

978. Production of SARS Coronavirus-Like Particles That Bind Host Cells and Serve as Vaccine Antigen

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Severe Acute Respiratory Syndrome (SARS) is a deadly form of pneumonia caused by a recently identified coronavirus (SARS-CoV). Viral particles of SARS-CoV comprise three virion structural proteins, including spike (S), membrane (M), and envelope (E). To efficiently express S, M, and E proteins of SARS-CoV simultaneously in one cell, we constructed a plasmid harboring the three genes with GFP (green fluorescence protein) fusion to the M protein. Expression of the three genes is engineered under regulation of *tet* operon. Stable transfection of this plasmid into Vero E6 cells creates cell lines showing inducible expression of virus-like particles (VLPs). Expression and packaging of VLP in cytoplasm is observed by confocal microscopy. We then purified the secreted VLPs from cell culture medium through ultra-centrifugation. Containment of each protein in VLPs was assured by coomassie blue staining and western blotting. Interestingly, two types of particles were secreted by one of our producing cell lines. One contains M dominantly, the other contains S dominantly. Electron microscopy reveals homogenous particles of both VLPs. We have further demonstrated binding capability of M dominant VLP to Vero E6 cells as host by flow cytometry and confocal microscopy analyses. Immunization of the two VLPs induced antibodies against both VLPs in ELISA and western blot, suggesting a promising immunogenicity of both VLPs as vaccine antigen. The genetic engineered VLPs bearing resemblance to the authentic SARS-CoV as well as their antibodies are important tools toward development of effective vaccine against highly contagious infectious disease like SARS.

979. Antigen-Transduced T Cell Blasts Specifically Delete Antigen-Specific Cytotoxic T Cells and Induce a CD4+CD25+ Regulatory T Cell Response

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Introduction: Antigen presentation by T cells has been shown to induce anergy and apoptosis in responding antigen-specific T cells. This phenomenon might be useful in targeting antigen-specific T cells for the treatment of autoimmune disease and allogeneic organ transplantation. Therefore, we examined the immune response to antigen-presenting T cell blasts (T-APC) transduced with known antigens (CMV pp65, EBV-LMP2 or adenovirus hexon protein).

Methods: T-APC were prepared by stimulating PBMC on OKT3 and anti-CD28-coated plates and transducing with the MSCV retroviral vector encoding CMVpp65, EBV-LMP2 or adenovirus hexon protein. T-APC were subsequently expanded with high dose IL-2. PBMC were then stimulated on a weekly basis with irradiated antigen-expressing autologous T-APC. Responding cells were analyzed using MHC class I multimers for pp65, LMP2 and hexon proteins, annexin-V, and antibodies to CD4, CD8, CTLA4, GITR and LAG-3 by flow cytometry. Analysis of FoxP3 expression was performed using real-time quantitative PCR (Q-PCR) normalized to GAPDH. To test regulatory activity, responder cells cultures were separated into CD4+CD25+ and CD4+CD25-

populations and examined for their ability to inhibit proliferation of PBMC against pp65 or LMP2-transduced EBV-lymphoblastoid B cell lines (LCL) in secondary co-culture experiments.

Results: T-APC were readily expanded (>100-fold) and transduced by retroviral constructs (>90% GFP+). Antigen-transduced T-APC were capable of stimulating an antigen-specific CD8+ T cell response as evident by a temporary increase in the frequency of pp65, LMP2 and hexon-specific T cells when analyzed by multimers. However, the frequencies of responding antigen-specific CD8+ T cells decreased during multiple T-APC stimulations (7 to 21 days) and were positive for annexin-V indicating apoptosis. This effect could not be rescued by restimulation with antigen-transduced professional APCs such as EBV-LCL. T-APC stimulated cultures (n=23) showed a significant increase in the frequency of CD4+CD25+ T cells that were FoxP3, CTLA4, GITR and LAG-3 positive when compared to LCL (n=10) stimulated cultures (*P* < .05). CD4+CD25+ T cells inhibited (up to 95% inhibition) secondary cultures stimulated with LCL in a cell-contact dependent manner, indicating a potential regulatory function.

Conclusions: T-APC transduced with antigen induced antigen-specific anergy/apoptosis in CD8+ T cells and an increase in CD4+CD25+ regulatory T cells in vitro. Vaccination with T-APC expressing auto-immune antigens may be useful in targeting auto-reactive T cells and inducing regulatory T cells in diseases such as multiple sclerosis or type I diabetes.

980. Specific Immune Response Against the *Plasmodium falciparum* CS Protein Mediated by Baculovirus Vectors

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Malaria is the most common tropical disease worldwide. More than 300 million people are infected and about 2-3 million die annually. The most pathogenic form, Malaria tropica, is caused by *Plasmodium falciparum*. This enormous world-health problem is compounded by parasites becoming resistant to drugs which are commonly used for treatment and also the complete lack of an effective vaccine. Recombinant baculovirus vectors derived from the *Autographa californica* nuclear polyhedrosis virus (AcNPV) are commonly used as a tool for high-level expression of recombinant proteins of interest in insect cells. Baculovirus vectors can mediate an immune response against an antigen when it is displayed on the viral surface, or when it is expressed from the baculovirus backbone using promoters which are active in mammalian cells. Their ability to accommodate very large inserts of foreign DNA, the lack of a preexisting/neutralizing immunity to baculoviruses in humans and the easy generation of high titer virus stocks are further clear advantages of this vector system for the delivery of a vaccine.

In order to use baculoviruses for an induction of a specific immune response we inserted a *P. falciparum* circumsporozoite (CS) protein expression cassette, driven by the strong CMV promoter, into the baculoviral genome (AcNPV-CSFVI). An expression of the CS protein in antigen presenting cells (APC) should activate CD8+ T cells via MHC I presentation. The display of a second, recombinant CS peptide in the viral envelope was achieved by fusion of the CS gene to the main baculovirus surface protein gp64 (AcNPV-CS/gp64). This envelope-modified baculovirus mimics the parasite to induce CD4+ T cells and CS specific antibodies following uptake into APC and antigen presentation by MHC II. A third vector (AcNPV-CSFVI-CS/gp64) was constructed to combine expression and presentation of CS antigens in mammalian cells.