Scientific Basis for a Live Attenuated *nef* – deleted HIV-1 Therapeutic Vaccine

Hypothesis

It is postulated by the inventor that a *nef* deficient live attenuated HIV-1 constructed with a large deletion in the *nef* and in which the remaining open reading frames, particularly *tat, pol, gag, env* and *vpr* are preserved, when injected in an individual infected with a wild-type HIV-1, is therapeutic as a result of one or more of the following mechanisms:

1. By allowing normal interleukin-2 (IL2), gamma interferon (IFNγ) and other unknown activating agent/chemokine production in T_{HELPER} cells thus restoring signaling and activation of specific cytotoxic ("killer") T lymphocytes (CTL) recognizing HIV antigen displaying cells in addition to activating B lymphocytes,
2. By preventing anergy of cytotoxic T lymphocytes produced by inhibition of a second chemokine activating signal in specific binding signaling pairs -

    a. VCAM-1(Vascular Cell Adhesion Molecule-1) : VLA-4 (Very Late integrin Antigen-4),
    b. ICAM-1/2 (InterCellular Adhesion Molecule –1/2) : LFA-1 (Lymphocyte Function Associated Molecule-1),
    c. LFA-3 : CD2 (Clusters of Differentiation 2) ,
    d. B7 (B cell mediated coactivator –7) : CTLA-4 (Cytotoxic T Lymphocyte Activator –4),

e. ? : HSA (Heat Stable Antigen) or heretofore unknown lymphokines

by the Nef protein. This second signal is required for activation of a T cell that has MHC-I (Major Histocompatibility Complex Class I):TCR (T-Cell Receptor) binding and lack of it creates anergy,

2. By continually activating, stimulating and maintaining a cell mediated immune response to wild-type HIV via lines of specific CTLs which appear to be crucial in controlling and/or eliminating HIV in exposed individuals that didn't seroconvert, in "non-progressors" and in cross-immunity provided by HIV-2.
3. By eliminating the protection offered by Nef to infected cells mediated via a downregulation of MHC class I on the cell surface.
5. and, in a minor way by competing with the wild-type HIV for potential hosts.

Using a therapeutic vaccine *after* exposure is possible only when the disease has a long incubation period before death ensues. One vivid example is use of live attenuated duck-embryo vaccine against rabies. This vaccine is given *after* exposure or suspected exposure and is extremely effective because rabies has an incubation period of 60 days to 1 year. Since the incubation period in HIV infection leading to AIDS and death is even longer, a post-exposure vaccination is extremely promising.

Scientific rationale behind Contre Vir™

There is currently no specific immunological treatment utilizing specific immunodynamics for the treatment of Human Immunodeficiency Virus (HIV) infection. The two main sub types HIV-1 and HIV-2 are members of a group of closely related human and non-human primate lentiviruses which are RNA retroviruses. Several attempts at prevention and treatment have

been made by using virus envelope (gp120, gp160 & gp41) and *gag* (p24 & p15) proteins to develop humoral (antibody or B-cell mediated immunity) all of which have so far been unsuccessful because the virus engages in cell to cell propagation and largely escapes the neutralizing antibodies. There are other vaccines currently being studied that would use vectors such as canarypox or vaccinia viruses expressing HIV envelope and one or two *gag* proteins primarily to induce both B and T cell immunity against those proteins or presenting cells strictly for prophylaxis. These are in Phase I studies and their efficacy as a prophylactic is unknown at this time. The HIV also mutates causing different serotypes of antigen in successive generation[21]. Phenotypic heterogeneity is found in replication kinetics, susceptibility to serum neutralization, anti-viral drug resistance, induction of cytopathicity and host-cell range specificity thus creating the need for a so called "polyvalent" vaccine that will be effective against all the known and unknown clades of HIV-1 . Moreover, some viral proteins inhibit the induction of IL-2 mRNA in infected cells thus defeating the T cell mediated immunity.

Infection by HIV leads to progressive deterioration of cell-mediated immunity via loss of T_{HELPER} cells bearing CD_4 receptors. This makes the victim susceptible to opportunistic infections such as *pneumocystis carinii* pneumonia, cytomegalovirus, *toxoplasma gondii* and *Mycobacterium tuberculosis* infections. Tumors such a Kaposi's sarcoma also commonly occur. Once the CD_4 counts drop to near zero, death ensues rapidly.

