

RMA-S is a low H-2 expressor mutant of the Rauscher virus-induced T cell lymphoma RBL-5 of C57BL/6 (H-2^b) origin [9]. Full H-2^b expression can be induced by adding

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H-2^b affine peptides or incubating the cells at low temperature [10–12]. It is resistant to CTL specific for internally processed antigens [15]. The antigen-processing defect could be corrected by the transfection of the ATP-dependent transport protein gene HAM-2 into the RMA-S cells [16]. The H-2 D^d gene, when transfected into RMA-S cells was subject to the same peptide loading defect as the endogenous H-2^b molecule, and could be up-regulated by appropriate peptides or low temperature in a similar way (Franksson et al., to be published). The .174/T2 hybrid line was kindly provided by Dr. P. Cresswell, Howard Hughes Medical Institute, Section of Immunobiology, Yale University School of Medicine, New Haven, CT. It was derived by fusion of the .174 mutant LCL and the CEM^R T lymphoma and selected for the loss of chromosome 6 of CEM^R as described [17–21]. It does not express B5 and its HLA-A2.1 expression is about 70%–80% lower than that of the wild-type LCL 721. Its peptide loading deficiency is most probably due to the deletion of the RING4 and RING11 genes [22].

2.2 Indirect immunofluorescence assay for MHC-peptide interaction and monoclonal antibodies

Aliquots of 2.5×10^5 RMA-S or .174/T2 cells were incubated with peptide in 250 µl RPMI 1640 for 24 h at 37 °C. The final peptide concentration was 100 µM. MHC class I levels were detected by indirect immunofluorescence using FACS IV (Becton Dickinson, Mountain View, CA). The results were calculated on the basis of fluorescence intensity (FI), $FI_{sample} : FI_{control}$, where sample means peptide-treated cells and control means cells without peptide.

Living cells were stained for MHC class I antigens by indirect immunofluorescence. The first layer was the 28-14-8S (anti H-2 D^b, mouse $IgG_2\varkappa$ [23], the 28-13-3S anti K^b, mouse IgM [24], the 34-5-8S anti H-2 D^d, mouse $IgG_{2a}\varkappa$ [25] or the BB7.2 anti HLA-A2 α1 domain, mouse IgG_{2b} [26]. All the reagents were culture supernatants of hybridomas obtained from American Type Culture Collection (Rockville, MD), used at 10–15 µg/ml. The second layer was a fluorescein isothiocyanate (FITC)-labeled affinity-purified rabbit anti-mouse antibody (Sigma, Saint Louis, MO) at a 1:20 dilution. Samples were analyzed by cytofluorimetry using a fluorescence-activated cell sorter (FACS IV, Becton Dickinson).

2.3 Peptide synthesis

A series of 41 peptides spanning the HIV-1 gag polyprotein precursor molecule were synthesized. The peptides are 24 amino acids (aa) long (with the exception of the 22–23 aa long peptides 1, 14, 15, 17 and 25 aa long peptide 27 and 30) overlap in 12 (in some cases 11 or 13) aa with both neighboring peptides (Table 1). In addition, 33 peptides derived from the EBV-encoded proteins EBNA-1, 2, 3, 4, 5, 6, LMP, BZLF2, BILF2, BSLF2, BALF4 and BcLF1 LMP (Table 2) were synthesized. The majority of the peptides derived from EBV-encoded proteins were originally synthesized in order to produce monospecific anti-peptide sera to identify the corresponding EBV gene products in Western blots or similar immunoassays. The peptides derived from EBNA-3 (aa334–346 and 339–352) have been identified as human T cell epitopes in advance [27].

