REVIEW ARTICLE

Severe acute respiratory syndrome vaccine development: experiences of vaccination against avian infectious bronchitis coronavirus

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Vaccines against infectious bronchitis of chickens (Gallus gallus domesticus) have arguably been the most successful, and certainly the most widely used, of vaccines for diseases caused by coronaviruses, the others being against bovine, canine, feline and porcine coronaviruses. Infectious bronchitis virus (IBV), together with the genetically related coronaviruses of turkey (Meleagris gallopavo) and ring-necked pheasant (Phasianus colchicus), is a group 3 coronavirus, Severe acute respiratory syndrome (SARS) coronavirus being tentatively in group 4, the other known mammalian coronaviruses being in groups 1 and 2. IBV replicates not only in respiratory tissues (including the nose, trachea, lungs and airsacs, causing respiratory disease), but also in the kidney (associated with minor or major nephritis), oviduct, and in many parts of the alimentary tract—the oesophagus, proventriculus, duodenum, jejunum, bursa of Fabricius, caecal tonsils, rectum and cloaca, usually without clinical effects. The virus can persist, being re-excreted at the onset of egg laying (4 to 5 months of age), believed to be a consequence of the stress of coming into lay. Genetic lines of chickens differ in the extent to which IBV causes mortality in chicks, and in respect of clearance of the virus after the acute phase. Live attenuated (by passage in chicken embryonated eggs) IBV strains were introduced as vaccines in the 1950s, followed a couple of decades later by inactivated vaccines for boosting protection in egg-laying birds. Live vaccines are usually applied to meat-type chickens at 1 day of age. In experimental situations this can result in sterile immunity when challenged by virulent homologous virus. Although 100% of chickens may be protected (against clinical signs and loss of ciliary activity in trachea), sometimes 10% of vaccinated chicks may not respond with a protective immune response. Protection is short lived, the start of the decline being apparent 9 weeks after vaccination with vaccines based on highly attenuated strains. IBV exists as scores of serotypes (defined by the neutralization test), cross-protection often being poor. Consequently, chickens may be re-vaccinated, with the same or another serotype, two or three weeks later. Single applications of inactivated virus has generally led to protection of <50% of chickens. Two applications have led to 90 to 100% protection in some reports, but remaining below 50% in others. In practice in the field, inactivated vaccines are used in laying birds that have previously been primed with two or three live attenuated virus vaccinations. This increases protection of the laying birds against egg production losses and induces a sustained level of serum antibody, which is passed to progeny. The large spike glycoprotein (S) comprises a carboxy-terminal S2 subunit (approximately 625 amino acid residues), which anchors S in the virus envelope, and an amino-terminal S1 subunit (approximately 520 residues), believed to largely form the distal bulbous part of S. The S1 subunit (purified from IBV virus, expressed using baculovirus or expressed in birds from a fowlpoxvirus vector) induced virus neutralizing antibody. Although protective immune responses were induced, multiple inoculations were required and the percentage of protected chickens was too low (<50%) for commercial application. Remarkably, expression of S1 in birds using a non-pathogenic fowl adenovirus vector induced protection in 90% and 100% of chickens in two experiments. Differences of as little as 5% between the S1 sequences can result in poor cross-protection. Differences in S1 of 2 to 3% (10 to 15 amino acids) can change serotype, suggesting that a small number of epitopes are immunodominant with respect to neutralizing antibody. Initial studies of the role of the IBV nucleocapsid protein (N) in immunity suggested that immunization with
bacterially expressed N, while not inducing protection directly, improved the induction of protection by a subsequent inoculation with inactivated IBV. In another study, two intramuscular immunizations of a plasmid expressing N induced protective immunity. The basis of immunity to IBV is not well understood. Serum antibody levels do not correlate with protection, although local antibody is believed to play a role. Adoptive transfer of IBV-infection-induced αβ T cells bearing CD8 antigen protected chicks from challenge infection. In conclusion, live attenuated IBV vaccines induce good, although short-lived, protection against homologous challenge, although a minority of individuals may respond poorly. Inactivated IBV vaccines are insufficiently efficacious when applied only once and in the absence of priming by live vaccine. Two applications of inactivated IBV are much more efficacious, although this is not a commercially viable proposition in the poultry industry. However, the cost and logistics of multiple application of a SARS inactivated vaccine would be more acceptable for the protection of human populations, especially if limited to targeted groups (e.g. health care workers and high-risk contacts). Application of a SARS vaccine is perhaps best limited to a minimal number of targeted individuals who can be monitored, as some vaccinated persons might, if infected by SARS coronavirus, become asymptomatic excretors of virus, thereby posing a risk to non-vaccinated people. Looking further into the future, the high efficacy of the fowl adenovirus vector expressing the IBV S1 subunit provides optimism for a live SARS vaccine, if that were deemed to be necessary, with the possibility of including the N protein gene.

