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Human CD4$^+$ T lymphocytes recognize a highly conserved epitope of human T lymphotropic virus type 1 (HTLV-1) *env* gp21 restricted by HLA DRB1*0101

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SUMMARY

HTLV-1 causes two distinct human diseases, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T cell leukaemia/lymphoma (ATL). Persistently infected individuals carry a risk of < 1% of developing either disease. These basic epidemiological data imply that virus–host interactions, especially immunogenetic factors, influence the outcome of infection. Several studies showed that the HLA class II DR1 DQ5 haplotype is over-represented in HAM/TSP, but rare in ATL. Therefore, we selected four patients with HAM/TSP and one seronegative control who all carried the HLA DR1 DQ5 haplotype. We analysed the CD4$^+$ T lymphocyte response against eight synthetic peptides of HTLV-1 envelope (*env*) glycoprotein gp21, a crucial target antigen in HAM/TSP. The first of two immunodominant epitopes corresponded to a domain of the HTLV-1 envelope protein which had previously been shown to be essential for HTLV-1 envelope function. The second immunodominant epitope overlapped a highly conserved sequence of the retroviral transmembrane envelope protein. DR1 (DRB1*0101)-restricted T lymphocytes were activated by the conserved peptide sequence in nanomolar concentrations. In contrast, this conserved sequence can also induce non-specific, cAMP-mediated immunosuppressive effects on T cells when added in micromolar concentrations to culture media, as shown by Haraguchi S, Good RA, James-Yarish M, Cianciolo GJ, Day NK, Proc Natl Acad Sci USA 1995; **92**:5568–71. Hence, HTLV-1 *env* gp21 might exert either stimulating immunological or immunosuppressive effects in HTLV-1-infected individuals, depending on the level of its expression and the presence of HLA DRB1*0101.

Keywords HTLV-1 T lymphocytes epitope HLA

INTRODUCTION

HTLV-1 infection is associated with two distinct diseases, a neoplastic disease, adult T cell leukaemia/lymphoma (ATL) [1], and a chronic inflammatory disease of the spinal cord, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [2,3]. Either disease occurs in a small percentage of HTLV-1-infected individuals. The relatively rare occurrences of diseases in virus-infected humans and the different patterns of clinical diseases are thought to be linked to immunogenetic host factors. HAM/TSP patients suffer from spastic paraparesis and urinary symptoms, furthermore there is a frequent association with sicca syndrome, uveitis or arthritis. HAM/TSP is regarded as being an immunomediated disease.

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Certain HLA antigens and haplotypes are segregated between patients with ATL and HAM/TSP [4–8]. The HLA haplotypes predominant in HAM/TSP are associated with a high immune responsiveness against HTLV-1 [4]. The most significant association between HAM/TSP and HLA is represented by HLA-DR1 (HLA DRB1*0101) [6,8], which is known to be in strong linkage disequilibrium with HLA-DQ5 (HLA DQB1*0501).

HLA class II antigens have a strong effect on the epitope specificities of the CD4$^+$ T cell immune response. To date, there is evidence of a significant CD4$^+$ T cell response against HTLV-1 antigens [9–11], and also against central nervous system (CNS) autoantigen in HAM/TSP [12], but little is known about CD4$^+$ T cell epitopes apart from one established epitope of HTLV-1 gp46 [9]. Furthermore, there is a lack of knowledge regarding the relation of particular HLA class II molecules to CD4$^+$ T cell epitopes of HTLV-1 proteins. We found that there are far more

Table 1. Synthetic peptides of HTLV-1 *env* gp21 used to generate human T lymphocyte lines.

Synthetic peptides	Amino acid numbers	Sequence*
sp332	332–352	GGITGSMSLASGKSLLHEVDKD
sp343	343–363	GKSLLHEVDKDISQLTQAIVK
sp350	350–386	VDKDISQLTQAIVKNHKNLLKIAQYAAQNRRGLDLLF
sp378	378–398	NRRGLDLLFWEQGGLCKALQE
sp382	382–403	LDLLFWEQGGLCKALQEQCRFP
sp400	400–426	CRFPNITNSHVPILQERPPLENRVLTG
sp426	426–448	GWGLNWDLGLSQWAREALQTGIT
sp458	458–488	LAGPCILRQLRHLPSRVRYPHYSLIKPESSL

* Single-letter code for amino acids.

$CD4^+$ T cell epitopes in the transmembrane part of the HTLV-1 envelope (*env*) glycoprotein gp21 than in the extramembrane part of the envelope protein gp46, when we analysed the $CD4^+$ T cell repertoire in peripheral blood mononuclear cells (PBMC) from HTLV-1^-, 'naive' individuals with different HLA haplotypes [13]. Furthermore, in HAM/TSP, the B cell immune response against HTLV-1 is directed against a diversity of epitopes, both in the systemic and CNS compartment [14], but HTLV-1 gp21-specific antibodies were found frequently in those patients carrying HAM/TSP-associated HLA haplotypes [15].

