Vaccine 26 (2008) 5182–5187

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Cross-clade immune responses to Gag p24 in patients infected with different HIV-1 subtypes and correlation with HLA class I and II alleles

L. Gudmundsdotter[a,b,*], D. Bernasconi[e], B. Hejdeman[c], E. Sandstrom[c], A. Alaeus[d], K. Lidman[d], B. Ensoli[e], B. Wahren[a,b], S. Buttò[e]

[a] Swedish Institute for Infectious Disease Control, Solna, Sweden
[b] Department of Microbiology, Tumor and Cell biology, Karolinska Institutet, Solna, Sweden
[c] Venhälsan, Department of Infectious Diseases, Karolinska University Hospital, Solna, Sweden
[d] Department of Infectious Diseases, Karolinska University Hospital, Solna, Sweden
[e] National AIDS Center, Istituto Superiore di Sanità, Rome, Italy

ARTICLE INFO

Article history:
Available online 22 April 2008

Keywords:
HIV-1
Cross-clade responses
Gag p24
HLA class I
HLA class II

ABSTRACT

Individuals infected with different subtypes of HIV-1 (A, B, C, D, CRF01_AE and CRF02_AG) were analyzed for their antigen-specific immune response with respect to their HLA genetics. The p24 Gag protein was selected for analysis, since previous studies of the same cohort of patients had shown that almost 80% of these individuals responded to Gag peptides of subtypes A, B and/or C. A large number of Gag antigen-specific responses were recorded. Both previously recognized as well as new epitopes were identified, assumed to bind HLA classes I and/or II. Fifteen individuals showed class I cellular responses to T cell epitopes irrespective of the infecting virus subtype. For five individuals infected with subtypes A, B, D and CRF02_AG, new T cell epitopes are described. Responses related to the patient's class I alleles are frequent, and several new putative class II responses were found.

1. Introduction

There is a critical need for an effective Human Immunodeficiency virus type-1 (HIV-1) vaccine, in particular in developing countries where HIV infection is spreading at high rates. However, HIV-1 viruses circulating in these geographical areas show considerable genetic and antigenic variations, which may be a limiting factor to the development of an effective vaccine. As a consequence of virus variability, such a vaccine has to elicit immunological responses against a diverse array of viral variants both within and between viral clades. Several studies have indicated that HIV-specific $CD4^+$ T-helper and $CD8^+$ cytotoxic T lymphocytes (CTLs) play a key role in controlling HIV-1 viremia and disease progression. In particular, CTLs appear to control the progression of HIV-1 infection, as well as to play a key role in determining the status of long-term non-progressors [1–6]. In addition, potent HIV-1-specific CD4+ T cell proliferative responses have been shown to be inversely related to viral load and to be present in long term non-progressing individuals (LTNP) [7–10].

The pattern of epitopes recognized by CTLs and $CD4^+$ T cells in response to viral infection is dependent on the class I and II human leukocyte antigen (HLA) alleles expressed by the individual. Several studies have associated specific HLA class I or class II determinants with decreased or increased risk of HIV infection and/or better control of infection or rapid progression upon infection. [11,12]. It has been reported that differences in the pattern of antigen recognition between ethnic groups can be related to the prevalence of HLA alleles within the population [13]. Selective pressure induced by HLA class I restricted CTL responses against HIV can also influence the evolution of the circulating viruses in a negative way by selecting for escape mutants [14]. Previous studies on HIV-1-specific cellular immune responses in HIV-1-infected individuals have shown cross-clade immune recognition [15–17], but the issue of whether an effective vaccine based on sequences from a single HIV-1 subtype could protect also against viruses from other subtypes or variants *in vivo* remains to be resolved.

In this context, we correlated the pattern of the antigenic epitopes recognized by HIV-infected individuals infected by different HIV-1 clades with the HLA class I and II alleles expressed by such individuals.

* Corresponding author at: Department of Virology, Swedish Institute for Infectious Disease Control, Nobelsv.18, Solna, Sweden. Tel.: +46 8 4572608; fax: +46 8 337272.
E-mail address: L.Gudmundsdotter@smi.ki.se (L. Gudmundsdotter).

