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BRIEF COMMUNICATION

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Genomic analysis of *Fas* and *FasL* genes and absence of correlation with disease progression in AIDS

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Abstract Apoptosis has been suggested as a major mechanism for the CD4+ T-lymphocyte depletion observed in patients infected with human immunodeficiency virus 1 (HIV-1). To evaluate the impact of genetic variations to apoptosis during progression of acquired immunodeficiency syndrome (AIDS), we have performed an extensive genetic analysis of *Fas* and Fas ligand (*FasL*) genes. The coding regions and promoters of these genes were resequenced in a cohort of 212 HIV-1-seropositive patients presenting extreme disease phenotypes and 155 healthy controls of Caucasian origin. Overall, 33 single nucleotide polymorphisms (SNPs) with an allele frequency >1% were identified and evaluated for their association with disease progression. Among them, 14 polymorphisms were newly characterized. We did not find any statistically significant association of *Fas* and *FasL* polymorphisms and haplotypes with AIDS progression.

Keywords *Fas*/*FasL* genes · AIDS progression · Single nucleotide polymorphism · Haplotype · GRIV cohort

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Apoptosis, or programmed cell death, is a key mechanism allowing multicellular organisms to tightly regulate cell growth, preventing pathological processes such as autoreactivity, cancer and immunodeficiency. In the pathogenesis of acquired immunodeficiency syndrome (AIDS), apoptosis is considered to play an important role in human immunodeficiency virus 1 (HIV-1)-dependent CD4+ T-lymphocyte depletion, induced and accelerated by the virus itself (Ameisen and Capron 1991; Gougeon et al. 1991; Laurent-Crawford et al. 1991; Terai et al. 1991; Westendorp et al. 1995; Zinkernagel 1995). Indeed, earlier studies showed a great fragility of CD4+ T cells leading to their apoptosis in patients infected with HIV-1 (Ameisen and Capron 1991; Groux et al. 1992). It was shown later that the degree of CD4+ T-cell apoptosis in patients prior to T-cell depletion tightly correlates with disease progression (Gougeon et al. 1996; Liegler et al. 1998).

Several different extrinsic pathways are hypothesized to induce death receptor-associated apoptosis in AIDS. Among them, accumulating evidences strongly indicate the direct involvement of the Fas/Fas ligand (FasL) pathway to the T-lymphocyte depletion. In T lymphocytes of HIV-1-positive individuals, the expression of *Fas* and *FasL* is significantly increased (Debatin et al. 1994; Mitra et al. 1996) and Fas-mediated apoptosis is accelerated by antibody cross-linking (Katsikis et al. 1995). Upregulated expression of FasL in macrophages infected with HIV-1 can induce apoptotic death of normal peripheral blood T cells in vitro, suggesting another death-signal transmission through macrophages in T-cell depletion (Badley et al. 1996). Moreover, a study using anti-Fas antibodies showed a significant decrease of Fas-induced cell death in T cells of patients with extremely slow progression as

