## Nef Mutations in Long-term Non-progressors from Former Plasma Donors Infected with HIV-1 Subtype B in China<sup>1</sup>

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**Objective** To study the specific amino acid variation in Nef that may be related to disease progression after infection with HIV-1 subtype B, a predominant strain circulating in China, and to determine whether changes in Nef secondary structure may influence different stages of AIDS development based on the concept that the Nef gene of HIV infection dramatically alter the severity of viral infection and virus replication and disease progression, and that long-term non-progressors (LTNP) of HIV infection are commonly associated with either a deletion of the Nef gene or the defective Nef alleles. **Methods** The study subjects were divided into LTNP<sub>1</sub>(n=14), LTNP<sub>2</sub> (n=16) and slow progressor (SP, n=19) groups for mutational analysis of the Nef sequence. The data were obtained by using Bioedit, MEGA, Anthewin and SAS software. **Results** Residues in Nef TA<sub>48/49</sub> and K<sub>151</sub> occurred more frequently in the LTNP group while AA<sub>48/49</sub> was more frequently observed in the SP group. Of the different stages of the secondary structure comparison using Nef consensus sequences of these three groups, one was roughly corresponding to the Nef<sub>48/49</sub> mutation site. **Conclusion** TA<sub>48/49</sub>, K<sub>151</sub>, and AA<sub>48/49</sub> in the Nef gene might be associated with the different stages of HIV infection, and there may be a link between the Nef secondary structure and the progression of HIV-1 infection.

Key words: HIV-1; Nef; Long-term nonprogressors; Sequence mutations; Secondary structure prediction

## REFERENCES

CD8<sup>+</sup> T cells: serial killers condemned to die? *Curr HIV Res* 2(2), 153-162.

- Valdez H, Carlson N L, Post A B, *et al.* (2002). HIV long-term non-progressors maintain brisk CD8 T cell responses to other viral antigens. *AIDS* 16(8), 1113-1118.
- 2. Paroli M, Propato A, Accapezzato D, *et al.* (2001). The immunology of HIV-infected long-term non-progressors--a current view. *Immunol Lett* **79**, 127-129.
- Guadalupe M, Reay E, Sankaran S, *et al.* (2003). Severe CD4<sup>+</sup> T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol 77(21), 11708-11717.
- Das S R, Jameel S (2005). Biology of the HIV Nef protein. Indian J Med Res 121(4), 315-332.
- Arold S T, Baur A S (2001). Dynamic Nef and Nef dynamics: how structure could explain the complex activities of this small HIV protein. *Trends Biochem Sci* 26(6), 356-363.
- Brenchley J M, Schacker T W, Ruff L E, *et al.* (2004). CD4<sup>+</sup> T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J Exp Med* 200(6), 749-759.
- 7. Petrovas C, Mueller Y M, Katsikis P D (2004). HIV-specific

- Zhuang Yan, Zhai Song, Sun Yong-tao, *et al.* (2006). Analysis of HIV-1 clade B Nef specific CD<sub>8</sub><sup>+</sup>T cell responses in Chinese population. *Chin J Microbiol Immunol* 26, 549-552.
- 9. Centers for Disease Control (1993). *Morb Mortal Wkly Rep* **41**, 1-19.
- 10.Min Wei, Hui Xing, Yiming Shao, et al. (2004). Biased G-to-A hypermutation in HIV-1 proviral DNA from a long-term non-progressor. AIDS 18(13), 1863-1865.
- 11. Masemola A, Mashishi T, Khoury G, et al. (2004). Hierarchical targeting of subtype C human immunodeficiency virus type 1 proteins by CD8<sup>+</sup> T cells: correlation with viral load. J Virol 78(7), 3233-3243.
- 12.Korber B, Walker B D, Brander C R, *et al.* (2005). HIV molecular immunology database. Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, N Mex.
- Geyer M, Fackler O T, Peterlin B M (2001). Structure--function relationships in HIV-1 Nef. *EMBO Rep* 2(7), 580-585.
- 14.Peng B, Robert-Guroff M (2001). Deletion of N-terminal myristoylation site of HIV Nef abrogates both MHC-1 and CD4 down-regulation. *Immunol Lett* 78(3), 195-200.
- Blagoveshchenskaya A D, Thomas L, Feliciangeli S F, et al. (2002). HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6 endocytic pathway. *Cell* 111(6), 853-866.

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- 16.Stumptner-Cuvelette P, Morchoisne S, Dugast M, et al. (2001). HIV-1 Nef impairs MHC class II antigen presentation and surface expression. *Proc Natl Acad Sci USA* 98(21), 12144-12149.
- 17.Stumptner-Cuvelette P, Jouve M, Helft J, et al. (2003). Human immunodeficiency virus-1 Nef expression induces intracellular accumulation of multivesicular bodies and major histocompatibility complex class II complexes: potential role of phosphatidylinositol 3-kinase. *Mol Biol Cell* 14(12), 4857-4870.
- 18.Schindler M, Wurfl S, Benaroch P, et al. (2003). Down-modulation of mature major histocompatibility complex class II and up-regulation of invariant chain cell surface expression are well-conserved functions of human and simian immunodeficiency virus nef alleles. J Virol 77(19), 10548-10556.
- 19.Bell I, Schaefer T M, Trible R P, et al. (2001). Down-modulation of the costimulatory molecule, CD28, is a conserved activity of multiple SIV Nefs and is dependent on histidine 196 of Nef. Virology 283(1), 148-158.
- Swigut T, Shohdy N, Skowronski J (2001). Mechanism for down-regulation of CD28 by Nef. *EMBO J* 20(7), 1593-1604.
- 21.Cohen G B, Rangan V S, Chen B K, et al. (2000). The human thioesterase II protein binds to a site on HIV-1 Nef critical for

CD4 down-regulation. J.Biol. Chem 275(30), 23097-23105.

- 22.Foster J L, Molina R P, Luo T, *et al.* (2001). Genetic and functional diversity of human immunodeficiency virus type 1 subtype B Nef primary isolates. *J Virol* **75** (4), 1672-1680.
- 23.Iafrate A J, Bronson S, Skowronski J, et al. (1997). Separable functions of Nef disrupt two aspects of T cell receptor machinery: CD4 expression and CD3 signaling. EMBO J 16(4), 673-684.
- 24.Kelleher A D, Long C, Holmes E C, et al. (2001). Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. J Exp Med 193(3), 375-386.
- 25. Kirchhoff F, Easterbrook P J, Douglas N, *et al.* (1999). Sequence variations in human immunodeficiency virus type 1 Nef are associated with different stages of disease. *J Virol* **73**(7), 5497-5508.
- 26.Miles L R, Agresta B E, Khan M B, et al. (2005). Effect of polypurine tract (PPT) mutations on human immunodeficiency virus type 1 replication: a virus with a completely randomized PPT retains low infectivity. J Virol **79**(11), 6859-6867.

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