EXPRESSION OF IBV SPIKE PROTEIN BY A VACCINIA VIRUS RECOMBINANT

Fiona Tomley, Matthew Binns, Mike Boursnell,
and Adrian Mockett

Institute for Animal Disease Research
Houghton Laboratory, Houghton, Huntingdon
PE17 2DA, UK

INTRODUCTION

The spike protein of IBV is a target for virus neutralising antibodies in the chicken (Cavanagh et al, 1984; Mockett, 1985). Vaccinia virus, a poxvirus, has been shown to be a useful and efficient eukaryotic expression vector. Here we describe the expression of the IBV spike from a cDNA clone inserted into vaccinia virus and demonstrate that mice vaccinated with the recombinant produce antibodies to the spike which can neutralise virus infectivity.

METHODS AND RESULTS

Construction of recombinant

The spike coding sequence of IBV strain Beaudette was excised from the previously described plasmid pMB179 (Binns et al, 1985) with TthIII and XbaI, end repaired and ligated into the SmaI site of plasmid pGS20 containing the vaccinia 7.5K promoter flanked by vaccinia thymidine kinase sequences (Mackett et al, 1984). A TK^- recombinant vaccinia virus containing the spike gene of IBV strain Beaudette was generated by transfection of CV-1 cells infected with wild type vaccinia virus with plasmid pSB1 using previously described methods (Mackett et al, 1984).

Immunofluorescence

To test for expression of the IBV spike gene we first used indirect immunofluorescence on CV-1 cells infected with wild type or recombinant vaccinia virus. Surface fixed monolayers were incubated with rabbit anti-IBV Beaudette serum followed by fluorescein-conjugated goat

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Table 1 Induction of anti-spike antibodies and virus neutralisation antibodies in mice inoculated with recombinant vaccinia virus. All titres are mean values from two individuals. Virus neutralisation titres are the dilutions of sera at which 100 median ciliostatic doses of IBV strain M-41 are neutralised in chicken tracheal organ cultures.

Immunising virus	ELISA titres on day				Neutralisation titres on day		
	0	21	35	49	0	21	35
Vaccinia (wild type)	0	0	0	0	10	10	10
vSP19-1 (recombinant)	0	180	820	1350	10	25	40

anti-rabbit antibody and photographed under ultraviolet illumination. Cells infected with wild type vaccinia virus did not show any fluorescence whereas cells infected with the recombinant vaccinia virus showed strong surface-specific fluorescence with the rabbit anti-IBV serum indicating that the spike protein was expressed and transported to the cell surface.

Immunoprecipitation

To analyse the polypeptide composition of the recombinant expressed spike, infected cells were radiolabelled with ^{35}S-methionine then lysed and immunoprecipitated with rabbit anti-IBV Beaudette serum using methods detailed by Smith et al, 1983. A polypeptide of approximate M_r 180K was immunoprecipitated from cells infected with the recombinant virus but not from wild type or mock infected cells. This corresponds in size to the primary translation product of the IBV spike and indicates that glycosylation occurs to a similar extent to normal IBV infection.

Animal vaccinations

Six week old Balb/C mice were vaccinated with wild type or recombinant virus ($5x10^7$ pfu per mouse) and revaccinated three weeks later with the same virus (10^8 per mouse). Serum samples were collected and the titre of antibody to IBV strain Beaudette determined by ELISA (Mockett & Cook, 1986). Virus neutralising antibody was determined in a ciliostasis test in chick embryo tracheal organ cultures using the ciliostatic IBV strain M-41 as a target antigen (Darbyshire et al,

1979). The results are shown in Table 1. Three weeks after the first vaccination with recombinant the mice had low but measurable anti-IBV antibody titres. These increased considerably after the second inoculations. Cross neutralisation between IBV strains Beaudette and the antigenically related M-41 is poor. However, despite this, sera taken from mice vaccinated with the recombinant vaccinia virus contained antibodies which could neutralise M-41 IBV infectivity as shown by the ciliostasis test. These data indicate that the IBV spike expressed by a recombinant poxvirus vector could potentially be used as a live IBV vaccine.

References

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