Antigen-specific release of β-chemokines by anti-HIV-1 cytotoxic T lymphocytes

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A major advance in understanding human immunodeficiency virus (HIV) biology was the discovery that the β-chemokines MIP-1α (macrophage inflammatory protein-1α), MIP-1β (macrophage inflammatory protein-1β) and RANTES (regulated on activation, normal T-cell expressed and secreted) inhibit entry of HIV-1 into CD4+ cells by blocking the critical interaction between the CCR5 coreceptor and the V3 domain of the viral envelope glycoprotein gp120 [1,2]. CD8+ lymphocytes are a major source of β-chemokines [3], but the stimulus for chemokine release has not been well defined. Here, we have shown that engagement of CD8+ cytotoxic T lymphocytes (CTLs) with HIV-1-encoded human leukocyte antigen (HLA) class I-restricted peptide antigens caused rapid and specific release of these β-chemokines. This release paralleled cytolytic activity and could be attenuated by naturally occurring amino acid variation within the HLA class I-restricted peptide sequence. Epitope variants that bound to appropriate HLA class I molecules but failed to stimulate cytolytic activity in CTLs also failed to stimulate chemokine release. We conclude that signalling through the T-cell receptor (TCR) following binding of antigen results in β-chemokine release from CTLs in addition to cytolytic activity, and that both responses can be abolished by epitope mutation. These results suggest that antigenic variation within HIV-1 might not only allow the host cell to escape lysis, but might also contribute to the propagation of infection by failing to activate β-chemokine-mediated inhibition of HIV-1 entry.

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Results and discussion

Release of the β-chemokines MIP-1α, MIP-1β and RANTES from HIV-1-specific CTL lines and clones (Table 1) was quantified by immunoassay and compared with lytic activity and interferon-γ (IFN-γ) production. In all assays, significant β-chemokine release required the appropriate peptide antigen bound to HLA class I; representative data are shown in Figure 1. This extends earlier work which demonstrated antigen-specific release of other cytokines by CTLs [4] and supports studies that correlated suppression of virus replication, as determined by bioassay, with lytic activity [5,6].

Despite increasing evidence that CTLs control HIV-1 infection *in vivo*, the significance of naturally occurring mutations within HIV-1 epitopes that reduce or abolish CTL recognition [7] remains uncertain. Recent work has suggested that evasion of CTL recognition contributes to both viral persistence following infection and progression to AIDS [8–10]. We therefore assessed the effect of naturally occurring amino acid variation within epitopes, represented in the experiments by synthetic peptides, on β-chemokine release by CTLs and compared this with the effect on cytolysis. In the donor 868 HLA-A2-restricted CTL line specific for the epitope SLYNTVATL (single-letter amino acid code), peptide variants with impaired ability to sensitize targets for lysis also failed to stimulate maximal β-chemokine release from CTLs when compared with wild-type peptide (Figure 2) [11]. Similar results were obtained for the 3F, 3L and 3F/5A peptide variants [11] with the HLA-A2-restricted SLYNTVATL-specific CTL clone from donor 003; the 6I variant [10] with the HLA-B27-restricted KRWIIMGLNK-specific CTL line from

