

REFERENCES

1. Los Alamos data base. 2002. The Circulating Recombinant Forms (CRFs). (Online). Available from: <http://www.amaassn.org/special/hiv/newslines/cdc/041700p1.htm>
2. Brown, T.; Sittitrai, W.; Vanichseni, S.; and Thisyakorn, U. The recent epidemiology of HIV and AIDS in Thailand. AIDS 1994; 8 Suppl 2: S131-141.
3. Ou, C. Y., et al. Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand. Lancet 1993; 341 (8854): 1171-1174.
4. Mason, C. J., et al. Nationwide surveillance of HIV-1 prevalence and subtype in young Thai men. J Acquir Immune Defic Syndr Hum Retrovirol 1998; 19 (2): 165-173.
5. Burton, D. R., and Moore, J. P. Why do we not have an HIV vaccine and how can we make one ? Nat Med 1998; 4 (5Suppl): 495-498.
6. Berman, P. W., et al. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Nature 1990; 345 (6276): 622-625.
7. Stott, E. J., et al. Evaluation of a candidate human immunodeficiency virus type 1 (HIV-1) vaccine in macaques: effect of vaccination with HIV-1 gp120 on subsequent challenge with heterologous simian immunodeficiency virus-HIV-1 chimeric virus. J Gen Virol 1998; 79 (Pt3):423-432.
8. Mellors, J. W.; Rinaldo, C. R. Jr.; Gupta, P.; White, R.M.; Todd, J. A.; and Kingsley, L. A. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996; 272 (5265): 1167-1170.
9. Riviere, Y.; Robertson, M. N.; and Buseyne, F. Cytotoxic T lymphocytes in human immunodeficiency virus infection: regulator genes. Curr Top Microbiol Immunol 1994; 189: 65-74.
10. van Baalen, C. A., et al. Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. J Gen Virol 1997; 78 (Pt8): 1913-1918.
11. Fisher, A. G., et al. The trans-activator gene of HTLV-III is essential for virus

- replication. Nature 1986; 320 (6060): 367-371.
12. Huang, L. M.; Joshi, A.; Willey, R.; Orenstein, J.; and Jeang, K. T. Human immunodeficiency viruses regulated by alternative trans-activator: genetic evidence for a novel non-transcriptional function of Tat in virion infectivity. EMBO J 1994; 13 (12): 2886-2896.
 13. Cafaro, A., et al. Control of SHIV-89.6P-infection of cynomolgus monkeys by HIV-1 Tat protein vaccine. Nat Med 1999; 5 (6): 643-650.
 14. Cafaro, A., et al. Vaccination with DNA containing tat coding sequences and unmethylated CpG motifs protects cynomolgus monkeys upon infection with simian/human immunodeficiency virus (SHIV89.6P). Vaccine 2001; 19 (20-22): 2862-2877.
 15. Pauza, C. D., et al. Vaccination with tat toxiod attenuates disease in simian/HIV-challenged macaques. Proc Nat Acad Sci USA 2000; 97 (7): 3515-3519.
 16. Berke, G. The CTL's kiss of death. Cell 1995; 81 (1): 9-12.
 17. Tschopp, J., and Hofmann, K. Cytotoxic T cells: more weapons for new targets? Trends Microbiol 1996; 4 (3): 91-94.
 18. Kagi, D., et al. Cytotoxicity mediated by T cells and natural killer cells in greatly impaired in perforin-deficient mice. Nature 1994; 369 (6475):31-37.
 19. Price, D. A.; Klenerman, P.; Booth, B. L.; Phillips, R. E.; and Sewell, A.K. Cytotoxic T lymphocytes, chemokines and antiviral immunity. Immunol Today 1999; 20 (5): 212-216.
 20. Kagi, D.; Ledermann, B.; Burki, K.; Zinkernagel, R. M.; and Hengartner, H. Molecular mechanisms of lymphocyte-mediated cytotoxicity and their role in immunological protection and pathogenesis in vivo. Annu Rev Immunol 1996; 14: 207-232.
 21. Levy, J. A.; Mackewicz, C. E.; and Barker, E. Controlling HIV pathogenesis: the role of noncytotoxic anti-HIV response of CD8+ T cells. Immunol Today 1996; 17 (5) 217-224.
 22. Kagi, D., and Hengartner, H. Different roles for cytotoxic T cells in the control of infections with cytopathic versus noncytopathic viruses. Curr Opin Immunol 1996; 8 (4): 472-477.
 23. Yang, O. O., and Walker, B. D. CD8+ cells in human immunodeficiency virus type I pathogenesis: cytolytic and noncytolytic inhibition of viral replication. Adv Immunol 1997; 66: 273-311.