Since B cell immune responses against peptide epitopes are controlled by the $CD4^+$ T cell immune response [16], it is worthwhile to look for a particular association: the $CD4^+$ T cell immune response against the HTLV-1 transmembrane envelope protein gp21, an important intrathecal target antigen [15] in HLA-$DR1^+$ individuals who are at a higher risk of developing HAM/TSP [6,8]. This study was designed to establish immunodominant $CD4^+$ T cell epitopes of HTLV-1 gp21, to analyse their functional importance for HLA binding, and to correlate them to the B cell epitopes of HTLV-1 gp21 established previously [14,15].

MATERIALS AND METHODS

Origin of lymphocytes and HLA typing

Four patients with HAM/TSP (diagnosed according to established guidelines [17]) and one control who was both HTLV-1 polymerase chain reaction (PCR)-negative and seronegative were selected, who all carried the HLA class II DR1 DQ5 haplotype. Three out of four HAM/TSP patients were homozygote for this haplotype.

HLA antigens of HAM/TSP patients and the control individual were determined serologically by the standard National Institutes of Health (NIH) microcytotoxicity test using sera which were standardized to the criteria and nomenclature of the 11th International Histocompatibility Workshop. The linkage of HLA 5 locus haplotypes in HAM/TSP patients was confirmed by family HLA studies as described [4,5]. In the seronegative control individual HLA phenotyping revealed a combination of HLA class II antigens, which also implicates the presence of the HLA class II DR1 DQ5 haplotype because of known linkage disequilibrium. Recently, our HLA haplotype data were confirmed and extended by HLA genotyping [8] using the PCR-restriction fragment length polymorphism (RFLP) method [18].

Microculture technique for selection of peptide-specific T cell lines

Eight synthetic peptides of the HTLV-1 gp21 amino acid sequence were provided by M. Nakamura (Kyushu University, Fukuoka, Japan) (Table 1). These peptides had been purified by high performance liquid chromatography (HPLC), and several had been used in serological studies, including our own studies of intrathecal antibody synthesis [14,15]. Furthermore, overlapping 15 mer synthetic peptides were made using amide resin chemistry as described [13], based on the known sequence of HTLV-1 gp21. The latter peptides were used to identify the minimal essential HTLV-1 gp21 amino acid sequences to stimulate antigen-specific T cell proliferation.

We used a T lymphocyte microculture approach which allows selection of antigen-specific T cell lines from PBMC and which was originally established to analyse the T lymphocyte repertoire against myelin basic protein in multiple sclerosis [19,20]. Briefly, PBMC were isolated from peripheral blood using standard density gradient centrifugation. PBMC were suspended in RPMI maintenance medium which contained 5% inactivated (56°C for 30 min) human serum, penicillin as well as streptomycin. Cells were exposed to the mixture of eight HTLV-1 gp21 peptides (Table 1), each at a concentration of 1 μg/ml. The cell suspension was distributed in round-bottomed 96-well culture plates (Nunc, Roskilde, Denmark, or Iwaki, Funabashi, Japan) at $2{\cdot}5\times10^4$, 5×10^4, 10^5, and 2×10^5 PBMC/well. For each experiment, at least 200 microcultures were set up and kept strictly separate. Four days after the start of culture, IL-2 (Takeda, Japan) was added to the microcultures to give a final concentration of 1 U/ml. Microcultures were supplemented with fresh medium containing IL-2 (final concentration 1 U/ml) every 2–4 days depending on culture growth.

On day 14 the cells in 96-well culture plates were centrifuged (Kubota cytocentrifuge; Tokyo, Japan) and washed once with PBS. After the last centrifugation step, PBS was replaced with RPMI maintenance medium. The eight gp21 peptides (each at a final concentration of 1 μg/ml RPMI maintenance medium) and antigen-presenting cells (APC), but no exogenous IL-2, were added to each microculture to restimulate T cells. DR1 DQ5-matched HTLV-1 PCR and seronegative donor PBMC treated by mitomycin C (2×10^5 PBMC/well) were used as APC. After another 4 days maintenance medium and exogenous IL-2 (final concentration 1 U/ml) were added. Fresh IL-2-containing medium was supplemented until day 28 of culture depending on lymphocyte proliferation.

On day 28 the cells in 96-well culture plates were centrifuged again and washed three times with PBS. After the last centrifugation

Table 2. Fine specificities of T lymphocyte lines against HTLV-1 *env* gp21 established from DR1 (DRB1*0101)-positive donors

	HLA class II				Proliferation*†								
Donor	DRB1	DQB1	T cell line	HLA restriction†	No Ag	sp332	sp343	sp350	sp378	sp382	sp400	sp426	sp458
Seronegative control	0101	0501											
	1301	0603											
			BK1C11	ND	306	283	260	307	17607	4153	257	258	5149
			BK2C7	ND	964	909	904	746	718	630	22689	570	616
			BK4E5	ND	316	282	291	272	287	368	7146	188	414
			BK4G11	ND	317	364	262	275	3954	771	347	329	323
HAM/TSP 1	0101	0501											
	0405	0401											
			910/6C5	ND	406	451	380	470	1785	626	390	239	433
			910/6F9	ND	883	620	687	721	4279	2199	5917	1254	1116
			910/8G7	DRB1*0101	376	257	204	401	34848	8186	1965	366	347
			910/5E5	ND	606	752	706	755	466	11108	4590	10813	798
			910/5C9	ND	1074	1291	1005	1066	3854	1382	4168	591	489
HAM/TSP 2	0101	0501											
			6D3	ND	1679	4139	2058	1905	2790	1475	2477	1888	1572
HAM/TSP 3	0101	0501											
			NB89	DRB1*0101	825	725	684	624	1523	1354	2147	648	2015
			NC9	DRB1*0101	78	121	96	98	5052	691	125	135	105
HAM/TSP 4	0101	0501											
			TG7	DRB1*0101	119	103	120	142	3472	869	239	172	235

