BACKGROUND PAPER

PROGRESS TOWARDS A CORONAVIRUS RECOMBINANT DNA VACCINE

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During the last decade recombinant DNA technology has been successfully applied to the development of 'new' vaccines. Although there are many examples of successful vaccines which have been developed using the 'classical approach' e.g. inactivated, attenuated and subunit vaccines, the demand for a new generation of vaccines is evident. The risk of the escape of a pathogen from a vaccine production facility, the difficulties associated with the production of viral subunits, the high mutation frequency observed in many RNA viruses and the possibility of genetic reassortment and recombination have stimulated researchers to explore new avenues of vaccine biotechnology.

With the cDNA cloning of several genes this approach has now also become feasible for coronaviruses. The aim of this chapter is to summarize recent advances towards the development of recombinant DNA vaccines against coronavirus induced diseases.

Which antigens should be chosen for the development of (bio)synthetic vaccines? Coronaviruses possess three major structural proteins: a nucleocapsid protein (N), a small integral membrane glycoprotein (M) and a large spike glycoprotein (S). While all coronaviruses contain these proteins, a subset (the MHV strains JHM and S, HEV, HCV-OC43 and BCV) is now recognized to possess an additional glycopolyptide (gp65 or HE) which is unrelated to S or M. The candidate antigen for a vaccine is often predicted on the basis of *in vitro* neutralization assays and passive transfer of antibodies followed by a virus challenge. However, a detailed understanding of both the humoral and cellular immune response in the natural disease is necessary to select the most effective antigen.

S and HE induce virus neutralizing antibody and in addition some anti-M antibodies neutralize virus in the presence of complement. The M protein of TGEV not only induces antibody which neutralizes in the presence of complement, but also induces the release of interferon by peripheral blood lymphocytes. Two anti-M MAbs were able to inhibit this induction.

Does *in vitro* neutralizing activity correlate with protection? Passive transfer of MAbs to animals followed by a challenge infection can give additional information on immune status. Some anti-M MAbs and anti-S MAbs, and a non-neutralizing anti-N Mab, were able to passively protect mice against lethal challenge. Complement does not play a role *in vivo* during protection mediated by passive transfer of an anti-M Mab since mice deficient in the C5 complement component were also protected and depletion of the C3 component did not affect the protective activity of this Mab.

A few attempts to protect animals using purified antigens from whole virus have been reported, but with limited success. Mice which had been inoculated intraperitoneally with purified S, but not N or M, from MHV-3 were protected from lethal challenge. The low level of protection induced by inactivated IBV was abolished after the removal of SI by urea treatment. A subunit, with a M_r of approximately 25K released from TGEV by sonication induced neutralising antibodies in pigs. This material protected against challenge with virulent TGEV and induced antibody in gilts which, when transferred to suckling offspring, resulted in reduced morbidity and mortality after challenge. Further characterization of the 25K entity has not been published.

Thus there is substantial evidence that the S protein can evoke a protective immune response. However in the case of feline infectious peritonitis (FIP), which is a progressive debilitating condition affecting domestic and wild Felidae, one must also consider the fact that S can induce a severe immunological reaction. The presence of antibody directed against FIP proteins often leads to an accelerated, fulminating course of the disease, i.e. clinical signs and lesions develop earlier and the mean survival time is significantly reduced ('early death'). These findings may be explained by antibody dependent enhancement (ADE) of virus infection, a phenomenon previously described for representatives of the flaviviridae, alphaviridae, bunyaviridae, poxviridae, rhabdoviridae, herpesviridae and reoviridae. Binding of antibody results in the formation of infectious virus-antibody complexes, which attach to the surface of the macrophage (via Fc-receptors) with higher efficiency than virus without antibody. Experimental evidence has recently been obtained that this mechanism is also involved in FIP. In a recent comparison of feline coronaviruses it was shown that virulent strains infect more macrophages, producing higher titers, and spread more readily to other macrophages; in addition, their infectivity was enhanced by antibody from sensitized cats (Stoddart 1988, personal communication). Immune enhancement is central to FIP pathogenesis and is due to anti-peptomer responses, as has been proven *in vivo* with the aid of a vaccinia virus recombinant (see article by Vennema *et al.*).

Research towards a recombinant DNA coronaviral vaccine is now conducted in several laboratories. Delineation of B-cell epitopes (see article by Laude *et al.*) and possibly in the near future also of T-cell epitopes will form the basis for the development of synthetic peptide, antigen and chimeric viral vaccines. The latter approach in which an epitope is genetically engineered into a carrier protein or carrier particle looks especially promising since the immunogenicity of these antigens is much better than a synthetic peptide chemically coupled to a carrier protein. Vaccination with synthetic peptides homologous to residue 993-1002 and 848-856 of the MHV S protein protected mice against lethal coronavirus-induced encephalitis. Although domain 848-856 of S of MHV binds to one neutralizing Mab, antibodies raised against the synthetic peptide did not neutralize MHV *in vitro*. Hence, protection against disease does not correlate with *in vitro* neutralization.

Mapping of B-cell epitopes and strain comparison on the level of the amino acid sequence of field isolates will also provide necessary data concerning antigenic variation. Immunological escape due to point mutations and/or recombination will certainly hamper the development of a vaccine against IBV. This problem may be solved by the identification of conserved B and T-cell epitopes.

A wide variety of expression systems have been developed for the production of antigens. Since the coronavirus spike gene encodes a highly glycosylated protein, eucaryotic expression systems have to be used to obtain recombinant protein that closely resembles the viral protein. Recombinant FIPV S protein isolated from a transformed mouse fibroblast cell line which was obtained after transfection with a bovine papilloma virus expression vector expressing the FIPV S gene under the control of the heat shock promoter, was fusogenic and induced neutralizing antibodies in mice. Experiments using virus-based transient expression systems like baculovirus recombinants (production in insect cells) and vaccinia virus recombinants (production in mammalian cells) are in progress, and some of the data are presented in other chapters (Taguchi *et al.*; Vennema *et al.*; Parker *et al.*).

The application of vectored live virus vaccines has been limited to vaccinia virus. Vaccinia virus recombinants expressing the IBV and FIPV S gene evoke an *in vitro* neutralizing immune response in chickens and kittens, respectively. The immunized chickens were only partially

protected (50%), whereas immunized kittens became sensitized (see above). Future research will focus on the use of DNA carrier viruses with a narrow host range e.g. fowlpox for protection against IBV, canine adenovirus for protection against canine coronavirus and feline herpesvirus for the protection against FIPV. Often a long-lasting immunity can only be achieved using a live attenuated virus vaccine. The main reason is that replicating immunogens evoke a broad spectrum of immune responses including local immunity. However, because of genetic instability the vaccine virus can regain virulence. The genetic stability of attenuated vaccines can possibly be increased by deletion mutagenesis. For RNA viruses this approach is only possible if an infectious cDNA clone is available and will certainly be an important area of future coronavirus research.