24. Borrow, P.; Lewicki, H.; Hahn, B. H.; Shaw, G. M.; and Oldstone, M. B. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994; 68 (9): 6103-6110.
25. Koup, R. A., et al. Temporal association of cellular immune responses with the initial control or viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994; 68 (7): 4650-4655.
26. Gotch, F. M.; Nixon, D. F.; Alp, N.; McMichael, A. J.; and Borysiewicz, L. K. High frequency of memory and effector gag specific cytotoxic T lymphocytes in HIV seropositive individuals. Int Immunol 1990; 2 (8): 707-712.
27. Ogg, G. S., et al. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. Science 1998; 279 (5359): 2103-2106.
28. Carmichael, A.; Jin, X.; Sissons, P.; and Borysiewicz, L. Quantitative analysis of the human immunodeficiency virus type 1 (HIV-1)-specific cytotoxic T lymphocyte (CTL) response at different stages of HIV-1 infection: differential CTL responses to HIV-1 and Epstein-Barr virus in late disease. J Exp Med 1993; 177 (2): 249-256.
29. Klein, M. R., et al. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. J Exp Med 1995; 181 (4): 1365-1372.
30. Rowland-Jones, S. L., et al. HIV-1 specific cytotoxic T-cell activity in an HIV-exposed but uninfected infant. Lancet 1993; 341 (8849): 860-861.
31. Stranford, S. A., et al. Lack of infection in HIV-exposed individuals is associated with a strong CD8(+) cell noncytotoxic anti-HIV response. Proc Nat Acad Sci U S A 1999; 96 (3): 1030-1035.
32. Clerici, M., et al. Cell-mediated immune response to human immunodeficiency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV-1. J Infect Dis 1992; 165 (6): 1012-1019.
33. Fowke, K. R., et al. Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya. Lancet 1996; 348 (9038): 1347-1351.
34. Rowland-Jones, S. L., et al. Cytotoxic T cell responses to multiple conserved HIV