AIDS and HIV infection initially involved homosexual men, intravenous drug users and hemophiliacs in the United States and Europe. However, heterosexual infection has become common and rampant in Africa (particularly Rwanda, Burundi, Zaire, Uganda, Kenya and Tanzania), Brazil, India, Myanmar and Thailand. According to the World Health Organization (WHO), in excess of 40,000,000 people currently harbor HIV infection worldwide.

Just like the viral protein vaccines tried thus far (and research abandoned by the National Institutes of Health due to poor results), pharmacological treatment with AZT (zidovudine), DDC (dideoxycytosine), DDI (dideoxyinosine) and protease inhibitors has also been frustrating due to development of resistant stains of the virus which continue the infection after, at most, a short break. At present, there is no definitive treatment that would effectively eliminate virus harboring cells and restore the cellular immune system. The protease inhibiting drugs have shown great promise and reduced mortality by lowering viral burdens, however, reservoir of pro-viral DNA in the neuroglia, development of resistance and serious sided effects such as diabetes, hypertension and cushingoid fat distribution make them to be far from panacea.

A variety of different approaches have been postulated which do not rely on either the B cell mediated response or on pharmacological intervention in viral synthesis. The late Professor Jonas Salk, in his commentary in Nature noted that as the disease progresses, titers of antibodies to gp41 and virus neutralizing antibody remain constant but the level of antibody which correlates with the presence of antibody dependent cell cytotoxicity (ADCC) and antibody to reverse transcriptase decline. He proposed treatment of symptomatic HIV infected patients with sera from asymptomatic HIV infected patients. He further hypothesized that HIV immunogens given to HIV infected patients would be protective[22].

Dead virions have been hypothesized but no researcher has yet tried whole dead virions either for prevention or treatment of HIV infection in humans in a meaningful manner. An inactivated gp120-depleted HIV-1 immunogen (Remune®) has been tried as a therapeutic uplift. While this immunogen increases the CTL activity directed at its own antigens, it cannot improve such activity significantly against other antigens.[66,67,68,69] Further research has revealed that this approach does not work.

Simian Immunodeficiency Virus (SIV) is a primate lentivirus with various strains that affect African green monkeys, macaque monkeys, sooty-mangabee monkeys, rhesus monkeys and chimpanzees. SIV infection in monkeys is widely used to study the physiology and pathology of the primate lentiviruses. A great deal of research as been done by attempting to infect monkeys with artificially created mutants of the SIV to determine their relative infectivity. Many of these studies focused on the role of the *nef* gene in the physiology of virus life cycle. The *nef* gene is present in all primate lentiviruses sequenced to date. The gene consists of an open reading frame beginning within or immediately after the 3' end of the *env* gene and overlaps the U3 portion of the 3' long terminal repeat. The gene was previously named F, 3'-orf or B-orf. It is expressed *in vivo* as determined by antibodies to the *nef* gene product in infected individuals. Luria et al have shown that at least some *nef* gene products block the induction of IL-2 mRNA in lymphoid cells triggered by activating agents phorbol myristate acetate (PMA), phytohemeagglutinin (PHA) and/or antibodies against CD3, TCR or CD2[15]. Kestler et al have found rapid reversion of stop codon point mutations in *nef* to open forms *in vivo*, demonstrating selective pressure for open, presumably functional forms of *nef* [12]. It was further shown that *nef* is necessary for vigorous virus replication in rhesus monkeys, for maintaining normal virus loads and for induction of the disease. Animals inoculated with *nef*-deletion mutants have remained disease free for at least 3 years while wild-type virus infected animals all developed AIDS and died[6]. This protection also extends to administration of SIV infected cells[1]. It has also been demonstrated that *nef* deletion increases viral replication but it is postulated that the responses to *nef* deletion are different *in vivo* and *in vitro*[10]. Additonal evidence, recently published shows a strong role of *nef* in T-cell signaling defects mediated via SH3, Lck, HLA-B7, NF-kappa pathways etc.[26,27,29,30,25,36,37,38,40,41,45,49,51]. Immunization with live attenuated *nef*- SIV mutants in macaques has shown a strong type 1 T_{HELPER} response and beta-chemokine production[74]. It has also been shown that HIV-1 Nef mediates lymphocyte

chemotaxis and activation by infected macrophages[70] and that the conserved core of HIV-1 Nef is essential for association with Lck and for enhanced viral replication in T-lymphocytes[72]. It has also been shown that the HIV-1 Nef alters Ca^{++} signaling in myelomonocytic cells through SH3-mediated protein-protein interactions, the significance of which is not fully understood[73]. The HIV-1 *nef* gene expression affects generation and function of human T cells but not dendritic cells[71].

