

P45 Autovaccines as therapeutic instruments to treat chronic bacterial infections

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Autovaccines (syn. autogenous vaccines) are rarely used to treat chronic bacterial infections, and, sometimes viral infections such as human papillomatosis. The power of autovaccines is obvious in cases, where infections are treated effectively even if the causative bacterium is resistant to antimicrobial drugs. These therapeutic instruments had been widely used during the first half of our century but, however, with the rise of the antibiotic era autovaccines became of minor interest. The mechanism by which autovaccines influence the immune system in cases of chronic disease has never been examined. Our hypothesis of how the immune system is influenced is a different activation of Th-subset cells compared to the chronic situation which leads from an inflammatory response with tissue damage towards a helper response.

OBJECTIVE: Application of autovaccines and examination of the immune response (pre- & postvaccination) in five human patients, who suffered from chronic bacterial infections (caused by *Pseudomonas aeruginosa* (2), *Escherichia coli* & *Enterococcus faecalis* (1), *Staphylococcus aureus* (1) and *Corynebacterium* spec. (1)).

METHODS: lymphocyte proliferation assays and flow cytometry.

RESULTS: Four out of the five patients recovered within 2 weeks after administering the first dose of their specific autovaccine, one failed to respond to the autovaccine.

CONCLUSIONS: The application of specific autovaccines to humans in case of chronic bacterial infection is a useful alternative at least in cases caused by bacteria exhibiting resistance to antibiotic drugs.

P46 A Group B Coxsackievirus is Attenuated by Site-Specific Mutations Within a Completely Conserved 5' Nucleotide Sequence in the 5' Non-Translated Region.

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With the worldwide eradication of the polioviruses, other enteroviruses will be explored as chimeric vaccines as gene-expression vectors. We are studying mutations within a 5 nucleotide tract of the coxsackievirus B3 (CVB3) 5' non-translated region (NTR), a tract that is completely conserved among all human enteroviruses, as an approach to generating attenuated vaccine strains. The sequence 5'-CGUUA is located at nt232-234 in the CVB3 map. Four mutants have been characterized: CVB3/0, a U to C mutation at nt234 (U234C); CVB3/234G, (U234G); CVB3/233C, (G233C), and double mutant CVB3/88, (G233C/A236U). Transfection of viral cDNAs at 37C resulted in barely detectable titers in early passages; a rapid re-acquisition of wild-type sequence and phenotype correlated with replicative vigor. Transfection at 33.5C however maintained the mutations. Temperature shift experiments demonstrate the mutations have little/no effect upon viral translation in HeLa cells. Preliminary data with mutations at nt234 demonstrated a lesion at the level of positive strand RNA synthesis and attenuation for disease in mice; the above CVB3 mutants are currently being analyzed for positive/negative viral RNA strand ratios and phenotype in mice. The data to date demonstrate that mutations in this completely conserved sequence attenuate CVB3 for virulence and replication. This approach, alone or in combination with other genetic approaches, such as chimeric genomes, should be useful for the design of more stably attenuated and safe enteroviral vectors.

P47 Application of Bioinformatics and Proteomics for Identification of Bacterial Gene Products As Potential Vaccine Candidates

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For vaccine development, we are using bioinformatics and genomic sequences from pathogenic bacteria to predict genes that may encode vaccine candidates, e.g. surface localized proteins. We are also using proteomics to rapidly identify such gene products, e.g. proteins in outer membrane preparations. We will demonstrate our bioinformatics approach using the published *Haemophilus influenzae* Rd genomic sequence and the identification of a putative vaccine candidate, the outer membrane lipoprotein, P6. This will include analysis of the genomic sequence to locate open reading frames (ORFs), annotation of the ORFs using BLAST, prediction of genes representing non-coding regions from %GC content and evaluation of each gene product for presence of signal peptide sequence characteristic of membrane proteins. To demonstrate the power of proteomics, we will show data on rapid and unequivocal identification of the P6 protein by peptide mass map analysis. This involves determination of mass spectrum of the peptide mixture obtained from digestion of P6 with a protease of known specificity. The peptide-mass fingerprint is definite enough to identify the protein uniquely by comparison with a database of peptide mass values calculated by applying the corresponding enzyme cleavage rules to entries in a sequence database using an appropriate scoring algorithm. We will also demonstrate the use of proteomics for identification of vaccine candidates for another pathogenic bacteria, *Helicobacter pylori* using two different approaches. The first involves rapid identification of a series of monoclonal antibody reactive proteins from N-terminal sequence tags. The other approach involves identification of proteins in outer membrane preparations by 2-D electrophoresis followed by trypsin digestion and mass map analysis.

P48 HARE: An Improved Measure of Efficacy for Phase III HIV-1 Vaccine Trials

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Introduction: In the summer of 1998, VaxGen began the first FDA-approved Phase III HIV-1 vaccine trial. The planning of such trials, even if randomized, double-blind and placebo-controlled, presents difficulties with respect to efficacy measurement. Traditional efficacy measures (EM) based on hazard ratios or cumulative incidence ratios are known to stray with time and may not provide the desired validity and precision due to unknown mode of vaccine action, heterogeneity and time-dependent forces of infection.

Objectives: To address the as yet unsettled issue of robust and unbiased vaccine efficacy (VE) estimation by presenting an improved EM candidate based on Hazard Regression (HARE) and Hazard Estimation (HEFT) log-spline models. Specifically, an EM that yields accurate VE estimates (independent of time) under various vaccinal characteristics and in the presence of different heterogeneities was developed.

Methods: The study employs a stochastic model simulating HIV transmission in a population in which clinical trial designs are embedded. Different design, population and vaccine characteristics are simulated. Knowing how data were generated, they can thereafter be used as benchmarks to evaluate EM based on HARE and HEFT.

Results: Based on simulation studies, HARE and HEFT measures provide valid VE estimation across different modes of vaccine action contrary to their traditional counterparts which demonstrate sensitivity and bias from one mode of action to another. HARE and HEFT measures also remain valid under different frailty effects and sexual mixing patterns.

Conclusion: In light of the global importance of AIDS and exorbitant costs associated with vaccine testing, it is important to have a reliable estimation framework to obtain the information we desire from phase III HIV vaccine trial data; HARE and HEFT are promising in this respect. Confidence intervals and sample size questions are forthcoming.