Vet. Res. 38 (2007) 281–297
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DOI: 10.1051/vetres:2006055

Review article

Coronavirus avian infectious bronchitis virus

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(Received 18 April 2006; accepted 22 September 2006)

Abstract – Infectious bronchitis virus (IBV), the coronavirus of the chicken (*Gallus gallus*), is one of the foremost causes of economic loss within the poultry industry, affecting the performance of both meat-type and egg-laying birds. The virus replicates not only in the epithelium of upper and lower respiratory tract tissues, but also in many tissues along the alimentary tract and elsewhere e.g. kidney, oviduct and testes. It can be detected in both respiratory and faecal material. There is increasing evidence that IBV can infect species of bird other than the chicken. Interestingly breeds of chicken vary with respect to the severity of infection with IBV, which may be related to the immune response. Probably the major reason for the high profile of IBV is the existence of a very large number of serotypes. Both live and inactivated IB vaccines are used extensively, the latter requiring priming by the former. Their effectiveness is diminished by poor cross-protection. The nature of the protective immune response to IBV is poorly understood. What is known is that the surface spike protein, indeed the amino-terminal S1 half, is sufficient to induce good protective immunity. There is increasing evidence that only a few amino acid differences amongst S proteins are sufficient to have a detrimental impact on cross-protection. Experimental vector IB vaccines and genetically manipulated IBVs – with heterologous spike protein genes – have produced promising results, including in the context of in ovo vaccination.

vaccines / tropism / host range / in ovo

Table of contents

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1. INTRODUCTION

Infectious bronchitis virus (IBV) is, by definition, the coronavirus of the domestic fowl (*Gallus gallus;* more commonly known as the chicken). Although it does indeed cause respiratory disease it also replicates at many non-respiratory epithelial surface, where it may cause pathology e.g. kidney, gonads [5] (reviewed in [20, 23]). Strains of the virus vary in the extent to which they cause pathology in non-respiratory organs. Replication at enteric surfaces is considered to not normally result in clinical disease, although it does result in faecal excretion of the virus. A genetically closely related coronavirus causes enteric disease in turkeys [22]. Tissue tropism is an aspect of IBV that has been neglected at the molecular level. An interesting facet of IBV that has received relatively little attention is the variation in IB disease severity amongst different breeds of domestic fowl.

Collectively the adverse effects make IBV the biggest single cause of infectious disease-related economic loss in the United Kingdom, and probably in other countries that have a similar disease spectrum and control measures as in the UK. Its distribution is virtually global. First described in the 1930s in the USA, IB research has been dominated by the extensive genetic variation exhibited by the surface spike (S) protein gene, recognised half a century ago as antigenic variation. Specifically, virus neutralisation tests revealed the existence of serotypes (currently numbering dozens), which are poorly cross-protective [9, 17, 21, 29, 31, 34, 36, 37, 40, 49, 61, 63, 82]. Consequently live and killed vaccines of various serotypes are in use, though never based on a sufficient number of serotypes, for economic reasons, to control the disease as well as one would like [4, 6, 21, 42]. Serological studies were followed by monoclonal antibody and sequencing approaches, to take us towards understanding the molecular basis of the antigenic variation [15, 39, 50, 56, 57]. This leaves us far short of understanding the molecular basis of cross-protection – or lack of it – as cellular immune responses play a role in protection, and these are poorly understood. We do know that the S protein alone is sufficient to induce good protective immunity [12, 20, 46, 51, 53, 93, 96]. There is increasing evidence that only a few amino acid differences amongst S proteins are sufficient to have a detrimental impact on cross-protection.

Live IB vaccines are at the heart of IB control. The development of reverse genetic ('infectious clone') systems for IBV has opened up the possibility of precisely modifying the IBV genome for vaccine development as well as for defining the roles of the virus proteins in pathogenicity – two interrelated areas of research [7, 10, 11, 46, 47, 104].