It is known that CTLs specific for epitopes in *env, gag, tat* and *pol* proteins can be detected quite early in the infection. It is a commonly held view that these cells, by killing virally infected cells prior to their production of additional infectious virus are responsible for the diminution in viral burden[24,25,31,44,48,60.]

Evidence has surfaced from a study of health care workers who had been exposed to HIV contaminated blood but did not seroconvert. When compared to individuals exposed to blood from uninfected individuals, 7 out of 20 persons exposed to HIV infected blood showed CTLs specific for HIV peptides in association with class I major histocompatibility complex (MHC) molecules. None of the individuals exposed to uninfected blood had similar CTLS[19]. The development of class I MHC- restricted and HIV specific CTLs is absolute proof the HIV infection occurred and then stopped. This is evident from the known cell biology of antigen presentation by class I MHC molecules because such a phenomenon generally requires endogenous production of protein from which the peptide is derived[9]. It is therefore quite plausible that the specific CTLs did eliminate the virus.

The recognition of "non-progressors" i.e. a set of HIV-1 infected individuals surviving for a prolonged period of time with essentially normal CD_4 counts and with no signs of disease suggest that an immune response is possible that actually control HIV[4,11,17.]

No specific information exists to explain this control of HIV in all of the non-progressors but in many cases, they possess CD_8 T cells that strikingly limit the capacity of HIV from their CD_4 T cells to infect peripheral blood mononuclear cells (PBMCs) that have been activated by phytohemagglutinin (PHA). Pure CD_4 cells from non-progressors are quite effective in transmitting their HIV to PHA-activated PBMCs but addition of their CD_8 T cells strikingly suppresses such infection. Even supernatants of CD_8 T cells can inhibit in vitro infection[16]. It has been postulated that a cell derived soluble factor from CD_8s distinct from any know cytokine may be responsible.

Spontaneously (via mutation) attenuated HIVs have been recovered from humans in whom infection has had a very benign course. Kirchhoff et al have described one individual with no sign of disease and a normal CD_4 cell count more than 10 years after infection[13]. Genomic analysis of this patient's HIV-1 virus at various times during this 10 year period has revealed a deletion in the *nef* gene that was sustained and extended. A set of individuals in Australia was infected from blood products obtained from a single infected donor with a similarly defective HIV-1 virus (The Sydney Blood Bank Cohort). All those infected had a benign, non-progressive course except for one individual who had systemic lupus erythematosus and was on high-dose glucocorticoid treatment[14]. Additional long-term survivors with defective *nef* genes in their HIV genome have been reported, [32,34,42,47,50,52,53,54,56]. A recent study has shown that in the Sydney Blood Bank Cohort, the effect of long-term infection with *nef*-defective attenuated HIV-1 resulted in an increase in $CD_{45}RO^+CD_4^+$ T lymphocytes and limited activation of CD_8^+ T lymphocytes[75].

Further evidence of CTL mediated immunity to HIV-1, this time via cross-reactivity to antigens of HIV-2, comes from an astounding series of observations. For example, a set of

commercial sex workers in Gambia infected with HIV-2 appeared to escape HIV-1 infection altogether in spite of exposure to several HIV-1 infected customers over a period of years. Many of these women have CTLs that recognize peptides shared by HIV-1 and HIV-2 which is suggestive of CTL mediated immune response to HIV–2 infection capable of cross-protection[20]. More support comes from an epidemiological study conducted on a group of commercial sex workers in Dakar, Senegal from 1985-1994. The subjects were segregated into HIV-2^+ and HIV-2^- groups. It was determined that over an extended period of observation, the risk of subsequent infection with HIV-1 of the HIV-2^+ group was about 2/3 less than the HIV-2^- women while the rate of other sexually transmitted diseases was virtually identical between the two groups[23]. Other observations have demonstrated similar results[60].

Evidence suggesting that an established HIV infection may not only be controlled but also actually eliminated comes from the study of one individual. HIV was isolated neonatally from a child but later, at age 5, the child was seronegative, had no clinical symptoms and had no recoverable virus. The infant had positive viral cultures on two separate occasions during the first year suggesting an infection from its mother[3]. So far, nothing is known about either the genomic nature of the virus infecting this child or about the anti-HIV immune response generated by it.