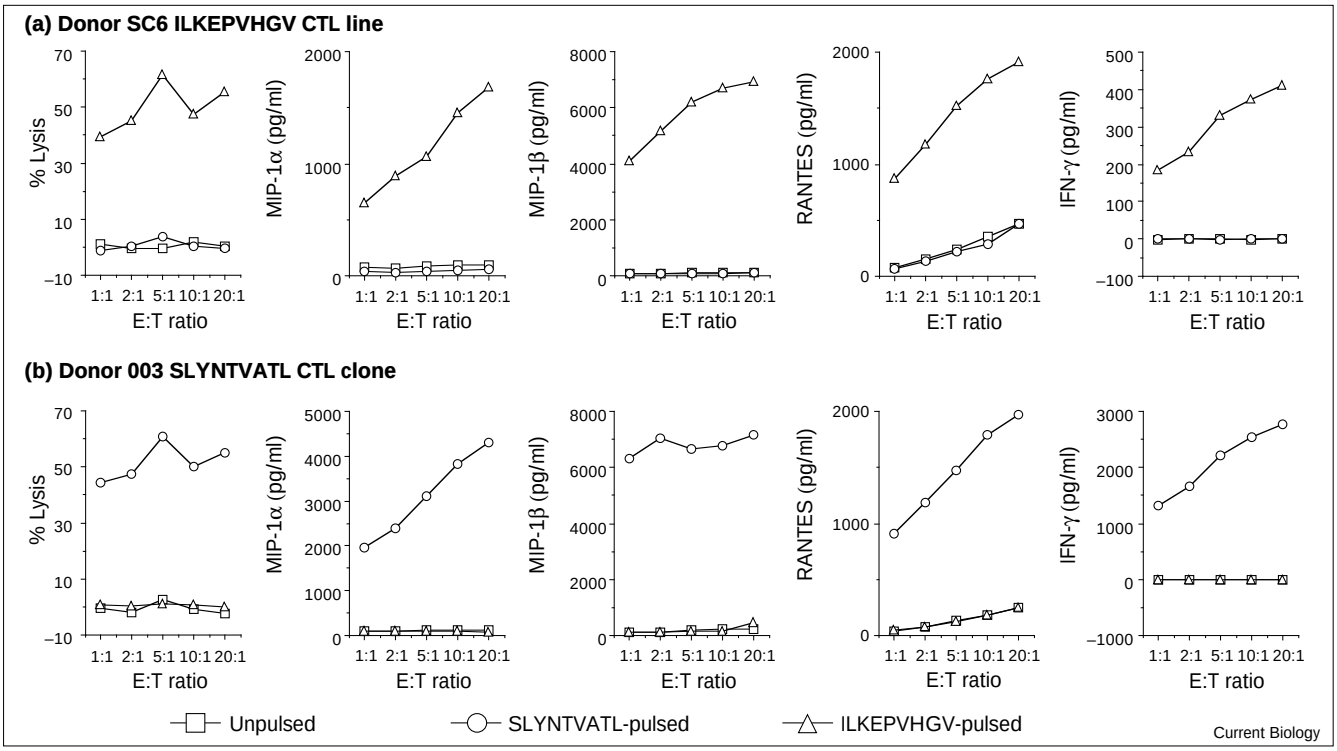
Table 1

CTL lines and clones used in this work.

Epitope	HIV-1 protein (amino acids)	HLA restriction	Donor	Line/clone
SLYNTVATL	p17 Gag 77–85	A2	868	Line
SLYNTVATL	p17 Gag 77–85	A2	868	Clone
SLYNTVATL	p17 Gag 77–85	A2	003	Clone
ISPRTLNAW	p24 Gag 147–155	B57	422	Line
KRWIIMGLNK	p24 Gag 263–272	B27	868	Line
VIYQYMDDL	Pol 346–354	A2	868	Clone
ILKEPVHGV	Pol 476–484	A2	SC6	Line
VRYPLTFGW	Nef 137–145	A24	106	Line

All epitope amino acids are numbered with reference to HIV-1 LAI amino acid numbering.

