Table 17: **Nef**

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(13–20)	Nef(13–20 LAI)	WPTVRERM	HIV-1 infection	human(B*0801,B8)	[Goulder (1997g)]
	• Unusual epitope for HLA-B8, but compatible with crystal structure predictions • Noted in Brander 1999, this database, to be B*0801				
Nef(62–81)	Nef(61–80)	EEEEVGFPVTPQVPLRPMTY	HIV infection	human( )	[Lieberman (1995)]
	• HIV-specific CTL lines developed by *ex vivo* stimulation with peptide				
Nef(62–81)	Nef(61–80 SF2)	EEEEVGFPVTPQVPLRPMTY	HIV infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 12 subjects had CTL that could recognize vaccinia expressed LAI Nef • Two of these 12 had CTL response to this peptide • The responding subjects were HLA-A11, A24, B8, B35, and HLA not determined				
Nef(62–81)	Nef(61–80 SF2)	EEEEVGFVTPQVPLRPMTY	HIV-1 infection	human( )	[Lieberman (1997b)]
	• CTL expanded *ex vivo* were later infused into HIV-1 infected patients				
Nef(66–80)	Nef(66–80 BRU)	VGFPVTPQVPLRMT	HIV-1 infection	human(A1, B8)	[Hadida (1992)]
	• HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients				
Nef(68–76)	Nef(72–80 SF2)	FPVRPQVPL	HIV-1 infection	human(B35)	[Shiga (1996)]
	• Binds HLA-B*3501				
Nef(68–76)	Nef(72–80 SF2)	FPVRPQVPL	HIV-1 infection	human(B*3501)	[Tomiyama (1997)]
	• A CTL clone responsive to this epitope was obtained • 3/7 B35 positive individuals had a CTL response to this epitope • An R to T substitution at position 4 abrogates specific lysis, but not binding to B*3501				
Nef(68–76)	Nef(68–76)	FPVTPQVPL	*in vitro* stimulation	human(B7)	[Wilson (1999b)]
	• Dendritic cells are the most potent for priming T cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors • Th1-biasing cytokines IL-12 or IFN alpha enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within • B7 and A2 Nef epitopes were studied FPVTPQVPL has a high affinity for B7				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(68–76)	Nef(68–76)	FPVTPQVPL	*in vitro* stimulation	human(B7)	[Wilson (1999b)]
	• Dendritic cells are the most potent for priming T cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors • Th1-biasing cytokines IL-12 or IFN alpha enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within • This epitope was just included in this study as a positive control				
Nef(68–77)	Nef(68–77 LAI)	FPVTPQVPLR	HIV-1 infection	human(B7,B*0702)	[Haas (1996)]
	• There was a high degree of variation in three CTL epitopes in Nef in four slow and non-progressors, and variant specific CTLs arose over time to eliminate variants, indicating immune selection • Noted in Brander 1999, this database, to be B*0702, B. Maier and B. Autran Pers. Comm.				
Nef(68–84)	Nef( )	FPVRPQVPLRPMTYKGA		human( )	[Jubier-Maurin (1999)]
	• 41 new HIV-1 strains describing envelope subtypes of HIV-1 A-H were genetically characterized in the nef region – 34 subtypes were classified in the same subtype in nef and env and 7 of the 41 strains were recombinants • This region was defined as a CTL epitope region that is conserved among HIV-1 M group subtypes				
Nef(71–79)	Nef(71–79 LAI)	TPQVPLRPM	HIV-1 infection	human(B*0702)	
	• Noted in Brander 1999, this database, to be B*0702, Pers. Comm. from P. Goulder				
Nef(71–81)	Nef(75–85 SF2)	RPQVPLRPMTY	HIV-1 infection	human(B35)	[Shiga (1996)]
	• Binds HLA-B*3501				
Nef(71–81)	Nef(75–85 SF2)	RPQVPLRPMTY	HIV-1 infection	human(B*3501)	[Tomiyama (1997)]
	• A CTL clone responsive to this epitope was obtained • 4/7 B35 positive individuals had a strong CTL response to this epitope • An R to T substitution at position 1 abrogates specific lysis, but not binding to B*3501 • An R to H substitution at position 7 did not alter reactivity				
Nef(72–91)	Nef(71–90 SF2)	PQVPLRMTYKAAVDLSHFL	HIV-1 infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • Three of these 11 had CTL response to this peptide • The responding subjects were HLA-A3, A32, B51, B62; HLA-A11, A24, B8, B53				
Nef(72–91)	Nef(71–90 SF2)	PQVPLRPMTYKAAVDLSHFL	HIV-1 infection	human( )	[Lieberman (1997b)]
	• CTL expanded *ex vivo* were later infused into HIV-1 infected patients				
Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK		human(B27)	[Culmann(1998)]
	• Optimal epitope mapped by peptide titration				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Durali (1998)]
	• Cross-clade CTL response was studied by determining the CTL activity in seven patients from Bangui, (6 A subtype, and 1 AG recombinant infections) and one A subtype infection from a person living in France originally from Togo, to different antigens expressed in vaccinia • Pol reactivity: 8/8 had CTL to A subtype, and 7/8 to B subtype, and HIV-2 Pol was not tested • Gag reactivity: 7/8 reacted with A or B subtype gag, 3/8 with HIV-2 Gag • Nef reactivity: 7/8 reacted with A subtype, and 5/8 with B subtype, none with HIV-2 Nef • Env reactivity: 3/8 reacted with A subtype, 1/8 with B subtype, none with HIV-2 Env • One of the patients was shown to react to this epitope: QVPLRPMTYK			