Finally, an Australian study of non-progressors has conclusively shown that an HIV-1 infected blood donor and a cohort of six recipients (Sydney Blood Bank Cohort or SBBC) infected from this donor were infected with an HIV-1 with deletions in the *nef* gene. This *nef* deficient strain has not caused disease even in the members affected by the immunosuppressive effects of age, drug therapy and systemic lupus erythematosus (SLE)[7].

It has been shown that the regulation of the quality of the immune response is controlled mainly by the cytokines available at the time of priming in addition to the antigen dose. Specifically, interleukin-4 (IL-4) appears to inhibit priming for IFNγ production by T cells and IL-12 appears to strikingly enhance priming for IFNγ production[18].

Several researchers have been sounding cautionary notes based upon their own observations and those concerns must be addressed here. Dr. Ruth Ruprecht of Dana-Farber Cancer Institute, a highly respected and leading researcher first showed that a *nef* deleted SIV can cause disease in newborn infants in 1995, followed by a publication in 1999 in which her group conclusively showed that a multiply deleted construct of SIV caused AIDS in six out of eight newborn macaques and in 2 out of 16 adult macaques one of which died of simian AIDS[62]. Another concerning note comes from a follow-up of the Sydney Blood Bank Cohort[61]. Even though other researchers have found that even a small 12 base pair deletion in the SIVmac *nef* can protect from a simultaneous infection by wild-type SIV in cynologus monkeys followed for 68 months without causing disease[65], we concur with the warning issued by Dr. Ruprecht and her colleagues regarding using a gene-deficient viral construct as a ***prophylactic*** vaccine in <u>healthy</u> individuals and are not proposing a prophylactic use in healthy subjects. However, as a therapeutic vaccine, we are actually encouraged by the above findings. It must be noted that 25% of the newborns and 88% of the adults did not develop the disease. In fact, 75% of the adult macaques that were injected did not show any signs of the disease at all, despite a long-term follow-up. Moreover, even the adult macaques that developed signs of disease progression only did so after a considerably longer period than with a wild type SIV. As a therapeutic vaccine, if the proposed Phase I research reveals high levels of immunogenicity in 75% of the subjects it would be extremely encouraging indeed! In fact, a group from Stanford University led by Dr. Douglas Owens suggests that even a therapeutic vaccine that is effective in 75% of the recipients would extend lives, prevent deaths and save money[63]. The Sydney Blood Bank

Cohort had a relatively minor mutation in the *nef* and we believe that a substantial deletion of the *nef* as proposed here will have a more significant impact on reduction in the pathogenicity of the virus than seen in the Sydney Blood Bank Cohort (SBBC).

It is therefore postulated by the inventor that influencing the quality of the immune response might enhance its protective value. Since CTLs seem to be the most important factor thus far in control and/or elimination of HIV in instances where such control/elimination have been observed, control of the balance of cytokines that are generated and enhancement of the degree of expression of immunity at a cellular level are extremely important. It is very likely that the Nef protein prevents a second signal required for activation of T cells (in addition to the MHC-11:TCR binding) such as pairs VCAM-1:VLA-4, ICAM-1/2:LFA-1, LFA-3:CD2, B7:CTLA-4,?HSA or heretofore unknown lymphokines. The exact mechanism of how the Nef protein accomplishes this is unknown but there is evidence to suggest that there is interference in protein-tyrosine phosphorylation and the IL-2 transcription via interdigitating leucine hydrophobic bonds ("leucine zipper") between *Fos & Jun* protooncogene coded proteins. Promoting induction of HIV-1 specific IFNγ producing T cells and CTLs as opposed to T cells that produce other cytokines such as IL-4 and related cytokines such as IL-5 and IL-10 while eliminating probable hindrance to IL-2 production by deletion of the *nef* gene is a new approach for significantly increasing the potency and quality of the immune response in infected individuals. This is particularly true regarding development of potent specific CTL lines, which seem to have played a major role in non-progressors and HIV-2 infected commercial sex workers. Another study has recently shown that Nef protected HIV-1 infected cells by reducing the epitope density on their surfaces allowing evasion of CTL lysis by them. This mechanism appears to be mediated via Nef driven down-regulation of MHC class I and could be overcome by adding an excess of the relevant HIV-1 epitope as a soluble peptide[64].

References

1. Almond N, Kent K, Cranage M, Rud E, Clarke B, and Stott EJ (1995). Protection by attenuated simian immunodeficiency virus in macaques against challenge with virus-infected cells. Lancet 346, 1342-1344.

2. Apte, SN et al. Live attenuated nef HIV-1 suspension as a therapeutic vaccine for the treatment of HIV-1 infection – In preparation.