- epitopes in HIV-resistant prostitutes in Nairobi. J Clin Invest 1998; 102 (9) : 1758-1765.
35. Evans, D. T., et al. Virus-specific cytotoxic T-lymphocyte response select for amino-acid variation in simian immunodeficiency virus Env and Nef. Nat Med 1999; 5 (11):1270-1276.
 36. Wilson, C. C., et al. Frequent detection of escape from cytotoxic T-lymphocyte recognition in perinatal human immunodeficiency virus (HIV) type 1 transmission: the ariel project for the prevention of transmission of HIV from mother to infant. J Virol 1999; 73 (5): 3975-3985.
 37. Ensoli, B., et al. Release, uptake, and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. J Virol 1993; 67 (1): 277-287.
 38. Chang, H.C.; Samaniego, F.; Nair, B.C.; Buonaguro, L.; and Ensoli, B. HIV-1 Tat protein exits from cells via a leaderless secretory pathway and binds to extracellular matrix-associated heparan sulfate proteoglycans through its basic region. AIDS 1997; 11 (12): 1421-1431.
 39. Frankel, A. D., and Pabo, C. O. Cellular uptake of the tat protein from human immunodeficiency virus. Cell 1988; 55 (6): 1189-1193.
 40. Huang, L. M.; Joshi, A.; Willey, R.; Orenstein, J.; and Jeang, K. T. Human immunodeficiency viruses regulated by alternative trans-activators: genetic evidence for a novel non-transcriptional function of Tat in virion infectivity. EMBO J 1994; 13 (12): 2886-2896.
 41. Goldstein, G. HIV-1 Tat protein as a potential AIDS vaccine. Nat Med 1996; 2 (9): 960-964.
 42. Collins, K. L.; Chen, B. K.; Kalams, S. A.; Walker, B. D.; and Baltimore, D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. Nature 1998; 391 (6665): 397-401.
 43. Addo, M. M., et al. The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals. Proc Nat Acad Sci U S A 2001; 98 (4): 1781-1786.
 44. Tahtinen, M., et al. DNA vaccination in mice using HIV-1 nef, rev and tat genes in self-replicating pBN-vector. Vaccine 2001; 20 (1-2): 12-15.
 45. Morris, C. B.; Thanawastain, A.; Sullivan, D. E.; and Clements, J. D. Identification of a peptide capable of inducing an HIV-1 Tat-specific CTL

- response. Vaccine 2001; 20 (1-2): 12-15.
46. Altfeld, M. A., et al. Identification of dominant optimal HLA-B60- and HLA-B61-restricted cytotoxic T-lymphocyte (CTL) epitopes: rapid characterization of CTL response by enzyme-linked immunospot assay. J Virol 2000; 74 (18): 8514-8519.
 47. Pavlakis, G. N. AIDS: Biology, diagnosis, treatment and prevention. Philadelphia: Leppencott-Raven, 1997.
 48. Kablian, L., et al. Detection of intracellular expression and secretion of interferon- γ at the single-cell level after activation of human T cells with tetanus toxoid *in vitro*. Eur J Immunol 1990; 20: 1085-1089.
 49. Kumar, A.; Weiss, W.; Tine, J. A.; Hoffman, S.L.; Rogers, W. O. ELISPOT assay for detection of peptide specific Interferon- γ secreting cells in rhesus macaques. J Immuno Methods 2001; 47: 49-60.
 50. Currier, J. R., et al. A panel of MHC class I restricted viral peptides for use as a quality control for vaccine trial ELISOPT assays. J Immuno Methods 2002; 260: 157-172.
 51. Tobery, T. W., et al. A simple and efficient method for monitoring of antigen-specific T cell responses using peptide pool arrays in a modified ELISpot assay. J Immuno Methods 2001; 254: 59-66.
 52. Scheibenbogen, C., et al. Quantitation of antigen-reactive T cells in peripheral blood by IFN γ -ELISPOT assay and chromium-release assay: a four-centre comparative trial. J Immuno Methods 2002; 260: 157-172.
 53. Kern, F., et al. Analysis of CD8 T cell reactivity to cytomegalovirus using protein-spanning pools of overlapping pentadecapeptides. Eur J Immunol 2000; 30: 1676-1682.
 54. Larsson, M., et al. A recombination vaccinia virus based ELISOPT assay detects high frequencies of Pol-specific CD8 T cell in HIV-1-positive individuals. AIDS 1999; 13: 767-773.
 55. Folks, T. M., and Hart, C. E. The Life Cycle of Human Immunodeficiency Virus Type 1. 4 th ed. Philadelphia: Lippencott-Raven , 1997.
 56. Pavlakis, G. N. The Molecular Biology of Human Immunodeficiency Virus Type 1. Philadelphia: Leppencott-Raven , 1997.
 57. Luciw, P. A. Fields Virology, 3 rd ed. Philadelphia: Leppencott-Raven, 1996.