HIV-1 gag and EBNA-1 C term., EBNA-2a, EBNA-3/EBV.1, EBNA2-EBV.2, Lydma-1, 4, and 5 and the EBV lytic cycle protein peptides (Table 2) were synthesized with a 9050 PepSynthesizer (Milligen, Eschborn, FRG) using Fmoc (9-fluorenylmethyloxycarbonyl)-protected amino acids as described earlier [28, 29]. The peptides were lyophylized and purified by reverse-phase high-performance liquid chromatography (HPLC) using a C2/C18 copolymer column (PepS, Pharmacia, Freiburg, FRG) and a gradient of 0%–70% acetonitrileF in 0.1% trifluoroacetic acid. The peptide containing fractions were lyophilized and characterized by amino acid sequencing (Applied Biosystems, Weiterstedt, FRG). Peptides 68, 68b, 77 and KEHVIQNAFRK, kindly provided by Dr. D. Moss, Queensland Institute of Medical Research, Brisbane, Australia, were synthesized as described earlier [30]. The remaining EBV peptides were synthesized using t-Boc amino acids (Bachem, Bubendorf, Switzerland) and p-methylbenzhydrylamine resin (Fluka, Buchs, Switzerland) according to the multiple solid-phase peptide synthesis method as described earlier [30–32] kindly provided by Dr. J. Dillner, Dept. of Virology, Karolinska Institute, Stockholm, Sweden.

2.4 Medium

RPMI 1640 medium with L-glutamine (200 mM solution, 1% by volume), benzylpenicillin (100 IU/ml), streptomycin sulfate (100 µg/ml), Hepes buffer (0.1 mM) and 10% heat-inactivated FCS was used for cell culturing.

3 Results

3.1 HIV-1 gag peptides affecting HLA-A2.1 expression on .174/T2 cells

Peptides which caused elevation of MHC class I fluorescence intensity (FI) by a factor of 2 or more, calculated with the formula given in Sect. 2.2, were considered as positive. We detected 6/39 positive peptides on HLA-A2.1. The quantified cytofluorimetric results are shown in Table 1 and Fig. 1 a.

3.2 HIV-1 gag peptides affecting H-2 D^b, K^b or D^b expression on RMA-S cells

Of 39 peptides 6 elevated the expression of D^b molecules on RMA-S cells by a factor of two or more (Table 1 and Fig. 1 b). None of these positive peptides was identical or overlapping with those elevating the A2.1 levels on the .174/T2 line. Of 39 peptides 8 were found positive on K^b (Table 1, Fig. 1 c). Three of these, gag 14, 21 and 22 were positive on the HLA-A2.1 as well and one, gag 12 was positive also for D^b and D^d. It is noteworthy that the latter peptide appeared to be cross-reactive with all the four MHC class I antigens as it was on the borderline of positivity (1.8) also for A2.1.

We found 2 of 39 peptides that elevated D^d expression at least with factor 2 (Table 1). One of these, gag 27 enhanced the expression of the D^d molecule with a factor of 2.9 but did not affect the other two murine MHC molecules at all and elevated only weakly the expression of the A2.1 molecules (1.4).