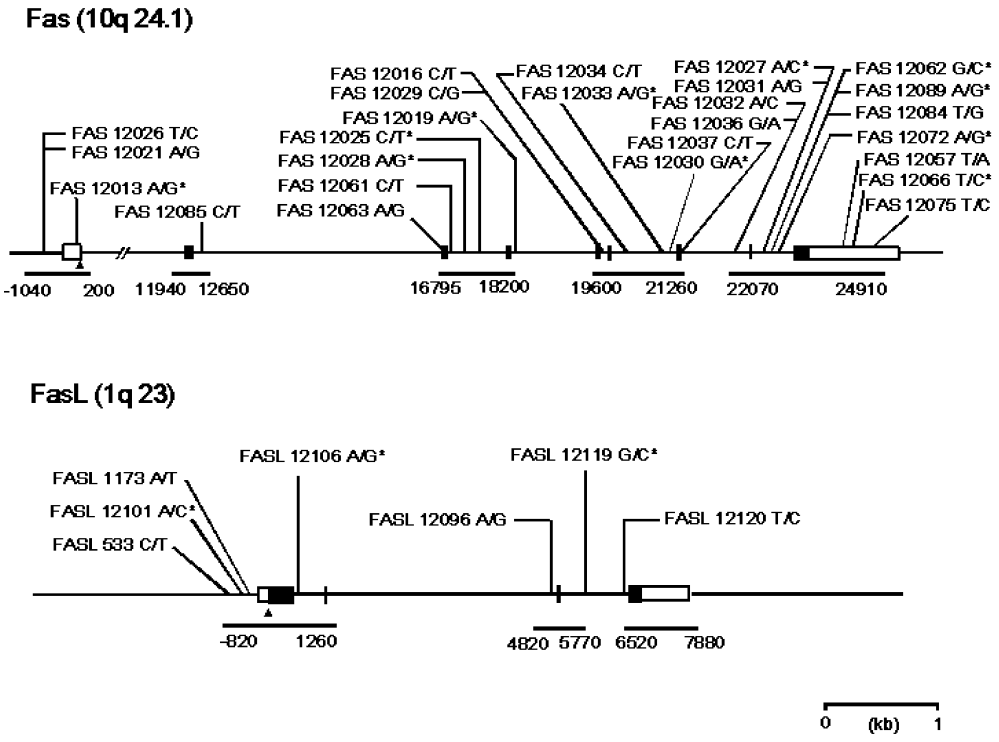

Fig. 1 Organization of *Fas* and *FasL* genes. Coding and untranslated regions of each gene are, respectively, indicated by *solid rectangles* and *open rectangles*. Positions of the 33 SNPs with frequencies of more than 1% are indicated with the name and nucleotide changes. The *asterisks* correspond to SNPs that were newly characterized in this study. Sequenced regions are shown by the *horizontal line* below each gene with start and end positions according to the first nucleotide of the initiation codon as +1 (*black triangle*). The genomic sequences used for the alignment are: *Fas*, NT_008769.11 and *FasL*, NT_029874.5. For experimental details, see Vasilescu et al. (2003)

well as in one of their uninfected parents (Bottarel et al. 2001). This indicates the inherited alteration of the Fas-signaling pathway resulting in the quantitative difference in the degree of apoptosis, and thus the disease progression.

In the present study, we focused on genetic variations of *Fas* and *FasL* genes to clarify whether genetic variations of these apoptosis-related genes could influence disease onset and progression. To perform this study, we employed the GRIV cohort consisting of two sub-populations of Caucasian HIV-1-positive individuals with extreme phenotypes: 154 asymptomatic individuals (SP) with a $CD4^+$ cell count above 500/mm^3 for 8 or more years after seroconversion and 58 patients with rapid progression (RP) showing a drop in their $CD4^+$ cell count below 300/mm^3 in less than 3 years after the last seronegative test (for more details, see Flores-Villanueva et al. 2003; Huber et al. 2003; Rappaport et al. 1997). We also employed 155 seronegative control (CTR) subjects from the same ethnic origin.

We systematically screened the *Fas* and *FasL* genes for polymorphisms by resequencing the exons, flanking regions and promoter regions. We identified 46 and 30 polymorphisms in *Fas* and *FasL* genes, respectively (Fig. 1). Out of these 76 polymorphisms identified, 33 single nucleotide polymorphisms (SNPs) have an allele frequency of 1% or greater in at least one of SP, RP and CTR groups. Fourteen among these 33 SNPs are newly identified in this study. As shown in Fig. 1, 6 SNPs are located in exons. The SNPs *Fas_12063* and *Fas_12037* introduce synonymous changes, while the other four are located either in the 5′UTR (*Fas_12013*) or the 3′UTR (*Fas_12057*, *Fas_12066* and *Fas_12075*). Table 1 summarizes the frequency of each SNP in the SP, RP and CTR populations, their association with AIDS progression and the relevant information known to date for each SNP. The SNP distributions in cases and controls were in Hardy-Weinberg equilibrium.

We performed statistical analysis to test whether these polymorphisms or deduced SNP haplotypes are associated with disease progression. We failed to obtain any significant association between polymorphisms in *Fas* and *FasL* genes and AIDS progression by Fisher's exact test (the lowest *P* value is 0.096 in *FasL_533* between RP and SP). Similarly, the same test using estimated haplotypes (Fig. 2) did not find any significant association between particular haplotypes of *Fas* or *FasL* genes and AIDS progression (Table 2). The lowest *P*-value was 0.102 for the haplotype 3 of the *Fas* gene obtained by comparing RP with SP.