* Significant results are underlined, i.e. the stimulation index is > 2.
† ND, Not done; no Ag, no antigen (control cultures).

step, PBS was replaced with maintenance medium without IL-2. Two suspensions of APC were prepared from mitomycin C-treated PBMC of the DR1 DQ5-matched HTLV-1$^-$ donor. One was preincubated for 1 h with the HTLV-1 gp21 peptides at final concentrations of 1 μg/ml for each peptide, while the other suspension acted as a control and was not exposed to peptides. The original washed microcultures were split into two wells in new microculture plates and cocultured with one or the other APC suspension. These duplicate microcultures were incubated for another 4 days and examined daily for microscopic evidence of antigen-specific cell proliferation, i.e. proliferation only in the presence of antigen. Microcultures which showed proliferation both with and without antigen were discarded. Precursor frequencies were estimated following established principles [21] as described previously [13]. According to the screening procedure, positive microcultures were selected, transferred to 24- or 12-well culture plates and further propagated in two to three cycles of restimulation in the presence of APC and antigen, followed by IL-2-driven proliferation (concentrations as described above).

T lymphocyte proliferation assay

After antigen-specific T cell lines had been washed three times in PBS, their specificities were assayed in the presence of APC and different peptides. Microcultures were subjected to the standard ^{3}H-thymidine incorporation assay by pulsing with 1 μCi ^{3}H-thymidine (specific activity 25 Ci/mmol) per well and harvesting after 16 h. In all experiments DR1 DQ5-matched HTLV-1$^-$ donor PBMC treated by mitomycin C (2×10^5 PBMC/well) were used as APC. Concentrations of antigens and MoAbs were as described in the figures and tables. The following anti-HLA antibodies were used: anti-HLA-ABC (Dako, Glostrup, Denmark), anti-HLA-DR, anti-HLA-DQw1/3 and anti-HLA-DP (Becton Dickinson, Mountain View, CA). Recombinant HTLV-1 p21 (amino acids 326–433; Intracel Corp., Cambridge, MA) was employed as a positive control. Experiments were performed in triplicate. Antigen-specific proliferation was regarded to be significant if the stimulation index (SI; mean of antigen-stimulated cultures/mean of control cultures without antigen) was > 2. The s.d. of triplicates was < 15% of the mean, except in control cultures (without antigen) showing a very low spontaneous proliferation.

Search for homologies and phylogenetic analysis

In order to detect possible cross-reactivities with other proteins, the SWISSPROT database was searched for homologies of the newly defined HTLV-1 gp21 epitope sequences (using the server of the Computational Biochemistry Research Group, ETH Zürich, Switzerland). After finding retroviral homologous proteins, a multiple alignment was performed by selection of envelope sequences from different retroviral genera [22].

In order to find expressed homologous human sequences, a similarity search of the HTLV-1 gp21 amino acid sequences was performed using the TBLASTN algorithm and the Genebank database of expressed sequence tags, GeneBank EST (at the server of the NCBI, Bethesda, MD).

RESULTS

HTLV-1 env gp21-specific T cell lines

Using the T cell microculture approach and HTLV-1 non-infected, HLA-DR1 -DQ5-matched PBMC as APC, T lymphocyte lines

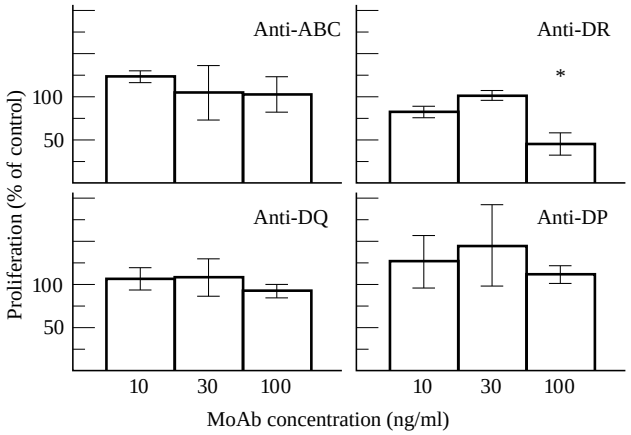

Fig. 1. Inhibition of proliferation by anti-MHC MoAbs. In a representative experiment using T cell line 910/8G7, there was a significant inhibition of antigen-specific proliferation by anti-HLA-DR antibodies (*). Data are expressed as mean + s.d.

were established against HTLV-1 gp21 synthetic peptides (Table 2). These T cell lines had the $CD3^+CD4^+$ phenotype (results not shown), as expected because of the selection procedure. In the presence of HLA-DR1 -DQ5-matched APC and antigen, proliferation of T cell lines could be blocked by a MoAb against HLA-DR, but not against other HLA class II or against HLA class I (as shown in a representative experiment in Fig. 1; summarized in Table 2). Under the conditions of the microculture approach, we estimated the precursor cell frequencies in the seronegative control and the non-homozygote HAM/TSP patient: 1 out of 10^6 PBMC and 1 out of 6×10^4 PBMC, respectively.

