DEPARTMENT for ENVIRONMENT, FOOD and RURAL AFFAIRS

Research and Development

CSG 15

Final Project Report

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Project title	New strategies for vaccines against infectious bronchitis and the elucidation of pathogenicity and immunity
DEFRA project code	**OD0712**
Contractor organisation and location	Institute for Animal Health Compton
Total DEFRA project costs	£ 971,300
Project start date	01/04/99
Project end date	31/03/03

Executive summary (maximum 2 sides A4)

Infectious bronchitis virus (IBV, a coronavirus) is the major respiratory pathogen of chickens in Great Britain (GB). In meat-type birds the disease causes some mortality in chicks and slows growth. It also predisposes them to secondary bacterial infection, with additional mortality, whilst some strains cause kidney disease. In layers and breeders the virus grows in the oviduct as well as in respiratory and other tissues, infection resulting in drops in the number and quality of eggs. Although live attenuated and inactivated vaccines are used to control IB the existence of many serotypes of IBV undermines prophylaxis; a vaccine of one serotype does not induce satisfactory protection to all other serotypes. The major protection-inducing protein of IBV is the large surface spike protein, S. If the S protein of a vaccine could be replaced with that from a new serotype, then the modified vaccine might be fully efficacious against the new serotype.

A disadvantage of live attenuated vaccines, made by conventional means, against many pathogens is that they revert to virulence. Genetic manipulation has the potential for modifying the genome (the complete set of genes) of IBV such that it is attenuated in a non-reversible way; a genetically stable vaccine. By virtue of genetically modifying a vaccine it is also 'marked' as such, which is helpful for diagnosis and epidemiological studies.

The purpose of this project was to develop ways of manipulating the IBV genome, to make a modified IBV (recombinant IBV) and to investigate its biological behaviour. The project has been extremely successful. We have constructed a full-length DNA copy of the IBV genome (which is made of RNA) and cloned it into the genome of vaccinia virus. From this we were able to recover infectious IBV. This system enabled us to modify IBV genes at the DNA stage and to insert the modified genes into the genome of IBV for experimental vaccine development.

Our initial attempt at novel vaccine development involved using a defective IBV RNA to produce proteins. The defective RNA was much smaller (6,100 nucleotides) than the full IBV genome (27,600 nucleotides) and could be replicated with the help of normal IBV. The defective RNA was packaged into virus-

like particles. We showed that genes could be inserted into the defective RNA and that the proteins encoded by these genes were produced (Stirrups, et al. 2000a. *Journal of General Virology*, 81, 791-801; Stirrups, et al.. 2000b. *Journal of General Virology*, 81, 1687-1698; Evans, *et al.* 2000. *Journal of General Virology*, 81, 2855-2865; Dalton, *et al.* 2001. *Journal of Virology*, 75, 125-133.) The defective RNA-containing particles could also be propagated in embryonated eggs, which are used to make IB vaccines, and proteins were produced, including interferon-γ, in them (Hackney et al. 2003. *Journal of Virology*, 77, 5694-702.) However, the defective RNAs with inserted genes were not stable; the gene was lost when the virus was propagated repeatedly. An internal ribosome entry site of encephalomyelitis virus was inserted into the defective RNA to increase protein production. Although the IRES worked *in vitro*, it did not work when the defective RNA was with standard (helper) virus in cells; the IRES interfered with replication of the defective RNA.

The defective RNA was also used in a number of ways to introduce modified genes into IBV by recombination. However, none of these approaches was successful. Nonetheless, the experience that we had gained in assembling DNA copies of large parts of the IBV genome enabled us to attempt to construct a DNA copy of the entire genome of the Beaudette strain. In this we were successful. We then cloned the full length DNA into the genome of vaccinia virus, under the control of a T7 promoter, the basis for recovering (rescuing) live IBV. We introduced the vaccinia virus DNA into cells by transfection; also transfected with a plasmid containing the IBV nucleoprotein gene (N), which we found to be essential for rescue of IBV; infected the cells with a recombinant fowlpox virus containing the T7 polymerase gene, that we had made earlier (Britton *et al.* 1996. *Journal of General Virology*, 77, 963-967). T7 polymerase from the latter resulted in the production of a full-length RNA copy of the IBV genome and a messenger RNA from which N protein was produced. All the viral proteins needed for replication were produced from the full-length IBV RNA, and new infectious IBV was released from the cells (Casais *et al.*, 2001. *Journal of Virology*, 75, 12359-12369.) The procedure is highly reproducible and is currently the only 'infectious DNA clone' or 'reverse genetic' approach for IBV.

The S protein of some other coronaviruses has been shown to be a determinant of tissue tropism and pathogenicity. It was possible that if the S protein of a virulent IBV (donor strain) was used to replace S of our non-pathogenic Beaudette strain (receiver) that the latter would become pathogenic, clearly undesirable from a vaccine point of view. To address this issue we reconstructed the DNA copy of Beaudette, except that we replaced its S gene with that from the virulent M41 strain, to produce BeauR-M41(S). Initially we examined its biological properties using cell cultures (Casais *et al.* 2003. *Journal of Virology*, in press). Beaudette and M41 grow equally well in chick kidney cells; the BeauR-M41(S) grew likewise, as expected. Whereas Beaudette grows in Vero cells, BHK cells and chick embryo fibroblasts, M41 replicates very poorly or not at all; BeauR-M41(S) grew like M41. Thus the Beaudette strain was unable to grow in these cells when its S protein had been replaced by that of M41, showing that the S protein was a determinant of the host cell range of IBV.

To see if the Beaudette strain had become virulent after acquisition of the S protein of virulent M41, chickens were inoculated with the viruses. BeauR-M41(S) was non-pathogenic; acquisition of the M41 S protein had <u>not</u> made it pathogenic. This was very promising for vaccine development. It suggests that S protein genes from virulent field strains could be swapped into the genome of a vaccinal strain without making the latter pathogenic.

An experiment was then performed to see if vaccination with BeauR-M41(S) would induce immune responses that would protect chickens from infection by virulent M41. Chickens were vaccinated by eye-drop and intra-nasally. Three weeks later the chickens were challenged with virulent M41 by the same routes to assess the degree of immunity. Vaccination with both Beaudette and BeauR-M41(S) induced protection against clinical signs but the protection was much greater with BeauR-M41(S). For example, on the basis of protection of the trachea from ciliostasis caused by the challenge virus, Beaudette induced a low level of protection, and then in only 1 of 9 birds. In contrast, BeauR-M41(S) had induced good tracheal protection in 7 of 9 chickens. The S proteins of Beaudette and M41 differ by only approximately 5% of their amino acids. The results indicate that some important protection-inducing epitopes are associated with some of the amino acids that differ between these two viruses.

We have recently introduced the transient dominant selection approach for making recombinant IBVs, as this is technically less exacting. After modifying an IBV gene as desired it is cloned into a plasmid with a guanine phosphoribosyltransferase (gpt) gene for selection mechanism. This is transfected into cells that have been infected with vaccinia virus containing the DNA copy of the IBV genome. Recombination results in the generation of vaccine virus containing cloned IBV with the desired alteration. These vaccinia viruses are selected away from the non-altered vaccinia virus, based on the gpt selection system, after which recombinant IBV is recovered in our established system. In this way we have swapped an S gene and made mutations to

genes 3 and 5. We are now using our infectious clone system to investigate further the rational (specific, knowledge led) modification of IBV for vaccine development.