A general case-control study using 283 HIV-positive patients and 111 control subjects (Cascino et al. 1998) on two SNPs in the *Fas* gene (*Fas_12063* and *Fas_12037*) did not show significant association between combination of two SNPs and AIDS. The more specific study shown here using two sub-populations of AIDS patients with extreme phenotypes did not provide any further genetic evidence that *Fas* and *FasL* genes are major genetic determinants in the onset and pathogenicity of HIV-1 infection.

Increased apoptosis of T cells has been reported after Epstein-Barr virus infections (Akbar et al. 1993; Uehara et al. 1992), varicella-zoster infections (Akbar et al. 1993) and infections induced by *herpesviridae* (Razvi and

Table 1 Summary of the polymorphisms of *Fas* and *FasL* genes

Gene	SNP			Freq A1			*P*-values for test statistics[a]		References to previous studies of the variant and new IDs
	ID	A1	A2	CTR	SP	RP	CvRvS	RvS	
Fas	*12026*	T	C	0.87	0.90	0.89	0.617	0.746	NCBI (rs2234768)
	12021	A	G	0.53	0.51	0.53	0.942	1.000	(Huang et al. 1997)
	12013	A	G	0.98	0.97	1.00	1.000	1.000	NEW (ss 20399338)
	12085	C	T	0.58	0.64	0.61	0.316	0.643	NCBI (rs2296603)
	12063	A	G	0.96	0.94	0.97	0.352	0.304	(Cascino et al. 1998)
	12061	C	T	0.89	0.89	0.88	0.970	0.851	(Bolstad et al. 2000)
	12028	A	G	0.96	0.94	0.97	0.312	0.428	NEW (ss 20399339)
	12025	T	C	0.57	0.62	0.57	0.373	0.440	NEW (ss 20399340)
	12019	A	G	0.99	0.97	1.00	0.141	0.353	NEW (ss 20399341)
	12016	C	T	0.98	0.98	1.00	0.787	0.566	NCBI (rs3218620)
	12029	C	G	0.58	0.63	0.55	0.302	0.192	(Bolstad et al. 2000)
	12034	C	T	0.28	0.34	0.30	0.435	0.588	NCBI (rs1571020)
	12033	A	G	0.96	0.93	0.95	0.403	0.791	NEW (ss 20399342)
	12030	G	A	0.84	0.87	0.91	0.199	0.421	NEW (ss 20399343)
	12037	T	C	0.30	0.31	0.31	0.992	1.000	(Cascino et al. 1998)
	12036	A	G	0.58	0.64	0.58	0.291	0.345	NCBI (rs3752986)
	12032	A	C	0.54	0.58	0.53	0.627	0.560	NCBI (rs3752985)
	12031	A	G	0.56	0.57	0.56	0.981	0.908	NCBI (rs1571019)
	12027	C	A	0.89	0.89	0.88	0.953	0.848	NEW (ss 20399344)
	12062	G	C	0.96	0.93	0.95	0.264	0.613	NEW (ss 20399345)
	12089	A	G	1.00	1.00	0.99	0.965	0.265	NEW (ss 20399346)
	12084	T	G	0.56	0.64	0.60	0.232	0.545	NCBI (rs1977389)
	12072	G	A	0.98	0.97	0.96	0.468	0.741	NEW (ss 20399347)
	12057	A	T	0.90	0.88	0.84	0.267	0.381	NCBI (rs1051070)
	12066	T	C	0.96	0.94	0.97	0.274	0.303	NEW (ss 20399348)
	12075	C	T	0.89	0.88	0.88	0.853	1.000	NCBI (rs1468063)
FasL	*533*	C	T	0.63	0.59	0.70	0.226	0.096	(Nolsoe et al. 2002)
	12101	A	C	1.00	1.00	0.99	0.964	0.260	NEW (ss 20399349)
	1173	A	T	0.84	0.84	0.84	0.988	1.000	(Nolsoe et al. 2002)
	12106	A	G	0.90	0.89	0.93	0.482	0.253	NEW (ss 20399350)
	12096	A	G	0.84	0.83	0.84	0.940	0.879	(Bolstad et al. 2000)
	12119	G	C	0.54	0.54	0.52	0.956	0.902	NEW (ss 20399351)
	12120	T	C	0.00	0.01	0.01	0.959	1.000	NCBI (rs2639653)

