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SHORT COMMUNICATION

CD8+ cytotoxic T lymphocytes of a cynomolgus macaque infected with simian immunodeficiency virus (SIV) mac32H-J5 recognize a nine amino acid epitope in SIV Gag p26

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A detailed analysis of simian immunodeficiency virus (SIV)-specific cytotoxic T lymphocyte (CTL) responses and the identification of the proteins and epitopes they target may improve the design of immunotherapeutic interventions and provide insights into AIDS pathogenesis. Here, we identified a new CTL epitope in the SIV Gag protein, recognized by CD8+ and MHC class I-restricted CTL clones from a long-term asymptomatic cynomolgus macaque (*Macaca fascicularis*) infected with SIVmac32H-J5. Using overlapping synthetic peptides, the optimal minimal epitope was characterized as a nine amino acid peptide representing amino acids 242–250 of p26 (SVDEQIQWM). CTL recognition was shown to be abolished by amino acid substitutions observed within homologous human immunodeficiency virus (HIV)-1 and HIV-2 sequences.

Infection of macaques with several strains or clones of simian immunodeficiency virus (SIV) shows remarkable similarities with human immunodeficiency virus (HIV) infection of humans and provides a valuable model for investigating the role of cytotoxic T lymphocyte (CTL) immunity in the host defence against lentiviruses (Desrosiers, 1990; Letvin *et al.*, 1994). A number of studies have shown that detailed analysis of SIV-specific CTL responses and the identification of the proteins and epitopes they target may help the design of immunotherapeutic interventions and provide insights into AIDS pathogenesis (Chen *et al.*, 1992; Yasutomi *et al.*, 1995; Hulskotte *et al.*, 1995). Extending our previous studies of HIV-1-infected humans (Van Baalen *et al.*, 1996), we present here a minimally defined CTL epitope in SIV Gag, recognized by CTL of the $CD8^+$ and MHC class I-restricted phenotype. The epitope was identified using CTL clones and bulk cultures of peripheral blood mononuclear cells (PBMC) isolated from a cynomolgus macaque (designated K71) during the first 4 months of infection with the pathogenic molecular clone SIVmac32H-J5 (Rud *et al.*, 1994). Following the initial virus burst at week 2 after infection, the monkey showed effective virus containment (Hullskotte *et al.*, 1995), and remained asymptomatic with stable $CD4^+$ cell counts during the subsequent 22 months of observation (not shown).

Effector cells were generated from PBMC under limiting dilution conditions. Cells in R-10 medium (RPMI 1640 containing 10% FCS) were cultured with autologous paraformaldehyde-fixed B-lymphoblastoid cell lines (B-LCL) (10^4 per well) infected with a recombinant vaccinia virus (rVV) expressing Gag p55 of SIVmac32H, and with autologous irradiated (2500 rad) feeder PBMC (10^4 per well), as described (Geretti *et al.*, 1996). Cultures received recombinant interleukin-2 (rIL-2, 10 U/ml) from day 3, and were restimulated on day 7. On day 14, they were tested in split-well assays against autologous ^{51}Cr-labelled B-LCL either infected with SIV Gag rVV, or infected with a vaccinia virus control (186-poly, containing a polyclonal site without insert; Transgene), or incubated with medium alone. Individual positive cultures of dilutions showing $< 33\%$ positive wells underwent a third cycle of SIV Gag-specific stimulation and were subsequently subcloned by non-specific stimulation with PHA-L (1 μg/ml), irradiated (2500 rad) allogenic PBMC (10^5 per well), irradiated (3500 rad) allogenic human B-LCL (10^4 per well) (Van de Griend, 1984) and rIL-2 (50 U/ml). These CTL are operationally referred to as lines. Eleven CTL lines were established and maintained in culture for at least 3 months by alternate (every 14–21 days) cycles of specific and non-specific stimulation. The CTL lines' surface phenotype was $CD2^+$ $CD8^+$ $CD4^-$ (not shown). Their fine specificities were determined with three sets of 20-mer synthetic peptides, overlapping by 10 amino acids, together spanning the p26 (ADP714/1-22), p17 (ADP775/1-13) and p15 (ADP776/1-14) sequences of