Coronaviruses, genetically similar to IBV, are being increasingly detected in avian species. Evidence is increasing that IBV has a wider host range than was previously thought – and not only in galliform (chicken-like) birds e.g. the peafowl but also in non-galliform birds e.g. the teal, which is a duck [66]. Indeed, some of the long-known mammalian coronaviruses have a wider host range than their name

would suggest, and SARS-CoV is a case in point. Moreover, host range can be determined by the surface spike glycoprotein (S). For example, a genetically manipulated murine coronavirus with the S protein of feline coronavirus replicates in feline cells [44].

Recombination is undoubtedly a feature of the replication and evolution of IBV [19, 58, 61] and other coronaviruses. However, to my mind this is a property that was given too much prominence when SARS-CoV emerged in humans. The circumstantial evidence for recombination, derived from gene sequence comparisons, is probably telling us of events that occurred a very long time ago. That said, the conditions for recombination amongst IBV strains in the field are there: extremely large numbers of chickens (a global population of some 40 billion per annum), most kept at high density; ease of spread of the virus; cocirculation of serotypes [9], including proof of co-infection with more than one serotype in a given flock [18].

2. BRIEF MOLECULAR BIOLOGICAL ASPECTS OF CORONAVIRUSES

Coronaviruses are enveloped, pleiomorphic, with a mean diameter of approximately 120 nm, and have large (20 nm), club-shaped surface projections – the heavily glycosylated spike glycoprotein, S. The composition of coronavirus particles has been reviewed [23, 62, 101]. Virions form by budding at internal cell membranes, not at the cell surface.

The S protein is a dimer or trimer. It has two known functions: to attach the virus to receptor molecules on host cells, and to activate fusion of the virion membrane with host cell membranes, to release the viral genome into the cell. IBV and other, though not all, coronaviruses, have the S protein in a cleaved form, as two subunits, amino-terminal S1 and carboxy-terminal S2. For IBV these comprise a little over 500 and 600 amino acids, respectively. The bulbous head of the S protein is believed to be formed largely by the S1 subunit. The S protein is anchored in the membrane by the carboxyterminal portion of S2.

The cleavage site is usually associated with one or more pairs of basic amino acids. For example, Arg-Arg-Ser-Arg-Arg is a common S1-S2 connecting peptide of IBV [15]. Whether cleaved or not, it is the S1 part that is responsible for attachment of the virus to cells [10], whilst S2 is responsible for membrane fusion. The location of the receptor binding domain (RBD) within S1 is not known for IBV, and varies amongst other coronaviruses (reviewed by [23, 101]).

Apart from the S protein, all coronaviruses have a large copy number of a smaller, integral membrane glycoprotein (M; approximately 230 amino acids) and low amounts of a very small, membrane-associated, non-glycoslyated protein, E (approximately 100 amino acids). Both of these proteins are required for virus particle formation. The S protein interacts with the transmembrane region of M. Closely associated with the RNA genome (to form a ribonucleoprotein, RNP) is the nucleocapsid protein (N; approximately 420 amino acids).

The coronavirus genome is a single-stranded, positive-sense RNA of 27 000 to 32 000 nucleotides (27.6 kb in the case of IBV). They all have the same general genome organisation [23, 62, 101].

5'UTR - polymerase gene – structural protein genes (S-E-M-N) – UTR 3' where the UTR are untranslated regions (each ~ 500 nucleotides in IBV).

Gene 1 encodes 15 (IBV) or 16 non-structural proteins (nsp), which are associated with RNA replication and transcription. In addition to these ns proteins there are, interspersed amongst the structural protein genes, one or more genes that

encode small non-structural proteins. IBV has two of these genes, called genes 3 and 5. Gene 3 has three open reading frames (ORFs), encoding proteins 3a, 3b and 3c, where 3c is the E protein. Gene 5 encodes two proteins, 5a and 5b. These genes are located thus:

-S-3a,b,c(E)-M-5a,b-N-

Coronaviruses have been assigned to three groups, IBV being in Group 3. The groups were initially devised on the basis of a lack of antigenic relationships between the species of different groups (reviewed by [20, 41]). Sequencing has largely confirmed these groupings [41]. The location of the non-gene 1 ns protein genes has also been used as a property said to be group-specific. However, as more coronaviruses are discovered and analysed, this criterion gets decreasingly valid. For example, until SARS-CoV was discovered, IBV and closely related viruses were unique in having an ns protein gene between the M and N genes. Indeed, SARS-CoV has more ns protein genes interspersed amongst the structural protein genes than any other coronavirus (reviewed by [100]). Currently Group 3 members are exclusively from avian species (Tab. I).

