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MOLECULAR HOMOLOGY BETWEEN ALPHA-DEFENSIN AND SCRUB TYPHUS RICKETTSIA ORIENTIA TSUTSUGAMUSHI 56K TYPE SPECIFIC ANTIGEN

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INTRODUCTION

The challenge of an HIV vaccine is not yet solved, but new areas were appearing with advances coming from unexpected directions, such as amelioration of HIV infection in some (about 40%) but not all patients infected by scrub typhus in Thailand (Watt G., 2001, 2000). In the same time, elucidation of the mysterious protective factor in long-term nonprogressors conducted to the discovery of a-defensin (Zhang L., 2002). The crucial Nef protein was in fact an a-defensin-like homolog (Tran M.K.G., 2003). What became evident then is the strength of molecular mimicry as an unique method for unifying these 3 discoveries, at the level of amino acid sequences (as we shall see further), at the cross road of these 3 apparently independent data.

ANTI-HIV PROTECTIVE FACTOR: THE a-DEFENSIN (comment in: Cohen J, 2002)

Zhang L.(2002) discovered an a-defensin ***isotype*** [differing by isoleucine 28 (Ileu 28 or I 28), instead of phenylalanine (Phe or F) in usual, classical, commercially available a-defensin] as the natural protective factor against HIV-1 in long-term nonprogressors remaining alife without treatment. This isotype was 20 fold more protective than the common, commercially available a-defensin. The a-defensin mechanism of action was at 2 levels: directly inactivating virus particles and affecting the ability of target CD4 cells to replicate the virus (Mackewicz C.E., 2003).

SCRUB TYPHUS (Watt G., 2003; Seong S-Y, 2001; Silpapojakul K, 1997)

Caused by the gram-negative bacillus *Orientia Tsutsugamushi (Or.Tsu.*)[formerly *Rickettsia* (Tamura A., 1995, 1991)], scrub typhus, a mite-borne disease is transmitted by chiggers (Leptotrombidium hexapod larvae) that fell off rodents and then live in the scrub bush. Scrub typhus is endemic in the geographic triangle of South East Asia. The number of *Or.Tsu.* strains is high, about more than a hundred different strains, classified in several families (see a phylogenetic tree in: Enatsu T., 1999). Clinical features were characterized by fever, rash, eschar, regional lymphadenopathy, pneumonitis, meningitis and disseminated intravascular coagulation, and vary in severity from mild and self-limiting to fatal. The disease responds generally to doxycycline.

THE ODD INTERSECTION BETWEEN HIV AND SCRUB TYPHUS (comment in: Cohen J., 2000; James J.S., 2000)

Watt G. (2001, 2000) remarked a beneficial effect of sera from scrub typhus *Or.Tsu.*-infected HIV-1+ patients on HIV-1 replication, suggesting a protective cross-reactive antibody. He found in Thaïland that scrub typhus infection by *Or.Tsu.* was beneficial for HIV-1+ patients, by a cross-reactive immunity involving antibodies to a common epitope between *Or.Tsu.* and HIV-1. In cell cultures, sera from patients and from *Or.Tsu.* inoculated mice bloked HIV-1 replication and induced reversion of S.I. (syncytium inducing, more dangerous) viruses to N.S.I. (n on-s yncytium inducing, more benign) phenotypes. *Or.Tsu.* was isolated less often in blood samples from 37/72 (48,6%) patients without than with HIV-1 infection [2/14 (14,3%)]($p < .05$) (Kantipong P., 1996). The HIV-1 serotype is important to consider, as in Thailand it is generally the clade AE which is implicated: Convalescent plasma infusions (Watt G., 2001) decreased HIV-1 viral loads in 7/10 patients. A striking molecular match was found between *Or.Tsu.* Karp strain 47 kD antigen 433 TLREIVTNIK 442 and HIV envelope gp120 post-V3 loop 214 TLRQIVTNLK 223. However, owing to HIV-1 hypervariability, Thailand AE strains were less homologous. (The protective role discovered was caused by antibodies, as Moriuchi M. (2003) observed a HIV-1 reactivation in 4/6 cases by direct in vitro *Or.Tsu.* stimulation of peripheral blood mononuclear cells).

MOLECULAR HOMOLOGY BETWEEN HIV-1 NEF AND a-DEFENSIN

Tran M.K.G. (2003) described a tridimensional (3D) molecular homology between HIV-1 Nef and a-defensin: Nef was found mimicking a member of the scorpion venom family: Alpha-defensin (Tran M.K.G., 2003), the natural protective protein in long-term non-progressors (Zhang L., 2002). It is noteworthy that Zhang's a-defensin variant (I 28) is *20 times* more protective against HIV-1 than commercial a-defensin (F 28), pointing to an important role for this isoleucine 28 residue.