3. Bryson YJ, Pang S, Wei LS, Dickover R, Diagne A, and Chen IS (1995). Clearance of HIV infection in a perinatally infected infant. N. Engl, J. Med. 332, 833-838.

4. Cao Y, Qin L, Zhang L, Safrit J, and Ho DD (1995). Virologic and Immunologic characterization of long-term survivors of Human Immunodeficiency Virus Type 1 infection. N. Engl, J. Med. 332, 201-208.

5. Coffin JM (1995). HIV population dynamics in vivo: Implication for gentic variation, pathogenisis, and therapy. Science 267, 483-489.

6. Daniel MD, Kirchhoff F, Czajak SC, Sehgal PK, and Desrosiers RC (1992). Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. Science 256, 1938-1941.

7. Deacon NJ, Tsykin A, Solomon A, Smith K, Ludford-Menting M, Hooker JD et al (1995). Genomic Structure of an Attenuated Quasi Species of HIV-1 from a Blood Transfusion Donor and Recipients. Science 270, 988-991.

8. Dullege AM (1994). Status and future of biocin HIV-RGP 120SF2 subunit vaccines: the experience of the Biocine Company, Neuvieme Colloque des Cent Gardes 301-306.

9. Germain RN and Marguiles DH (1993). The biochemistry and cell biology of antigen processing and presentation. Annu. Rev. Immunol. 11, 403-460.

10. Gibbs JS and Desrosiers RC (1993); in Human Retroviruses, Cullen BR,ed, Oxford University Press, NY 1993.

11. Huang Y, Zhang L, and Ho DD (1995). Characterization of nef sequences in long-term survivors of human immunodeficiency virus type 1 infection. J. Virol. 69, 93-100.

12. Kestler HW et al (1991); Cell, 65:651.

13. Kirchhoff F, Greenough TC, Brettler DB, Sullivan JL, and Desrosiers RC (1995). Brief report: absence of intact nef sequences in a long-term survivor with nonprogressive HIV-1 infection. N. Engl. J. Med. 332, 228-232.

14. Learmont J, Tindall B, Evans L, Cunniingham A, Cunningham P, Wells J, Penny R, Kaldor J, and Cooper DA (1992). Long-term symptomless HIV-1 infection in recipients of blood products from a single donor. Lancet 340, 863-867.

15. Luria S, Chambers I, Berg P (1991); Proc Natl Acad Sci USA 88:5326.

16. Mackewicz CE, Ortega H, and Levy JA (1994). Effect of cytokines of HIV replication in CD4 lymphocytes, lack of identity with CD8 cell antiviral factor. Cell Immunol. 160, 329-343.

17. Pantaleo G, Menzo S, Vaccarezza M, Graziosi C, Cohen OJ, Damarest JP, Montefiori D, Orenstein JM, Foc C, Schrager LK, et al (1995). Studies in sufjects with long-term nonprogressive human immunodeficiency virus infection. N. Engl. J. Med. 332, 209-216.

18. Paul WE, and Seder RA (1994). Lymphocyte responses and cytokines. Cell 76, 241-251.

19. Pinto LA, Sullivan J, burzotaky JA, Oleriol M, Kussler HA, Landay AL and Shearer GM, (1995). Env-specific cytotoxic T lymphocyte responses in HIV seronegative heath care workers occupationally exposed to HIV-contaminated body fluids. J. Clin Invest., in press.

20. Rowland-Jones S, Sutton J, Arlyoshi K, Dong T, Gotch F, McAdam S, Whitby D, Sabally S, Allimore A, Corran T, Takiguchi M, Schultz T, McMichael A, and Whittle H (1995). HIV-specific cytotoxic T-cells in HIV-exposed but uninfected gambian women. Nature Med. 1, 59-84.

21. Saag MS, Hahn BH, Gibbons J, Li Y, Parks ES, Parks WP, and Shaw GM (1988). Extensive variations of human immunodeficiency virus type-1 in vivo. Nature 334, 440-444.

22. Salk J (1987); Prospects for the control of AIDS by immunizing seropositive individuals. Nature327:473-476.

23. Travers K, Mboup S, Marlink R, Gueye-Ndiaye A, Siby T, Thior I, Dieng-Sarr A, Sankale JL, Mullins C, Ndoye I, Hsieh CC, Essex M, and Kanki P, (1995). Natural protection against HIV-1 infection provided by HIV-2. Science 268, 1612-1615.