Nef(73–82)	Nef(73–82 NL43)	QVPLRPMTYK	HIV-1 infection	human(A*0301)	[Koenig (1990)]
	• Tyr is critical for binding to A3.1 • C. Brander notes that this is a A*0301 epitope in the 1999 database				
Nef(73–82)	Nef(73–82 BRU)	QVPLRPMTYK	HIV-1 infection	human(A3, A11, B35)	[Culmann (1991)]
	• Nef CTL clones from HIV+ donors				
Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Robertson (1993)]
	• Development of a retroviral vector (pNeoNef) to generate autologous CTL targets • [Hunziker (1998)] suggests that HLA-A2 does not in fact present this epitope • The initial assignment of HLA-A2 presentation for this epitope was based on a serological HLA typing. Subsequently, the authors revisted the issue with genetic HLA typing and found that HLA-A11 was the correct presenting molecule (Dr. Florence Buseyne, Pers. Comm., 2000)				
Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Couillin (1994), Goulder (1997a)]
	• Mutational variation in HIV epitopes in individuals with appropriate HLA types can result in evasion of CTL response • [Goulder (1997a)] is a review of immune escape that summarizes this study				
Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A11)	[Couillin (1995)]
	• Mutations found in this epitope in HLA-A11 positive and negative donors were characterized				
Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Goulder (1997b), Goulder (1997a)]
	• Identical twin hemophiliac brothers were both infected with the same batch of factor VIII • Both had a response to this epitope • [Goulder (1997a)] is a review of immune escape that summarizes this study				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV-1 infection	human(A3)	[Lubaki (1997)]
	• 82 HIV-1-specific CTL clones from 5 long term non-progressors were isolated and analyzed for breadth of response • A sustained Gag, Env and Nef response was observed, and clones were restricted by multiple HLA epitopes, indicating a polyclonal response • An A3+ subject had a strong response to this epitope, with 10/11 CTL clones being specific for this epitope, isolated at two time points, 1 year apart				
Nef(73–82)	Nef(73–82)	QVPLRPMTYK	HIV infection	human( )	[Garcia (1997)]
	• The anti-Nef CTL line P1 specific for this epitope is able to kill target cells via two mechanisms • First: Ca^{2+}-dependent, perforin-dependent Nef-specific lysis • Second: Ca^{2+}-independent, CD95-dependent apoptosis that could also kill non-specific targets • Findings indicate that the two mechanisms are not mutually exclusive in human CTL, as they are in mice • CTL mediated CD95-dependent apoptosis may play a role in pathogenesis				
Nef(73–82)	Nef(73–82 LAI)	QVPLRPMTYK	HIV-1 infection	human(A3.1)	[Koenig (1995)]
	• Alanine substitutions L76A, R77A, M79A, T80A significantly decreased immunogenicity of peptide • Nef CTL clones (4N225) were infused into an HIV-1 infected volunteer to evaluate effects of infusion on viral load/patient health • Infusion led to outburst of escape variants which resulted in higher viral load/accelerated disease progression				
Nef(73–82)	Nef(73–82 LAI)	SVPLRPMTYK	HIV-1 infection	human(B35 or C4)	[Buseyne (1993a)]
	• Vertical transmission of HIV ranges from 13% to 39% • Primary assays showed cytotoxic activities against at least one HIV protein was detected in 70% of infected children • Epitopes recognized in five children were mapped using synthetic peptides and secondary cultures • Patient EM13, who had a CTL response to three epitopes in Nef, was infected via blood transfusion after birth and went from CDC stage P2A to P2E during the study				
Nef(74–81)	Nef(73–82 LAI)	VPLRPMTY	HIV-1 or HIV-2 infection	human(B*3501,B35)	[McMichael & Walker(1994), Culmann (1991)]
	• Review of HIV CTL epitopes – defined by B35 motif found within a larger peptide • Noted by C. Brander *et al*., this database 1999, to be a B*3501 epitope				
Nef(74–81)	Nef(73–82 LAI)	VPLRPMTY	HIV-1 or -2 infection	human(B35)	[Rowland-Jones (1995)]
	• VPLRPMTY also recognized by CTL from HIV-2 seropositives, epitope is conserved				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(74–81)	Nef( )	VPLRPMTY	HIV-1 exposure	human(B35)	[Rowland-Jones (1998a)]
• A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating • The A and D subtype consensus are identical to the B clade epitope					
Nef(74–81)	Nef(75–82)	VPLRPMTY	none	human(B35)	[Lalvani (1997)]