58. Tumer, B. G., and Summers, M. F. Structural biology of HIV. J Mol Biol 1998; 285: 1-32.
59. Gelderblom, H. R. Assembly and morphology of HIV: potential effect of structure on viral function. AIDS 1991; 5: 617-638.
60. Bukrinsky, M. I., et al. Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. Proc Nat Acad Sci USA 1992; 89: 6580-6584.
61. Kondo, E.; Mammano, F.; Cohen, E. A.; and Gottlinger, H. G. The p6^{gag} domain of human immunodeficiency virus type I is sufficient for the incorporation of Vpr into heterologous viral particles. J Virol 1995; 69: 2759-2764.
62. Kohl, N.E., et al. Active human immunodeficiency virus protease is required for viral infectivity. Proc Nat Acad Sci USA 1988; 85:4648-4653.
63. Dayton, A.I., et al. The trans-activator gene of the human T-cell lymphotropic virus type III is required for replication. Cell 1986; 44: 941-947.
64. Fisher, A.G., et al. The transactivator gene of HTLV-III is essential for virus replication. Nature 1986; 320: 367-371.
65. Wright, C. M.; Felbar, B. K.; Paskalis, H.; and Pavlakis, G. N. Expression and characterization of *trans*-activator of HTLV-III/LAV virus. Science 1986; 234: 988-1005.
66. Aruya, S. K., et al. Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science 1985; 229: 69-75.
67. Sodroski, J., et al. Location of the trans-activating region on the genome of human T-cell lymphotropic virus type III. Science 1985; 229: 74-82.
68. Rice, A.P., and Mathews, M. B. Transcriptional but not translational regulation of HIV-1 by the *tat* gene product. Nature 1988; 332: 551-559.
69. Laspia, M.; Rice, A.; and Mathews, M. HIV-1 *tat* protein increases transcriptional initiation and stabilizes elongation. Cell 1989; 59:283-287.
70. Marciniak, R. A.; Calnam, B. J.; Frankel, A. D.; and Sharp, P. A. HIV-1 *tat* protein trans-activates transcription in vitro. Cell 1990; 63: 791-802.
71. Feinberg, M.; Baltimore, D.; and Frankel, A. The role of Tat in the human immunodeficiency virus life cycle indicates a primary effect on transcriptional elongation. Proc Natl Acad Sci USA 1991; 88: 4045-4059.
72. Kato, H., et al. HIV-1 *tat* acts as a processivity factor in vitro in conjunction with cellular elongation factors. Genes Dev 1992; 6: 655-666.

73. Daly, T. J., et al. Specific binding of HIV-1 recombinant Rev-responsive element in vitro. Nature 1989; 342: 816-819.
74. Avert. 2002. World Wide HIV and AIDS Statistics Information 2001. (Online).
Available from: <http://www.avert.org/worlstatinfo.htm>.
75. Charneau, P., et al. Isolation and envelope sequence of a highly divergent HIV-1 isolate: definition of a new HIV-1 group. Virology 1994; 205: 247-253.
76. Simon, F., et al. Identification of a new human immunodeficiency virus type 1 distinct from group M and group O. Nat Med 1998; 4: 1032-1037.
77. Sharp, P. M.; Robertson, D. L.; Gao, F.; and Hahn, B. Origins and diversity of human immunodeficiency viruses. AIDS 1994; 4 (suppl 1): s27-s42.
78. Korber, B. T. M.; Allen, E. E.; Farmer, A. D.; and Myers, G. L. Heterogeneity of HIV-2. AIDS 1995; 9 (suppl A): s5-s18.
79. Delwart, E.L.; Sheppard, H. W.; Walker, B. D; Goudsmit, J.; and Mullins, J. I. Human immunodeficiency virus type 1 evolution in vitro tracked by DNA heteroduplex mobility assay. J Virol; 68: 6672-6683.
80. Louwagie, I., et al. Genetic diversity of the envelope glycoprotein from human immunodeficiency virus type 1 isolates of African origin. J virol 1995; 69: 263-271.
81. Townsend, A., and Bonmer, H. Antigen recognition by class I restricted T lymphocytes. Annu Rev Immunol 1989; 7: 601-624.
82. Wange, R., and Samelson, L. Complex complexes: signaling at the TCR. Immunity 1996; 5: 197-205.
83. Wiess, A., and Littman, D. Signal transduction by lymphocyte antigen receptors. Cell 1994; 76: 263-274.
84. Purbhoo, M., et al. Copresentation of natural HIV-1 agonist fails to induce the T cell receptor signaling cascade. Proc Natl Acad Sci USA 1998; 95: 4527-4532.
85. Couillin, I., et al. Impaired cytotoxic T lymphocyte recognition due to genetic variations in the main immunogenic region of the human immunodeficiency virus 1 NEF protein. J Exp Med 1994; 180: 1129-1134.
86. McAdam, S., et al. Immunogenic HIV variant peptides that bind to HLA-B8 can fail to stimulate cytotoxic T lymphocyte responses. J Immunol 1995; 155: 2729-2736.