(a) HLA 2.1 binding peptides

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL

LETSEGCRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA

LDKIEEEQNK SKKKAQQAAA DTGHSSQVSQ NYPIVQNIQG QMVHQAISPR

TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNTMLNT VGGHQAAM

QMLKETINEEAA EWDRVHPVHA GPIAPGQMRE PRGSDIAGTT STLQEQIG

WMTNNPPIPVGE IYKRWIILGL NKIVRMYSPT SILDIRQGPK EPFRDYVDRF

YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTILKALGPA ATLEEMMT

AC QGVGGPGHKA RVLAEMSQV TNTATIMMQR GNFRNQRKMV KCFNCG

KEGH TARNCRAPRK KGCWKCGKEG HQMKDCTERQ ANFLGKIW

PS YKGRPGNFLQ SRPEPTAPPF LQSRPEPTAP PEESFRSGVE

TTTPPQKQEP IDKELYPLTS LRSLFGNDPS SQ

(b) H-2 D^b binding peptides

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL

LETSEGCRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA

LDKIEEEQNK SKKKAQQAAA DTGHSSQVSQ NYPIVQNIQG QMVHQAISPR

TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNTMLNT VGGHQAAM

QM LKETINEEAA EWDRVHPVHA GPIAPGQMRE PRGSDIAGTT STLQEQIG

WM TNNPPIPVGE IYKRWIILGL NKIVRMYSPT SILDIRQGPK EPFRDYVDRF

YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTILKALGPA ATLEEMMTAC

QGVGGPGHKA RVLAEMSQV TNTATIMMQR GNFRNQRKMV KCFNCGKE

GH TARNCRAPRK KGCWKCGKEG HQMKDCTERQ ANFLGKIWPS YKGR

PGNFLQ SRPEPTAPPF LQSRPEPTAP PEESFRSGVE TTTPPQKQEP IDKE

LYPLTS LRSLFGNDPS SQ

3.3 Sensitivity of the method as compared with other techniques

We have found that 15%, 21% and 15% of 39 HIV-1 gag peptides were positive on D^b, K^b and A2.1, respectively, as detected by immunofluorescence. Using a quantitative assembly assay (immunoprecipitation with conformation-dependent mAb), Elvin et al. [6] found that 22%, 15% and 14% of 49 HIV-1 gag peptides (of 15 aa average length, showing a 5 aa overlap with the neighboring ones) gave positive signals on D^b, K^b and A2.1, respectively. This suggests that the sensitivities of the two methods are similar, and that they are more discriminative than the solid-phase plate binding assay which suggested that 44% of the HIV-1 gag peptides, identical to those used by Elvin et al., bound to A2.1 [4].

(c) H-2 K^b binding peptides

MGARASVLSG GELDRWEKIR LRPGGKKKYK LKHIVWASRE LERFAVNPGL

LETSEGCRQI LGQLQPSLQT GSEELRSLYN TVATLYCVHQ RIEIKDTKEA

LDKIEEEQNK SKKKAQQAAA DTGHSSQVSQ NYPIVQNIQG QMVHQAISPR

TLNAWVKVVE EKAFSPEVIP MFSALSEGAT PQDLNTMLNT VGGHQAAM

QM LKETINEEAA EWDRVHPVHA GPIAPGQMRE PRGSDIAGTT STLQEQ

IGWM TNNPPIPVGE IYKRWIILGL NKIVRMYSPT SILDIRQGPK EPFRDYVD

RF YKTLRAEQAS QEVKNWMTET LLVQNANPDC KTILKALGPA ATLEEMM

TAC QGVGGPGHKA RVLAEMSQV TNTATIMMQR GNFRNQRKMV KCFNCG

KEGH TARNCRAPRK KGCWKCGKEG HQMKDCTERQ ANFLGKIWPS YKG

RPGNFLQ SRPEPTAPPF LQSRPEPTAP PEESFRSGVE TTTPPQKQEP

IDKELYPLTS LRSLFGNDPS SQ

Figure 1. Ability of overlapping synthetic peptides to enhance expression of MHC class I molecules. (a) HLA-A2.1; (b) H-2 D^b; (c) K^b, assessed by indirect immunofluorescence (solid symbols above the sequence) and by the *in vitro* assembly assay [6] (open symbols below the sequence). Peptides which caused elevation of MHC class I fluorescence intensity (FI) by a factor of 2 or more, calculated with the formula given in Sect. 2.2 and peptides increasing the concentration of the folded heavy chains more than three standard deviations from the control were considered as positive in the immunofluorescence and *in vitro* assembly assays, respectively. Bold letters mark motifs of peptides binding to the relevant MHC class I molecules, predicted by computer searching based on published motifs [33]. Asterisks mark the first amino acid of the motifs.

3.4 EBV peptides affecting HLA-A2.1 expression on the .174/T2 cells

Thirty-two peptides derived from EBV-associated proteins were screened for the capacity to interact with HLA-A2.1. One of them, the EBNA-1-derived peptide 107 elevated the expression of HLA-A2.1 molecules by more than factor 2 (Table 2).

3.5 EBV peptides affecting H-2 D^b, K^b or D^d expression on RMA-S cells

Of the 16 screened peptides 7 were found positive for induction of D^b expression. One of them was derived from EBNA-2, 4 from EBNA-3, 1 from EBNA-6 and 1 derived from LMP (Table 2). Five of them elevated the expression of K^b as well, but had no effect on A2.1 and D^d. Of 16 peptides screened on K^b 5 were positive. One was derived from EBNA-2, 3 from EBNA-3 and 1 from EBNA-6. They elevated also D^b expression, but had no effect on A2.1 and D^d (Table 2). Seventeen peptides were also tested on the RMA-S D^d transfectant. One, BSLF2 1–13 was positive without cross-reactivity for the other included class I antigens (Table 2).