Figure 1

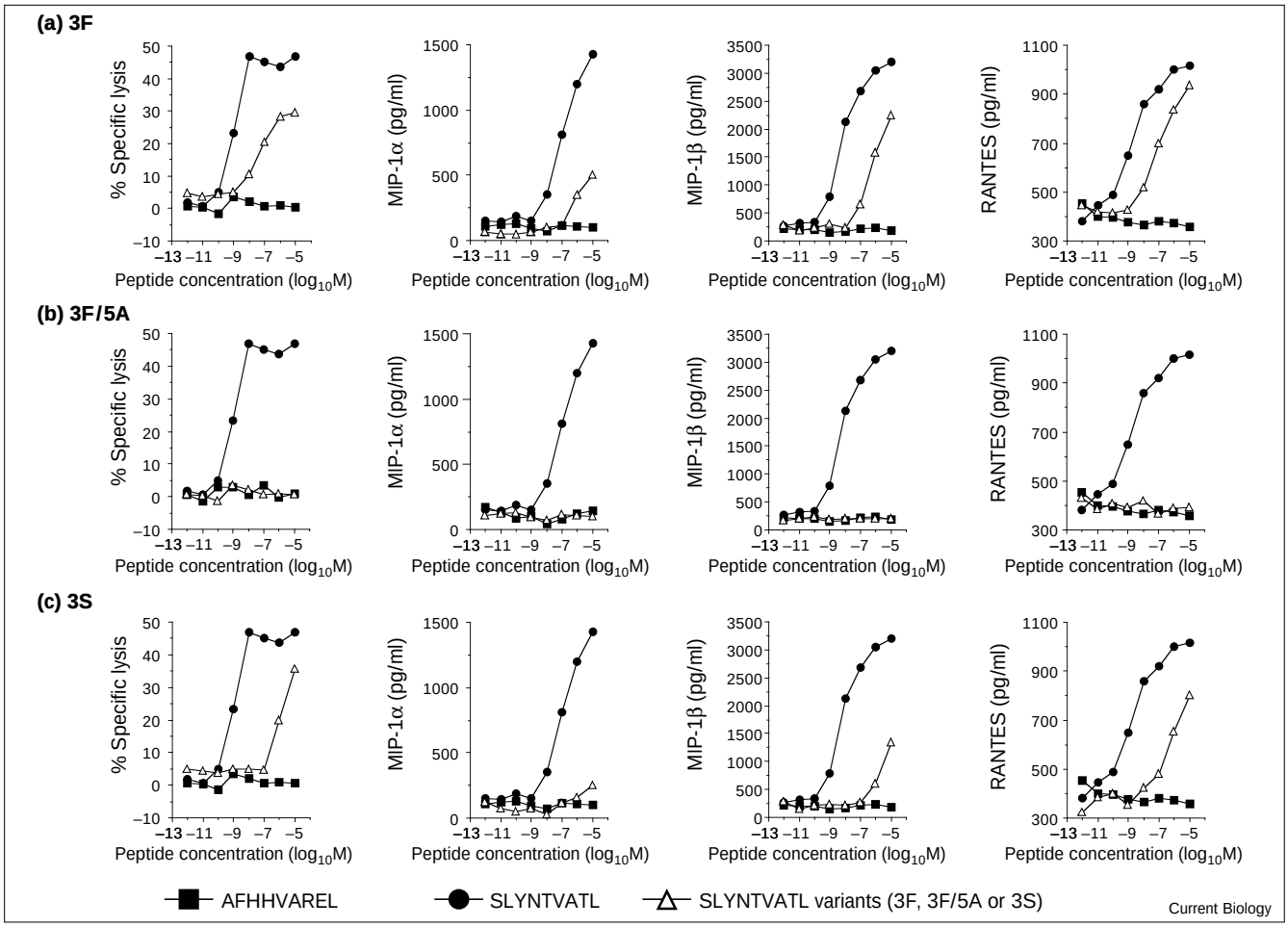


Production of β-chemokines and IFN-γ by HIV-1-specific CTLs: (a) donor SC6 ILKEPVHGV CTL line (10 days post-restimulation) and (b) donor 003 SLYNTVATL CTL clone (9 days post-restimulation). The percentage lysis and production of β-chemokines and IFN-γ (in pg/ml) are plotted against a range of effector:target ratios (E:T). Results are shown for unpulsed targets, and for targets pulsed with the peptides SLYNTVATL and ILKEPVHGV. Similar results were obtained with the CTL lines and clones shown in Table 1 (data not shown). Autologous or HLA-matched B-lymphoblastoid cell lines (B-LCL) were used as targets in all assays. Targets were pulsed with 50 μM peptide, or mock-pulsed with medium alone, for 90 min and washed twice prior to assay. Lysis and cytokine release were determined at 4 h. Background production of cytokines by target cells was minimal. IFN-γ release is known to occur following TCR signalling and its measurement in these initial assays was included as a methodological control. A degree of cross-reactivity was observed with recombinant interleukin-2 in the RANTES immunoassay. Although free peptide was not present in these assays, a contribution to chemokine release from bystander lysis (that is, lysis of neighbouring CTLs in the absence of cognate recognition through the TCR) cannot be excluded [28]; CTLs lysed with water (in the presence of broad spectrum protease inhibitors) prior to antigen exposure, however, did not release significant amounts of MIP-1α or MIP-1β, suggesting that simple cytoplasmic release from CTLs acting as targets in assay cultures does not account for the observed coupling of cytolytic activity with the release of these β-chemokines. RANTES (as determined by immunoassay) was detected in CTL lysates (data not shown). In time course experiments, MIP-1α, MIP-1β and IFN-γ production was detected only after a lag phase of approximately 90 min following antigen presentation, consistent with regulation at the level of transcription; RANTES release, in contrast, was observed within several minutes. These observations suggest that RANTES is released from a pre-formed store within the CTLs. Chemokine concentrations achieved by TCR triggering of CTLs generally ranked MIP-1β > MIP-1α/RANTES. Background release of β-chemokines (that is, release in the absence of peptide, or with an unrelated peptide) by CTLs was most significant for RANTES, and decreased with time from restimulation.