Coronaviruses undergo recombination; if a cell is infected by two strains of a given species of coronavirus, then progeny with sequence(s) derived from both parents may result. This has been demonstrated experimentally for IBV [58] whilst sequencing of many field strains has provided convincing evidence that many, possibly all, IBV strains are recombinants between different IBV strains [16, 24, 52, 98, 99].

3. PATHOGENESIS OF IBV

IBV initially infects the upper respiratory tract, where it is restricted to the ciliated and mucus-secreting cells (reviewed by [35]). Titres of live virus are maximal in the nose and trachea within three days and remain so for two to five days further [1, 48]; reviewed by [20]. Similar virus titres occur in the lungs and airsacs. Small areas of pneumonia may be observed in the lungs, although IBV is not considered to cause pneumonia [35]. Deciliation of the ciliated epithelia of the nose and trachea follows infection. Infection is commonly followed by secondary bacterial infections, which can be the main cause of the most debilitating disease, including mortality [97].

In addition to replicating in many respiratory tissues (including nose, trachea, lungs and airsacs, causing respiratory disease), IBV grows at many other epithelial surfaces, including in kidney (associated with minor or major nephritis), oviduct, testes, and many parts of the alimentary tract – oesophagus, proventriculus, duodenum, jejunum, bursa of Fabricius, caecal tonsils (near the distal end of the tract), rectum and cloaca (the common opening for release of eggs and faeces) (reviewed by [20, 35]) (Fig. 1). Infection of enteric tissues usually does not manifest itself clinically. Nephritis is not uncommon in a proportion of naturally IBV-infected meat-type birds. Some IBV strains are intrinsically nephropathogenic i.e. they reproducibly cause nephritis when inoculated experimentally into specific pathogen free chickens, causing mortality [30, 63, 64, 81]. IBV infects mainly the lower nephron down to the collecting duct epithelial cells [25, 26]. An ultrastructural investigation revealed that the virus replicated in all segments of tubules and ducts, but more frequently in the epithelial cells of the collecting ducts, collecting tubules, distal convoluted tubules and Henle's loops [25]. Modest to high titres of IBV in the kidney do not necessarily correlate with overt kidney disease. For example, the Moroccan G strain replicated to similar titres in kidney as in trachea (Fig. 1), though no gross kidney changes were observed [1].

Table I. Species from which Group 3 coronaviruses have been isolated[a] or detected[b] (by RT-PCR).

Host species[f]		Virus name
Domestic fowl (chicken)[c]	*Gallus gallus*	Infectious bronchitis virus[a]
Turkey[c]	*Meleagris gallopavo*	Turkey coronavirus[a]
Pheasant[c]	*Phasianus colchicus*	Pheasant coronavirus[a]
Guinea fowl[c]	*Numida meleagris*	[a,d]
Peafowl[c]	*Pavo cristatus*	[a,d]
Partridge[c]	*Alectoris* sp.	[a,d]
Blue-winged teal (a duck)	*Anas* sp.	[a,d]
Pigeon	*Columbia livia*	[b,e] Pigeon coronavirus
Mallard duck	*Anas platyrhynchos*	[b,e] Duck coronavirus
Greylag goose	*Anser anser*	[b,e] Goose coronavirus

[a] These viruses have been isolated and propagated in embryonated domestic fowl eggs following inoculation into the allantoic cavity.

[b] Isolates were not recovered following inoculation into the allantoic cavity of embryonated domestic fowl eggs [54].

[c] Members of the order Galliformes (chicken-like).

[d] The genome organisation, gene sequences and biological properties of these isolates were such that it is possible that they were actually IBVs; domestic fowl were kept nearby. The peafowl isolate of Liu et al. [66] had > 99% genome sequence identity with the IB vaccine strain H120. The teal duck isolate caused disease when experimentally inoculated into chickens [66].

[e] These viruses had nucleocapsid (N) gene and 3' UTR sequences similar to those of the other coronaviruses in Table I, indicating that they were Group 3 coronaviruses. They also had one or two additional ORFs between the N gene and the 3' UTR [54].