• A peptide based protocol was optimized for restimulation of CTLp using optimized peptide and IL-7 concentrations – importantly this protocol does not stimulate a primary response, only secondary – peptide-specific CTLp counts could be obtained via staining with peptide-Class I tetramers • This peptide was one of the B35 presented test peptides used in control experiments showing that the assay gave no activity using lymphocytes from 21 healthy B35 seronegative donors					
Nef(74–81)	Nef(75–82)	VPLRPMTY	no CTL shown	human(B*3501)	[Smith (1996)]
• Crystal structure of VPLRPMTY-class I B allele HLA-B*3501 complex					
Nef(74–81)	Nef(74–82)	VPLRPMTY		human(A3)	[Carreno (1992)]
• Included in HLA-A3 binding peptide competition study					
Nef(74–81)	Nef( )	VPLRPMTY	HIV-1 exposure	human(B35)	[Rowland-Jones (1998b)]
• HIV specific-CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection • Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world • Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes • This epitope is conserved among A, B, and D clade viruses					
Nef(74–81)	Nef( )	VPLRPMTY		human(B35)	[Rowland-Jones (1999)]
• CTL responses in seronegative highly HIV-exposed African female sex workers in Gambia and Nairobi were studied – these women had no delta 32 deletion in CCR5 • In Gambia there is exposure to both HIV-1 and HIV-2, CTL responses to B35 epitopes in exposed uninfected women are cross-reactive, • HIV-2 version of this epitope is conserved: VPLRPMTY, and CTLs are cross-reactive – one of five B35 CTL epitopes that are cross-reactive, see also [Rowland-Jones (1995)]					

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(74–82)	Nef(73–82)	VPLRPMTYK	no CTL shown	human(A11)	[Zhang (1993)]
	• Exploration of A11 binding motif				
Nef(75–82)	Nef(75–82 LAI)	PLRPMTYK	HIV-1 infection	human(A*1101)	[McMichael & Walker(1994)]
	• Review of HIV CTL epitopes • C. Brander notes that this is a A*1101 epitope in the 1999 database				
Nef(77–85)	Nef(77–85 LAI)	RPMTYKAAL	HIV-1 infection	human(B*0702)	[Bauer (1997)]
	• Structural constraints on the Nef protein may prevent escape • Noted in Brander 1999, this database, to be B*0702				
Nef(82–91)	Nef(82–91 LAI)	KAAVDLSHFL	HIV-1 infection	human(C*0802)	[Nixon (1999)]
	• A patient who made a mono-specific CTL response to this Nef specific epitope was given effective anti-retroviral therapy within 90 days of infection, reducing the antigenic stimulous • Within 7 days of therapy, his CTLp frequency dropped from 60 to 4 per million PBMC, as his viremia dropped • The patient went from having a activated effector population (detected by CTLp and clone specific RNA) to a non-activated quiescent population (detected by the CTL-clone specfic DNA)				
Nef(82–101)	Nef(81–100 SF2)	KAAVDLSHFLKEKGGLEGLI	HIV-1 infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • Three of these 11 had CTL response to this peptide • The responding subjects were HLA-A1, A2, B8, B14; HLA-A11, A24, B8, B53				
Nef(83–94)	Nef(83–94 BRU)	AAVDLSHFLKEK	HIV-1 infection	human(A11)	[Culmann (1991)]
	• Epitope defined by boundaries of overlapping peptides that stimulate Nef CTL clones				
Nef(84–91)	Nef(84–91 LAI)	AVDLSHFL	HIV-1 infection	human(Bw62)	[Culmann-Penciolelli (1994)]

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(84–92)	Nef(84–92 LAI)	AVDLSHFLK	HIV-1 infection	human(A11)	[McMichael & Walker(1994)]
	• Review of HIV CTL epitopes • C. Brander notes that this is a A*1101 epitope in the 1999 database				
Nef(84–92)	Nef(84–92 LAI)	AVDLSHFLK	HIV-1 infection	human(A11)	[Couillin (1994), Goulder (1997a)]
	• Mutational variation in HIV epitopes in individuals with appropriate HLA types can result in evasion of CTL response • [Goulder (1997a)] is a review of immune escape that summarizes this study				
Nef(84–92)	Nef(84–92 LAI)	AVDLSHFLK	HIV-1 infection	human(A11)	[Couillin (1995)]
	• Mutations found in this epitope in HLA-A11 positive and negative donors were characterized				
Nef(86–94)	Nef(84–92 LAI)	DLSHFLKEK	HIV-1 infection	human(A3.1)	[McMichael & Walker(1994)]
	• Review of HIV CTL epitopes				
Nef(86–100)	Nef(86–100 LAI)	DLSHFLKEKGGLEGL	HIV-1 infection	human(B35)	[Buseyne (1993b)]
Nef(86–100)	Nef(86–100 LAI)	DLSHFLKEKGGLEGL	HIV-1 infection	human(A2)	[Robertson (1993)]
	• Development of a retroviral vector (pNeoNef) to generate autologous targets				
Nef(86–100)	Nef(86–100 LAI)	DLSHFLKEKGGLEGL	HIV-1 infection	human(B35 or C4)	[Buseyne (1993a)]
	• Vertical transmission of HIV ranges from 13% to 39% • Primary assays showed cytotoxic activities against at least one HIV protein was detected in 70% of infected children • Epitopes recognized in five children were mapped using synthetic peptides and secondary cultures • Patient EM13, who had a CTL response to three epitopes in Nef, was infected via blood transfusion after birth and went from CDC stage P2A to P2E during the study				