**Introduction**

Severe acute respiratory syndrome (SARS) emerged in humans in Guangdong Province, China, in late 2002 and subsequently spread to other continents early the following year (Chan-Yeung & Yu, 2003; Lee et al., 2003; Peiris et al., 2003; Poutanen et al., 2003; Tsang et al., 2003). Shortly after the spread of the virus beyond China the causative agent was quickly identified as being a species of coronavirus (SARS coronavirus) that was previously unknown (Drosten et al., 2003; Ksiazek et al., 2003; Marra et al., 2003; Peiris et al., 2003; Rota et al., 2003). Indeed, the proteins of SARS coronavirus had such low amino acid identity with those of known coronaviruses that it has been tentatively assigned to a new coronavirus group 4. The three existing coronavirus groups (Table 1) were initially devised on the basis of a lack of antigenic relationships between the species of different groups (Pedersen et al., 1978; Sánchez et al., 1990; Enjuanes et al., 2000; González et al., 2003). It is probable that the vast majority of the human population is susceptible to SARS coronavirus. The SARS epidemic was contained at only 8460 cases by the enormous effort, and sacrifice, of a large number of individuals associated with many public authorities. So severe were the infections, in a fully susceptible population, that 808 died; doubtless a greater proportion would have died had it not been for the medical attention focussed upon them. Sadly, a large proportion of the deaths in some countries were sustained by health care workers. In addition to the human misery, there were dire economic consequences for those countries most affected.

The last SARS cases of the early 2003 epidemic were in June 2003. The disease might arise again by further transmission from the suspected animal reservoir, or transmission from human, possibly asymptomatic, carriers. There is evidence from studies of avian infectious bronchitis virus (IBV) and feline coronavirus that coronaviruses establish persistent infections. Chicks that had been experimentally infected with IBV at 1 day of age excreted virus at around 19 weeks of age (Jones & Ambali, 1987). It is suspected that the stressor of the start of egg production caused the release of the virus. Approximately 10% of cats that had been naturally infected with feline coronavirus became asymptomatic carriers, excreting virus for over 1 year (Addie & Jarrett, 2001; Addie et al., 2003). Others excreted virus for periods of several months.

The World Health Organisation has called for the development of vaccines against SARS. This short review looks at the experiences with IBV vaccines over half a century, which might be instructive with regard to the development of SARS vaccines.

| Group | Coronavirus species |
|-------|---------------------|
| Group 1 | Porcine transmissible gastroenteritis coronavirus |
|        | Canine enteric coronavirus |
|        | Feline coronavirus |
|        | Porcine epidemic diarrhoea coronavirus |
|        | Human coronavirus 229E |
| Group 2 | Murine hepatitis coronavirus |
|        | Human coronavirus OC43 |
|        | Bovine coronavirus |
|        | Canine respiratory coronavirus |
|        | Porcine haemagglutinating encephalomyelitis coronavirus |
| Group 3 | Infectious bronchitis coronavirus |
|        | Turkey coronavirus |
|        | Pheasant coronavirus |
| Group 4 | SARS coronavirus* |

*SARS coronavirus* has provisionally been placed in group 4, based on the criteria previously used to place the other coronaviruses into groups (i.e. extremely low amino acid identity between its proteins and those of the other three groups, and nature and organization of its non-structural protein genes).
Infectious bronchitis

As suggested by its name, IBV causes respiratory disease, although its replication is not limited to the respiratory tract. It is the major respiratory virus of the chicken (the domestic fowl), as it is endemic in probably all countries that raise chickens. Its host range is considered to be limited to the chicken (Cavanagh & Naqi, 2003), although genetically very similar coronaviruses cause disease in turkeys (enteric disease; Guy, 2000; Cavanagh et al., 2001) and pheasants (respiratory and kidney disease; Lister et al., 1985; Gough et al., 1996; Cavanagh et al., 2002). IBV occurs globally, both in chickens kept on a large and a small scale (Wunderwald & Hoop, 2002). It exists as scores of serotypes/genotypes (Cavanagh, 2001; Meulemans et al., 2001; Farsang et al., 2002), which are problematic with regard to prophylaxis.