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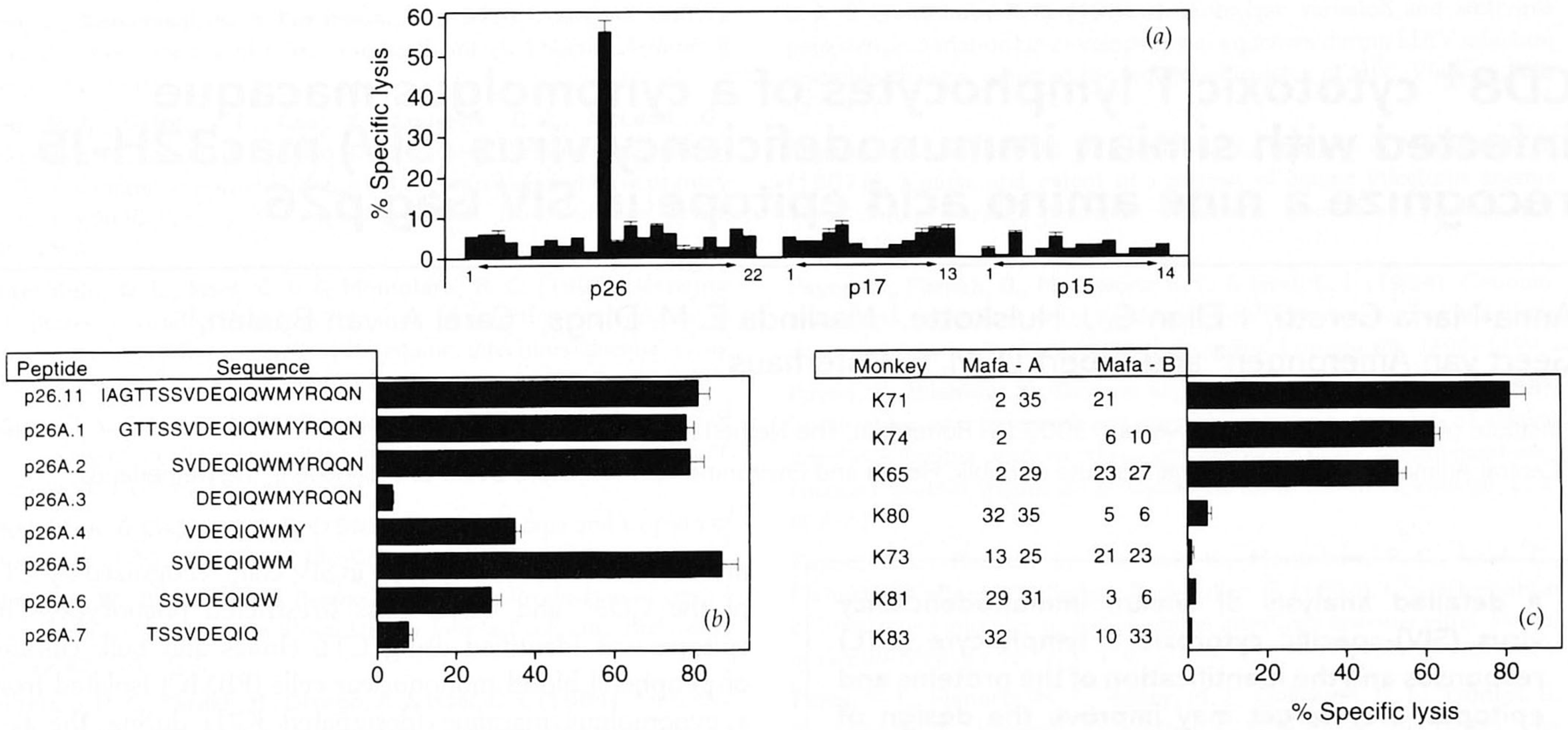

Fig. 1. Fine mapping of an MHC class I-restricted epitope in SIV Gag p26. CTL line K71/E26 was tested against: (*a*) autologous target B-LCL either pulsed with 30 μM synthetic peptides (20 residues long, with a 10 amino acid overlap) covering the p26 (peptides 1–22), p17 (peptides 1–13) and p15 (peptides 1–14) sequences of SIVmac251; (*b*) autologous target B-LCL sensitized with 30 μM synthetic peptides (9–20 residues long) spanning amino acids 236–255 of p26; (*c*) autologous, MHC class I mismatched and partially matched target B-LCL pulsed with 30 μM peptide p26A.5. Results are expressed as mean percentage specific lysis with standard errors from duplicate well estimations at an E:T ratio of either 5:1 (*a*) or 10:1 (*b*, *c*).

Table 1. CD8$^+$ cytotoxic T lymphocytes expanded by stimulation with peptide p26A.5

Effector cells generated by stimulation with autologous, peptide p26A.5-sensitized B-LCL were separated into positively isolated CD8$^+$ (CD8$^+$, > 99% CD8$^+$ cells) and CD8-depleted (CD8$^-$, < 2% CD8$^+$ cells) fractions immediately before testing for cytotoxicity at different E:T ratios. Target cells were autologous B-LCL, either sensitized with 30 μM of peptide p26A.5, or infected with SIV Gag rVV or vaccinia virus (vv) control, or incubated with medium alone. Results are expressed as mean percentage specific lysis from duplicate well estimations with standard error < 10%.