[f] A coronavirus has been isolated from a parrot. On the basis of very limited sequence data, it is not clear in which, if any, of the three coronavirus groups that this virus would be placed [43].

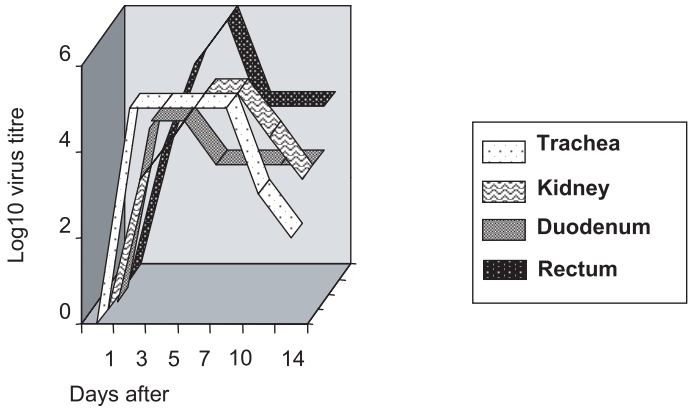

Figure 1. Replication of the Moroccan G strain of IBV in chickens following inoculation by eye-drop and in the nose. Infectious virus was titrated in chick embryo tracheal organ cultures. Titres are shown to the nearest whole log10 number. From [1], reviewed in [20].

Infection of the oviduct is believed to contribute to diminished egg production. The virus can also replicate in the testes [5]. Birds have a small lymphoid organ, the Harderian gland, in the eye-socket which is a major contributor to locally produced antibody for protecting oculonasal mucosae [35]. IBV replicates in this organ, and also in another lymphoid organ, the bursa of Fabricius [1, 87].

Interestingly, IBV can establish persistent infections in chickens. In experiments involving the inoculation of chicks, virus was no longer detectibly excreted after the initial period of replication. However, excretion re-started at around the time that egg production started (~ 19 weeks of age) [55]. It is suspected that the stressor of the start of egg production caused the release of the virus.

4. DETERMINANTS OF PATHOGENICITY

Virtually nothing is known about the determinants of pathogenicity for IBV.

4.1. Coronavirus spike protein as a determinant of pathogenicity

It has been shown for some coronaviruses that the S protein is a determinant of tissue tropism [60]. Rottier and colleagues exchanged the S protein gene of murine hepatitis virus (MHV) with that of feline coronavirus (FCoV). Unlike the wild-type MHV, the 'felinized' MHV was able to replicate in feline cells, presumably by virtue of recognising receptor molecules on the surface of the feline cells [44]. Differences in the S protein, sometimes as few as one or two amino acids, of porcine transmissible gastroenteritis (TGEV) determine whether the virus is enteropathogenic or essentially non-pathogenic in pigs [3, 59]. Genetically manipulated MHVs, with different tropisms with regard to disease in the liver and nerve tissue, have tropisms reflective of the strain from the S gene was derived [75, 76]. We have shown that the S protein is an important factor with regard to the host cell range of IBV, at least in vitro. Replacement of the S protein gene of the Beaudette strain (able to replicate in chick embryo fibroblast, CEF, cells, and in mammalian Vero and BHK cells) with that from the M41 strain (poor growth in CEFs and no productive replication in Vero and BHK cells) resulted in virus with the inferior host cell range of the S protein donor, M41 [10]. Whether the S protein is a determinant of IBV pathogenicity is an open question. The spike-swapped recombinant just referred to was non-pathogenic in chickens. Thus having the S protein of a pathogenic strain is not sufficient for pathogenicity to be expressed.

Studies with TGEV have led Enjuanes and colleagues [59, 85] to suggest that two domains on the S protein might be involved in attaching to enteric cells, one for binding to porcine aminopeptidase N. This receptor is present in lung tissue as well as in enteric tissue; binding to this does not account for the different tropisms. The other domain might be involved in the binding to a coreceptor, not defined, essential for the enteric tropism; differences in this domain affected the tropism of TGEV. A further complication is involved. Binding of TGEV to neuraminic acid increases the efficiency of binding to cultured cells, though it is not an absolute requirement [85]. That said mutants that were unable to bind to neuraminic acid were no longer enteropathogenic [59, 86].