0264-410X/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2008.03.094

2. Materials and methods

2.1. Study subjects

Blood samples were collected from 60 individuals, recruited at the Karolinska University Hospital, Solna, infected with several subtypes of HIV-1 and at different stages of infection. Fifty percent of the subjects were Caucasian, 43% African, 3.5% Hispanic and 3.5% Asian. Median viral load was 50 RNA copies/ml blood [ranges: <50–91,000 RNA copies/ml for HAART treated patients and <50–137,000 RNA copies/ml for non-treated patients] and the absolute $CD4^+$ T cell count was 539 cells/mm^3 blood. The patient cohort included 7 patients infected with subtype A viruses, 21 with subtype B, 19 with subtype C, 7 with subtype D, 1 with subtype G infection, 4 with subtype CRF_01AE infection and 1 with subtype CRF_02AG infection [18]. The viral subtypes were determined by genotyping of the envelope and *p17* genes [19]. Thirteen of the 60 patients were treatment naive and the remaining 47 received continuous highly active antiretroviral treatment (HAART). Twenty eight of the samples were investigated for reactivity to overlapping peptides representing the entire sequence of p24 included in the plasmid DNA vaccine constructs used for prophylactic and for therapeutic vaccination [20].

2.2. $CD4^+$ T cell counts, plasma HIV-1 RNA levels and HLA classes I and II typing

$CD4^+$ T cell number in blood was measured by flow cytometry. Viral load was measured by Amplicor HIV-1 monitor test (detection limit 50 copies/ml), Roche Diagnostic Systems, Inc., Branchburg, NJ. Patients HLA type was determined to the oligo-allelic level using Dynal RELITM Reverse Sequence-Specific Oligonucleotide low resolution kits for the classes I and II HLA (HLA-A, -B, -C, -DQ, -DR, -DPA1 loci) (Dynal, Norway). DPB1 locus was characterised by HLA SSO analysis according to procedures previously described [21]. Typing was performed by sequence-specific primers SSP-PCR (Olerup SSP, GenoVision, Austria).

2.3. HIV-1 peptides for determination of peptide-specific responses

Lyophilized 15mer peptides, overlapping by 10 amino acids, covering the HIV-1 Gag p24 protein from subtypes A, B and C, were obtained from the EU Programme EVA/AVIP Centralized facility for AIDS reagents NIBSC, UK and Thermo Hybaid (Germany).

2.4. Interferon (IFN)-γ ELISpot assay

ELISpot assay was performed on both total peripheral blood mononuclear cells (PBMC) and CD8-depleted PBMC. PBMC were separated from whole blood by density gradient centrifugation on Ficoll-Paque (GE Healthcare, Uppsala, Sweden) and plated onto a 96-well plate (Millipore, MA, USA) (200,000 cells/well) that had been pre-coated with 0.5 mg/ml of anti-IFN-γ monoclonal antibody and 1-DIK (Mabtech, Stockholm, Sweden). For CD8-depleted ELISpot, $CD8^+$ cells were separated by positive $CD8^+$ T cell depletion using magnetic beads (DynaBeads, Dynal, Norway). Peptides from Gag p24 from subtypes A and B were used in a matrix containing 19 pools with 9–10 peptides in each pool. Peptides from Gag p24 subtype C were used in a matrix containing 14 pools with 6–7 peptides in each pool. In the matrix system each peptide

Table 1
Distribution HLA-DR alleles in the study population were divided by ethnicity

Caucasian					Black				
Subject ID	HLA-DR[a]		Second HLA-DR[b]		Subject ID	HLA-DR		Second HLA-DR	
	DRB1	DRB1	DR	DR		DRB1	DRB1	DR	DR
6004	*01	*13	DRB3	–	6120	*01	*13	DRB3	–
5998	*01	*09	DRB4	–	6051	*0102	*1503	–	–
6044	*01	*08	–	–	5994	*03	*1204	DRB3	–
6030	*01	*11	–	–	6033	*03	*0415	DRB3	DRB4
6073	*01	*13	DRB3	–	5986	*03	*1001	–	DRB3
6029	*01	*13	–	–	6152	*0301	*1101	–	–
5980	*01	*09	DRB4	–	6053	*07	*13	DRB3	DRB4
5979	*01	*13	–	–	6041	*0701	*0802	–	–
6048	*0101	*0301	–	–	6098	*0701	*0901	–	–
5815	*0103	*11	DRB3	–	5984	*08	*11	DRB3	–
6024	*03	*03	DRB3	–	6116	*11	*0801	–	–
6061	*03	*1309	DRB3	–	6013	*11	*13	DRB3	–
6062	*04	*07	DRB4	–	6022	*11	*15	DRB3	DRB5
6002	*04	*07	–	–	6045	*12	*15	DRB3	DRB5
5977	*0401	*1101	–	–	6037	*13	*15	DRB3	DRB5
6036	*07	*08	DRB4		5992	*13		DRB3	–
6147	*09	*11	DRB3	DRB4	6019	*13	*07	DRB3	DRB4
6097	*09	*11	DRB3	DRB4	6047	*13	*15	DRB3	DRB5
6042	*1001	*1301	–	–	6043	*13	*13	–	–
6035	*1101	*1303	–	–	6031	*13	*07	DRB3	DRB4
6034	*13	*07	DRB3	DRB4	6082	*13	*07	DRB3	DRB4
5985	*13	*15	DRB3	DRB5	6102	*1301	*11	–	–
6114	*13	*15	DRB3	DRB5					
6077	*1301	*1501	–	–					
6094	*15	*12	DRB3	DRB5					
6100	*15	*04	DRB4	DRB5					
6139	*15	*1001	DRB5	–					
5820	*15		DRB5	–					
6103	*1501	*1501	–	–					
6038	*1122		DRB5*01						