OBJECTIVE

We tried to elucidate the mechanism of protection against HIV-1 infection induced by scrub typhus, and to determine precisely the protective cross-reactive epitope(s) common to HIV-1 and *Or.Tsu.* Such discovery would be important in the design of an anti-HIV-1 vaccine. Watt G (2001) has found a previous match between HIV gp120 V1-V3 loop C-terminus and *Or.Tsu.*
47 Kd antigen, but the implicated Thailand AE strains were less homologous. Thus we decided to focus primarily on AE strains. Nef has not been explored. Nef is very important quantitatively, as it is very abundant in severe dementia astrocytes (Ranki A., 1995) and constitutes about 77% of lymphocytes and macrophages intra-cellular viral mRNA, far before Rev (20%) or Tat (< 2 %) (Robert-Guroff M., 1990).

METHODS

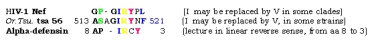
We compared the amino acid (aa) sequences and 3D structure of HIV-1 proteins (Los Alamos 1998-2003 databanks), HIV-1 Nef (Lee C-H, 1996), a-defensin [(I 28) (Zhang L., 2002) and F28 (Hill C.P., 1991) and Rick.*Or.Tsu.* tsa 56 (about 100 sequences in Genbank, nucleotide). The other defensins were included: a4-,a5-,a6-, a-defensin (NP009916, NP066290, Q01524, Sawai M.V., 2000: PDBid:1fqq).

RESULTS

Aligned R24 and K172 are both basic residues

Interestingly, the sequences of other defensines (a-, a4-, a5-, a6-defensins) were alignable

neither with Nef nor Risckettsia (negative controls). Between clade E Thailand HIV-1 Nef and Rick.Or.Tsu. 56 Kd type specific antigen (tsa 56) (513-521), we found a common motif centered on the tetrapeptide GIRY (or GVRY):



P and S are commonly found on the tip of a loop; L and F are hydrophobic semi-conserved, A and G are space providing aa. The length is 9 aa, which is significant. Interestingly, this epitope is located exactly in the Nef region which mimicks a-defensin (Tran M.K.G., 2003), suggesting a crucial functional role.

Watt G. (2003) found also a protective effect of acute **dengue** infection, with a decrease of HIV viral load. Thus, we screened also the dengue virus amino acid sequence and found the typical motif AWL common to a-defensin and
Rick.Or.Tsu.
tsa56. Interestingly, other arboviruses (Yellow fever, Tick-borne encephalitis, Japanese encephalitis) did not contain AWL and were not homologous (negative controls). This new data may not be simply coincidental, as it is very coherent with the previous alignments:

DISCUSSION and CONCLUSIONS

Rick.Or.Tsu. tsa 56 is a perfect molecular mimicry of Zhang's a-defensin on the tetrapeptide IAWL, with a significant homology spanning 8 residues, suggesting that protection may be mediated by a cross-reactive antibody against HIV-1 Thailand AE clade Nef sequence IWKFDSA. The trilogy (Thailand clade AE HIV-1 Nef, Zhang's a-defensin and *Rick. Or.Tsu.*
tsa 56) defines 2 homologous linear epitopes (centered respectively on IAWL and GIRY, another 8 residues epitope) located in proximity in a 3D space. For some clade B and Thailand clade AE, vaccines with these Nef epitopes, or an anatoxine with
Rick.Or.Tsu.
tsa 56, and passive immunotherapy (sera, monoclonal antibodies, Fab, Fv,...) could be designed as an interesting anti-HIV-1 therapy, as this Nef region is relatively conserved inside each geographical clade. A protective geographic specific vaccine or immunotherapy strategy may be developed, with a better fitness between the corresponding HIV-1 Thai Nef and
Or.Tsu.
strains than a blind serotherapy (as did Watt G. in 2001). The relatively conserved Nef sequence may be presented by heat shock proteins to dendritic cells (Srivastava P, 2002). This also confirms our scorpion venom model of AIDS and the efficacy of Tacrine, a modifier of scorpion venom's receptor (the Na+ voltage-dependent channel). What was evident and very surprising is the strength of molecular mimicry as an unique method for unifying these 3

independent discoveries (a-defensin, HIV-1 Thai AE nef, amelioration of HIV infection by scrub typhus), at the level of amino acid sequences, at the cross road of these 3 apparently disparate data.

BIBLIOGRAPHY

- Ariyoshi K, Whittle H; HIV-1 viral load and scrub typhus; Lancet 2000, 356 (9243): 1766; author reply 1766-7. Comment on: Lancet 2000, 356 (9228): 475-9.
- Cohen J; AIDS research. The odd intersection of HIV and scrub typhus. Science 2000, 289: 1278.
- Cohen J; Mystery anti-HIV factor unmasked? Science 2002, 297: 2188-9.
- Enatsu T, Urakami H, Tamura A; Phylogenetic analysis of Orientia tsutsugamushi strains based on the sequence homologies of 56-kDa type-specific antigen genes. FEMS Microbiol Lett 1999, 180: 163-9.
- Hill CP, Yee J, Selsted ME, Eisenberg D; Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization. Science 1991, 251: 1481-5.