Table 4. Alignment of the conserved retroviral sequence with homologous, expressed sequence tags (EST) of human origin

Sequence*	Origin of mRNA	Alignment
Q82234	HTLV-1 env gp21	QNRRGLDLLFWEQGGLCKAL
T95945	Fetal liver and spleen	QNRRGLDMLTAAQGGICLAL
T9210	Fetal liver and spleen	QNHRGLDLLTVEKGGLCTFL
R24496	Placenta	QNHRGLDLLTAEKGGLCIFL
H53521	Fetal liver and spleen	QNHRGLDLLTAEKGGLCIFL
R32560	Placenta	QNHRGLDLLTAEKGGLCIFL
R66837	Placenta	QNHRGLDLLTAEKGGLCIFL
R67128	Placenta	QNHRGLDLLTAEKGGLCIFL
R71049	Placenta	QNHRGLDLLTAEXGGLCIFX

A TBLASTN search of the GeneBank EST database was performed. Plus strand nucleotide sequences were translated into amino acid sequences.

* GeneBank accession number.

Table 3. Alignment of conserved retroviral sequences corresponding to HTLV-1 *env* amino acids 377–396

Retrovirus*	Protein	Homologous sequence
HTLV-BLV group		
HTLV-1	gp21	QNRRGLDL**LFWEQG GLCK**AL
HTLV-2	gp21	QNRRGLDL**LFWEQG GLCK**AI
STLV	gp21	QNRRGLDL**LFWEQG GLCK**AL
BLV	gp30	QNRRGLDW**LYIRLGFQSLCP**TI
MLV-related viruses		
FENV	p15	QNRRGLDL**LFLQEG GLCA**AL
MLV	p15	QNRRGLDL**LFLKEG GLCA**AL
AVIRE	gp22	QNRRGLDL**LTAEQG GICL**AL
Type D retrovirus group		
BAEVM	p20	QNRRGLDL**LTAEQG GICL**AL
MPMV	gp20	QNRRGLDL**LTAEQG GICL**AL
Lentivirus group		
HIV-1		No strong homology
HIV-2		No strong homology
Mammalian type B oncovirus group		
MMTV		No strong homology
Foamy virus group		
FOAMV		No strong homology

Apart from members of the HTLV-BLV group, members of five other retrovirus groups have been included [20].

* HTLV-1, Human T cell leukaemia virus type 1 (strain ATK); HTLV-2, human T cell leukaemia virus type 2; STLV, simian T lymphotropic virus type 1 (isolate A212); BLV, bovine leukaemia virus (American isolate FLK); FENV, feline endogenous virus ECE1; MLV, friend murine leukaemia virus (isolate 57); AVIRE, avian reticuloendotheliosis virus; BAEVM, baboon endogenous virus (strain M7); MPMV, simian Mason-Pfizer virus (MPMV); HIV-1, human immunodeficiency virus type 1 (ARV2/SF2 isolate); HIV-2, human immunodeficiency virus type 2 (isolate BEN); MMTV, mouse mammary tumour virus (strain BR6); FOAMV, human spumaretrovirus (foamy virus).