The ability of IBV to replicate at many respiratory, enteric and other epithelial surfaces may be related in part to the fact that attachment of IBV to host cells is dependent on N-acetylneuraminic acid (sialic acid) at the cell surface. Studies of haemagglutination by IBV had demonstrated the role of neuraminic acid in

the attachment process. Moreover, IBV attached preferentially when the linkage of the neuraminic acid to the body of the oligosaccharide was α2,3 [84]. More recent studies have confirmed that α2,3-linked N-acetylneuraminic acid is also preferentially used by IBV for attachment to host cells in which the virus replicates [102]. Firstly, the number of cells infected was greatly reduced by prior treatment with neuraminidase from Vibrio cholerae. Secondly, it was demonstrated that the cell types that were susceptible to IBV strain Beaudette, including Vero cells, had α2,3-linked N-acetylneuraminic acid. In contrast, the Vero E6 subline was not susceptible to IBV – and did not express α2,3-linked N-acetylneuraminic acid. Whilst the affinity for α2,3-linked N-acetylneuraminic acid helps to explain the pan-tropic nature of IBV within the chicken, it cannot be the only determinant of susceptibility; such neuraminic acid is present on cells that are not infected by IBV. It may be that binding to neuraminic acid is a primary step, efficient infection requiring further contacts to be made, involving a more specific, less generally distributed, secondary receptor. Moreover, there may be more than one secondary receptor. This notion might explain why although the Beaudette and M41 strains, and a chimaera of Beaudette with the spike gene of M41, replicate equally well in chick kidney cells in vitro, only the Beaudette strain replicates in Vero and BHK cells [10]; the Beaudette S protein, but not that of M41, probably recognizes a receptor on Vero and BHK cells.

4.2. Role of other coronavirus proteins in pathogenicity

The pathogenic nature of coronaviruses is not determined solely by the S protein; other 'background' genes can play a role [76]; reviewed by [101]. For example, the A59 and JHM strains are hepatotropic and non-hepatotropic, respectively. When the S gene of JHM was replaced with that of A59, the recombinant virus caused minimal infection of the liver and induced hepatitis very poorly [76].

As mentioned above, coronaviruses have one or more genes, interspersed amongst the structural protein genes, that encode relatively small proteins. With the exception of one of these proteins of SARS coronavirus, these small genes encode non-structural proteins whose function is unknown. What we do know is that they are not required for replication per se. For example, genetically manipulated IBVs that were unable to produce proteins 3a and 3b [47] or 5a and 5b [11] reached normal titres in vitro and in ovo. They did likewise in tracheal organ cultures (ex vivo), although mutants unable to produce some of these non-structural proteins declined in titre earlier than wild-type virus. It is conceivable that the organ cultures mounted more effective innate immune responses than in cell culture and in ovo. If that is the case, then it would suggest that the function of one or more of the small non-structural proteins would be to combat innate immunity. This is pure conjecture at the moment. Others have suggested the same in respect of non-structural protein genes of other coronaviruses, though there is no evidence yet to support this hypothesis. Shen et al. [89] and Youn et al. [104] have also demonstrated that proteins 3b and 5a, respectively, are not required for replication in vitro. Deletion of all the non-gene 1 non-structural protein genes of MHV produced virus that replicated in mice but which, unlike the wild-type virus, was non-lethal [33].

Inactivation (whether by deletion or other modification) of individual non-structural protein genes does not necessarily result in reduced pathogenicity. FCoV unable to make the ORF 7b protein was still lethal for cats [45]. Removal of

gene 3 of TGEV did not diminish its enteropathogenicity [92].

The non-gene 1-encoded accessory proteins are probably not the only nsp that have an influence on pathogenicity. Thus single amino acid substitutions in nsp14 encoded by gene 1 of MHV attenuated pathogenicity for mice [94].