87. Kaslow, R., et al. A1, Cw7, B8, DR3 HLA antigen combination associated with rapid decline of T-helper lymphocytes in HIV-1 infection. A report from the Multicenter AIDS Cohort Study. Lancet 1990; 335: 927-930.
88. Steel, C., et al. HLA haplotype A1 B8 DR3 as a risk factor for HIV-related disease. Lancet 1988; 1: 1185-1188.
89. Carrington, M., et al. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science 1999; 283: 1748-1752.
90. Su, B., et al. Human granzyme B is essential for DNA fragmentation of susceptible target cells. Eur J Immunol 1994; 24: 2073-2080.
91. Kagi, D., et al. Fas and perforin pathways as major mechanisms of T cell-mediated cytotoxicity. Science 1994; 265: 528-530.
92. Kojima, H., et al. Two distinct pathways of specific killing revealed by perforin mutant cytotoxic T lymphocytes. Immunity 1994; 1: 357-364.
93. Nagata, S. and Golstein, P. The Fas death factor. Science 1995; 267: 1449-1456.
94. Betts, MR., et al. Human Immunodeficiency Virus Type 1-Specific Cytotoxic T Lymphocyte Activity Is Inversely Correlated with HIV Type1 Viral Load in HIV Type1-Infected Long-Term Survivors. AIDS Res Hum Retroviruses 1999; 15(13): 1219-1228.
95. Price, D. A., et al. The Influence of Antigenic Variation on cytotoxic T lymphocytes responses in HIV-1 infection. J Mol Med 1998; 76: 699-708.
96. Gallimore, A., et al. Early Soppresion of SIV replication by CD8+ nef-specific cytotoxic T cells in vaccinated macaques. Nat Med 1995; 1: 1167-1173.
97. Schmitz, J., et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999; 283: 857-860.
98. Letvin, N. L., et al. Cytotoxic T lymphocytes specific for the simian immunodeficiency virus. Immunol Rev 1999; 170: 127-134.
99. Kuroda, M., et al. Emergence of CTL coincides with clearance of virus during primary simian immunodeficiency virus infection in rhesus monkeys. J Immunol 1999; 162: 5172-5183.
100. Villada, I., et al. Positive role of macaque CTL during SIV infection. FEMS Immunol Med Microbiol 1997; 19: 81-87.
101. Ruben, S., et al. Structural and function. J Virol 1989; 63: 1-8.
102. Novitsky, V., et al. Identification of Human Immunodeficiency Virus Type 1 Subtype C Gag-, Tat-, Rev-, and Nef-Specific Elispot-Based Cytotoxic T-

Lymphocyte Responses for AIDS Vaccine Design. J Virol 2001; 75: 9210-9228.