donor 868; the 3F/5A variant [11] with the HLA-A2-restricted SLYNTVATL-specific CTL clone from donor 868; and the 3C variant [12,13] with the HLA-A2-restricted VIYQYMDDL-specific CTL clone from donor 868 (data not shown). The observation that β-chemokine production parallels cytolytic activity with both wild-type and variant epitopes over a range of peptide concentrations suggests that naturally occurring epitope mutation facilitates HIV-1 evasion of both lytic and suppressor functions of CTLs.

Individuals that are homozygous for a mutant allele of CCR5 in which a 32 base-pair deletion causes a frameshift resulting in a non-functional receptor have been shown to be resistant to HIV-1 infection despite repeated exposure [2]; in addition, there is some evidence that HIV-1 seropositive heterozygotes progress more slowly to AIDS [2]. As cellular CCR5 expression levels correlate with infectability by macrophage-tropic HIV-1 [14], receptor blockade by endogenous β-chemokines might inhibit the propagation of infection within infected individuals, thereby slowing disease progression. The capacity of β-chemokines to control HIV-1 infection *in vivo* remains unknown, however; inhibition of virus entry does not necessarily correlate with suppression of viral replication [15]. Furthermore, CCR5

Figure 2



Effects of natural epitope variants on cytolysis and β-chemokine production by the donor 868 HLA-A2-restricted SLYNTVATL-specific CTL line (12 days post-restimulation). An E:T ratio of 6:1 was used in each experiment. Lysis and β-chemokine release were measured at 6 h and are plotted against peptide concentration. Results are shown for the SLYNTVATL variants (**a**) 3F, (**b**) 3F/5A and (**c**) 3S and for the peptides AFHHVAREL (HLA-A2-restricted peptide epitope corresponding to amino acids 190–198 of Nef, included as a specificity control) and SLYNTVATL in (a–c). Background production of chemokines by the B-LCL targets in these assays was significant only for MIP-1α (510 pg/ml) and was subtracted from experimental values. These assays were performed in the presence of free peptide, conditions under which CTL–CTL lysis may be significant [28]. Some β-chemokine release may therefore be due to CTLs acting as targets in culture, either throughout the assay (RANTES), or following activation by antigen (MIP-1α and MIP-1β). Similar patterns were observed with targets prepulsed with peptide (concentration 1 μM) and with free peptide in the absence of targets (that is, with CTLs presenting antigen to each other). Release of β-chemokines also paralleled cytolysis with the 3C, 3L, 6I, 3F/6I, 6I/8V, 3F/8V and 3F/6I/8V peptide variants (data not shown); these variants have been shown to bind HLA-A2 [11]. Differences in the peptide sensitivity of these CTL responses might reflect varying thresholds of detection, but previous work has demonstrated that different CTL functions might be observed at different levels of TCR occupancy [29].

receptor blockade does not account fully for $CD8^+$ lymphocyte-mediated inhibition of HIV-1 replication [2,5,16–20]. As yet poorly characterized secreted or cell-associated factors with more potent suppressor activity may have a greater effect on disease progression [16].

Chemokines play a central role in orchestrating host cellular defences through their pro-inflammatory, chemoattractant and cytomodulatory properties as well as inhibiting entry of HIV-1 into cells [21–24]. Their release by virus-specific CTLs following the recognition of an infected host cell may be essential for the coordination of an effective antiviral immune response [23]. Subversion of these functions by antigenic variation could seriously impair antiviral immunity, and so provide a potent mechanism of immune evasion.