5. VARIATION IN HOST SUSCEPTIBILITY TO IBV

Experiments have shown that the outcome of infection with IBV depends on the breed of chicken. Although IBV replicates to similar levels in the trachea in all the breeds [78], supported by experiments involving tracheal explants, the extent of mortality varies greatly. This has been studied using IBV alone, and also in conjunction with *Escherichia coli* [28, 91]. The virus predisposed the birds to infection with the bacterium, resulting in increased mortality. Bumstead et al. [8] continued with this IBV/*E. coli* infection, showing marked variation in mortality amongst several inbred lines of White Leghorn chickens. Otsuki et al. [79] studied two lines in more detail, finding that virus titres declined much more slowly in the susceptible line. It is suspected that the underlying cause of the difference is immunological. There is evidence for the MHC haplotype of chickens influencing genetic resistance to IBV [2].

The extent to which infection by a nephropathogenic strain caused mortality was also dependent on the breed of chicken [81].

6. NATURE OF IMMUNE RESPONSES TO IBV

It is the humoral immune response to IBV vaccination that has been studied the most, by measurement of antibody levels in serum, using ELISA, VN or haemagglutination-inhibition (HAI) tests (reviewed by [20, 34]), and also in lachrymal secretions (tears; [38]). However, there have also been a few studies of IBV antibodies in nose and trachea. The basis of immunity to IBV is not well understood. Serum antibody levels do not correlate with protection, though local antibody is believed to play a role in protection of the respiratory tract [51, 83].

The profile of the serum antibody response depends on the method used to detect it. Following infection of chickens with a virulent strain of IBV, specific antibody was first detected by ELISA (plates coated with IBV), and later by VN and HI tests [28, 70–72].

Following infection with a live IBV vaccinal strain there was a good primary IgM response [68, 73]. As expected, the primary IgM response peaked, and declined, before that of the IgG response [68]. The secondary IgM response i.e. in response to a second (challenge) infection, peaked at the same time as that of IgG, but declined faster.

Collisson and colleagues [80, 87] have shown that cytotoxic T-cell (CTL) responses in chickens to IBV infection correlated with initial decreases in infection and clinical signs. CTL activity was major histocompatibility complex restricted, and lysis was mediated by CD8+ CD4- cells. Adoptive transfer of IBV-infection-induced alphabeta T cells bearing CD8 antigen protected chicks from challenge infection [88]; reviewed in [27]. Earlier work has been reviewed in [35].

Following infection of chickens by IBV, interferon was detected in trachea and lung, and at lower levels in plasma, kidney, liver and spleen [78]. Chicken interferon type I reduced replication of IBV in chick kidney cell cultures, and in tracheal organ cultures [80]. Moreover, intravenous or oral application of type I interferon

delayed the onset of disease in chickens and its severity.

Very much more remains to be done with regard to elucidating the innate and adaptive immune responses to IBV [35].

7. EPIDEMIOLOGY AND CLINICAL SIGNS

IB is virtually a global disease [24, 39, 40, 65, 67, 89, 90]. The virus is highly infectious, presumed to spread by aerosol as well as by mechanical means. Several serotypes can co-circulate in a region [9]. As serotypes cross-protect poorly, chickens can be productively infected several times including more than once within the short, six-week life of a meat-type chicken [18]. Chickens, especially young ones, exhibit nasal discharge, snicking (similar to sneezing), râles (a vibration emanating from lower in the respiratory tract watery), watery eyes and lethargy [21, 35]. Young chicks may die directly from IBV infection but a greater number die following secondary bacterial infection [21]. Juvenile and mature birds suffer less from IBV infection although the economic consequences can be high. Infection of meat-type birds results in growth retardation. In layers, there is a drop in egg production, which might never return to normal, and in quality of eggs.

8. ANTIGENIC DIVERSITY

IBV exists as scores, maybe hundreds, of serotypes. Most differ from each other by 20 to 25% of S1 amino acids [19, 24, 29, 31, 37, 40, 50, 65, 70]. The S2 polypeptides differ by less than half that amount, to an extent similar to that found in the other structural proteins (10 to 15%; (reviewed by [23])). However, some serotypes differ by approximately 50% of S1 amino acids [17, 39, 60]. The differences between the S1 proteins undoubtedly have a selective advantage; generally speaking, the immunity induced by inoculation with one serotype protects poorly against infection with heterologous serotypes (reviewed by Cavanagh [20]), as it is the S protein that is the major inducer of virus neutralizing (VN) antibody and protection [13, 14, 51, 56, 57, 96].