[a] The study subject were typed for their HLA-DRB1 molecules.
[b] The three second molecules (DRB3, DRB4, DRB5) that are genetically associated with HLA-DRB1 are also indicated.

was represented in two different pools allowing identification of a unique reactivity to each peptide included. The peptide pools were diluted in RPMI-1640 medium and used in a final concentration of 5 μg/ml. RPMI medium without peptides was use as a negative control. Positive controls included Cytomegalovirus, Epstein Barr virus and Influenza peptides (CEF peptides from National Institutes of Health, US), and Phytohemagglutinin (PHA, Orion Diagnostica, Sweden). Responses >2-times background value and ≥50 SFC/10^6 PBMC above background were considered as positive.

3. Results

3.1. Frequency of HLA class I and II alleles

DNA-defined HLA typing distinguished 16, 18 and 17 class I HLA-A, -B, or -C alleles respectively. In this population, the most frequent alleles were A*02(02, 03, 11, 23, 29, 31, 33, 68, 74) (35%), B*07 (07, 08, 14, 15, 35, 37, 40, 50, 56) (23.3%), C*02(03, 04, 06, 07, 12, 18) (16.7%), A*03(03, 11, 24, 26, 32, 33, 34, 68, 74) (15%), C*03(04, 06, 07, 08, 16) (14.6%), B*15 (15, 18, 35, 37, 49, 50) (14%) and B*08 (08, 14, 15, 35, 44) (11.6%). The distribution of class II HLA-DR alleles in the major ethnical groups (Caucasian and black) within the study population is shown in Table 1. Several HLA class II alleles were found at high frequencies and the most frequent alleles were DRB3 (49%), DRB5 (17%), DRB01*13 (21%), DRB01*15 (14%), DRB1*01 (13%), DQB1*05 (39%), DQB1*06 (24%), DQB1*02 (21%), DPA1*0103 (67%).

3.2. Identification of unique and common peptide reactivities

Cell samples from patients in the cohort were tested for IFN-γ ELIspot. Five patients were infected with subtype A or recombinant forms of subtype A (CRF_01AE and CRF_02AG) viruses, 14 with subtype B, 3 with subtype C, and 5 with subtype D viruses. Cells from the rest of the patients in the cohort were not analysed for either of the two following reasons; they did not show any reactivity to p24; or they were used in a cross-clade analysis of whole peptide pools covering Gag p24 A, B and Env A, B, C [18]. The response to the peptide mapping is shown in Fig. 1. Reactivity was demonstrated against 58 reactive 15 mer peptides, in total, 20 out of 46 peptides for subtypes A and B libraries, and 18 out of 46 peptides for the subtype C library. The most important finding was, that lymphocytes from different patients cross-reacted to peptides representing different subtypes and that the reactivity was distributed along all the p24 protein. All patients included here reacted with at least one peptide and the individuals with the broadest response reacted with up to six different peptides.

Peptides to which the majority of patients cross-reacted were located in the region located between peptides 25 and 35 for