IBV causes deciliation of the ciliated epithelia of the nose and trachea, clinical signs being similar to those of the common cold caused in humans by the group 1 and group 2 human coronaviruses (HCoV-229E and HCoV-OC43, respectively; Cavanagh, 2000, 2004). Chickens, especially those of only a few days or weeks of age, exhibit nasal discharge, sneezing (similar to sneezing), watery eyes and lethargy (Dhinakar Raj & Jones, 1997; Cavanagh & Naqi, 2003). Some chickens exhibit râles, a vibration emanating from lower in the respiratory tract, and small areas of pneumonia may be observed in the lungs (Dhinakar Raj & Jones, 1997). Notwithstanding this, and the fact that titres of virus in the lungs can be similar to those in the nose and trachea, bronchitis is not considered to cause pneumonia.

From a welfare point of view the hardest hit are chicks of only a few days or weeks of age. Some may die directly from the viral infection but a greater number die following secondary bacterial infection. An overall slowing down of growth causes further economic losses. Juvenile and mature birds suffer less from IBV infection although the economic consequences of infection in egg-laying stock can be disastrous, as egg production drops precipitously and usually does not rise back to normal in the flock as a whole.

As discussed further later, IBV grows at many epithelial surfaces beyond the respiratory tract (reviewed by Dhinakar Raj & Jones, 1997). Although many alimentary tract tissues are susceptible to IBV, infection of enteric tissues usually does not manifest itself clinically. Recently a strain of IBV has been associated with disease of the proventriculitis (the cranial glandular compartment of the stomach, adjacent to the gizzard), which is the caudal muscular compartment (Yu et al., 2001a). Nephritis is not uncommon in a proportion of naturally IBV-infected meat-type birds, environmental factors playing a role. Some IBV strains are intrinsically nephropathogenic; that is, they repro-ducingly cause nephritis when inoculated experimentally into specific pathogen free chickens (Lambrechts et al., 1993; Pensaert & Lambrechts, 1994; Cook et al., 2001; Li & Yang, 2001). The oviduct is also susceptible to IBV, which may contribute to diminished egg production.

Infectious bronchitis virus

IBV contains the same number of structural proteins (i.e. those present in virions) as the group 1 coronaviruses; that is, a nucleocapsid protein associated with the 27.6 kb single-stranded, positive-sense RNA genome, a large spike glycoprotein (S), a smaller integral membrane glycoprotein (M) and a few molecules of the envelope protein (Lai & Cavanagh, 1997; Enjuanes et al., 2000; González et al., 2003). It does not contain a haemagglutinin esterase glycoprotein such as is found in group 2 coronaviruses. The coronavirus S protein is a dimer or trimer (Cavanagh, 1983; Gallagher & Buchmeier, 2001; Lewicki & Gallagher, 2002). The S glycopolypeptide of IBV, like that of group 2 coronaviruses, is cleaved into amino-terminal S1 (approximately 520 amino acid residues) and carboxy-terminal S2 (approximately 625 residues) glycopolypeptides (Lai & Cavanagh, 1997). The IBV S protein has been demonstrated to be a determinant of the host cell range in vitro. When the S protein gene of an infectious cDNA clone of the Beaudette strain (Casais et al., 2001), which replicates in mammalian Vero and BHK cells, was replaced by that of the M41 strain, which does not replicate in those cell lines, the recombinant virus was unable to replicate in them (Casais et al., 2003). The recombinant did replicate in primary chick kidney cells, as did both the Beaudette and M41 strains. Whether the S protein of IBV is a determinant of tissue tropism in vivo remains to be determined.

Tissue tropism of IBV

IBV initially infects the upper respiratory tract. Titres of live virus are maximal in the nose and trachea by 3 days post inoculation (by eye-drop and intranasally) and remain so for 2 to 5 days further, depending on the strain (Table 2) (Ambali & Jones, 1990; Hofstad & Yoder, 1966). Thereafter the titre falls to below detectable levels, although virus was still detectable at 14 days in some studies (Ambali & Jones, 1990). The virus is restricted to the ciliated and mucus-secreting cells (reviewed by Dhinakar Raj & Jones, 1997). Similar virus titres occur in the lungs and airsacs, sometimes peaking a little after those in the nose and trachea. Small areas of pneumonia may be observed in the lungs (Dhinakar Raj & Jones, 1997).

