

P25 The Cytoplasmic Tail of CD86 Costimulatory Molecule Involved in Anti-HIV CTL Responses During DNA Vaccination.

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Lately we reported that co-administration of CD86, but not CD80 cDNA along with DNA antigens dramatically increased anti-HIV-specific CTL responses. We suggest the following mechanisms to explain the functional differences between CD80 and CD86 molecules after DNA vaccination. First, the triggering of receptors with CD80 and CD86 molecules may supply different signals to T-cells. Second, the ligation of CD86, but not CD80 on the surface of APCs could provide important signal to these cells and in turn induce production of certain molecules (for example the cytokines/ lymphokines/ chemokines), which are important for T cell activation. To evaluate these hypotheses we propose using DNA immunization as a model system to investigate the functional region or regions of human CD86/CD80 molecule involved in enhancement of cellular immune responses against HIV-1. We immunized mice with DNA encoding gp120 of HIV-1 and DNA encoding different CD80/CD86 chimeric or truncated molecules. We determined that both CD80 and CD86 V-domains were equally potent for activation of virus-specific CTL responses. More importantly our data demonstrated the critical role of cytoplasmic tail of CD86 in anti-HIV CTL activation. Thus, plasmids encoding certain forms of CD80/86 could be used as molecular adjuvants during nucleic acid vaccination.

P26 Effect of Promoter Strength on Cellular and Humoral Immune Responses Elicited by HIV-1 Multigenic DNA Vaccines
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Nucleic acid vaccination can induce humoral and cellular immune responses, both of which may be important for protection against HIV-1. The effect of promoter strength on immune responses generated by non-HIV DNA vaccines has been analyzed in small animals. However, the effects have not been studied in primates using HIV-1 DNA vaccines. In order to examine the effects of two different promoters in an HIV-1 DNA vaccine, we constructed two DNAs which included the HIV-1 *gag*, *env*, *rev* and *vpu* under the regulation of the AKV murine leukemia viral LTR (pAKV-NL_{APol}) or the human CMV-IE promoter (pCMV-NL_{APol}). In *in vitro* studies, it was found that the CMV promoter expressed CAT 10-20 times more strongly than the AKV-LTR. This increase in expression was reflected in HIV-1 protein levels seen in DNA vaccine-transfected cell lines. To examine the *in vivo* effects of the different promoters, the HIV-1 DNA vaccines were injected into rhesus macaques and pig-tailed macaques. Both DNA vaccines induced persistent Gag antibodies and intermittent Env-specific antibodies in rhesus macaques. The pCMV-NL_{APol} generated stronger humoral responses with fewer injections and lower doses of vaccine DNA than pAKV-NL_{APol}. In the pig-tailed macaques, similar results were obtained with pCMV-NL_{APol}. On the other hand, the T-cell proliferative responses generated to Gag and Env were not significantly different. Thus, the promoter strength may have little effect on the intensity of the cellular responses generated by an HIV-1 DNA vaccine, whereas the humoral responses seen *in vivo* correlated with the promoter strength.

P27 Humoral and Cellular Immune Response Elicited by HIV-1 *Env* DNA Constructs in Mice

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DNA segment encoding *env* gene of HIV-1 (BRU-2) was amplified and cloned into mammalian expression vector. *In vitro* expression was studied by western blot analysis of transfected He La cells. Mice were injected I/M or I/D with 100ug of DNA construct per dose in normal saline at biweekly intervals. A low antibody level was detected as determined by ELISA after 5 doses. Subsequent inoculations failed to increase the antibody titers significantly. The mice were boosted with a mixture of two recombinant vaccinia viruses, vPE8 and vPE16, expressing gp120 and gp160 respectively. A transient increase in antibody response was observed which was independent of the route of DNA inoculation. Once the antibody response started falling additional inoculation with recombinant vaccinia antigen failed to boost the antibody responses. T lymphocyte response was studied by Lymphocyte Proliferation Assays on the spleen cells from the immunized mice after 3 doses of 100 ug of DNA construct. The data showed the presence of T cell response second week onwards after the third dose and was present even at the end of 24 weeks.

P28 HIV-1 Vaccine Trials at the Vaccine Trial Centre (VTC), Bangkok

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The first HIV-1 vaccine project at VTC began in 1993 using AIDSVAXTMB monovalent vaccine. The vaccine was found to be safe and equally immunogenic in Thai as in compared to the US volunteers. This trial led to the development of combined bivalent B/E vaccines. A Phase I/II trial to evaluate the safety and immunogenicity of AIDSVAXTMB/E vaccine was started in late 1997. Volunteers(n=92) were enrolled from both the general and IVDU population. The results after two doses showed that the vaccine was safe. The study also demonstrated that the immunogenicity of bivalent rgp120 based vaccines is similar to monovalent rgp120 based vaccines. Like monovalent rgp120 vaccines, greater than 95% seroconversion was induced by both of the gp120 antigens at the 6 week time-point. Moreover the magnitude of the antibody response at this time was similar to that obtained with the monovalent vaccine. Based on these results, A Phase III efficacy trial has been planned in Bangkok and was approved in Jan. 99. At the same time as a part of The Thai AIDS Vaccine Evaluation Group, a trial "A Phase I/II, Double-blind, Placebo-controlled Study of the Chiron HIV Thai E gp120/MF59 Vaccine Administered Alone or Combined with the Chiron HIV SF2 gp120 Antigen in Healthy HIV-Seronegative Thai Adults" was also started. The objectives were to determine the safety and immunogenicity of the vaccine candidate in healthy. VTC is one of 4 sites and has completed enrollment of 99 volunteers includes 6 who were in the open label part. 98 have completed the 3 doses immunization. The vaccine was safe, the immunological result of this study are pending. Thus, the Vaccine Trial Centre of Mahidol University is actively engagement HIV vaccine both phase I&II and III.