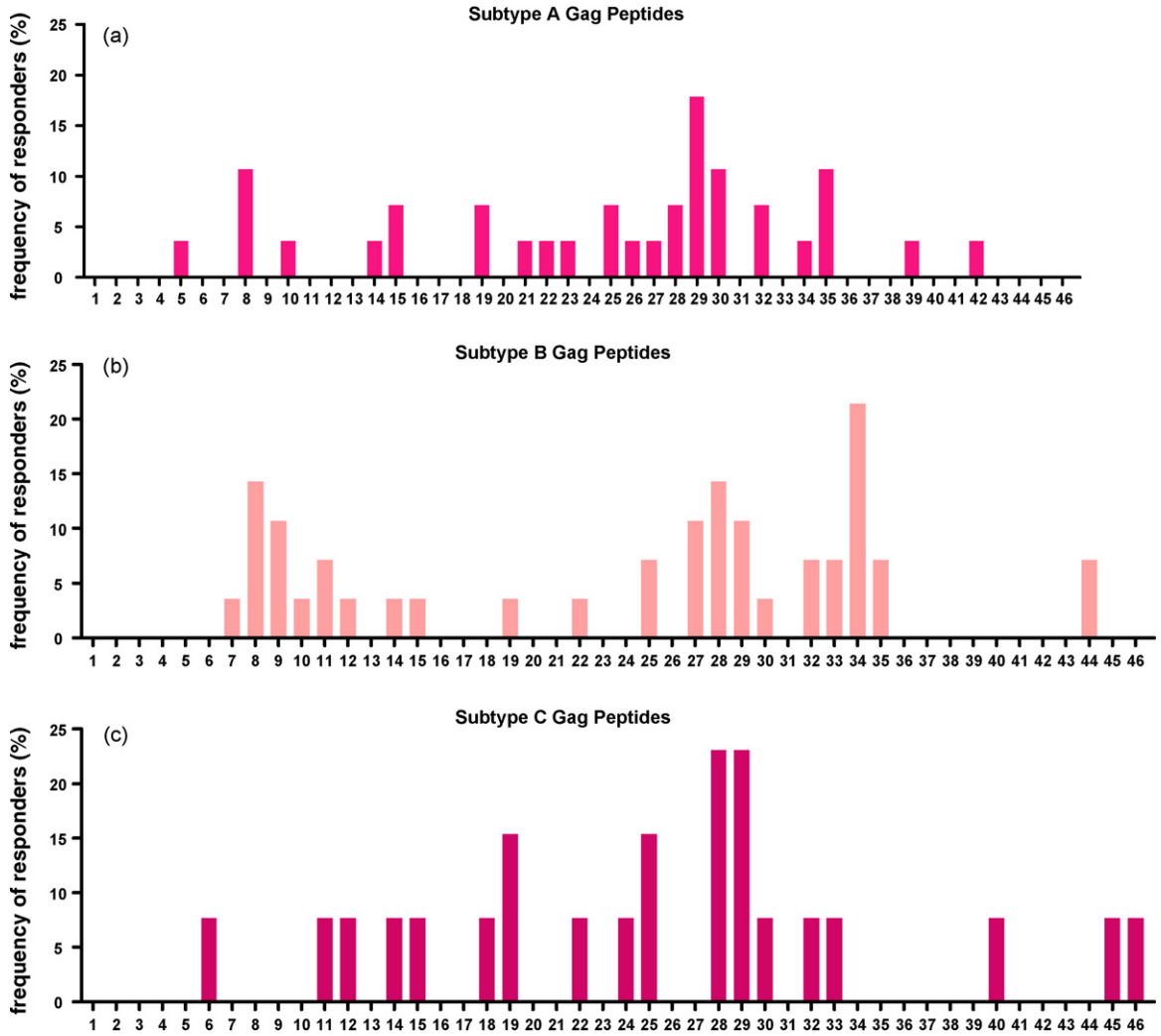

Fig. 1. Identification of individual reactive HIV-1 Gag p24 peptides. Percentage of patients responding is shown on the *y*-axis as the positive responder frequency, for Gag A and B, $n = 28$ and for Gag C, $n = 13$.