3.6 Comparison of the positive peptides with motifs for the respective MHC class I molecules

We made a computer searching for A2.1, D^b and K^b binding nonamers in the HIV-1 gag protein based on the published

Table 1. Effect of overlapping peptides spanning the HIV-1 gag protein on the expression of MHC class I molecules on cells of the peptide loading deficient human .174/T2 (HLA-A2.1) line, murine RMA-S line (H-2 D^b and K^b) and its transfectant with the D^d gene

Peptide	MHC class I expression A2.1	D^b	K^b	D^d
1 MGARASVLSGGELDRWEKIRLR	1.0[a)]	1.3	1.6	1.0
2 ELDRWEKIRLRPGGKKKYKLKHIV	1.3	1.8	1.9	0.9
3 PGGKKKYKLKHIVWASRELERFAV	1.3	1.9	2.2	1.0
4 WASRELERFAVNPGLLETSEGCRQ	1.1	1.8	1.8	0.7
5 NPGLLETSEGCRQILGQLQPSLQT	1.7	1.0	1.5	0.7
6 ILGQLQPSLQTGSEELRSLYNTVA	1.1	2.1[b)]	1.4	1.0
7 SEELRSLYNTVATLYCVHQRIEIK	1.5	1.2	1.3	1.5
8 LYCVHQRIEIKDTKEALDKIEEEQ	1.9	1.2	1.5	1.4
9 TKEALDKIEEEQNKSKKKAAAA	Toxic			
10 NKSKKKAQQAAADTGHSSQVSQNY	2.2	1.3	1.9	1.2
11 TGHSSQVSQNYPIVQNIQGQMVHQ	1.9	1.9	1.5	0.6
12 PIVQNIQGQMWHQAISPRTLNAWVK	1.8	2.4	2.5	2.2
13 AISPRTLNAWVKVVEEKAFSPEVI	2.2	1.1	1.4	0.8
14 VVEEKAFSPEVIPMFSALSEGAT	2.3	1.0	2.0	0.7
15 MFSALSEGATPQDLNTMLNTVGG	1.9	1.2	1.9	0.6
16 PQDLNTMLNTVGGHQAAMQMLKET	1.8	1.1	1.6	1.0
17 HQAAMQMLKETINEEAAEWDRVH	1.9	1.4	2.0	1.0
18 INEEAAEWDRVHPVHAGPIAPGQM	1.5	1.2	1.8	0.8
19 PVHAGPIAPGQMREPRGSDIAGTT	0.8	1.1	1.9	1.4
20 REPRGSDIAGTTSTLQEQIGWMTN	1.7	1.2	2.2	1.2
21 STLQEQIGWMTNNPPIPVGEIYKR	2.3	1.0	2.1	0.8
22 NPPIPVGEIYKRWIILGLNKIVRM	2.0	1.2	2.0	1.1
23 WIILGLNKIVRMYSPTSILDIRQG	1.6	1.2	2.0	1.0
24 YSPTSILDIRQGPKEPFRDYVDRF	1.3	1.3	1.3	1.4
25 PKEPFRDYVDRFYKTLRAEQASQE	1.1	1.3	1.0	1.8
26 YKTLRAEQASQEVKNWMTETLLVQ	1.1	1.1	1.3	1.4
27 KNWMTETLLVQNANPDCKTILKALG	1.4	1.0	1.0	2.9
28 ANPDCKTILKALGPAATLEEMMTA	1.5	0.8	0.8	1.0
29 AATLEEMMTACQGVGGPGHKARVL	1.4	0.8	0.8	1.5
30 GVGGPGHKARVLAEAMSQVTNTAT	0.7	1.1	1.1	1.1
31 AEMSQVTNTATIMMQRGNFRNQR	0.5	1.1	1.8	0.9
32 MMQRGNFRNQRKMVKCFNCGKEGH	1.2	2.5	1.2	1.2
33 MVKCFNCGKEGHTARNCRAPRKKG	Toxic			
34 TARNCRAPRKKGCWKCGKEGHQMK	0.2	1.0	1.2	0.8
35 WKCGKEGHQMKDCTERQANFLKGI	1.1	1.1	0.9	1.4
36 CTERQANFLGKIWPSYKGRPGNFL	2.8	1.1	0.8	1.0
37 PSYKGRPGNFLQSRPEPTAPPELQ	1.4	1.0	1.2	1.5
38 SRPEPTAPPFLQSRPEPTAPPEES	1.5	1.0	1.2	1.2
39 RPEPTAPPEESFRSGVETTTPPQK	1.4	2.0	1.0	1.3
40 RSGVETTTPPQKQEPIDKELYPLT	1.6	2.0	1.1	1.2
41 EPIDKELYPLTSLRSLFGNDPSSQ	1.8	2.0	1.0	1.1
MP 57-68 K62 KGILGKVFTLTV[c)]	4.9	1.0	1.1	0.9
NP 345-360 SFIRGTKVSPRGKLST[d)]	1.0	1.0	3.9	1.1
NP 365-279 IASNENMDAMESSTL[e)]	1.2	5.6	1.1	1.0