Nef(87–102)	Nef( )	FSHFLKEKGGLEGLIY		human( )	[Jubier-Maurin (1999)]
	• 41 new HIV-1 strains describing envelope subtypes of HIV-1 A-H were genetically characterized in the nef region – 34 subtypes were classified in the same subtype in nef and env and 7 of the 41 strains were recombinants • This region was defined as a CTL epitope region that is conserved among HIV-1 M group subtypes				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(90–97)	Nef(89–97 LAI)	FLKEKGGL	HIV-1 infection	human(B*0801,B8)	[Price (1997)]
	• CTL escape variants appeared over time in HLA-B8 HIV-1+ individual, providing evidence for immune escape • Most variants appear at position 5, an anchor residue • FLKE(ENQ)GGL showed reduced binding efficiency and recognition • Double mutants (FIKENGGL, FLEENGGL, and FLKGNGGL) completely escaped recognition • [Goulder (1997a)] is a review of immune escape that summarizes this study in the context of CTL escape to fixation • Noted in Brander 1999, this database, to be B*0801				
Nef(90–97)	Nef( )	FLKEKGGL	Multi-epitope gene in VVA	human(B8)	[Hanke (1998b), Hanke (1998a)]
	• This epitope was shown to be processed and presented to appropriate CTL clones upon infection of human target cells with vaccinia virus Ankara (VVA) carrying 20 HIV-1 epitopes recognized by humans				
Nef(90–97)	Nef(88–95)	FLKEKGGL	HIV-1 infection	human(B8)	[Goulder (1997g)]
	• Natural variants for this epitope have been observed in several donors • Substitutions Q5, N5, E5 that alter anchor position 5 are not well recognized • Substitution I2 binds well to B8 and is recognized				
Nef(90–97)	Nef(90–97)	FLKEKGGL	HIV-1 infection	human(B8)	[Dyer (1999)]
	• CTL specific responses were measured over a 1.5- to 1.3-year period in members of the Sydney Blood Bank Cohort (SBBC) who had been infected with a natural attenuated strain of HIV-1 which was nef-defective. Some of these patients had prolonged high levels of CTL effector and memory cells despite low viral load.				
Nef(92–101)	Nef(92–101)	KEKGGLEGL		human(B*4001,B60)	
	• Noted by C. Brander *et al*., this database 1999, to be a B*4001,B60 epitope, Pers. Comm. P. Goulder and M. Altfeld				
Nef(93–106)	Nef(93–106 BRU)	EKGGLEGLIHSQRR	HIV-1 infection	human(A1, B8)	[Hadida (1992)]
	• HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients				
Nef(102–115)	Nef(102–115 LAI)	HSQRRQDILDLWIY	HIV-1 infection	human(B7)	[Goulder (1997b), Goulder (1997a)]
	• Identical twin hemophiliac brothers were both infected with the same batch of factor VIII • One had a strong response to this peptide, the other did not • [Goulder (1997a)] is a review of immune escape that summarizes this study				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(102–121)	Nef(101–120 SF2)	HSQRRQDILDLQIYHTQGYF	HIV-1 infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • Two of these 11 had CTL response to this peptide • The responding subjects were HLA-A2, A3, B8, B62 and HLA-A2, B21				
Nef(103–127)	Nef(103–127 PV22)	SQRRQDILDLWIYHTQG-YFPDWQNY	HIV-1 infection	human(B13)	[Jassoy (1993)]
	• HIV-1 specific CTLs release γ-IFN, and α- and β-TNF				
Nef(105–114)	Nef(105–114 LAI)	RRQDILDLWI	HIV-1 infection	human(B*2705)	[Goulder (1997e)]
	• Defined as optimal epitope from within reactive peptide HSQRRQDILDLWIYHTQGYF [Nef(102-121 LAI)] • HLA-B*2705 is associated with slow HIV disease progression • The HLA-B*2705 binding motif includes R at position 2, and L in the C-term position				
Nef(112–133)	Nef(111–132)	LWIYHTQGYFPDWQNYT-PGPGV	HIV infection	human( )	[Lieberman (1995)]
	• HIV-specific CTL lines developed by *ex vivo* stimulation with peptide				
Nef(112–133)	Nef(111–132 SF2)	LWIYHTQGYFPDWQNYT-PGPGV	HIV infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • Four of these 11 had CTL response to this peptide • The responding subjects were HLA-A2, B21; HLA-A1, A3, B7, B15; HLA-A2, A26, B7, B38				
Nef(112–133)	Nef(111–132 SF2)	LWIYHTQGYFPDWQNYT-PGPGV	HIV-1 infection	human( )	[Lieberman (1997b)]
	• CTL expanded *ex vivo* were later infused into HIV-1 infected patients				
Nef(113–125)	Nef(113–125 BRU)	WIYHTQGYFPDWQ	HIV-1 infection	human(B17)	[Culmann (1989)]
	• Nef CTL clones from HIV+ donors				
Nef(113–126)	Nef( )	VYHTQGYFPDWQNY	HIV-1 infection	human( )	[Jubier-Maurin (1999)]
	• 41 new HIV-1 strains describing envelope subtypes of HIV-1 A-H were genetically characterized in the nef region – 34 subtypes were classified in the same subtype in nef and env and 7 of the 41 strains were recombinants • This region was defined as a CTL epitope region that is conserved among HIV-1 M group subtypes				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(113–128)	Nef(113–128 BRU)	WIYHTQGYFPDWQNYT	HIV-1 infection	human(A1)	[Hadida (1992)]
	• HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients				
Nef(115–125)	Nef(115–125 BRU)	YHTQGYFPDWQ	HIV-1 infection	human(B17)	[Culmann (1991)]
	• Nef CTL clones from HIV+ donors				
Nef(116–125)	Nef(116–125 BRU)	HTQGYFPDWQ	HIV-1 infection	human(B*5701,B57)	[Culmann (1991)]
	• Nef CTL clones from HIV+ donors, optimal peptide mapped • Described as B*5701 in C. Brander *et al*., this database, 1999				
Nef(117–127)	Nef(117–127 LAI)	TQGYFPDWQNY	HIV-1 infection	human(B*1501,Bw62)	[Culmann(1998)]
	• Optimal peptide defined by titration • Noted in Brander 1999, this database, to be B*1501, Pers. Comm. B. Culmann				
Nef(117–128)	Nef(117–128 BRU)	TQGYFPDWQNYT	HIV-1 infection	human(B17, B37)	[Culmann (1991)]
	• Nef CTL clones from HIV+ donors				
Nef(118–127)	Nef(118–127 LAI)	QGYFPDWQNY		human(Bw62)	[McMichael & Walker(1994)]
	• Review of HIV CTL epitopes				
Nef(120–128)	Nef(120–128 LAI)	YFPDWQNYT	HIV-1 infection	human(B*3701,B37, B*5701,B57)	[Culmann(1998)]
	• Nef CTL clones from HIV+ donors – optimum peptide mapped by titration • Noted by C. Brander *et al*., this database 1999, to be a B*3701 epitope and a B*5701 epitope				
Nef(120–128)	Nef(120–128 IIIB)	FFPDWKNYT	HIV-1 infection	human(B15)	[Wilson (1999a)]
	• This study describes maternal CTL responses in the context of mother-to-infant transmission • Detection of CTL escape mutants in the mother was associated with transmission, but the CTL susceptible forms of the virus tended to be found in infected infants • LFPDWKNYT is an escape mutant				
Nef(120–144)	Nef(120–144 SF2)	YFPDWQNYTPGPGIRYP-LTFGWCYK	HIV-1 infection	human(A24)	[Jassoy (1992)]
	• Epitope recognized by CTL clone derived from CSF				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(122–141)	Nef(121–140 SF2)	PDWQNYTPGPGVRYPLTFGW	HIV-1 infection	human( )	[Lieberman (1997a)]
• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • Three of these 11 had CTL response to this peptide • The responding subjects were HLA-A2, B21; HLA-A3, A24, B7, B38					
Nef(123–137)	Nef(123–137 IIIB)	QWQNYTPGPGVRYPL	HIV-1 infection	human( )	[Wilson (1996)]
• Epitope defined in the context of the Pediatric AIDS Foundation ARIEL Project, a mother-infant HIV transmission study • FFPDYTPGPGTRFPL and FFPDYKPGPGTRFPL, naturally occurring variants, were found in mother and are not recognized • LFPDYKPGPGTRFPL and FFPDYKPGPGTRFPL, naturally occurring variants, were found in infant and are not recognized					
Nef(126–138)	Nef(126–138 BRU)	NYTPGPGVRYPLT	HIV-1 infection	human(B7)	[Culmann (1991)]
• Nef CTL clones from HIV+ donors					
Nef(128–137)	Nef(128–137 LAI)	TPGPGVRYPL	HIV-1 infection	human(B*0702,B7)	[Haas (1996), Haas (1997)]
• There was a high degree of variation in three CTL epitopes in Nef in four slow and non-progressors, and variant specific CTLs arose over time to eliminate variants, indicating immune selection • The epitope position was taken from [Haas (1997)] • Noted in Brander 1999, this database, to be B*0702					
Nef(128–137)	Nef( )	TPGPGVRYPL	HIV-1 exposure	human(B7)	[Rowland-Jones (1998a)]
• A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating • The D subtype consensus is identical to the B clade epitope • The A subtype consensus is TPGPGIRYPL					
Nef(128–137)	Nef( )	TPGPGVRYPL	HIV-1 exposure	human(B7(*8101))	[Rowland-Jones (1998b)]
• HIV specific-CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection • Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world • Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes • Clade A version of the epitope: TPGPGIRYPL, clade D version: TPGPGIRYPL					

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(128–137)	Nef( )	TPGPGVRYPL	HIV-1 exposure	human(B7)	[Rowland-Jones (1998b)]
	• HIV specific-CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection • Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world • Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes • This epitope is conserved among B and D clade viruses • The Clade A version of the epitope: TPGPGIRYPL				
Nef(128–137)	Nef(128–137)	TPGPGVRYPL	*in vitro* stimulation	human(B7)	[Wilson (1999b)]
	• Dendritic cells are the most potent for priming T cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors • Th1-biasing cytokines IL-12 or IFN alpha enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within • CTL from a B7 donor displayed no reactivity to this epitope, although it had been immunodominant in another study [Haas (1996)]				
Nef(128–137)	Nef(128–137 LAI)	TPGPGVRYPL		human(B*4201,42)	
	• Noted in C. Brander *et al*., 1999, this database, to be B*4201, P. Goulder, Pers. Comm.				