Table 2
Peptides containing epitopes associated with known HLA class I alleles

Peptide#	Peptide sequence*	Subject ID	Subject Subtype	HLA class I restricting allele
5	**VT**SPRTLNAWVKV**I**E[a]	**6114**	**D**	A02
	VTSPRTLNAWVKVIE[a]			B7
6	TLNAWVKV**I**EE**K**AFS[c]	**6114**	**D**	A2
7	VKV**V**EE**K**AFSPEVIP[b]	**5984**	**D**	B4501
8	E**K**AFSPEVIPMFSAL[a,b]	**6021**	**B**	B58
	E**R**AFSPEVIPMFSAL[a,b]	**6082**	**C**	B57
9	PEVIPMFSALSEGAT[d]	**5985**	**B**	Cw1
11	SEGATP**Q**DLN**T**MLN**T**[b,c]	**6030**	**B**	B14
	SEGATP**Q**DLN**T**MLN**T**[b,c]	**6114**	**D**	B7
12	P**Q**DLNTMLN**T**VGGHQ[b]	**6030**	**B**	B14
14	VGGHQAAM**E**MLK**D**TI[a]	**5998**	**B**	A2
	VGGHQAAM**Q**MLK**D**TI[c]	**6114**	**D**	A2
18	EWDR**V**HP**V**HAGP**I**AP[b]	**6133**	**B**	A2,
	EWDR**V**HP**V**HAGP**I**AP[b]			B7
19	HP**V**HAGP**V**APGQMRE[b]	**5998**	**B**	B35
	HP**V**HAGP**I**APGQMRE[c]	**6114**	**D**	B35
	HP**V**HAGP**V**APGQMRE[b]	**6133**	**B**	B7
24	STLQEQI**G**WMT**N**NPP[b]	**6097**	**B**	A2
27	**I**PVG**E**IYKRWIILGL[a,b,c]	**6133**	**B**	B8
28	IYKRWIILGLNKIVR[a,b,c]	**5984**	**D**	A3
	IYKRWIILGLNKIVR[a,b,c]	**6082**	**C**	A3
	IYKRWIILGLNKIVR[a,b,c]	**6103**	**AE**	A24
	IYKRWIILGLNKIVR[a,b,c]			A3
	IYKRWIILGLNKIVR[a,b,c]	**6061**	**B**	A24
29	II**L**GLNKIVRMYSP**T**[b]	**5998**	**B**	A3
	II**L**GLNKIVRMYSP**V**[a]	**6094**	**A**	A3,
	II**L**GLNKIVRMYSP**V**[a]			B15
	I**V**LGLNKIVRMYSP**V**[b]	**6097**	**B**	B15
33	**R**QGPKEPFRDYVDRF[b]	**5985**	**B**	A0201
34	EPFRDYVDRF**Y**K**T**LR[b]	**5998**	**B**	A2
	EPFRDYVDRF**Y**K**T**LR[b]	**6073**	**D**	A0201
	EPFRDYVDRF**F**K**A**LR[a]	**6094**	**A**	A3,
	EPFRDYVDRFFKALR[a]			B15
35	YVDRF**F**K**A**LRAEQA**T**	**5997**	**AE**	Cw0303
	YVDRF**Y**K**T**LRAEQA**S**[a,b]	**6030**	**B**	B14
40	LLVQNANPDCK**T**IL**K**[b,c]	**6030**	**B**	B35
	LLVQNANPDCK**T**IL**K**[b,c]	**6139**	**AE**	B7
43	ATLEEMMTACQGVGG[b]	**6073**	**D**	A2
46	QGVGGP**G**HKARVL[c]	**6139**	**AE**	B7

* Differences in amino acid sequence between subtype A, B and C peptide library are shown in bold. The reacting epitope and its association with the presenting HLA class I allele are shown by underlined peptide sequence.
[a] Reacting peptide derived from Gag subtype A library.
[b] Reacting peptide derived from Gag subtype B library.
[c] Reacting peptide derived from Gag subtype C library.

subtype A, in the regions comprised between peptides 7–15 and peptides 25–35 for subtype B and in the region comprised between peptides 28–33 for subtype C (Fig. 1). In the subtype A peptide sequence (Fig. 1a), the most recognised peptide was peptide 29, corresponding to the sequence IILGLNKIVRMYSPV, that was recognised by cells from 5 patients (1 infected with subtype A, 4 with subtype B). In the subtype B peptide sequence (Fig. 1b), the most recognised peptide was peptide 34 corresponding to the sequence EPFRDYVDRFYKTLR that reacted with lymphocytes from 6 patients (1 infected with subtype A, 3 with subtype B and 2 with subtype D). In the subtype C peptide sequence (Fig. 1c), the 2 peptides most frequently recognised were 28 and 29 corresponding to the sequences IYKRWIILGLNKIVR and IILGLNKIVRMYSPV that were recognised by 3 patients all infected with subtype B.

3.3. Association of HIV-1 peptide responses with HLA class I alleles

Cell reactivity to p24 peptides was associated to the known HLA class I and II alleles. Fifteen individuals recognised peptides containing epitopes that have been previously described to bind the HLA class I alleles expressed by the patients listed in Table 2. The epitope associations are defined by information from the Los Alamos HIV database (http://www.hiv.lanl.gov/content/immunology/tables/ctl_summary.html). Within these known epitope/allele associations we found no significant correlation between the infecting virus subtype and the subtype of the recognized peptide sequence. Importantly, seven patients showed cross-reactivity and reacted with either two or three variants of the same peptide. Five individuals displayed reactivity to a peptide that contains more than one epitope that could be restricted

Table 3
Peptides containing epitopes not previously associated with these particular HLA class I alleles