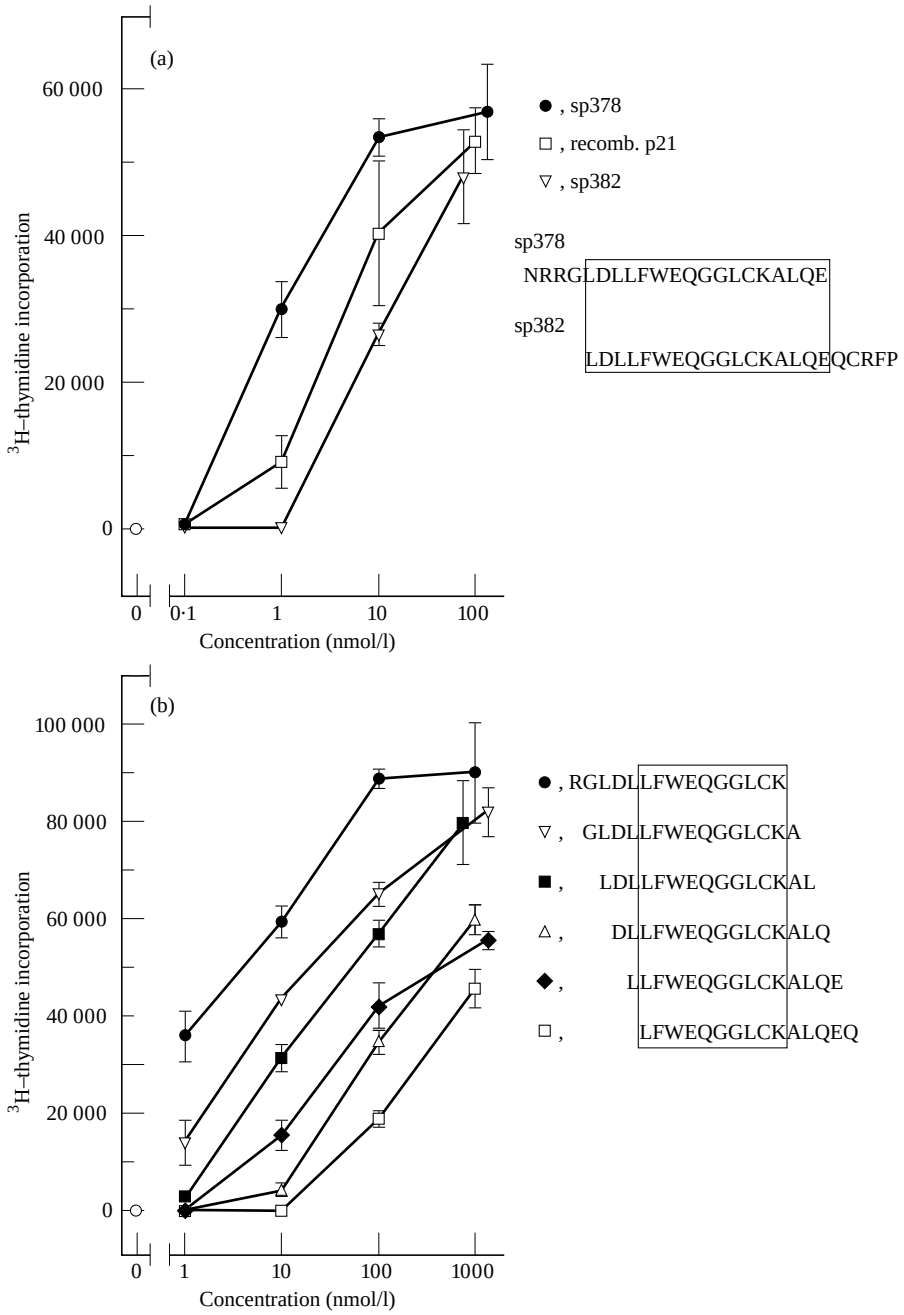

Fig. 2. Proliferation of a HTLV-1 gp21 sp378/sp382-specific CD4$^+$ T cell line (BK/1C11) depending on concentration and amino acid sequences of peptides. (a) Response to two long overlapping synthetic peptides, sp378 and sp382, which were used for selection of T cell lines, and to recombinant HTLV-1 p21. (b) Epitope analysis using short overlapping synthetic peptides with one amino acid shift. Values are expressed as mean + s.d. of triplicated data per point.

Fine specificities of T cell lines

Some T cell lines showed a proliferative response against more than one peptide, others against a single peptide (Table 2). Two specificities were most common: against the overlapping synthetic peptides sp378/sp382 and against sp400 (Table 2). These epitopes were further analysed using shorter synthetic peptides. An essential sequence of the sp378/sp382 epitope was 10 amino acids long (LFWEQGGLCK; Fig. 2), but T cell proliferation was greatly enhanced by a stretch of the N-terminal amino acids (Fig. 2b). A peptide lacking the leucine at the N-terminal end (FWEQGGLCKALQEQC) did not activate the T cell lines (data not shown). With respect to the sp400 epitope, the essential sequence consisted of 11 amino acids (RPPLENRVLTG; Fig. 3).

Homology analysis of HTLV-1 gp21 T cell epitopes

SWISSPROT database screening for homologies and subsequent phylogenetic analysis revealed that the sp378/sp382 epitope is localized in a highly conserved part of the retroviral envelope sequence, but there are no significant homologies to lentivirus, spumaretro-

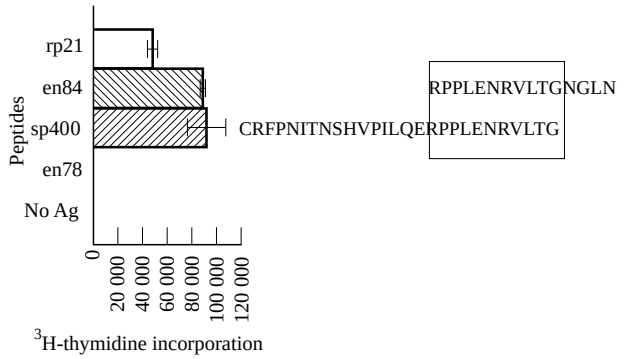

Fig. 3. Analysis of epitope specificity of T cell line BK/2C7 for HTLV-1 gp21 using different overlapping peptides. Only recombinant p21 (rp21), peptides sp400 (amino acids 400–426) and en84 (amino acids 416–430) elicited a strong proliferative response, whereas all other peptides, including peptide sequences 396–410, 401–415, 406–420, 411–425, were negative (for clarity only one negative peptide is shown). Peptide concentrations were 1 μmol*l*. Data are expressed as mean + s.d.

virus and MMTV envelope proteins (Table 3). The sp400 epitope has a very low homology to the same retroviral genera and BLV, but a high homology to HTLV-2 and STLV (results not shown).