Nef(130–143)	Nef(130–143 LAI)	GPGVRYPLTFGWCY	HIV-1 infection	human(B*57)	[Goulder (1996b)]
	• CTL response to this epitope observed in 4 long term survivors • Peptide defined on the basis of B*5801 binding motif, yet not cross-restricted except at high concentrations				
Nef(131–143)	Nef( )	GIRYPLTFGWCFK		human( )	[Jubier-Maurin (1999)]
	• 41 new HIV-1 strains describing envelope subtypes of HIV-1 A-H were genetically characterized in the nef region – 34 subtypes were classified in the same subtype in nef and env and 7 of the 41 strains were recombinants • This region was defined as a CTL epitope region that is conserved among HIV-1 M group subtypes				
Nef(132–147)	Nef(132–147 BRU)	GVRYPLTFGWCYKLVP	HIV-1 infection	human(A1, B8)	[Hadida (1992)]
	• HIV-1 specific CTLs detected in lymphoid organs				
Nef(132–147)	Nef(132–147 BRU)	GVRYPLTFGWCYKLVP	HIV-1 infection	human(B18)	[Culmann (1991)]
	• Nef CTL clones from HIV+ donors				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(133–148)	Nef(133–148 LAI)	VRYPLTFGWCYKLVPV		human(B57)	[Brander & Walker(1997a)]
	• P. Goulder, pers. comm.				
Nef(134–141)	Nef(134–141 LAI)	RYPLTFGW		human(B27)	[Culmann(1998)]
	• Optimal peptide defined by titration				
Nef(134–143)	Nef(138–147 SF2)	RYPLTFGWCF	HIV-1 infection	human(A*2402)	[Ikeda-Moore (1997)]
	• Defined using reverse immunogenetics – 59 HLA-A*2402 binding peptides were predicted by searching for A*2402 anchors in HIV proteins, (Tyr at 2, and Phe, Leu or Ile at the C term) – 53 of the 59 peptides bound A*2402 • This peptide induced CTL in 3/4 HIV-1+ people tested • RYPLTFGWCF bound to A*2402 strongly, the epitope can be processed in a vaccinia construct and presented – two specific CTL clones were obtained				
Nef(134–144)	Nef(134–144 LAI)	RYPLTFGWCYK	HIV-1 infection	human(B18)	[Couillin (1994), Goulder (1997a)]
	• Mutational variation in HIV epitopes in individuals with appropriate HLA types can result in evasion of CTL response • [Goulder (1997a)] is a review of immune escape that summarizes this study				
Nef(135–143)	Nef(139–147 SF2)	YPLTFGWCF	HIV-1 infection	human(B35)	[Shiga (1996)]
	• Binds HLA-B*3501				
Nef(135–143)	Nef( )	YPLTFGWCY	HIV-1 exposure	human(B49)	[Rowland-Jones (1998a)]
	• A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating • The A subtype consensus is identical to the B clade epitope • The D subtype consensus is YPLTFGWCf				
Nef(135–143)	Nef(135–143 LAI)	YPLTFGWCY	HIV-1 exposure	human(B*1801,B18)	[Culmann (1991), Culmann-Penciolelli (1994)]
	• Nef CTL clones from HIV+ donors • Noted in Brander 1999, this database, to be B*1801				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(135–143)	Nef( )	YLPTFGWCY	HIV-1 exposure	human(B49)	[Rowland-Jones (1998b)]
• HIV specific-CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection • Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world • Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes • This epitope is conserved among A and B clade viruses • The Clade D version of the epitope, YPLTFGWCF, was preferentially recognized by CTL					
Nef(136–145)	Nef(136–145)	PLTFGWCFKL	HIV-1 infection	human(A2)	[Durali (1998)]
• Cross-clade CTL response was studied by determining the CTL activity in seven patients from Bangui, (6 A subtype, and 1 AG recombinant infections) and one A subtype infection from a person living in France originally from Togo, to different antigens expressed in vaccinia • Pol reactivity: 8/8 had CTL to A subtype, and 7/8 to B subtype, and HIV-2 Pol was not tested • Gag reactivity: 7/8 reacted with A or B subtype gag, 3/8 with HIV-2 Gag • Nef reactivity: 7/8 reacted with A subtype, and 5/8 with B subtype, none with HIV-2 Nef • Env reactivity: 3/8 reacted with A subtype, 1/8 with B subtype, none with HIV-2 Env • Patient B18 had the greatest breadth and diversity of response, and recognized Gag SLYNTVATL and Nef PLTFGWCFKL					
Nef(136–145)	Nef(136–145)	PLTFGWCYKL	*in vitro* stimulation	human(A*0201)	[Wilson (1999b)]
• Dendritic cells are the most potent for priming T cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors • Th1-biasing cytokines IL-12 or IFN alpha enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within • B7 and A2 Nef epitopes were studied and the relative binding affinity of A2 epitopes for A2 was: PLTFGWCYKL greater than VLEWRFDSRL much greater than AFHHVAREL • Noted in Brander *et al*., 1999 this database, to be A*0201					
Nef(162–181)	Nef(161–180)	TSLLHPVSLHGMDDPEREVL	HIV infection	human( )	[Lieberman (1995)]
• HIV-specific CTL lines developed by *ex vivo* stimulation with peptide					
Nef(162–181)	Nef(161–180 SF2)	TSLLHPVSLHGMDDPEREVL	HIV infection	human( )	[Lieberman (1997a)]
• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • One of these 11 had CTL response to this peptide					

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(162–181)	Nef(101–120 SF2)	TSLLHPVSLHGMDDPEREVL	HIV-1 infection	human( )	[Lieberman (1997b)]