		% Specific lysis			
			SIV Gag		
Effector cells	E:T	p26A.5	rVV	vv	Medium
Total	50:1	72	63	13	13
	25:1	65	55	10	11
	12:1	55	34	8	7
CD8$^+$	50:1	76	59	10	9
	25:1	61	53	8	8
	12:1	55	35	7	5
CD8$^-$	50:1	8	5	6	6
	25:1	5	4	6	4
	12:1	5	1	2	0

SIVmac251. As shown in Fig. 1(*a*) for the CTL line designated K71/E26, of the 49 peptides tested, only peptide p26.11, covering amino acids 236–255 (IAGTTSSVDEQIQWMYR) of p26, sensitized targets for lysis by the CTL lines. The two contiguous peptides p26.10 and p26.12 were not recognized, indicating that the CTL epitope was contained in the central region of peptide p26.11. To define the minimal epitope within peptide p26.11, the CTL lines were tested for recognition of synthetic peptides spanning amino acids 236–255 of p26 and varying in length from 20 to 9 residues (European Veterinary Laboratory, Woerden, The Netherlands). As shown in Fig. 1(*b*) for CTL line K71/E26, the 9-mer peptide p26A.5, representing amino acids 242–250 (SVDEQIQWM) of p26, optimally sensitized targets for lysis by the CTL lines. Two truncated peptides lacking either the N-terminal S residue (p26A.4: VDEQIQWMY) or the C-terminal M residue (p26A.6: SSVDEQIQW) were considerably less efficient. Further truncation at either terminus abolished recognition, probably by destroying MHC anchor residues. To study MHC class I restriction, the CTL lines were tested against MHC class I mismatched and partially matched B-LCL pulsed with peptide p26A.5. As shown in Fig. 1(*c*) for CTL line K71/E26, lysis was restricted to targets sharing the Mafa-A2 allele, indicating recognition in the context of this macaque MHC class I molecule. It should be noted, however, that MHC class I alleles were defined by serological techniques, and one-dimensional