Furthermore, we searched the database of expressed human sequence tags (GeneBank EST database) for homologies with the conserved retroviral sequence which includes the sp378/sp382 epitope. Several human sequences could be identified which had previously been found to be expressed in human fetal tissues (Table 4).

DISCUSSION

In our study, we employed a microculture approach to select HTLV-1 transmembrane envelope protein gp21-specific $CD4^+$ T cell lines from PBMC of four HTLV-1-infected and one non-infected individual who all carried the HLA class II DR1 DQ5 haplotype. Since HTLV-1 gp21-specific T cells could be isolated from the HTLV-1 non-infected donor, this T cell response appears to be part of the normal human T cell repertoire. T cell lines against the two common HTLV-1 gp21 epitopes were restricted by HLA-DR1 (HLA DRB1*0101). Thus, we report the immunodominant peptide motifs of HTLV-1 gp21 restricted by HLA-DR1 which have been established by T cell repertoire analysis. These peptide motifs were recognized by T cells when synthetic peptides bound to HLA were presented by APC to T cells, but also after antigen processing of a recombinant HTLV-1 p21 protein by APC (Figs 2a and 3).

The two immunodominant epitopes of HTLV-1 gp21 were further analysed by short overlapping synthetic peptides. Two unique T cell epitopes were defined (LFWEQGGLCK and RPPLENRVLTG) which showed special features compared with the established body of evidence regarding peptide sequences binding to HLA-DR1 (HLA DRB1*0101) [23–26]. In the epitope LFWEQGGLCK, three hydrophobic amino acids leucine, phenylalanine and tryptophan (underlined) are at the N-terminal end, which probably includes the main anchor position [23]. Furthermore, there are possible minor anchor positions five amino acids from the main anchor positions, i.e. two glycines [23]. We hypothesize that this epitope contains a strong HLA-DR1 binding motif. The enhancement of T cell proliferation by prolonging the peptide at the N-terminal end (Fig. 2b) might be related either to different folding of the longer peptide in water, or to enhanced binding to HLA-DR1 before or after proteolytic cleavage [26]. Next, we asked the question why this unique epitope is immunodominant although HTLV-1 gp21 contains a high number of possible HLA-DR1 binding sequences [13,23]. Surprisingly, we found that this epitope corresponds to a highly conserved retroviral envelope sequence shared by many retroviruses, as shown in Table 3. Indeed it might be possible that the interaction of the retroviral envelope sequence, HLA and T cell receptor might be shaped by mammalian phylogeny. An immune response which is effective against a variety of retroviruses is likely to be conserved during phylogeny. There is another possibility: a similar sequence might be part of an endogenous retroviral sequence in the human genome. Indeed, two retroviral envelope sequences in Table 3 are derived from mammalian endogenous retroviruses. There is evidence that human cells express similar sequences (Table 4) which are probably derived from endogenous retroviral genes integrated in the human genome. To date it is unknown whether these sequences which are expressed in early life might induce tolerance or an autoreactive immune response in later life. Experimental studies on these endogenous conserved retroviral sequences need to be conducted to clarify their origin, expression in differentiated tissues and immunogenicity both in HTLV-1-infected and non-infected individuals.

There is another interesting feature of this highly conserved peptide sequence. It has been found to be suppressive for several immune functions if added as a synthetic peptide in micromolar concentrations to the culture medium of mononuclear cells [27,28]. This suppressive effect is non-specific and mediated via intracellular cAMP [28]. The HLA-DR1-restricted specific immune response against the epitope at concentrations in the nanomolar range (Fig. 2) might protect the individual from the deleterious effects of this HTLV-1 gp21 domain at much higher concentrations. Thus, the $CD4^+$ T cell response might contribute to the protective effect of HLA-DR1 against ATL, since HLA-DR1 is very rare in patients with ATL [8].

The other immunodominant epitope RPPLENRVLTG lacks similarity to the established HLA-DR1 binding motif [23–26]. For this epitope it is very difficult to define the main anchor position for DR1 [23]. The sequence corresponds to another important domain of HTLV-1 gp21, a functional domain of the retrovirus as established by a peptide inhibition assay for syncytium formation [29]. Indeed, the function of this sequence might be important to make it the second immunodominant epitope. This sequence is conserved only in the HTLV and STLV retroviruses, but has a low homology to other retroviruses. To date, there is no evidence of a homologous endogenous retroviral sequence in the human genome.

At this stage of our studies of the $CD4^+$ T cell response against HTLV-1 we used HTLV-1 gp21-specific $CD4^+$ T cell lines cultured from PBMC to identify immunodominant epitopes (Table 2). In further experiments we need to select these lines into peptide-specific T cell clones in order to analyse their particular functions and to clarify if some of the clones are infected with HTLV-1.