Lucio & Fabricant (1990) investigated the growth of several isolates of IBV in chickens.
Although they did not quantify the virus that they recovered, they showed that infectious virus was recovered from the trachea, bronchus, lung, oesophagus, proventriculus, duodenum, jejunum, caeca (caecal tonsils), kidney and cloaca. The longest periods of isolation were in respect of the kidney and caecal tonsils, the latter being at the distal end of the alimentary tract in birds.

Ambali & Jones (1990) used another strain (strain G) of IBV that produced high titres in the kidney, similar to those in the trachea (Table 1). Various alimentary tract tissues (proventriculus, duodenum, jejunum, ileum, caecal tonsils, bursa of Fabricius and rectum) had peak titres the same as in the trachea and kidney, or within 10-fold of that amount (Table 1). Fluorescence revealed the location of the virus, in epithelial cells. Several other isolates were compared with the G strain; they were isolated much less frequently or not at all from kidney and rectal contents (El-Houadfi et al., 1986). Organ culture studies showed that while all strains studied by Bhattacharjee & Jones (1997) grew in the proventriculus, bursa and kidney explants, some did not grow in the caecal tonsil or rectal explants.

In the experiments of Hofstad & Yoder (1966), titres in the kidney and bursa of Fabricius were approximately two orders of magnitude lower than in the respiratory tissues for a number of IBV strains of low passage number in embryos. High egg-passaged virus (85 passages) was almost undetectable in the kidney and bursa, and gave reduced titres in the trachea and lung.

The most intensively studied tropism, apart from the upper respiratory tract, has been that for the kidney. Although only a small proportion of IBV isolates exhibit high nephropathogenicity, these can cause up to 44% mortality within 3 weeks of intratracheal inoculation (Pensaert & Lambrechts, 1994) in the absence of vaccination. Histopathological and immunochemical studies of the process showed that the MA-87 strain of virus attacked mainly the lower nephron down to the collecting duct epithelial cells (Chen & Itakura, 1996; Chen et al., 1996). 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 (Chen, 1996). The extent of mortality is age related (greatest in chicks; Lambrechts et al., 1993) and varies according to the line of bird; for example, 18% and 44% of an egg layer-type bird and a meat-type bird, respectively (Pensaert & Lambrechts, 1994).

Birds have a small lymphoid organ, the Harderian gland, in the eye-socket that is a major contributor to locally produced antibody for protecting ocuonasan mucosae (reviewed by Dhinakar Raj & Jones, 1997). Inoculation of a mild vaccinal strain of IBV by eye-drop resulted in replication of virus in the gland and partial damage to it (Toro et al., 1996). There was an increase in the number of plasma cells and enlargement of lymphoid foci. IBV has also been isolated from another lymphoid organ, the bursa of Fabricius (Table 1) (El-Houadfi et al., 1986; Ambali & Jones, 1990), with gross and histopathological lesions occurring, even after infection with vaccinal strains.

Most recently, Yu et al. (2001a) have studied the pathogenesis of three isolates of IBV from the proventriculus of chicken flocks that had affected proventriculi. Experimental infections with these strains resulted in age-dependent mortality (75 to 100% in 2-week-old birds, 0 to 25% in 16-week-old chickens). Necropsy of sick chickens revealed that the mucosa of the proventriculus was thickened and exuded a milky fluid when squeezed, and the entire proventriculus was enlarged.

Thus, IBV strains as a group productively infect a large range of epithelial surfaces, literally from the top to the bottom of the chicken. Isolates differ in their extent of replication in the non-respiratory

| Table 2. Titres of IBV (strain G) in respiratory and non-respiratory tissues following intranasal and eye-drop inoculation of 1-day-old chicks (from Ambali & Jones, 1990) |
|---|---|---|---|---|---|---|
| Tissue | 1 day | 3 days | 5 days | 7 days | 10 days | 14 days |
| Non-enteric | | | | | | |
| Trachea | 5 | 5 | 5 | 5 | 3 | 2 |
| Kidney | 3 | 4 | 5 | 5 | 4 | 3 |
| Upper gut | | | | | | |
| Proventriculus | 5 | 3 | 3 | 3 | 3 | 3 |
| Duodenum | 4 | 4 | 3 | 3 | 3 | 3 |
| Jejunum | 0 | 2 | 2 | 0 | 0 | 0 |
| Lower gut | | | | | | |
| Rectum | 3 | 5 | 6 | 4 | 4 | 4 |
| Bursa | 2 | 3 | 5 | 4 | 3 | 3 |
| Caecal tonsil | 2 | 3 | 4 | 4 | 4 | 4 |
| Ileum | 3 | 5 | 3 | 3 | 3 | 3 |