103. Buranapraditkun S. HIV-1 gag/pol cross-clade specific cytotoxic T lymphocytes and gag epitope mapping in asymptomatic HIV-1-infected Thai patients. Master's thesis, Inter-Departmental Program in Medical Microbiology, Graduate School, Chulalongkorn University.

APPENDICES

APPENDIX I

CHEMICAL AGENTS AND INSTRUMENTS

A. Chemical Substances

Alkaline phosphatase substrate (Bio Rad Labs., Hercules, CA)
Anti-IFN- γ mAb 1-D1K (Mabtech, Stockholm, Sweden)
Anti-IFN- γ mAb 7-B6-1 (Mabtech, Stockholm, Sweden)
Clorox
DMSO (Sigma, UK)
Fetal Bovine Serum (Bio Whittaker, Maryland, USA)
Glutamine (Sigma, UK)
Isoprep (Robbins Scientific, Norway)
PBS (Sigma, UK)
Penicillin (General Drugs House, Thailand)
RPMI medium 1640 (GIBCO, USA)
Streptavidin-alkaline phosphatase conjugate (Mabtech, Stockholm, Sweden)
Streptomycin (General Drugs House, Thailand)
Truncated Tat peptide (Natural and Medical Sciences Institute at the University of Tuebingen, Germany)
Trypan blue (Sigma, UK)

B. Instruments

6-well flat plate (Costar, USA)
24-well flat plate (Costar, USA)
96-well polyvinylidene difluoride backed plates (Millipore, Bedford, MA)
Analytical balance (Precise, Swiss)
Automatic pipette (Gilson, USA)
Centrifuge

Conical tube 50, 15 mL (Falcon, USA)
Counting chamber
Cover slip
Cryotube (Sarstedt, Germany)
Freezer -70°C
Glove
Heparin tube (Becton-Dickinson, USA)
Incubator (Forma Scientific, USA)
Microcentrifuge (Eppendorf, USA)
Microtube 250 µl
Mixer-Vortex-Genic (Scientific industries, USA)
Multichannel pipette
Pipette tip
Serological pipette 25, 10, 5, 2, 1 mL (Costar, USA)
Stereomicroscope (Olympus, Japan)
Water bath (Shel-lab, USA)

APPENDIX II

AMINO ACID SEQUENCE OF TAT PEPTIDE

1. Abbreviation for amino acids

A alanine	L leucine
R arginine	K lysine
N asparagine	M methionine
D aspartic acid	F phenylalanine
C cysteine	P proline
Q glutamine	S serine
E glutamic acid	T threonine
G glycine	W tryptophan
H histidine	Y tyrosine
I isoleucine	V valine

2. HIV-1 CRF01_AE Tat peptides

Peptide No.	Region	Amino acid length	Amino acid sequence
Pool 1			
1	Tat 1-21	21	MELVDPNLEPWNHPGSQPTTA
2	Tat 12-29	18	NHPGSQTTACSKCYCKK
3	Tat 20-39	20	TACSKCYCKKCCWHCQLCFL
4	Tat 30-49	20	CCWHCQLCFLKKGLGISYGR
5	Tat 40-56	17	KKGLGISYGRKKRKHRR
Pool 2			
6	Tat 47-65	19	YGRKKRKHRRGTPQSSKDH
7	Tat 56-74	19	RGTPQSSKDHQNPIPKQPL
8	Tat 65-85	21	HQNPIPKQPLPIIRGNPTDPK
9	Tat 76-95	20	IIRGNPTDPKESKKEVASKA
10	Tat 86-102	17	ESKKEVASKAETDPCDA

BIOGRAPHY

Miss Sunantha Kosonsiriluk was born on July 7, 1978 in Bangkok, Thailand. She received a Bachelors' degree in Biology in 1998 from the Faculty of Science, Srinakarinwirote University, Bangkok, Thailand. She enrolled at Chulalongkorn University in a graduate program for a Masters' Degree in Medical Science in 1999.