Strikingly, differences of as few as 2 to 3% of S1 amino acid residues (10 to 15 residues) can result in a change in serotype, defined as lack of cross-neutralization using convalescent sera [15, 56, 57]. These few differences may contribute to diminished cross-protection in challenge experiments in chickens [17]. Monoclonal antibody analysis has revealed that many of the amino acids involved in the formation of VN epitopes are located within the first and third quarters of the linear S1 polypeptide [34, 56, 57], which is where closely related strains (> 95% amino acid identity in S1) also differ [4, 37]. Thus these parts of S1 are very tolerant of amino acid changes, changes that probably confer a selective advantage.

9. PREVENTION

The extent to which infection is an economic problem will depend on many factors, including the strain of virus, age of chicken at infection, nutrition, and the environment both within the poultry house e.g. ammonia levels, and outside e.g. temperature [21, 28, 63, 65, 81, 97]. In areas where there are many poultry farms, it is virtually impossible to keep chickens free of IBV. Biosecurity is likely to be insufficient, as the virus is spread readily. Consequently vaccination is commonly practised. Whilst the humoral response to IB vaccination has been measured for many years, very little is known about the cellular immunity induced by IB vaccines or field strains.

10. CONTROL BY VACCINATION

10.1. Live vaccines

Vaccination to control IB has been practiced for over a half a century [4, 20, 21]. Field strains are universally attenuated by passage in embryonated domestic fowl eggs.

Live vaccines are usually applied to meat-type chickens at one day of age, in the hatchery. In experimental situations this can result in sterile immunity when challenged by virulent homologous virus within three weeks of vaccination. Sometimes, even in closely controlled experimental situations, 10% of vaccinated chicks do not respond with a protective immune response against challenge with the homologous strain [17, 29, 49, 77, 82, 103]. These results show that chickens (out-bred, though with restricted sets of parental breeding stocks) are not uniform in their response to IBV vaccination. This is a factor to be kept in mind not only with respect to vaccination of domestic animals but also if vaccines against coronaviruses in humans, e.g. SARS-CoV, are to be contemplated.

In this context there is another aspect of IB vaccination to be kept in mind; protection is short-lived, the start of the decline being apparent nine weeks after vaccination [32, 42]. Consequently commercial egg layers, which are kept for a year or more, are vaccinated several times with live vaccine, perhaps with more than one serotype. Even broilers, which are processed at only six or so weeks of age, may be revaccinated if IB is very problematic in an area. Revaccination may be with a different serotype, as this approach sometimes gives protection against a broader range of serotypes [29].

The efficacy of vaccination with live vaccine varies amongst inbred lines of chickens i.e. genetic differences between individuals affects the efficacy of the immune response [28, 79, 81, 91].

Vaccines have not been developed commercially with nephropathogenic IBV strains in mind. However, this has been studied experimentally. Vaccination, by coarse spray, with the homologous attenuated strain completely protected against mortality upon challenge four weeks later with the wild-type nephropathogenic virus [80]. Challenge virus in the kidney was assessed by immunofluorescence. By this criterion the number of chicks with detectable IBV in the kidney was reduced by 84% by vaccination with the homologous vaccine, and not at all by the heterologous vaccines.

10.2. Inactivated virus and subunit vaccines

Inactivated oil-emulsion IBV vaccines were developed during the 1960s and 1970s. The objective was to make a vaccine that would give long-lasting immunity to the hen bird, to protect against drops in egg production. Single applications of inactivated virus induced little or no protection against egg loss [6, 69, 74], and no protection against loss of ciliary activity in the trachea [68].

Other studies, using purified, inactivated IBV, have been successful in getting a degree of protection against respiratory tract protection, though usually at a rate of $< 59\%$ [12, 51, 93]; reviewed in more detail by [20]. Rather better protection with inactivated IBV has been achieved against losses in egg production [69]. Notwithstanding, common practice is to vaccinate egg layers with live vaccine at two or three weeks of age, followed by more live vaccinations until shortly before the birds start laying eggs they are given killed vaccine [21].