24. Walker BD, (1990). Cytotoxic T-Lymphocyte responses to HIV-1 infected individuals. AIDS Vaccine Research and Clinical Trials, S.D. Putney and D.P. Bolognesi, eds. (New York: Marcel Dekker), pp. 179-194.

25. Walker BD, Flexner C, Birch LK, Fisher L, Paradis TJ, Aldovini A, Young R, Moss B, and Schooley RE (1989). Long-term culture and fine specificity of human cytotoxic T-lymphocyte clones reactive with human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA 86, 9514-9518.

26. Arold S, Franken P, Strub MP, et al (1997).The crystal structure of HIV-1 nef protein bound to the Fyn kinase SH3 domain suggest a role for this complex in altered T cell receptor signaling. Structure 15, 5(10):1361-72.

27. Bandres JC, Ratner L. (1994) Human immunodeficiency virus type 1 nef protein down-regulates transcription factors NF-kappa B and AP-1 in human T cells in vitro after T-cell receptor stimulation. J Virol 68:3243-9

28. Bandres JC, Shaw AS, Ratner L, (1995) HIV-1 nef protein down regulation of CD4 surface expression: relevance of 1ck binding domain of CD4. Virology 207, 338-41.

29. Bauer AS, Sawai ET, Dazin P, Fantl WJ, Cheng-Mayer C, Peterlin BM (1994) HIV-1 nef leads to inhibition of activation of T cells depending on its intracellular localization. Immunity 1, 373-84.

30. Bauer M, Lucchiari-Hartz M, Maier R, Haas G, Autran B, Eichmann K, Frank R, Maier B, Meyerhans A, et al (1997) Structural Constraints of HIV-1 nefd may curtail escape from HLA-B7 restricted CTL recognition. Immuno. Lett 55 (2): 119-22.

31. Betoletti A, Cham F, McAdam S, Rostron T, Rowland-Jones S, Sabally S, Corrah T, Ariyoshi K, whittle H (1998) Cytotoxic T cells from human immunodeficiency virus type-2 infected patients frequently cross-reacted with different human immunodeficiency virus type 1 clades. J Virl 72(3):2439-48.

32. Blaak H, Bouwer M, Rain Lj, de Wolfe F, Shuitemaker H, (1998) In vitro replication kinetics of human immunodeficiency virus type 1 (HIV-1) variants in relation to virus load in long-term survivors of HIV-1 infection [In Process Citation]. J Infect Dis 177(3):600-10.

33. Cao H, Kenki P, Sankale JL, Dieng-Sarr A, Mazzara GP, Kalams SA, Korber B, Mboup S, waler BD (1997) Cytotoxic T-lymphocyte cross-reactivity among different human immunodeficiency virus type 1 clades: implications for vaccine development. J Virol 71(11):8615-23.

34. Cohen J (1995) new clues found to how some people live with HIV[news;comment]. Science 270:917-8.

35. Collete Y, Dutarte H, Benziance A, Romas-Morales, Benarous R, Harris M, Olive D (1996) Physical and functional interaction of nef with Lck. HIV-1 nef-induced T-cell signaling defects. J Biol. Chem 271:6333-41.

36. Collete Y, Dutarte H, Benziane A, Olive D (1997) The role of HIV-1 nef in T-cell activation: nef impairs induction of TH1 cytokines and interacts with the Src family tyrosine kinase Lck. Res Virol 148:52-8.

37. Collete Y, Mawas C, Olive D (1996) Evidence for intact CD28 signaling in T cell hyporesponsiveness induced by the HIV-1 nef gene. Eur J Immunol 26:1788-93.

38. Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D (1998) HIV-1 nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. Nature 22,391(6665):397-401.

39. Cranage MP, Whatmore AM, Sharpe SA, Cook N, Polyanskaya N, Leech S, Smith JD, Rud Ew, Dennis MJ, Hall GA (1997) Macaques infected with live attenuated SIVmac are protected against superinfection via the rectal mucosa. Virology 229:143-54.

40. De SK, Marsh JW, (1994) HIV-1 nef inhibits a common activation pathway in NIH-3T3 cells. J Biol Chem 269:6656-60.

41. Fiala M, Rhodes RH, Shapshak P, Nagano I, Martinez-Maza O, Diagna A, Baldwin G, Graves M(1996) Regulations of HIV-1 infection in astocytes: expression of nef, TNFF-alpha and IL-6 is enhanced in coculture of astocytes with macrophages. J Neurovirol 2:158-66.