a) The results were calculated on the basis of fluorescence intensity (FI): $\frac{FI_{sample}}{FI_{control}}$ where sample means peptide treated cells and control means cells without peptide.
b) Peptides which elevated the fluorescence intensity with a factor of 2 or more were classed as positives (underlined).
c) Influenza matrix protein peptide K62, included as control for HLA-A2.1 binding.
d) Influenza nucleoprotein peptide, control for K^b binding.
e) Influenza nucleoprotein peptide, control for D^b binding.

motifs for these allele products [33]. Thirty-one A2.1 binding motifs were found, distributed in 23 peptides in our HIV-1 gag peptide panel (Fig. 1 a). Two of the six peptides which elevated A2.1 expression with factor 2 or more included such motifs. Of the 23 peptides including motif(s)14 (61%) caused elevation of A2.1 expression with a factor of 1.5 or more. On the other hand 6 of the 16 peptides (38%) without motif elevated A2.1 expression 1.5 or more. The finding that both the overlapping HIV-1 gag 11 and 12 peptides elevated A2.1, could be explained by the relevant motif included into the overlapping part of the both peptides.

Six D^b binding motifs were predicted in the HIV-1 gag protein distributed in 5 peptides in our panel (Fig. 1b). One of the 6 peptides in our panel, which elevated D^b

Table 2. Effect of peptides derived from EBV-encoded proteins on the expression of MHC class I molecules on cells of the deficient human .174/T2 (HLA-A2.1) line, murine RMA-S line (H-2 D^b and K^b) and its D^d transfectant

Peptide		MHC class I expression			
		A2.1	D^b	K^b	D^d
Latent Proteins					
EBNA-1					
EBNA-1 C-term	AIKDLVMTKPAPTCNIRV-TVCSFDDGV	1.4[a)]	0.9	1.1	1.5
107	AGAGGGAGGAGAGGG-AGGAGG	2.1	–[b)]	–	–
108	CRARGRGRGAGEKRPM	1.1	–	–	–
EBNA-1, 5	RGGSRERARGRGRGR-GEKRP	1.1	–	–	0.8
EBNA-1, 6	RGRGRGEKRPRSPSSQSSSS	1.0	–	–	0.9
EBNA-2					
EBNA2a	PRRPEPNTSSPSMPELSPVLG-LHQGQ	1.0	–	–	–
116 (213-230C)	PPRPTRPTTLPPIPLLIC	1.0	–	–	–
TB3 (305-317)	PPGVINDLHHL	1.2	5.5	2.0	–
TB1 (454-465)	LDESWDYEFETT	1.5	1.7	0.9	–
EBNA-3					
68B	AWNAGFLRGRAYGID	1.8	3.9	1.5	0.8
68G	AWNAGFLRGRAYGLD	1.3	4.4	2.9	0.8
EBNA-3/EBV.1 (334-348)	AWNAGFLRGRAYG	0.9	2.3	2.4	1.1
EBNA-3/EBV.2 (339-353)	FLRGRAYGHDLLRTE	0.9	2.9	2.3	1.1
EBNA-4					
203	PAPQAPYGYQEP	1.3	–	–	–
EBNA-5					
186	PRGDRSEGPGPTRPGPPG	1.6	–	–	–
187	GQEPRRVRRRVLV	1.3	–	–	–
188	EEEEVVSGSPSG	1.0	–	–	–
EBNA-6					
	KEHVIQNAFRK	0.7	–	–	0.9
77	EENLLDFVRFMGVMSSCNNP	–	4.1	3.2	0.7
LMP					
Lydma-1 (42-53C)	IVMSDWTGGALLC	1.6	1.7	1.6	1.5
405 (43-53)	VMSDWTGGALL	1.4	–	–	–
Lydma-4 (125-132)	LLEMLWRLGATL	0.8	1.7	1.7	–
Lydma-5 (159-171)	YLNWWTLLVDL	0.8	1.5	1.0	0.9
TB2 (321-331)	PQLTEEVENRG	1.2	2.4	1.5	–
TB2a (321-331)	PQLTEEVENR	1.4	0.5	1.0	–
1-18	MEHALERGPGPRRPPGRC	1.0	–	–	–
138 (188-205C)	GQRHSDEHHHHDDSLPHPQC	1.3	–	–	–
Proteins of the lytic cycle					
BcLF1 (327-352)	VMRTFGEHMARIVDSPEIC-AGSTKSDL	0.7	0.9	1.1	1.1
BILF2 (K4, 48-69)	VSRIELGRGYTPC	1.1	1.2	1.2	1.0
BZLF1 2. exon	LEIKRYKNRVASKC	1.1	1.0	1.0	1.0
BSLF2 (1-13)	HVPSQLRSRTSSL	1.1	–	–	2.0
BALF4 (266-278)	GTNPQGERRATLDC	0.6	–	–	0.9
BALF4 (675-688)	RKDLDNAVSNGRNQC	1.2	–	–	1.0