[a] Since the nominal *P*-values results were not significant, we did not perform the Bonferroni corrections

Fas

	Freq	12026	12021	12013	12085	12063	12061	12028	12025	12019	12016	12029	12034	12033	12030	12037	12036	12032	12031	12027	12062	12089	12084	12072	12057	12066	12075
H1	0.305	T	A	A	T	A	C	A	C	A	C	G	T	A	G	C	G	C	G	C	G	A	G	G	A	T	C
H2	0.131	T	G	A	C	A	C	A	T	A	C	C	C	A	G	T	A	A	A	C	G	A	T	G	A	T	C
H3	0.097	T	G	A	C	A	C	A	T	A	C	C	C	A	G	T	A	A	A	C	G	A	T	G	T	T	C
H4	0.085	T	G	A	C	A	T	A	T	A	C	C	T	A	G	C	A	A	A	A	G	A	T	G	A	T	T
H5	0.078	C	A	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	A	C	G	A	T	G	A	T	C
H6	0.063	T	A	A	C	A	C	A	T	A	C	C	C	A	G	T	A	A	A	C	G	A	T	G	A	T	C
H7	0.042	T	G	A	C	G	C	G	T	A	C	C	T	G	G	C	A	C	G	C	C	A	T	G	A	C	C
H8	0.036	T	G	A	T	A	C	A	C	A	C	G	T	A	G	C	G	C	G	C	G	A	G	G	A	T	C
H9	0.018	C	A	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	A	C	G	A	T	A	A	T	C
H10	0.014	T	G	G	T	A	C	A	C	A	C	G	T	A	G	C	G	C	G	C	G	A	G	G	A	T	C
H11	0.014	T	G	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	A	C	G	A	T	G	A	T	C
H12	0.011	T	G	A	T	A	C	A	C	A	T	G	T	A	G	C	G	C	G	C	G	A	G	G	A	T	C
H13	0.011	T	A	A	C	A	C	A	T	A	C	C	T	A	A	C	A	A	A	C	G	A	T	G	A	T	C
H14	0.011	T	G	A	C	A	C	A	T	G	C	C	C	A	G	T	A	A	A	C	G	A	T	G	T	T	C

FasL

	Freq	533	12101	1173	12106	12096	12119	12120
H1	0.413	C	A	A	A	A	G	C
H2	0.176	C	A	A	A	A	C	C
H3	0.148	T	A	T	A	G	C	C
H4	0.108	T	A	A	A	A	C	C
H5	0.085	T	A	A	G	A	G	C
H6	0.016	T	A	A	A	A	G	C

Fig. 2 Summary of polymorphisms combinations. Haplotype frequencies using SNPs with frequencies >1% for each gene were estimated with the EM algorithm (Laird 1993). The haplotypes were sorted by estimated frequency among population

Welsh 1993). So the identification of 14 novel SNPs with relatively high frequency (>1%) and haplotype information on these genes should prove useful for the genetic study of patients with other virus infections or with cancer. In another hand, more extensive genetic studies on additional genes related to Fas/FasL-mediated apoptosis will clarify the role of this pathway in AIDS.

Table 2 Summary of polymorphism combinations and estimated frequencies for haplotypes in the *Fas* and *FasL* genes. *CTR* Seronegative control subjects, *SP* asymptomatic individuals with a $CD4^+$ cell count above 500/mm^3 for 8 or more years after seroconversion, *RP* rapid progression individuals showing a drop in their $CD4^+$ cell count below 300/mm^3 in less than 3 years after the last seronegative test