	• CTL expanded *ex vivo* were later infused into HIV-1 infected patients				
Nef(162–181)	Nef(161–180 SF2)	TSLLHPVSLHGMDDPEREVL	HIV infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • One of these 11 had CTL response to this peptide				
Nef(172–191)	Nef(171–190 SF2)	GMDDPEREVLEWRFDSRLAF	HIV-1 infection	human( )	[Lieberman (1997a)]
	• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • One of these 11 had CTL response to this peptide • The responding subject was HLA-A2, B21				
Nef(180–189)	Nef(180–189 LAI)	VLEWRFDSRL	HIV-1 infection	human(A*0201)	[Haas (1996), Haas (1997)]
	• There was a high degree of variation in three CTL epitopes in Nef in four slow and non-progressors, and variant specific CTLs arose over time to eliminate variants, indicating immune selection • Noted in Brander *et al*., 1999 this database, to be A*0201				
Nef(180–189)	Nef(180–189)	VLEWRFDSRL	*in vitro* stimulation	human(A2)	[Wilson (1999b)]
	• Dendritic cells are the most potent for priming T cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors • Th1-biasing cytokines IL-12 or IFN alpha enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from withi • B7 and A2 Nef epitopes were studied and the relative binding affinity of A2 epitopes for A2 was: PLTFGWCYKL greater than VLEWRFDSRL much greater than AFHHVAREL				
Nef(182–198)	Nef(182–198 BRU)	EWRFDSRLAFHHVAREL	HIV-1 infection	human(A1, B8)	[Hadida (1992)]
	• HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients				
Nef(182–198)	Nef(182–198 BRU)	EWRFDSRLAFHHVAREL	HIV-1 infection	human(A25)	[Cheynier (1992)]
	• CTL isolated in children born to HIV-1 positive mothers				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(182–198)	Nef(182–198 LAI)	EWRFDSRLAFHHVAREL	HIV-1 infection	human(B35)	[Hadida (1995)]
• The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions					
Nef(182–198)	Nef(182–198 LAI)	EWRFDSRLAFHHVAREL	HIV-1 infection	human(A1, A25(10))	[Hadida (1995)]
• The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions					
Nef(182–198)	Nef(182–198 LAI)	EWRFDSRLAFHHVAREL	Rec Mengo virus-HIV 1 Nef 65-206	murine(H-2^d)	[Van der Ryst (1998)]
• Macaca mulatta did not have a detectable response to this vaccine • Balb/c mice had a weak response to this epitope in the Mengo virus construct – in contrast, HIV-1 Nef induces a strong CTL response in mice when presented in a vaccinia background					
Nef(182–201)	Nef(191–205 SF2)	EWRFDSRLAFHHVARELHPE	HIV-1 infection	human( )	[Lieberman (1997a)]
• Of 25 patients, most had CTL specific for more than 1 HIV-1 protein • 11 subjects had CTL that could recognize vaccinia expressed LAI Nef • One of these 11 had CTL response to this peptide • The responding subject was HLA-A2, B21					
Nef(186–193)	Nef(186–193 LAI)	DSRLAFHH	HIV-1 infection	human(B35)	[Hadida (1995)]
• The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions					
Nef(186–194)	Nef(186–194 BRU)	DSRLAFHHV		human(B51)	[Connan (1994)]
• Resulted in the assembly of HLA-B51					
Nef(188–196)	Nef(188–196 LAI)	RLAFHHVAR	HIV-1 infection	human(B52)	[Hadida (1995)]
• The C-terminal region of Nef (182-205) contains multiple CTL epitopes with 5 distinct HLA restrictions					
Nef(188–201)	Nef(188–201 LAI)	RLAFHHVARELHPE	HIV-1 infection	human(B35 or C4)	[Buseyne (1993a)]
• Vertical transmission of HIV ranges from 13% to 39% • Primary assays showed cytotoxic activities against at least one HIV protein was detected in 70% of infected children • Epitopes recognized in five children were mapped using synthetic peptides and secondary cultures • Patient EM13, who had a CTL response to three epitopes in Nef, was infected via blood transfusion after birth and went from CDC stage P2A to P2E during the study					

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef(190–198)	Nef(190–198 LAI)	AFHHVAREL	HIV-1 exposure	human(A2)	[Rowland-Jones (1998a)]
	• CTL recognition reported in the context of HLA-B52 and A2.1, A2.2 and A2.4 • A CTL response was found in exposed but uninfected prostitutes from Nairobi using previously defined B clade epitopes that tended to be conserved in A and D clades – such cross-reactivity could protect against both A and D and confer protection in Nairobi where both subtypes are circulating • The A subtype consensus is ALKHRAYEL • The D subtype consensus is AfEHKAREm • [Hunziker (1998)] suggests that HLA-A2 does not in fact present this epitope, and notes that it does not promote A2 assembly [Connan (1994)] – also see [Brander (1998b)] • [Hunziker (1998)] maintains that HLA-A2 does not present this epitope contrary to an earlier report [Hadida (1995)], (also see [Brander (1998a)]) – despite the position of Hunziker *et al*., Rowland-Jones and colleagues are confident that this epitope in its A clade form is presented by HLA-A*0201 and A*0202, and it is one of the most common responses seen in both seropositive and exposed-uninfected donors from Nairobi (Rupert Kaul, Pers. Comm.)			