Induction of immunity by the S1 spike subunit has been studied with S1 prepared from purified virus [12, 51] and expression using baculovirus [93]. Although protective immune responses were induced,

multiple inoculations of subviral material were required and the percentage of protected chickens was too low (< 50%) for commercial application.

10.3. Vector vaccines

S1 has also been expressed in birds using fowl pox virus [99] and fowl adenovirus vectors [53]. Remarkably, expression of S1 in birds using a nonpathogenic fowl adenovirus vector induced protection in 90% and 100% of chickens in two experiments. This was the case after only a single application of the vector. Success may have been related to the fact that the vector replicated well in the respiratory tract of the birds.

10.4. Small differences amongst S1 proteins may reduce cross-protection

As might be expected, cross-protection tends to diminish as the degree of amino acid identity between the S1 proteins of two IBV strains decreases [17, 40]. Some recent findings have relevance not only to the control of IB but also for control of coronavirus diseases in other domestic animals and humans. It has been mentioned above that 10% of chickens, such as outbred Rhode Island Red, may not develop a protective immune response after careful vaccination [17]. Another finding is that small differences between S proteins of vaccinating and challenge virus may reduce the efficacy of the former. We have known for some time that differences in S1of 2 to 3% (10 to 15 amino acids) can change serotype, suggesting that a small number of epitopes are immunodominant with respect to neutralising antibody. The experiments of Cavanagh et al. [17] suggested that such differences could adversely affect cross-protection, though the results were not statistically significant.

As described above, we have made a chimaera comprising the genome of the non-pathogenic Beaudette strain of IBV except that it had the S gene of the pathogenic M41 strain [10]. Both strains are of the Massachusetts serotype, with 95.0% S1 amino acid identity. When chickens were inoculated with Beaudette, M41 or spike-swapped recombinant IBV and challenged with M41, Beaudette induced almost no tracheal protection when assessed by tracheal ciliary activity and poor protection when assessed by snicking. In contrast, the recombinant IBV induced protection almost as well as the M41 strain. This was not due to an increased virulence of the receiver strain, as the spike-swapped recombinant was as non-pathogenic as the Beaudette strain, and both replicated poorly. As Beaudette and recombinant virus had identical proteins except for the spike protein, it would appear that the poor immunity induced by Beaudette against the donor strain was due to some of the 5.0% of amino acid differences (27 different residues) in S1. This supported the suggestions of earlier work that small differences in S1 can contribute to poor cross-protection [17, 77].

These results are interesting in the context of identifying the protection-inducing epitopes of the S protein and of explaining poor vaccine performance in the field. They indicate that a vaccine might perform suboptimally even against challenge with virus of the same serotype, if critical amino acids differ. This is something that should be kept in mind if development of vaccines against other coronaviruses is being contemplated. It would be as well to acquire knowledge of the diversity of the S protein as rapidly as possible.

10.5. Conclusions: prospects for IB vaccine development

The existence of infectious clone/reverse genetic systems for IBV and other

coronaviruses has the potential for the development of a new generation of live vaccines. As described above, these have already been used to identify genes that are not required for replication per se and which are believed to aid virus survival in vivo; these genes are targets for elimination or modification – for rational, as opposed to the current empirical, attenuation of pathogenicity.

An objective specific to the poultry industry is the production of vaccines that can be administered in ovo i.e. to embryos of 18 days of age, three days prior to hatch. The technology exists to apply vaccines very precisely in this way, more efficiently than vaccinating hatched birds by spray or drinking water. To date this objective has been achieved only in respect of Marek's disease virus. Vaccine strains of other viruses, including IBV, adversely affect hatchability. Our molecularly cloned IBV is remarkably benign when given to 18-day-old embryos; hatchability is unaffected. Furthermore, preliminary experiments have shown that our spike-swapped chimaeras induce protective immunity following in ovo vaccination [95].

The existence of scores of IBV serotypes will remain, meaning that the occasional production of new vaccines with appropriate S protein genes is likely to be continued for a long time. Since our reverse genetic system enables us to swap S protein genes [7, 10, 47], the prospects for a new era of IB vaccines are good.

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