In general, the functional and regulatory properties of $CD4^+$ T cells are related to helper effects for B cells [16] and supportive effects for the cytotoxic T cell response [30]. The significance of 'helper' $CD4^+$ T cells for the B cell immune response in HAM/TSP can be linked to our own studies of the systemic and intrathecal humoral immune response [14,15]. Interestingly, the sequence sp382 contains an immunodominant T cell epitope and a

frequently recognized B cell epitope [14,15]. The sequence sp458 frequently binds antibodies in samples from HLA-DR1$^+$ HAM/TSP patients [15], it does not contain an immunodominant T cell epitope restricted by HLA-DR1 (as shown), but two HLA-DR1-restricted CD4$^+$ T cell epitopes have been identified in the proximity, i.e. amino acids 436–450 and 451–465 [13]. HTLV-1 gp21 is an important target antigen both for the T cell and the B cell immune response. The sites of immunodominant epitopes for the CD4$^+$ T cell and B cell receptors tend to overlap or to be in close proximity. The same applies to HTLV-1 gp46 as shown by Jacobson *et al*. [9]. Using data from our previous studies [14,15], we could compare the B cell and CD4$^+$ T cell specificities in HAM/TSP patients 1 and 2. In HAM/TSP patient 1 the systemic and intrathecal humoral immune responses were directed against peptides sp332, sp350, sp382 and sp458, but only sp382 elicited a significant CD4$^+$ T cell response. In HAM/TSP patient 2 there was a significant systemic humoral immune response only against peptide sp350, but the CD4$^+$ T cell response was directed against sp332.

To date there is little knowledge of the interactions of B and T cell immune responses and of the distribution of B and T cell epitopes in viral infections. In measles [31] and influenza [32] virus infections there is evidence for adjacent or overlapping B and T cell epitopes. In a persisting viral infection, as in other secondary immune responses, B cells might act as highly efficient APC which present antigen, provide a second signal for T cell activation and secrete cytokines [33,34]. Proximity of T cell and B cell epitopes in a protein antigen might facilitate the specific activation of both T cells and B cells for the same antigen [9,13,31–34].

Regarding the cellular immune responses in HAM/TSP, recently the main research focus has been on HTLV-1 tax-specific CD8$^+$ T cells [35–38]. The functional potential and data on high precursor frequencies of HTLV-1 tax-specific cytotoxic T cells (CTL) in HAM/TSP and asymptomatic carriers remain controversial (as reviewed in [38]). So far, there are no experimental data on the precursor frequencies and epitope specificities of HTLV-1 gp21-specific CD8$^+$ CTL. Future studies should also evaluate CD8$^+$ HTLV-1 gp21-specific T cells in HAM/TSP, as well as in asymptomatic HTLV-1 carriers and seronegative individuals.

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REFERENCES

1 Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematological features of 16 cases. Blood 1977; **50**:481–92.

2 Osame M, Usuku K, Izumo S *et al*. HTLV-I associated myelopathy. A new clinical entity. Lancet 1986; **1**:1031–2.

3 Gessain A, Barin F, Vernant JC *et al*. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 1985; **2**:407–10.

4 Usuku K, Sonoda S, Osame M *et al*. HLA haplotype-linked high immune responsiveness against HTLV-I in HTLV-I-associated myelopathy: comparison with adult T-cell leukemia/lymphoma. Ann Neurol 1988; **23**; (Suppl.):S143–50.

5 Sonoda S, Yashiki S, Fujiyoshi T *et al*. Immunogenetic factors involved in the pathogenesis of adult T-cell leukemia and HTLV-I- associated myelopathy. Gann Monograph Cancer Res 1992; **39**:81–93.

6 Nishimura Y, Okubo R, Minato S *et al*. A possible association between HLA and HTLV-1-associated myelopathy (HAM) in Japanese. Tissue Antigens 1991; **37**:230–1.

7 Usuku K, Nishizawa M, Matsuki K *et al*. Association of a particular amino acid sequence of the HLA DRβ1 chain with HTLV-I-associated myelopathy. Eur J Immunol 1990; **20**:1603–16.

8 Sonoda S, Fujiyoshi T, Yashiki S. Immunogenetics of HTLV-I/II and associated diseases. J Acquir Immune Defic Syndr 1996; **13** (Suppl. 1):S119–23.

9 Jacobson S, Reuben JS, Streilein RD, Palker TJ. Induction of CD4$^+$, human T lymphotropic virus type-1-specific cytotoxic T lymphocytes from patients with HAM/TSP. Recognition of an immunogenic region of the gp46 envelope glycoprotein of human T lymphotropic virus type-1. J Immunol 1991; **146**:1155–62.

10 Manca F, Li Pira G, Fenoglio D *et al*. Recognition of human T-leukemia virus (HTLV-1) envelope by human CD4$^+$ T-cell lines from HTLV-1 seronegative individuals: specificity and clonal heterogeneity. Blood 1995; **85**:1547–54.

11 Katahira Y, Yashiki S, Fujiyoshi T *et al*. *In vitro* induction of cytotoxic T lymphocytes against HTLV-I-infected T-cells from adult T-cell leukemia patients, asymptomatic HTLV-I carriers and seronegative healthy donors. Jpn J Cancer Res 1995; **86**:21–27.

12 Nagai M, Yashiki S, Fujiyoshi T *et al*. Characterization of a unique T-cell clone established from a patient with HAM/TSP which recognized HTLV- I-infected T-cell antigens as well as spinal cord tissue antigens. J Neuroimmunol 1996; **65**:97–105.

13 Yamano Y, Kitze B, Yashiki S *et al*. Preferential recognition of HTLV-I gp21 envelope protein restricted by HLA-DRB1 alleles of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). J Neuroimmunol 1997; **76**:50–60.