Peptide #	Peptide sequence	Subject ID	Subject subtype	HLA class I	HLA class II
10	MFSALSEGATP**H**DLN[a]	6114	D	A*02/*11; B*07/*35, Cw*04/*07	DRB1*13/*15; DRB3; DRB5; DQB1*06/*06; DPA1*0103/*0103; DPB1*0201/*0401
15	AAM**Q**MLK**D**TINEEAA[b,c]	6133	B	A*02/*03; B*07/*08; Cw*07/*16	DRB1*1301/*1501; DQB1*06/*06; DPA1*0103/*0202, DPB1(ND)
25	QI**G**WMTNNPPIPVG**E**[a,b]	6102	A/G	A*23/*23; B*15/*49; Cw*02/*07	DRB1*1301/*11; DQB1*05/*05; DPA1*0201/*0201; DPB1*0101/*1301
30	NKIVRMYSP**V**SILDI[a]	6102	A/G	A*23/*23; B*15/*49; Cw*02/*07	DRB1*1301/*11; DQB1*05/*05; DPA1*0201/*0201; DPB1*0101/*1301
32	SILDIKQGPKEPFRD[a]	6094	A	A*0308/*24; B*15/*18, Cw(ND)	DRB1*15/*12; DRB3; DRB5; DQB1*06/*03; DPA1*0103/*0103; DPB1(ND)
33	**R**QGPKEPFRDYVDRF[a,b]	6102	A/G	A*23/*23; B*15/*49; Cw*02/*07	DRB1*1301/*11; DQB1*05/*05; DPA1*0201/*0201; DPB1*0101/*1301
42	KSIL**RG**LG**AG**ATLEE[a]	6094	A	A*0308/*24; B*15/*18, Cw(ND)	DRB1*15/*12; DRB3; DRB5; DQB1*06/*03; DPA1*0103/*0103; DPB1(ND)
46	QGVGGP**G**HKARVL[b,c]	6030	B	A*02/*33; B*14/*35; Cw*04/*1214	DRB1*01/*11; DQB1*05/*05; DPA1*01/DPA1*0201; DPB1*0201/*1001

Difference in amino acid sequence between subtype A, B and C peptide library are shown in bold.
[a] Reacting peptide derived from Gag subtype A library.
[b] Reacting peptide derived from Gag subtype B library.
[c] Reacting peptide derived from Gag subtype C library.

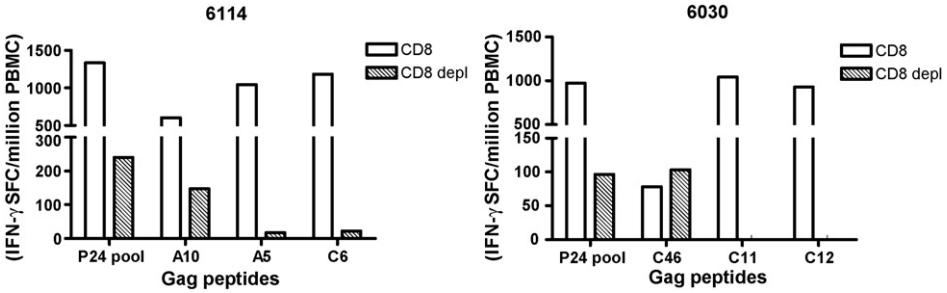

Fig. 2. IFN-γ ELISpot responses to gag 15 mer peptides in two individuals, identification of $CD8^+$ and $CD4^+$-specific T cell responses were distinguished by depletion of CD8 T cells.

by the patients HLA alleles. To pin point the exact epitope/allele association a further analysis has to be performed.

3.4. Putative HLA class II reactivities

Five individuals (6114, 6133, 6102, 6094 and 6030) infected with virus subtypes A, B, D and CRF02_AG, reacted with 8 peptides harbouring epitopes not previously associated with their particular HLA alleles (Table 3). To further substantiate the reactivities PBMC from subjects 6114 and 6030 were evaluated for specific $CD4^+$ and $CD8^+$ T cell reactivity by IFN-γ ELISpot (Fig. 2). Both subjects show $CD4^+$ and $CD8^+$ T cell responses towards the whole pool of Gag p24 (Fig. 2a and b). *Ex vivo*, both patients 6030 and 6114 show HIV-specific $CD8^+$ reactivity to peptides A5, C6, C11 and C12 and no $CD4^+$ T cell response and therefore confirming the peptide/HLA class I association described in Table 2. In addition, subjects 6114 and 6030 show both $CD4^+$ and $CD8^+$ T cell responses against peptides 10 (from subtype A) and 46 (from subtype C). This suggests that these peptides harbour epitopes restricted by the patient's HLA class I alleles but the epitopes are also situated within a $CD4^+$ region restricted by the patient's HLA class II alleles described in Table 3.