Gene	Haplotype[a]	CTR		SP		RP		*P*-values	
		Counts	Frequency	Counts	Frequency	Counts	Frequency	CvSvR	SvR
Fas	H1	100/286	0.350	86/268	0.321	34/102	0.333	0.732	0.804
	H2	43/286	0.150	39/268	0.146	15/102	0.147	0.989	1.000
	H3	26/286	0.091	26/268	0.097	16/102	0.157	0.178	0.102
	H4	26/286	0.091	26/268	0.097	9/102	0.088	0.983	1.000
	H5	29/286	0.101	23/268	0.086	4/102	0.039	0.140	0.178
	H6	14/286	0.049	23/268	0.086	7/102	0.069	0.240	0.675
	H7	9/286	0.031	18/268	0.067	4/102	0.039	0.157	0.460
	H8	13/286	0.045	8/268	0.030	4/102	0.039	0.627	0.742
	H9	4/286	0.014	4/268	0.015	3/102	0.029	0.595	0.398
	H10	5/286	0.017	3/268	0.011	2/102	0.020	0.754	0.618
	H11	6/286	0.021	3/268	0.011	1/102	0.010	0.688	1.000
	H12	4/286	0.014	2/268	0.007	2/102	0.020	0.575	0.303
	H13	3/286	0.010	4/268	0.015	1/102	0.010	0.707	1.000
	H14	4/286	0.014	3/268	0.011	0/102	0.000	0.690	0.565
FasL	H1	129/294	0.439	115/282	0.408	51/106	0.481	0.495	0.205
	H2	58/294	0.197	48/282	0.170	21/106	0.198	0.701	0.551
	H3	44/294	0.150	47/282	0.167	15/106	0.142	0.777	0.642
	H4	31/294	0.105	36/282	0.128	11/106	0.104	0.672	0.603
	H5	26/294	0.088	29/282	0.103	6/106	0.057	0.359	0.231
	H6	6/294	0.020	7/282	0.025	2/106	0.019	0.795	0.679

[a] Haplotypes with frequency greater than 1% were used for the statistical analysis, which was performed as previously described (Vasilescu et al. 2003)

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References

Akbar AN, Borthwick N, Salmon M, Gombert W, Bofill M, Shamsadeen N, Pilling D, Pett S, Grundy JE, Janossy G (1993) The significance of low *bcl-2* expression by CD45RO T cells in normal individuals and patients with acute viral infections. The role of apoptosis in T-cell memory. J Exp Med 178:427–438

Ameisen JC, Capron A (1991) Cell dysfunction and depletion in AIDS: the programmed cell death hypothesis. Immunol Today 12:102–105

Badley AD, McElhinny JA, Leibson PJ, Lynch DH, Alderson MR, Paya CV (1996) Upregulation of Fas ligand expression by human immunodeficiency virus in human macrophages mediates apoptosis of uninfected T lymphocytes. J Virol 70:199–206

Bolstad AI, Wargelius A, Nakken B, Haga HJ, Jonsson R (2000) Fas and Fas ligand gene polymorphisms in primary Sjogren's syndrome. J Rheumatol 27:2397–2405

Bottarel F, Bonissoni S, Lucia MB, Bragardo M, Bensi T, Buonfiglio D, Mezzatesta C, DiFranco D, Balotta C, Capobianchi MR, Dianzani I, Cauda R, Dianzani U (2001) Decreased function of Fas in patients displaying delayed progression of HIV-induced immune deficiency. Hematol J 2:220–227

Cascino I, Ballerini C, Audino S, Rombola G, Massacesi L, Colombo G, Scorza Smeraldi R, d'Alfonso S, Momigliano Richiardi P, Tosi R, Ruberti G (1998) *Fas* gene polymorphisms are not associated with systemic lupus erythematosus, multiple sclerosis and HIV infection. Dis Markers 13:221–225

Debatin KM, Fahrig-Faissner A, Enenkel-Stoodt S, Kreuz W, Benner A, Krammer PH (1994) High expression of APO-1 (CD95) on T lymphocytes from human immunodeficiency virus-1-infected children. Blood 83:3101–3103

Flores-Villanueva PO, Hendel H, Caillat-Zucman S, Rappaport J, Burgos-Tiburcio A, Bertin-Maghit S, Ruiz-Morales JA, Teran ME, Rodriguez-Tafur J, Zagury JF (2003) Associations of MHC ancestral haplotypes with resistance/susceptibility to AIDS disease development. J Immunol 170:1925–1929

Gougeon ML, Olivier R, Garcia S, Guetard D, Dragic T, Dauguet C, Montagnier L (1991) Demonstration of an engagement process towards cell death by apoptosis in lymphocytes of HIV-infected patients. CR Acad Sci III 312:529–537