14 Kitze B, Usuku K, Izumo S *et al*. Diversity of intrathecal antibody synthesis against HTLV-I and its relation to HTLV-I associated myelopathy. J Neurol 1996; **243**:393–400.

15 Kitze B, Usuku K, Yashiki S *et al*. Intrathecal humoral immune response in HAM/TSP in relation to HLA haplotype analysis. Acta Neurol Scand 1996; **94**:287–93.

16 Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; **7**:145–73.

17 Osame M. Review of WHO Kagoshima Meeting and Diagnostic Guidelines for HAM/TSP. In: Blattner WA, ed. Human Retrovirology: HTLV. New York: Raven Press, 1990:191–7.

18 Ota M, Seki T, Fukushima H, Tsuji K, Inoko H. HLA-DRB1 genotyping by modified PCR-RELP method combined with group specific primers. Tissue Antigens 1992; **39**:187–202.

19 Kitze B, Pette M, Rohrbach E, Städt D, Kappos L, Wekerle H. Myelin specific T-lymphocytes in multiple sclerosis patients and healthy individuals. J Neuroimmunol 1988; **20**;:237.

20 Pette M, Fujita K, Kitze B *et al*. Myelin basic protein specific T lymphocyte lines from MS patients and healthy individuals. Neurol 1990; **40**:1770–6.

21 Lefkovits I, Waldmann H. Limiting dilution analysis of cells in the immune system. Cambridge: Cambridge University Press, 1979:38–82.

22 Coffin JM. Retroviridae. In: Francki RIB, Fauquet CM, Knudson DL, Brown F, eds. Classification and nomenclature of viruses—Fifth report of the international committee on taxonomy of viruses. New York: Springer, 1991:290–9.

23 Hammer J, Nagy ZA, Sinigaglia F. Rules governing peptide-class II MHC molecule interactions. Behring Inst Mitt 1994; **94**:124–32.

24 Hill CM, Liu A, Marshall KW *et al*. Exploration of requirements for peptide binding to HLA DRB1*0101 and DRB1*0401. J Immunol 1994; **152**:2890–8.

25 Brown JH, Jardetzky TS, Gorga JC *et al*. Three-dimensional structure of

the human class II histocompatibility antigen HLA- DR1. Nature 1993; **364**:33–39.
26 Engelhard VH. Structure of peptides associated with class I and class II MHC molecules. Annu Rev Immunol 1994; **12**:181–207.
27 Haraguchi S, Good RA, James-Yarish M, Cianciolo GJ, Day NK. Differential modulation of Th1- and Th2-related cytokine mRNA expression by a synthetic peptide homologous to a conserved domain within retroviral envelope protein. Proc Natl Acad Sci USA 1995; **92**:3611–5.
28 Haraguchi S, Good RA, James-Yarish M, Cianciolo GJ, Day NK. Induction of intracellular cAMP by a synthetic retroviral envelope peptide: a possible mechanism of immunopathogenesis in retroviral infections. Proc Natl Acad Sci USA 1995; **92**:5568–71.
29 Sagara Y, Inoue Y, Shiraki H, Jinno A, Hoshino H, Maeda Y. Identification and mapping of functional domains on human T-cell lymphotropic virus type 1 envelope proteins by using synthetic peptides. J Virol 1996; **70**:1564–9.
30 Stuhler G, Walden P. Collaboration of helper and cytotoxic T lymphocytes. Eur J Immunol 1993; **23**:2279–86.
31 Muller CP, Handtmann D, Brons NHC *et al*. Analysis of antibody response to the measles virus using synthetic peptides of the fusion protein: evidence of non-random pairing of T and B cell epitopes. Virus Res 1993; **30**:271–80.
32 Graham CM, Barnett BC, Hartlmeyer I *et al*. The structural requirements for class II (I-A^d)-restricted T cell recognition of influenza hemagglutinin: B cell epitopes define T cell epitopes. Eur J Immunol 1989; **19**:523–8.
33 Lanzavecchia A. Antigen-specific interaction between T and B cells. Nature 1985; **314**:537–9.
34 Pistoia V. Production of cytokines by human B cells in health and disease. Immunol Today 1997; **18**:343–50.
35 Elovaara I, Koenig S, Brewah AY, Woods RM, Lehky T, Jacobson S. High human T cell lymphotropic virus type 1 (HTLV-1)-specific precursor cytotoxic T lymphocyte frequencies in patients with HTLV-1-associated neurological disease. J Exp Med 1993; **177**:1567–73.
36 Elovaara I, Utz U, Smith L, Jacobson S. Limited T cell receptor usage by HTLV-I tax-specific, HLA class I restricted cytotoxic T lymphocytes from patients with HTLV-I associated neurological disease. J Neuroimmunol 1995; **63**:47–53.
37 Daenke S, Kermode AG, Hall SE *et al*. High activated and memory cytotoxic T-cell responses to HTLV-1 in healthy carriers and patients with tropical spastic paraparesis. Virol 1996; **217**:139–46.
38 Bangham CRM, Kermode AG, Hall SE, Daenke S. The cytotoxic T-lymphocyte response to HTLV-I: the main determinant of disease? Sem Virol 1996; **7**:41–48.