*CD, ciliostatic dose; the virus was titrated in tracheal organ cultures, the presence of virus being indicated by cessation of ciliary activity. For simplicity, titres have been rounded to the nearest log value.
tissues, and some produce clinical disease in non-respiratory tissues, most notably the kidney and proventriculus. The pantropism of IBV might be the case for SARS coronavirus, as the latter has not only been associated with pneumonia, but also with diarrhoea (although it remains to be demonstrated whether the SARS virus is replicating in enteric tissues; Peiris et al., 2003). A point of difference is that whereas SARS virus is associated with severe clinical signs in both the respiratory and enteric tracts, IBV is usually limited to disease in the respiratory tract.

**IBV exists as dozens of serotypes**

It is not appropriate in this short review to go into detail about the extensive antigenic variation exhibited by IBV (Darbyshire et al., 1979; Ignjatovic & Mcwaters; 1991; Cook et al., 1999; Cavanagh, 2001; Meulemans et al., 2001; Farsang et al., 2002); it is an issue that may not arise with SARS coronavirus. That said, only a few amino acid differences in the S1 protein of two strains of IBV are sufficient to change the serotype.

The large spike glycoprotein (S) comprises a carboxy-terminal S2 subunit (approximately 625 amino acid residues), which anchors S in the virus envelope, and an amino-terminal S1 subunit (approximately 520 residues), believed to largely form the distal bulbous part of S (Cavanagh, 1995; Lai & Cavanagh, 1997). It is the S1 subunit that induces virus-neutralizing (VN) antibody. This has been demonstrated in various ways: failure to induce VN by virus from which S1 had been removed (Cavanagh et al., 1986); induction of VN antibody by S1 released from virions by treatment with urea (Cavanagh et al., 1986), by non-ionic detergent, followed by affinity chromatography (Ignjatovic & Galli, 1994) and by expression from a vaccinia virus recombinant (Tomley et al., 1987); by production of monoclonal antibody-resistant mutants, the mutations being in the S1 part of the spike protein gene (Cavanagh et al., 1988; Koch et al., 1990; Kant et al., 1992).

**Failure of serotypes to induce cross-protective immunity**

Experimental vaccination studies usually involve application of virus to the nostrils and by eye-drop, sometimes by direct application to the trachea. Protection is determined by challenge, 3 weeks after vaccination, the challenge virus being applied by the same route. Three main approaches to the assessment of protection have been: (1) observation of clinical signs (not commonly used); and removal of the trachea at 4 or 5 days after challenge followed by either (2) quantitative assessment of ciliary activity or (3) detection of live challenge virus, usually by inoculation of embryonated eggs.

The second and third methods result in similar deductions being made as regards protection (Marquardt et al., 1982), although in some studies challenge virus was isolated from some individuals whose ciliary activity was unaffected or little affected. Arguably, declaring a chicken as non-protected because of the recovery of small amounts of challenge virus, when ciliary activity is normal, is being too stringent.

Most IBV serotypes differ from each other by 20 to 25% of S1 amino acids (Adzhar et al., 1997; Kingham et al., 2000), although some differ by up to 50% (Cavanagh et al., 1997; Gelb et al., 1997). (Differences between the other IBV proteins are in the region of 10%, rarely exceeding 15%; Cavanagh et al., 2001, 2002.) Generally speaking, the immunity induced by inoculation with one serotype protects poorly against infection by heterologous serotypes.