42. Fowke KR, Nagelkerke NJ, Kimani J, Simonsen JN, Anzala AO, Bwayo JJ, MacDonald KS, Ngugi EN, Plummer FA (1996) Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. Lancet 348:1347-51.

43. Fujii Y, Otake K, Tashiro M, Adachi A (1996) Soluble nef antigen of HIV-1 cytotoxic for human CD4+ T cells. FEBS Lett 393:93-6.

44. Goldsmith MA, Warmerdam MT, Atchison RE, Miller MD, Greene WC (1995) Dissociation of the CD4 downregulation and viral infectivity enhancement functions of human immunodeficiency virus type 1 nef J Virol 69:4112-21.

45. Greenway A, Azad A, McPhee D (1995) Human immunodeficiency virus type 1 nef protein inhibits activation pathways in peripheral blood mononuclear cells and T cell lines. J Virol 69:1842-50.

46. Gulizia RJ, Collman RG, Levy JA, Trono D, Mosier DE (1997) Delection of nef slows but does not prevent CD4-postitive T-cell depletion in human immunodeficiency virus type 1-infected human-PBL-SCID mice. J Virol 71:4161-4164.

47. Harrer T, Harrer E, Kalams SA, Barbosa P, Trocha A, Johnson RP, Elbeik t, Feinberg MB, Buchbinder SP, Walker BD (1996) Cytotoxic T lymphocytes in asymptomatic long-term non-progressing HIV-1 infection. Breadth and specificity of the response and relation to in vivo viral quasispecies in a person with prolonged infection and low viral load. J Immunol 156:2616-23.

48. Harris M (1996) From negative factor to a critical role in virus pathogenesis: the changing fortunes of nef. J Gen Virol 77(Pt10):2379-92.

49. Iafrate AJ, Bronson S, Skowronski J (1997) Separable functions of nef disrupt two aspects of T cell receptor machinery: CD4 expression and CD3 signaling. EMBO J 16:673-84.

50. L'age M (1995) [Long-term survival with HIV infection. Virological-immunological studies of pathogenis]. Dtsch Med Wochenschr 120:1682-3.

51. Le Gall S, Prevost MC, Heard JM, Schwartz O (1997) Human immunodeficiency virus type 1 nef independently affects virion incorporation of major histocompatibilty complex class I molecules and virus infectivity. Virology 229:295-301.

52. Lucchiari-Hartz M, Bauer M, Niedermann G, Maier B, Meyehans A, Elchmann K (1996) Human immune response to HIV-1 nef II Induction of HIV-1/HIV-2 nef cross-reactive cytotoxic T lymophocytes in peripheral blood lymphocytes of non-infected healthy individuals. Int Immuno. 8:577-84.

53. Mariani R, Kirchhoff F, Greenough TC, Sullivan JL, Desrosiers RC, Sckowronski J (1996) High frequency of defective nef alleles in a long-term survivor with nonprogressive human immunodeficiency virus type 1 infection. J Virol 70:7752-64.

54. Michael NL, Chang G, d'Arcy LA, Ehrenberg PK, Mariani R, Busch MP, Birx DL, Schwartz DH (1995) Defective accessory genes in a human immunodeficiency virus type 1-infected long-term survivor lacking recoverable virus. J Virol69:4228-36.

55. Miller MD, Warmerdam MT, Page KA, Feinberg MB, Greene WC (1995) Expression of the human immunodeficiency virus type 1 (HIV-1) nef gene during HIV-1 production increases progeny particle infectivity independently of gp160 or viral entry. J Virol 69:579-84.

56. Premkumar DR, Ma XY, Maitra RK, Chakrabarti BK, Salkowitz J, Yen-Liberman B, Hirsch MS, Kestler HW (1996) The nef gene from a long-term HIV type 1 nonprogressor. AIDS Res Hum Retroviruses 12:337-45.

57. Price DA, Goulder PJ, Klenerman P, Sewell AK, Easterbrook PJ, Troop M, Bangham CR, Phillips RE (1997) Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. Proc natl Acad Sci USA4;94(5):1890-5.

58. Saito Y, Sharer LR, Epstein Lg, Michaels J, Mintz M, Louder M, Golding K, Cvetkovich TA, Blumberg BM (1994) Overexpression of nef as a marker for restricted HIV-1 infection of astocytes in postmortem pediatric central nervous tissues. Neurology 44:474-81.

59. Sinclair E, Barbosa P, Feinberg MB (1997) The nef gene products of both simian and human immunodeficiency viruses enhance virus infectivity and are functionally interchangeable. J Virol 71:3641-51.