- James JS; Scrub typhus: infection reduces viral load in some patients. AIDS Treat News 2000 Aug 18; (349): 3-4.
- Kantipong P, Watt G, Jongsakul K, Choenchitra C; Infection with human immunodeficiency virus does not influence the clinical severity of scrub typhus. Clin Infect Dis 1996, 23: 1168-70.
- Lee CH, Saksela K, Mirza UA, Chait BT, Kuriyan J; Crystal structure of the conserved core of HIV-1 Nef complexed with a Src family SH3 domain. Cell 1996, 85: 931-42.
- Mackewicz C.E., Yuan J, Tran P, Diaz L, Mack E, Selsted ME, Levy JA; Alpha-Defensins can have anti-HIV activity but are not CD8 cell anti-HIV factors. AIDS 2003, 17: F23-32. Erratum in: AIDS 2003, 17: F31.
- Moriuchi M, Tamura A, Moriuchi H; In vitro reactivation of human immunodeficiency virus-1 upon stimulation with scrub typhus rickettsial infection. Am J Trop Med Hyg 2003, 68: 557-61.
- Murata M, Yoshida Y, Osono M, Ohashi N, Oyanagi M, Urakami H, Tamura A, Nogami S, Tanaka H, Kawamura A Jr; Production and characterization of monoclonal strain-specific antibodies against prototype strains of Rickettsia tsutsugamushi. Microbiol Immunol 1986, 30: 599-610.
- Moss WJ, Ryon JJ, Monze M, Cutts F, Quinn TC, Griffin DE; Suppression of human immunodeficiency virus replication during acute measles. J Infect Dis. 2002, 185: 1035-42. Epub 2002 Mar 25.
- Ranki A, Nyberg M, Ovod V, Haltia M, Elovaara I, Raininko R, Haapasalo H, Krohn K; Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia. AIDS 1995, 9: 1001-8.
- Robert-Guroff M, Popovic M, Gartner S, Markham P, Gallo RC, Reitz MS. Structure and expression of tat-, rev-, and nef-specific transcripts of human immunodeficiency virus type 1 in infected lymphocytes and macrophages. J Virol 1990, 64: 3391-8.
- Seong SY, Choi MS, Kim IS; Orientia tsutsugamushi infection: overview and immune responses. Microbes Infect 2001, 3: 11-21. Review.

- Silpapojakul K; Scrub typhus in the Western Pacific region. Ann Acad Med Singapore. 1997, 26: 794-800. Review.
- Srivastava P; Interaction of heat shock proteins with peptides and antigen presenting cells: chaperoning of the innate and adaptive immune responses. Annu Rev Immunol 2002, 20: 395-425. Epub 2001 Oct 04. Review.
- Tamura A, Ohashi N, Urakami H, Miyamura S; Classification of Rickettsia tsutsugamushi in a new genus, Orientia gen. nov., as Orientia tsutsugamushi comb. nov. Int J Syst Bacteriol. 1995, 45: 589-91. Review.
- Tamura A, Urakami H, Ohashi N; A comparative view of Rickettsia tsutsugamushi and the other groups of rickettsiae. Eur J Epidemiol 1991, 7: 259-69. Review.
- Tran M.K.G., Caprani A., Nguyen D. X., Kirkiacharian S., Maurisson G., Fredj G; The scorpion venom model of AIDS : Alpha-defensin is homologous to HIV-1 Nef in three dimensions (3D). Tacrine with Silymarin as a rescue for failure and HIV-1 chemoresistence. E.A.C.S 9th European AIDS Conference, 1rst EACS Resistance & Pharmacology Worshop. Warsaw, Poland, October 25-29, 2003.
- Watt G, Kantipong P, Jongsakul K; Decrease in human immunodeficiency virus type 1 load during acute dengue fever. Clin Infect Dis 2003, 36: 1067-9. Epub 2003 Apr 04.
- Watt G, Chanbancherd P, Brown AE; Human immunodeficiency virus type 1 test results in patients with malaria and dengue infections. Clin Infect Dis. 2000 May;30(5):819.
- Watt G, Parola P; Scrub typhus and tropical rickettsioses. Curr Opin Infect Dis. 2003 Oct;16(5):429-36. Review.
- Watt G, Kantipong P, Jongsakul K, de Souza M, Burnouf T; Passive transfer of scrub typhus plasma to patients with AIDS: a descriptive clinical study. QJM. 2001, 94:599-607.

- Watt G, Kantipong P, de Souza M, Chanbancherd P, Jongsakul K, Ruangweerayud R, Loomis-Price LD, Polonis V, Myint KS, Birx DL, Brown AE, Krishna S; HIV-1 suppression during acute scrub-typhus infection. Lancet 2000, 356 (9228): 475-9. Comment in: Lancet 2000, 356 (9243): 1765-6; author reply 1766-7; Lancet 2000, 356 (9244): 1851.
- Zhang L., Yu W., He T., Caffrey R.E., Dalmasso S.F., Fu S., Pham T., Mei J., Ho J.J., Zhang W., Lopez P & Ho D.D; Contribution of human a-defensin-1, -2 and & -3 to the anti-HIV-1 activity of CD8 antiviral factor. Science 2002, 298 : 995-1000.