a) Results were calculated as in Table 1.
b) –: not tested.

expression with a factor 2 or more included two such motifs. Of the 5 peptides 2 (40%) which included motif(s) and 8 of the 34 peptides (24%) without motif(s) elevated D^b expression more than 1.5 times.

Four K^b-binding motifs were predicted, distributed in 4 peptides in our panel (Fig. 1c). Of the 8 peptides positive in our assessment 1 included K^b binding motif. Of the 4 peptides 2 (50%) including K^b motifs and 16 of the 31 peptides (52%) without motifs elevated K^b expression more than 1.5 times.

4 Discussion

Using immunofluorescence detection of elevated MHC class I levels as a method for assessing peptide binding, we have found that, for any tested H-2 or HLA allele, between 5% and 21% of the HIV-1 gag peptides, and between 6% and 43% of EBV peptides were scored as positive. The strength of the peptide-MHC class I interactions showed a continuous distribution. Three HIV-1 peptides interacted with two different MHC class I molecules and one elevated the expression of all the four tested MHC class I molecules. Five latent protein peptides (derived from EBNA-2, 3 and 6) gave positive results on two different MHC molecules and one (Lydma-1 derived from LMP) appeared to have a broad cross-reactivity, showing intermediate binding to all the four molecules. The peptide KRWIILGLNKIVRM has been shown to be B27 specific [34]. We now show, that the same sequence (HIV-1 gag peptide 22/23) binds to A2.1 and K^b, suggesting similar binding capacity of these human and murine MHC molecules. We have detected human mouse cross-reactivity also between A2.1 and K^b with the HIV-1 gag peptides 14, 21 and 22. It may be interpreted with similarities between these two MHC molecules. Peptide 14 but not 21 and 22 included both A2.1- and K^b-binding motifs. HIV-1 gag peptide 12 was found to bind to all the three murine MHC class I molecules investigated in this study and has shown binding to the human A2.1 also near to the criteria of positivity. This could reflect nondiscriminative binding properties or could suggest, that similarities in peptide binding characteristics between human/human, human/mouse or mouse/mouse may depend on the peptide sequence. No human/mouse cross-reactivity was detected with peptides from EBV proteins, included in this study, although the EBNA-3-derived 68B peptide which was positive for the murine D^b, elevated the expression of the human A2.1 1.8 times, near to the criterion of positivity. This peptide was previously shown to serve as T cell epitope for A-type virus specific, B8-restricted CTL [27]. For this peptide, we have detected a binding capacity to D^b and K^b molecules higher than the average of all the included peptides. Thorley-Lawsson et al. [35] has shown that one T cell clone generated with LMP 43–53 peptide was HLA-A1 restricted, which could suggest binding of this peptide to A1. With a near variant of this peptide, Lydma-1 (LMP 42–53) we have detected enhancement of all the four class I molecules. Thus, it appears that peptides 68B and LMP 42–53 show cross-reactivity between several human and mouse class I molecules. Of 16 EBV peptides 5 have shown mouse/mouse cross-reactivity between D^b and K^b.