60. Switzer WM, Wiktor S, Soriano V, Silva-Graca A, Manshiho K, Coulibaly IM, Ekpini E, Greenberg Ae, Folks TM, Heneine W (1998) Evidence of nef truncation in human immunodeficiency virus type 2 infection. J Infect Dis 177(1):65-71.

61. Learmont JC, Geczy AF, Mills J, Ashton LJ et al (1999) Immunologic and virologic status after 14 to 18 years of infection with an attenuated strain of HIV-1. A report from the Sydney Blood Bank Cohort. N Engl J Med 340(22):1715-22.

62. Baba TW, Liska V, Khimani AH et al (1999) Live attenuated, multiply deleted simian immunodeficiency virus causes AIDS in infants and adult macaques. Nat Med 5(2):194-203.

63. Owens DK, Edwards DM, Shachter RD (1998) Population effects of preventive and therapeutic vaccines in early and late stage epidemics. AIDS 12(9):1057-66.

64. Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected cells against killing by cytotoxic T lymphocytes. Nature (1998) 391(6665):397-401.

65. Titti F, Sernicola L, Geraci A, Panzini G, Di Fabio S et al. Live atenuated simian immunodeficiency virus prevents super-infection by cloned SIVmac251 in cynomolgus monkeys. J Gen Virol (1997) 78(pt 10):2529-39.

66. James JS HIV-specific immune responses restored by HIV immunogen plus antiretroviral suppression. AIDS Treat News (1998 Jul 10) (No 298):1-5

67. Moss RB, Giermakowska WK, Lanza P, Theofan G, Richieri SP, Jensen FC Carlo DJ. HIV-1-specific immune function after immunization with a gp120- depleted HIV-1

immunogen (REMUNE) in HIV-1 seropositive patients. HIV Pathog Treat Conf (1998) Mar 13-1996 (abstract no. 4064)

68. Moss R, Wallace MR, Lanza P, Giermakowska WK, Jensen FC, Theofan G, Chamberlin C, Richieri SP, Carlo DJ. P24 antigen stimulated immune responses after treatment with an inactivated gp 120-depleted HIV-1 immunogen (REMUNE) in HIV-1 seropositive subjects. Int Conf AIDS (1998) 12:525 (abstract no. 31151)

69. Moss RB, Trauger RJ, Giermakowska WK, Turner JL, Wallace MR, Jensen FC, Richieri SP, Ferre F, Daigle AE, Duffy C, Theofan G, Carlo DJ. Effect of immunization with an inactivated gp120-depleted HIV-1 immunogen on beta-chemokine and cytokine production in subjects with HIV-1 infection. J Acq Immune Def Syndr Hum Retrovirol 1997 Apr 1; 14(4):343-50

70. Swingler S, Mann A, Jacque J, Brichacek B, Sasseville VG, Williams K et al. HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. Nat Med 1999 Sep 5(9):997-103

71. Verhasselt B, Naessens E, Verhofstede C, De Smedt M, Schollen S, Kerre T et al. Human immunodeficiency virus nef gene expression affects generation and function of human T cells, but not dendritic cells. Blood 1999 Oct 15;94(8):2809-18

72. Cheng H, Hoxie JP, Parks WP. The conserved core of human immunodeficiency virus type 1 Nef is essential for association with Lck and for enhanced viral replication in T-lymphocytes. Virology 1999 Nov 10;264(1):5-15

73. Foti M, Cartier L, Piguet V, Lew DO, Carpentier JL, Trono D et al. The HIV Nef protein alters Ca^{++} signaling in myelomonocytic cells through SH3-mediated protein-protein interactions. J Biol Chem (1999) 274(49):34765-72

74. Gaudain MC, Glickman RL, Ahmad S, Yilma T, Johnson RP. Immuniztion with live attenuated simian immunodeficiency virus induces strong type 1 T helper responses and beta-chemokine production. Proc Natl Acad Sci (1999) 96(24):1031-6

75. Zaunders JJ, Geczy AF, Dyer WB, McIntyre LB, Cooley MA, Ashton LJ, Raynes-Greenow CH, Learmon J, Cooper DA, Sullivan JS. Effect of long-term infection with nef-defective attenuated HIV type 1 on CD4+ and CD8+ T lymphocytes: increased CD45RO+CD4+ T lymphocytes and limited activation of CD8+ T lymphocytes. AIDS Res Hum Retroviruses (1999) 15(17):1519-27