With regard to protection of the respiratory tract, in some experiments some heterologous challenge viruses broke through in 100% of the chickens (i.e. 0% cross-protection) (Hofstad, 1981; Marquardt et al., 1982; Picault et al., 1986). Other challenge viruses, known now to differ by 20% or so in S1 from the virus used to initially infect the chickens, broke through immunity in 30 to 70% of cases (Rosenberger et al., 1976; Hofstad, 1981). Collectively these and other reports show that cross-protection can range from very poor to moderate (poor for commercial purposes), although in some cases the differences among these reports may be also be due to differences in stringency by which protection was assessed (discussed later). When Winterfield et al. (1976) used clinical criteria to assess cross-protection, a vaccinal strain of the Massachusetts serotype was concluded to protect all chickens (i.e. 0% cross-protection) (Hofstad, 1981). Collectively these and other reports show that cross-protection can range from very poor to moderate (poor for commercial purposes), although in some cases the differences among these reports may be also be due to differences in stringency by which protection was assessed (discussed later). When Winterfield et al. (1976) used clinical criteria to assess cross-protection, a vaccinal strain of the Massachusetts serotype was concluded to protect all chickens (i.e. 0% cross-protection) (Hofstad, 1981). Collectively these and other reports show that cross-protection can range from very poor to moderate (poor for commercial purposes), although in some cases the differences among these reports may be also be due to differences in stringency by which protection was assessed (discussed later). When Winterfield et al. (1976) used clinical criteria to assess cross-protection, a vaccinal strain of the Massachusetts serotype was concluded to protect all chickens (i.e. 0% cross-protection) (Hofstad, 1981). Collectively these and other reports show that cross-protection can range from very poor to moderate (poor for commercial purposes), although in some cases the differences among these reports may be also be due to differences in stringency by which protection was assessed (discussed later). When Winterfield et al. (1976) used clinical criteria to assess cross-protection, a vaccinal strain of the Massachusetts serotype was concluded to protect all chickens (i.e. 0% cross-protection) (Hofstad, 1981). Collectively these and other reports show that cross-protection can range from very poor to moderate (poor for commercial purposes), although in some cases the differences among these reports may be also be due to differences in stringency by which protection was assessed (discussed later). When Winterfield et al. (1976) used clinical criteria to assess cross-protection, a vaccinal strain of the Massachusetts serotype was concluded to protect all chickens (i.e. 0% cross-protection) (Hofstad, 1981). Collectively these and other reports show that cross-protection can range from very poor to moderate (poor for commercial purposes), although in some cases the differences among these reports may be also be due to differences in stringency by which protection was assessed (discussed later).
some variants that differed by only 2 to 3% of S1 amino acids (10 to 15 residues) behaved as different serotypes (Cook, 1984; Cook & Huggins, 1986; Cavanagh et al., 1992).

Would small S1 differences between SARS coronavirus isolates be of practical significance?

It is conceivable that there are variants (e.g. with small differences in S1) of SARS coronavirus in the field (i.e. within its natural host(s)). If this were to be the case, and if variants infected humans, would the variants be able to break through the immunity induced by a vaccine based on a strain isolated from humans in early 2003? Unfortunately there have been few protection studies based on closely related variants of IBV.

Cavanagh et al. (1997) inoculated groups of 10 chickens with the virulent UK/6/82 isolate and challenged with isolates that differed by up to 4% of S1 amino acids (some of which were different serotypes; Cook, 1984; Cook & Huggins, 1986), protection being assessed on retention of ciliary activity in trachea. Challenge with two variants (98% S1 identity with UK/6/82) resulted in challenge scores (a relative measure of retention of ciliary activity) virtually the same as with the primary virus, but rather to heterogeneity of the immune response.

In other studies approximately 10% of chickens in a group, which had been inoculated and challenged with the same strain or with one of the same serotype, were not fully protected (Winterfield et al., 1976; Hofstad, 1981; Picault et al., 1986; Parsons et al., 1992; Cavanagh et al., 1997; Cook et al., 1999; Nix et al., 2000) (using virus isolation or tracheal ciliary activity as the criterion). These results show that chickens (out-bred, although with restricted sets of parental breeding stocks) are not uniform in their response to IBV vaccination. One would imagine that the human population might be even more heterogeneous in response to vaccination against SARS.

Nix et al. (2000) inoculated chickens with several virulent isolates that had 94% or greater amino acid identity in S1. All were assessed as being of the same serotype, Arkansas, but some cross-reacted in a VN test much less than others. Some isolates induced 100% protection, assessed by re-isolation of challenge virus, a stringent test, against challenge, including against challenge with a strain that was of lower serological identity. However, two other isolates induced cross-protection of only 55 to 58% of the chickens when the challenge virus was of lower serological identity.

Vaccination with live-attenuated vaccines

Meat-type chicks (broilers) are usually vaccinated on the day of hatch, in the hatchery, by spray. The birds are vaccinated at this very early age in part for logistical/economic reasons. Also, because IBV is endemic and ever-present in many poultry-rearing parts of the world, protection is needed as early
as possible. The consequences of IBV infection are greater for chicks than older birds. Also, the modern broiler goes for processing at only 6 weeks of age, so vaccination has to be early.