The lower proportion of HIV-1 peptides binding to A2.1 as compared to the solid-phase assay (3–5) is in accordance with the assembly assay with comformation-dependent antibody [6]. The two different sets of peptides derived from the HIV-1 gag protein screened by Elvin et al. [6] and our group opens the possibility of mapping shorter sequences from overlapping peptides found positive in the both assessments (Fig. 1a–c). More than the half of the positive peptides were identified as positives in both assays, but many were positive only in one assay. We have used 24 aa long HIV gag peptides which should be compared with reservation with those of 15 aa long peptides screened in the *in vitro* assembly assay by Elvin et al. *In vivo* presented "natural" peptides are 8–9 aa long [33, 36–38]. The suboptimal 24 aa length of our peptides may explain the observation that none of the HIV-1 gag peptides had as high capacity to enhance class I expression as the shorter control influenza peptides (2–2.9 times and 3.9–5.6 times elevation, respectively; Table 1). It may also explain why only two of the four known A2.1-restricted CTL epitopes derived from HIV gag protein [39], FLQSRPEPT and AMQMLKE, present in HIV-1 gag peptide 38 and 11, respectively, elevated A2.1 expression with a factor of 1.5 or more. The remaining two, PIAPQMRE and QMKDCTERQ, present in HIV-1 gag peptides 19 and 35 did not change A2.1 expression. The shorter sequence of the EBV peptides included in our study compared to the HIV-1 gag peptides may explain the higher average binding capacity of the positive EBV peptides (compare Tables 1 and 2).

Although HPLC-purified and sequenced peptides were used we can not exclude contamination by shorter sequences which may be responsible for the elevation of MHC class I levels. MHC molecules were indeed shown to accumulate the shorter byproducts of synthetic peptides [40, 41]. That could also explain why peptides overlapping positive ones often elevated class I expression. Cerundolo et al. [42] have shown that longer variants of an H-2 K^b-binding nanomer had 100-fold faster off-rates and Christinck et al. [43] have detected a dissociation rate for the natural influenza NP 366–374 peptide which was three orders of magnitude lower than that of the longer NP Y365–380 version. This does not exclude binding by longer peptides. It is also possible that the longer peptides used in this study had been trimmed to appropriate binding length after binding to the class I molecules as suggested [44, 45]. Recently, Sherman et al. [46] showed that proteolytic enzymes naturally present in serum may cleave longer synthetic peptides to shorter ones which have a class I binding capacity. On the other hand Henderson et al. and Wei et al. [47, 48] found recently that HLA-A2.1 bound peptides which are longer than nanomers can be eluted from the T2 cells. Such peptides bind to the A2.1 with affinities similar to those of nanomers [47].

Our results suggest that the presence of the published A2.1-, D^b- and K^b-binding motifs are not always sufficient for interaction of the peptide with the respective MHC molecules. Only one of the six D^b and one of the eight K^b-positive peptides included motif for the respective MHC molecule. A higher proportion of the HIV-1 gag peptides including D^b binding motifs elevated D^b expression with a factor of 1.5 or more than those without motifs (40% and 24%, respectively). This was not the case with peptides interacting with K^b (50% of peptides with motifs and 52% of peptides without motifs elevated K^b by a factor 1.5 or

more). Of the 16 peptides 6 (38%) elevated A2.1 expression with a factor of 1.5 or more. The suboptimal length of the peptides may explain that a proportional of peptides including motifs did not interact with the relevant MHC. The binding of peptides without motifs suggests, however, that further identification of motifs may be required.

The use of viable cells gave us a possibility to test the relevance of the results in cell lysates. The presented method may allow a first screening of large numbers of peptides to judge their feasibility as T cell epitopes.

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