Gougeon ML, Lecoeur H, Dulioust A, Enouf MG, Crouvoiser M, Goujard C, Debord T, Montagnier L (1996) Programmed cell death in peripheral lymphocytes from HIV-infected persons: increased susceptibility to apoptosis of CD4 and CD8 T cells correlates with lymphocyte activation and with disease progression. J Immunol 156:3509–3520

Groux H, Torpier G, Monte D, Mouton Y, Capron A, Ameisen JC (1992) Activation-induced death by apoptosis in $CD4^+$ T cells from human immunodeficiency virus-infected asymptomatic individuals. J Exp Med 175:331–340

Huang QR, Morris D, Manolios N (1997) Identification and characterization of polymorphisms in the promoter region of the human *Apo-1/Fas* (*CD95*) gene. Mol Immunol 34:577–582

Huber C, Pons O, Hendel H, Haumont P, Jacquemin L, Tamim S, Zagury JF (2003) Genomic studies in AIDS: problems and answers. Development of a statistical model integrating both longitudinal cohort studies and transversal observations of extreme cases. Biomed Pharmacother 57:25–33

Katsikis PD, Wunderlich ES, Smith CA, Herzenberg LA (1995) Fas antigen stimulation induces marked apoptosis of T lymphocytes in human immunodeficiency virus-infected individuals. J Exp Med 181:2029–2036

Laird N (1993) The EM algorithm. In: Rao CR (ed) Computational statistics. Handbook of statistics, vol 9. Elsevier, Amsterdam, pp 509–520

Laurent-Crawford AG, Krust B, Muller S, Riviere Y, Rey-Cuille MA, Bechet JM, Montagnier L, Hovanessian AG (1991) The cytopathic effect of HIV is associated with apoptosis. Virology 185:829–839

Liegler TJ, Yonemoto W, Elbeik T, Vittinghoff E, Buchbinder SP, Greene WC (1998) Diminished spontaneous apoptosis in lymphocytes from human immunodeficiency virus-infected long-term nonprogressors. J Infect Dis 178:669–679

Mitra D, Steiner M, Lynch DH, Staiano-Coico L, Laurence J (1996) HIV-1 upregulates Fas ligand expression in $CD4^+$ T cells in vitro and in vivo: association with Fas-mediated apoptosis and modulation by aurintricarboxylic acid. Immunology 87:581–585

Nolsoe RL, Kristiansen OP, Larsen ZM, Johannesen J, Pociot F, Mandrup-Poulsen T (2002) Complete mutation scan of the human Fas ligand gene: linkage studies in Type I diabetes mellitus families. Diabetologia 45:134–139

Rappaport J, Cho YY, Hendel H, Schwartz EJ, Schachter F, Zagury JF (1997) 32-bp *CCR-5* gene deletion and resistance to fast progression in HIV-1-infected heterozygotes. Lancet 349:922–923

Razvi ES, Welsh RM (1993) Programmed cell death of T lymphocytes during acute viral infection: a mechanism for virus-induced immune deficiency. J Virol 67:5754–5765

Terai C, Kornbluth RS, Pauza CD, Richman DD, Carson DA (1991) Apoptosis as a mechanism of cell death in cultured T lymphoblasts acutely infected with HIV-1. J Clin Invest 87:1710–1715

Uehara T, Miyawaki T, Ohta K, Tamaru Y, Yokoi T, Nakamura S, Taniguchi N (1992) Apoptotic cell death of primed $CD45RO^+$ T lymphocytes in Epstein-Barr virus-induced infectious mononucleosis. Blood 80:452–458

Vasilescu A, Heath SC, Ivanova R, Hendel H, Do H, Mazoyer A, Khadivpour E, Goutalier FX, Khalili K, Rappaport J, Lathrop GM, Matsuda F, Zagury JF (2003) Genomic analysis of Th1-Th2 cytokine genes in an AIDS cohort: identification of IL4 and IL10 haplotypes associated with the disease progression. Genes Immun 4:441–449

Westendorp MO, Frank R, Ochsenbauer C, Stricker K, Dhein J, Walczak H, Debatin KM, Krammer PH (1995) Sensitization of T cells to CD95-mediated apoptosis by HIV-1 Tat and gp120. Nature 375:497–500

Zinkernagel RM (1995) Are HIV-specific CTL responses salutary or pathogenic? Curr Opin Immunol 7: 462–470