Nef(190–198)	Nef(190–198 LAI)	AFHHVAREK	HIV-1 infection	human(A3)	[Hadida (1995)]
	• Naturally occurring L to K anchor substitution abrogates A2 binding, but permits HLA-A3 binding				
Nef(190–198)	Nef( )	AFHHVAREL	HIV-1 exposure	human(A2, A*0202, A*0201)	[Rowland-Jones (1998b)]
	• HIV specific-CTL were found in exposed seronegative prostitutes from Nairobi – these CTL may confer protection • Seroprevalence in this cohort is 90-95% and their HIV-1 exposure is among the highest in the world • Most isolated HIV strains are clade A in Nairobi, although clades C and D are also found – B clade epitopes are often cross-reactive, however stronger responses are frequently observed using A or D clade versions of epitopes • Clade A version of the epitope: ALKHRAYEL, Clade D epitope: AFEHKAREM • This epitope was recognized by two different exposed and uninfected prostitutes				
Nef(190–198)	Nef(190–198)	AFHHVAREL	*in vitro* stimulation	human(A2)	[Wilson (1999b)]
	• Dendritic cells are the most potent for priming T cell responses – DCs can stimulate autologous CTL responses from T-cells cultured from HIV negative donors • Th1-biasing cytokines IL-12 or IFN alpha enhance CTL responses *in vitro* whether the epitope is delivered by pulsing from peptide, or expressed from within • B7 and A2 Nef epitopes were studied and the relative binding affinity of A2 epitopes for A2 was: PLTFGWCYKL greater than VLEWRFDSRL much greater than AFHHVAREL				
Nef(192–206)	Nef(192–206 BRU)	HHVARELHPEYFKNC	HIV-1 infection	human(A1)	[Hadida (1992)]
	• HIV-1 specific CTLs detected in lymphoid organs of HIV-1 infected patients				

HXB2 Location	Author Location	Sequence	Immunogen	Species(HLA)	References
Nef( )	Nef( )		HIV-1 infection	human( )	[De Maria (1997)]
• CD3+ cells that also carry a natural killer cell receptor (NKR+) can exhibit down regulation of T-cell function • Anti-NKR IgM MAb masked this inhibitory function and increased HIV-1 specific CTL activity in phytohemagglutinin-activated PBMC cultured in the presence of IL-2 from 3/5 patients, and in one other case anti-NKR MAb brought HIV-1 specific CTL activity to detectable levels					
Nef( )	Nef( )		HIV-1 infection	human( )	[Buseyne (1998a)]
• This study showed a correlation with strong CTL memory and greater breadth of response in 7-12 months old infants, and remaining AIDS free for the first year of life, having higher absolute CD4 and CD8 cells, and lower viral load					
Nef( )	Nef( )		HIV-1 infection	human( )	[Buseyne (1998b)]
• In infants with positive CTL responses, most responses showed cross-clade reactivity with somwhat diminished recognition of epitopes from different subtypes					
Nef( )	Nef( )		Canary pox -HIV vaccine	human( )	[Evans (1999)]
• A Canary pox vaccine expressing gp120, gp41, Gag, Protease, Nef and Pol CL epitopes gave rise to CTL that could be detected in 61% of the volunteers – responses to Gag, Env, Nef and Pol were detected 3-6 months after the last vaccination					
Nef( )	Nef( )		HIV-1 infection	human( )	[da Silva & Hughes(1998)]
• CTL dense regions of Nef tend to lie in conserved domains with low non-synonymous substitution per site – authors consider that this may be due to a host adaptation to infection that focuses the CTL response to be directed against conserved functional domains [da Silva & Hughes(1998)]					
Nef( )	Nef( )		HIV infection	human( )	[Legrand (1997)]
• 17 recently infected patients were tested for CTL response to HIV proteins Env, Gag, Pol, Rev, Nef, Vif and Tat • An early response (within a month following PI) was noted in 87% of the subjects to Gag, 75% to Env, and 50% to Nef • Early responses to Pol, Rev, Vif and Tat were rare					
Nef( )	Nef( )		HIV infection	human( )	[Zerhouni (1997)]
• CTL responses to Env, Gag, Nef and RT were tested at various phases of disease progression – 10 asymptomatic patients generally had CTL responses to all proteins, 10 ARC patients responded well to all proteins except Nef, and AIDS patients had few responses to any proteins					
Nef( )	Nef( )		HIV-1 infection	( )	[Kuiken (1999)]
• A correlation between conserved regions of Nef and CTL epitope density was also noted in [Kuiken (1999)]. The authors suggest that this may be due to biological reasons such as the one described above, [da Silva & Hughes(1998)] or due to epitope processing, or may possibly be an artifact of experimental strategy for epitope definition such that conserved epitopes would tend to be identified because they would be more likely to be cross-reactive with the test reagents • Both p17 and Nef show a correlation between epitope density and conserved regions in the protein – in contrast, p24 is a more conserved protein and known epitopes are evenly distributed across p24					