RESEARCH PAPER ANALYSIS

CONSTRUCTION OF A LIVE ORAL CHOLERA VACCINE

One of the great promises offered by recombinant gene technology is the production of new and more effective vaccines[1]. In this issue of BIO/TECHNOLOGY, James Kaper and his colleagues at the Center for Vaccine Development (CVD, University of Maryland School of Medicine, Baltimore) describe the design and construction of a debilitated *Vibrio cholerae* that can be administered as a live oral cholera vaccine. Vibrio-associated diarrheas are a major cause of infant mortality in many developing countries and, despite years of effort, no satisfactory vaccine is available.

The organism Kaper modified is the agent of epidemic and endemic cholera, and is known to confer complete protective immunity following infection[2]. Any attenuated strain derived from it should, therefore, be a very effective vaccine. The approach of the CVD group was to remove 94% of the gene coding for the A1 subunit of the *V. cholerae* enterotoxin without deleting any other genes that might be important for establishing immunity. Since only the A1 subunit of the toxin is absent, the strain still elicits antibodies to the non-toxigenic B subunits. All other important antigens of the organism are left intact as well.

In order to delete the interior of the gene encoding the A1 subunit, the CVD group constructed a mobilizable plasmid vector carrying a toxin operon in which a 550 base pair fragment specifying most of the A1 peptide had been deleted by *in vitro* recombinant DNA techniques. This plasmid was then introduced into a strain containing a tetracycline resistance marker inserted into both copies of the chromosomal genes coding for the A1 peptide. Since the plasmid contains sequences flanking the ends of the deletion that are homologous to the chromosomal DNA, a recombination event that replaces the chromosomal A gene with the plasmid-borne deletion is possible. If both copies of the chromosomal A gene are removed, so are the tetracycline resistance markers; thus the resulting organisms will be tetracycline-sensitive. These cells can be significantly enriched in the population by using bacteriostatic concentrations of tetracycline in the presence of the bactericidal antibiotics ampicillin and cycloserine. This procedure greatly facilitates selection of the the desired recombinants. Since the A genes are almost completely deleted in this strain, there is no chance of reversion to toxigenicity: consequently the vaccine should be completely safe.

Using a similar approach, Mekalanos and coworkers at Harvard University[3] also successfully removed the toxin genes from cholera vibrios. However, their strains have not yet been tested in clinical trials. The CVD vaccine strain is curently being evaluated in human volunteers and the results thus far are extremely encouraging.

References

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