4. Discussion

A better understanding of the differences in HIV-1 immune response in populations from various ethnic groups is important for vaccine development. To address this need, we enrolled a cohort of 60 individuals in Sweden originating from different regions in the world and infected with HIV-1 of different subtypes. Surprisingly, when peptide pools representing the whole p24 from HIV-1 subtypes A, B and C, a broad cross-reactivity was detected, irrespective of their stage of disease or antiretroviral treatment. We found no significant correlation between the subtype of the infecting virus and the subtype of the peptide sequence recognized. On the contrary, the majority of the epitopes that elicited cross-reactive responses had identical amino acids in the anchor position of the epitope, hence even only partial sequence homology between subtypes in T cell immunogenic regions may account for the majority of cross-clade T cell responses. To a lesser extent, T cells may recognize different epitopes from more than one clade.

The HLA class II typing of the patients in this cohort revealed a spread among all different genotypes. Six individuals infected with subtypes A, B, D and CRF02_AG reacted to eight peptides harbouring epitopes not previously associated with those particular HLA class I alleles. Therefore reactivity to these peptides might be attributed to a HLA class II presentation. In two of these patients both $CD4^+$ and $CD8^+$ T cell responses were detected towards the described peptides. Due to lack of material, all patients could not be analysed in this way. There is no easy way to analyse the association between peptide epitopes and class II alleles and therefore no strong consistent genetic epidemiological effects of particular class II alleles have been identified in HIV-1 disease in sharp contrast to those observed for class I. New techniques to define the affinity and the avidity between class II monomers or tetramers and peptides are under development, although these techniques might not reveal whether the binding between peptide and HLA protein actually elicits a functional immune response. Both $CD8^+$ cytolytic and $CD4^+$ T-helper responses play impor-

tant roles in protection against HIV-1 infection and are necessary for maintaining an effective T-lymphocyte response against HIV [22–25] Obviously, studies of HLA class I and II allele frequencies and distribution must be extended, particularly in geographical regions with ethnic differences and high HIV-1 incidence rates.

In conclusion, we have demonstrated that Gag-specific cross-clade T cell responses can be detected in chronic HIV-1 infection and that subtype cross-reactive Gag responses dominate the late natural T cell response. We discovered a multitude of immunological responses to Gag peptides, which in turn could be associated with known epitopes of HLA classes I and II. Fifteen individuals had class I responses related to known epitopes and displayed cross-clade responses irrespective of subtype of virus infection. Previous studies have shown effective cross-recognition of several HIV-1 proteins [16,26,27]. Within these epitope/allele associations we found no significant correlation between the infecting virus subtype and the subtype of the recognized peptide sequence. This indicates that genetic vaccines representing a few circulating strains may act to induce immunity to a broad range of the HIV-1 viruses circulating worldwide.

References

[1] Wahren B, Morfeldt-Månsson L, Biberfeld G, Moberg L, Ljungman P, Nordlund S, et al. Impaired specific cellular response to HTLV-III before other immune defects in patients with HTLV-III infection. New Engl J Med 1986;315:393–4.

[2] Borrow P, et al. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary immunodeficiency virus type 1 infection. J Virol 1994;68:6103–10.

[3] Yang OO, Sarkis PT, Ali A, Harlow JD, Brander C, Kalams SA, et al. Determinant of HIV-1 mutational escape from cytotoxic T lymphocytes. J Exp Med 2003;197:1365–75.

[4] Ogg GS, Jin X, Bonhoeffer S, Dunbar PR, Nowak MA, Monard S, et al. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science 1998;279(5359):2103–6.

[5] Harrer E, Harrer T, Buchbinder S, Mann DL, Feinberg M, Yilma T, et al. HIV-1-specific cytotoxic T lymphocyte response in healthy, long-term nonprogressing seropositive persons. AIDS Res Hum Retroviruses 1994;10(Suppl. 2): S77–8.

[6] Hejdeman B, Bostrom AC, Matsuda R, Calarota S, Lenkei R, Fredriksson EL, et al. DNA immunization with HIV early genes in HIV type 1-infected patients on highly active antiretroviral therapy. AIDS Res Hum Retroviruses 2004;20(8):860–70.

[7] Rosenberg ES, Billingsley JM, Caliendo AM, Boswell SL, Sax PE, Kalams SA, et al. Vigorous HIV-1-specific $CD4^+$ T cell responses associated with control of viremia. Science 1997;278(5342):1447–50.

[8] Norris PJ, Rosenberg ES. Cellular immune response to human immunodeficiency virus. AIDS 2001;15(Suppl. 2):S16–21.