Materials and methods

Donors were either asymptomatic HIV-1-infected individuals from the Oxford Haemophilia Centre or genitourinary clinics in the Oxford

region, or individuals with primary HIV-1 infection from the Chelsea and Westminster Hospital, London. HLA type was determined by amplification refractory mutation system (ARMS)–PCR with sequence-specific primers [25]. Peptides were synthesized using standard fluorenylmethoxycarbonyl (Fmoc) techniques and were >90% pure as determined by high-pressure liquid chromatography (Research Genetics). Peptide-specific CTL lines and clones were derived from donor peripheral blood mononuclear cells as described previously [26,27]; the specificities and origins of the CTLs used in this work are described in Table 1. CTLs were washed twice in RPMI-1640 (Sigma) and diluted in fresh medium prior to assay. Cytolytic activity was determined using standard ^{51}Cr release methodology as described previously [27]. Supernatants were assayed for MIP-1α, MIP-1β, RANTES and IFN-γ with commercially available quantitative immunoassays (R&D Systems); standard calibration curves were constructed with each assay. All data points shown represent the mean of duplicate assays.

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References

1. Cocchi F, DeVico AL, Garzino-Demo A, Arya SK, Gallo RC, Lusso P: Identification of RANTES, MIP-1α, and MIP-1β as the major HIV-suppressive factors produced by CD8+ T cells. *Science* 1995, 270:1811-1815.
2. D'Souza MP, Harden VA: Chemokines and HIV-1 second receptors. *Nat Med* 1996, 2:1293-1300.
3. Conlon K, Lloyd A, Chattopadhyay U, Lukacs N, Kunkel S, Schall T, *et al.*: CD8+ and CD45RA+ human peripheral blood lymphocytes are potent sources of macrophage inflammatory protein 1α, interleukin-8 and RANTES. *Eur J Immunol* 1995, 25:751-756.
4. Jassoy C, Harrer T, Rosenthal T, Navia BA, Worth J, Johnson RP, *et al.*: Human immunodeficiency virus type 1-specific cytotoxic T lymphocytes release gamma interferon, tumor necrosis factor alpha (TNF-α), and TNF-β when they encounter their target antigens. *J Virol* 1993, 67:2844-2852.
5. Yang OO, Kalams SA, Trocha A, Cao H, Luster A, Johnson RP, *et al.*: Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class 1-restricted triggering of cytolytic and noncytolytic mechanisms. *J Virol* 1997, 71:3120-3128.
6. Buseyne F, Fevrier M, Garcia S, Gougeon ML, Riviere Y: Dual function of a human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte clone: inhibition of HIV replication by non-cytolytic mechanisms and lysis of HIV-infected CD4+ cells. *Virology* 1996, 225:248-253.
7. Phillips RE, Rowland-Jones S, Nixon DF, Gotch FM, Edwards JP, Ogunlesi AO, *et al.*: Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* 1991, 354:453-459.
8. Price DA, Goulder PJR, Klenerman P, Sewell AK, Easterbrook PJ, Troop M, *et al.*: Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. *Proc Natl Acad Sci USA* 1997, 94:1890-1895.
9. Borrow P, Lewicki H, Wei X, Horwitz MS, Peffer N, Meyers H, *et al.*: Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat Med* 1997, 3:205-211.
10. Goulder PJR, Phillips RE, Colbert RA, McAdam S, Ogg G, Nowak MA, *et al.*: Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat Med* 1997, 3:212-217.
11. Sewell AK, Harcourt GC, Goulder PJR, Price DA, Phillips RE: Antagonism of cytotoxic T lymphocyte-mediated lysis by natural HIV-1 altered peptide ligands requires simultaneous presentation of agonist and antagonist peptides. *Eur J Immunol* 1997, 27:2323-2329.