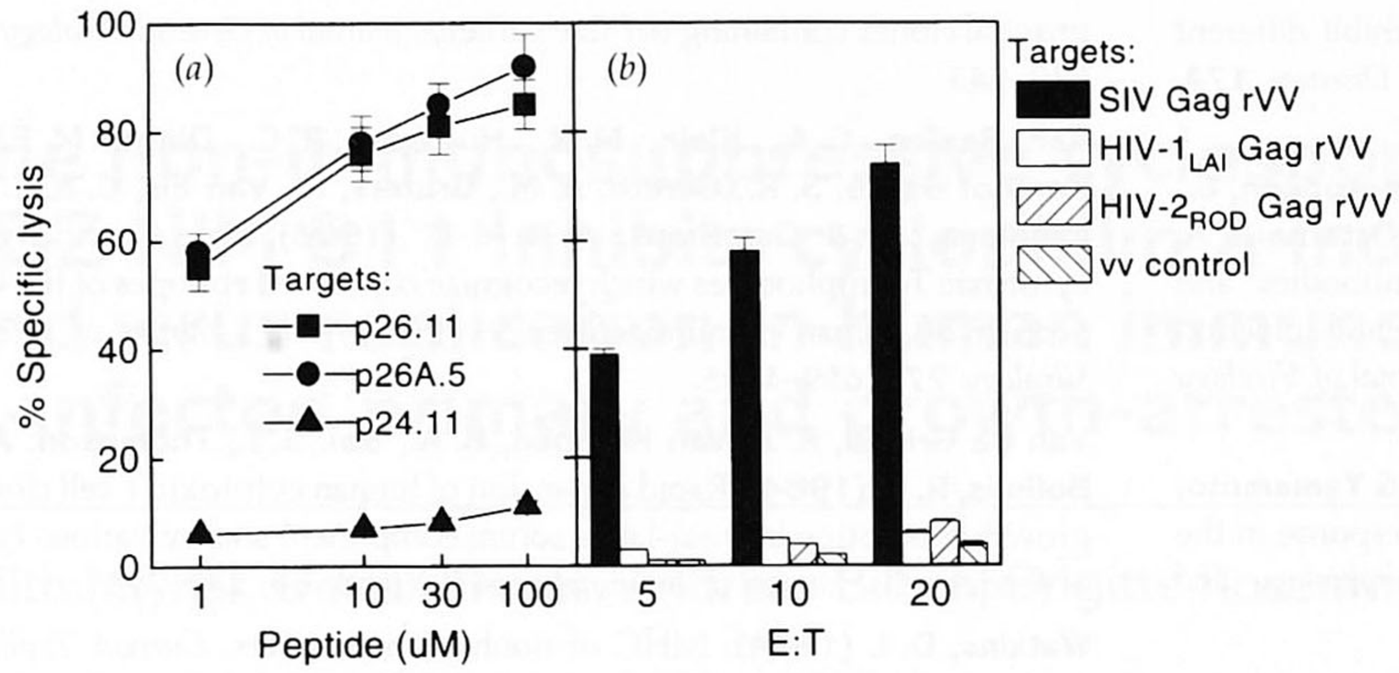

Fig. 2. Effects of sequence variation on CTL recognition. CTL line K71/E26 was tested against: (*a*) autologous target B-LCL pulsed with either peptide p26.11, or peptide p26A.5 or peptide p24.11 (GSDIAGTTSTLQEQIGWMTN, amino acids 235–254 of HIV-1_{SF2} p24), at an E:T ratio of 10:1; (*b*) autologous target B-LCL either infected with rVV expressing the Gag protein of SIVmac32H, or HIV-1_{LAI} or HIV-2_{ROD}, or infected with vaccinia virus (vv) control. Results are expressed as mean percentage specific lysis with standard errors from duplicate well estimations.

isoelectric focusing may be required to confirm the restriction element (Watkins, 1994).

In reciprocal experiments, PBMC (2×10^4 per well) were cultured for 10 days with autologous irradiated (5000 rad) B-LCL (10^4 per well) sensitized with 30 μM of peptide p26A.5, autologous irradiated feeder PBMC (10^4 per well) and rIL-2 (10 U/ml) from day 4. As a control, PBMC were stimulated with B-LCL sensitized with peptides p26.4, p26.6 or p17.6. No cytotoxic responses were mediated by these control cultures (data not shown), whereas effector cells expanded by peptide p26A.5 recognized autologous targets sensitized with the inducing peptide, as well as targets expressing endogenously processed antigen after infection with SIV Gag rVV (Table 1). The $CD8^+$ phenotype of peptide p26A.5-specific CTL was confirmed by depletion studies (Table 1), using anti-CD8 antibody-coated magnetic beads (Dynabeads M-450, Dynal) as described (Geretti *et al.*, 1993).

The amino acid region 242–250 of p26 partially overlaps with $CD4^+$ T helper epitopes previously identified in immunized cynomolgus macaques (Mills & Jones, 1994). In addition, the homologous consensus sequences of the HIV-1 A and B clades fulfil the requirements for binding the human HLA-A2.1 molecule (Brander *et al.*, 1995). The region is conserved among several strains of the HIV-2/SIV D clade, including SIVmac251, SIVmac32H, SIVmac1A11 and SIVmac239. The consensus sequence of the HIV-2/SIV D and C clades shows one amino acid substitution at position 242 (S → T), whereas the consensus sequence of the HIV-2/SIV A and B clades shows two amino acid substitutions, at positions 242 (S → T) and 244 (D → E) (Myers *et al.*, 1994). The homologous sequence is highly conserved among most HIV-1 clades (A–H). However, comparison of the SIVmac251 and HIV-1_{SF2} sequences reveals four amino acid substitutions at positions 242 (S → T), 243 (V → L), 244 (D → Q) and 248 (Q → G) (Myers *et al.*, 1994). As shown in Fig. 2(*a*), targets that were either pulsed with peptide p24.11 (ADP 788/11), covering amino acids 235–254 of HIV-1_{SF2} p24 (GSDIAGTTSTLQEQIGWMTN), or infected with an rVV expressing HIV-1_{LAI} Gag (TG1144; Transgene), were not recognized by CTL line K71/E26. These effector cells also failed to recognize targets infected with an rVV expressing HIV-2_{ROD} Gag (TG2112; Transgene), suggesting either lack of generation of the epitope, or that the two amino acid substitutions at positions 242 (S → T) and 244 (D → E) of HIV-2_{ROD} p26 were sufficient to abolish recognition.

These observations suggest that variations in the p26A.5 epitope may generate virus variants able to escape or antagonize the CTL response of monkey K71, thereby potentially affecting virus containment (Franco *et al.*, 1995). Longitudinal studies are in progress to address this hypothesis, providing an additional basis for investigating the role of CTL immunity in the control of lentiviral infections.

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