[9] Leandersson AC, Gilljam G, Fredriksson M, Hinkula J, Alaeus A, Lidman K, et al. Cross-reactive T-helper responses in patients infected with different subtypes of human immunodeficiency virus type 1. J Virol 2000;74(10): 4888–90.

[10] Heeney JL. The critical role of CD4(+) T-cell help in immunity to HIV. Vaccine 2002;20(15):1961–3.

[11] Carrington M, O'Brien SJ. The influence of HLA genotype on AIDS. Annu Rev Med 2003;54:535–51.

[12] Hendel H, Caillat-Zucman S, Lebuanec H, Carrington M, O'Brien S, Andrieu JM, et al. New class I and II HLA alleles strongly associated with opposite patterns of progression to AIDS. J Immunol 1999;162(11):6942–6.

[13] Goulder PJ, Brander C, Annamalai K, Mngqundaniso N, Govender U, Tang Y, et al. Differential narrow focusing of immunodominant human immunodeficiency virus gag-specific cytotoxic T-lymphocyte responses in infected African and Caucasoid adults and children. J Virol 2000;74(12):5679–90.

[14] Moore CB, John M, James IR, Christiansen FT, Witt CS, Mallal SA. Evidence of HIV-1 adaptation to HLA-restricted immune responses at a population level [see comment]. Science 2002;296(5572):1439–43.

[15] Ferrari G, Humphrey W, McElrath MJ, Excler JL, Duliege AM, Clements ML, et al. Clade B-based HIV-1 vaccines elicit cross-clade cytotoxic T lymphocyte reactivities in uninfected volunteers. Proc Natl Acad Sci USA 1997;94(4):1396–401.

[16] Coplan PM, Gupta SB, Dubey SA, Pitisuttithum P, Nikas A, Mbewe B, et al. Cross-reactivity of anti-HIV-1 T cell immune responses among the major HIV-1 clades in HIV-1-positive individuals from 4 continents. J Infect Dis 2005;191(9):1427–34.

[17] Malhotra U, Nolin J, Mullins JI, McElrath MJ. Comprehensive epitope analysis of cross-clade Gag-specific T-cell responses in individuals with early HIV-1 infection in the US epidemic. Vaccine 2007;25(2):381–90.

[18] Gudmundsdotter L, Sjodin A, Bostrom AC, Hejdeman B, Theve-Palm R, Alaeus A, et al. Therapeutic immunization for HIV. Springer Semin Immunopathol 2006;28(3):221–30.

[19] Leitner T, Albert J. The molecular clock of HIV-1 unveiled through analysis of a known transmission history. Proc Natl Acad Sci USA 1999;96:10752–7.

[20] Wahren B, Hejdeman B, Gotch F, Ensoli B, Sandström E. New measures for immunotherapy in HIV infection. Keystone conferences; 2004 [Abstract].

[21] Stewart CA, Horton R, Allcock RJ, Ashurst JL, Atrazhev AM, Coggill P, et al. Complete MHC haplotype sequencing for common disease gene mapping. Genome Res 2004;14(6):1176–87.

[22] Altfeld M, Rosenberg ES. The role of CD4(+) T helper cells in the cytotoxic T lymphocyte response to HIV-1. Curr Opin Immunol 2000;12(4):375–80.

[23] Kalams SA, Walker BD. The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. J Exp Med 1998;188(12):2199–204.

[24] Ndung'u T, Gaseitsiwe S, Sepako E, Doualla-Bell F, Peter T, Kim S, et al. Major histocompatibility complex class II (HLA-DRB and -DQB) allele frequencies in Botswana: association with human immunodeficiency virus type 1 infection. Clin Diagn Lab Immunol 2005;12(9):1020–8.

[25] Wahren J, Eriksson LS. The influence of a long-acting somatostatin analogue on splanchnic haemodynamics and metabolism in healthy subjects and patients with liver cirrhosis. Scand J Gastroenterol Suppl 1986;119:103–8.

[26] Butto S, Fiorelli V, Tripiciano A, Ruiz-Alvarez MJ, Scoglio A, Ensoli F, et al. Sequence conservation and antibody cross-recognition of clade B human immunodeficiency virus (HIV) type 1 Tat protein in HIV-1-infected Italians, Ugandans, and South Africans. J Infect Dis 2003;188(8):1171–80.

[27] Yu XG, Lichterfeld M, Perkins B, Kalife E, Mui S, Chen J, et al. High degree of interclade cross-reactivity of HIV-1-specific T cell responses at the single peptide level. AIDS 2005;19(14):1449–56.