12. Nunberg JH, Schleif WA, Boots EJ, O'Brien JA, Quintero JC, Hoffman JM, *et al.*: Viral resistance to human immunodeficiency virus type 1-specific pyridinone reverse transcriptase inhibitors. *J Virol* 1991, 65:4887-4892.
13. Richman D, Shih C-K, Lowy I, Rose J, Prodanovich P, Goff S, *et al.*: Human immunodeficiency virus type 1 mutants resistant to nonnucleoside inhibitors of reverse transcriptase arise in tissue culture. *Proc Natl Acad Sci USA* 1991, 88:11241-11245.
14. Wu L, Paxton WA, Kassam N, Ruffing N, Rottman JB, Sullivan N, *et al.*: CCR5 levels and expression pattern correlate with infectability by macrophage-tropic HIV-1, ***in vitro***. *J Exp Med* 1997, 185:1681-1691.
15. Schmidtmayerova H, Sherry B, Bukrinsky M: Chemokines and HIV replication. *Nature* 1996, 382:767.
16. Levy JA, Mackewicz CE, Barker E: Controlling HIV pathogenesis: the role of the noncytotoxic anti-HIV response of CD8+ T cells. *Immunol Today* 1996, 17:217-224.
17. Kinter AL, Ostrowski M, Goletti D, Oliva A, Weissman D, Gantt K, *et al.*: HIV replication in CD4+ T cells of HIV-infected individuals is regulated by a balance between the viral suppressive effects of endogenous β-chemokines and the viral inductive effects of other endogenous cytokines. *Proc Natl Acad Sci USA* 1996, 93:14076-14081.
18. Fauci AS: Host factors and the pathogenesis of HIV-induced disease. *Nature* 1996, 384:529-534.
19. Moriuchi H, Moriuchi M, Combadiere C, Murphy PM, Fauci AS: CD8+ T-cell-derived soluble factor(s), but not β-chemokines RANTES, MIP-1α, and MIP-1β, suppress HIV-1 replication in monocyte/macrophages. *Proc Natl Acad Sci USA* 1996, 93:15341-15345.
20. Paliard X, Lee AY, Walker CM: RANTES, MIP-1α and MIP-1β are not involved in the inhibition of HIV-1 SF33 replication mediated by CD8+ T-cell clones. *AIDS* 1996, 10:1317-1321.
21. Baggiolini M, Dewald B, Moser B: Interleukin-8 and related chemotactic cytokines — CXC and CC chemokines. *Adv Immunol* 1994, 55:97-179.
22. Schall TJ, Bacon K, Camp RDR, Kaspari JW, Goeddel DV: Human macrophage inflammatory protein α (MIP-1α) and MIP-1β chemokines attract distinct populations of lymphocytes. *J Exp Med* 1993, 177:1821-1826.
23. Cook DN, Beck MA, Coffman TM, Kirby SL, Sheridan JF, Pragnell IB, *et al.*: Requirement of MIP-1α for an inflammatory response to viral infection. *Science* 1995, 269:1583-1585.
24. Taub DD, Ortaldo JR, Turcovski-Corrales SM, Key ML, Longo DL, Murphy WJ: β chemokines costimulate lymphocyte cytolysis, proliferation, and lymphokine production. *J Leukoc Biol* 1996, 59:81-89.
25. Bunce M, O'Neill CM, Barnardo CNM, Krausa P, Browning MB, Morris PJ, *et al.*: Phototyping: comprehensive DNA typing for HLA-A, -B, -C, -DRB1, -DRB3, -DRB4, -DRB5 and -DQB1 by PCR with 144 primer mixes utilizing sequence-specific primers (SSP-PCR). *Tissue Antigens* 1995, 46:355-367.
26. Rowland-Jones SL, Powis SH, Sutton J, Mockridge I, Gotch FM, Murray N, *et al.*: An antigen processing polymorphism revealed by HLA-B8-restricted cytotoxic T lymphocytes which does not correlate with ***TAP*** gene polymorphism. *Eur J Immunol* 1993, 23:1999-2004.
27. Nixon DF, Townsend ARM, Elvin JG, Rizza CR, Gallwey J, McMichael AJ: HIV-1 gag-specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature* 1988, 336:484-487.
28. Suhrbier A, Burrows SR, Fernan A, Lavin MF, Baxter GD, Moss DJ: Peptide epitope induced apoptosis of human cytotoxic T lymphocytes. *J Immunol* 1993, 150:2169-2178.
29. Valitutti S, Mueller S, Dessing M, Lanzavecchia A: Different responses are elicited in cytotoxic T lymphocytes by different levels of T cell receptor occupancy. *J Exp Med* 1996, 183:1917-1921.