Many studies have been undertaken during the past 30 years or so to examine the degree of protection induced by single inoculations of various serotypes of IBV. In many of the studies referred to in the previous section, the initial inocula were virulent strains, not attenuated derivatives. This was an advantage in that the degree of protective immunity induced by a virus is in part dependent on its virulence. Notwithstanding, the overall conclusions reached, already described, were similar, irrespective of whether the initial inoculum virus was virulent or attenuated.

Duration of respiratory tract immunity following live attenuated vaccination

Protection of the respiratory tract following a single live attenuated virus vaccination has been found to be short-lived; Gough & Alexander (1979) and Darbyshire & Peters (1984) reported a decline in the number of protected chickens at 6 and 9 weeks after vaccination, respectively. In regions where a given serotype of IBV vaccine is consistently not giving acceptable protection against field challenge, the birds might be revaccinated 2 to 3 weeks after the first application. Sometimes this is with the same vaccine preparation, sometimes with another product of the same serotype, and sometimes with a vaccine of a heterologous serotype. The latter approach sometimes gives protection against a broader range of serotypes (Cook et al., 1991).

Protection against nephritis by live vaccines

Some strains of IBV are markedly nephropathogenic. For example, experimental tracheal infection of commercial meat-type birds with the B1648 nephropathogenic strain resulted in the death of approximately one-third of a group of 68 chickens within 9 days. Vaccination, by coarse spray, with the homologous attenuated strain completely protected against mortality upon challenge 4 weeks later (Pensaert & Lambrechts, 1994). Vaccination with non-homologous, commercially available vaccines markedly, although not completely, reduced mortality. The homologous vaccine had prevented detectable growth of the challenge virus in the trachea, as ascertained by virus isolation, but the heterologous vaccines did not do so. 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.

Vaccination with inactivated 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 (McDougall, 1969; Box et al., 1980; Muneer et al., 1987) and no protection against loss of ciliary activity in the trachea (Martins et al., 1991).

However, immunization of 11 chickens with a single dose of inactivated IBV strain M41 resulted in 36 to 45% (depending on the criterion used) developing respiratory tract protection (Cavanagh et al., 1986). A dose of 100 μg was not more efficacious than 5 μg. In a second experiment, 29% (based on retention of ciliary activity in the trachea) to 76% (based on not isolating challenge virus) of 17 chickens were protected after a dose of 10 μg. In a third experiment, 40% of 10 chickens were judged protected (retention of ciliary activity) following a single 10 μg dose. It is possible that larger amounts of virus was used in these three experiments than by previous workers, and that this was the reason for the partial success.

As part of a study of the role of the IBV nucleocapsid protein (N) protein in protective immunity (discussed later), Boots et al. (1992) immunized chickens with inactivated IBV at 4 weeks of age and again 6 weeks later. After challenge, 1/8 and 8/8 of the two groups of chickens, respectively, were protected, by assessment of ciliary activity in the trachea. The degree of protection was assessed as being slightly less (88%) when immunofluorescence was used to detect infected tracheal cells.

Ignjatovic & Galli (1994) gave four doses, each of 200 μg, of inactivated IBV. 40% and 20% of 10 birds had developed protection (assessed by non-re-isolation of challenge virus) in the trachea and kidney, respectively, after three doses. These percentages did not increase after four doses. Song et al. (1998) immunized chickens up to three times with an inactivated nephropathogenic strain. Protection (assessed by non-re-isolation of challenge virus) of the kidney and trachea was 50% and 13%, respectively, of eight chickens, after two inoculations, rising to 88% and 50%, respectively, after three inoculations.

To see whether two doses of inactivated vaccine would be protective against loss of egg production, McDougall (1969) vaccinated birds at an interval of 8 weeks and challenged them 18 weeks later. Only a few of the vaccinated birds had respiratory signs and these lasted for less than 3 days, compared with 8 days for the unvaccinated controls. The birds that had received the inactivated vaccine were largely protected against a drop in egg production. Gough et al. (1977) demonstrated a
poor serum antibody response to a single application of inactivated infectious bronchitis vaccine but titres increased markedly upon revaccination 10 weeks later.

The approach commonly used in the poultry industry today is to vaccinate young females two or more times with live vaccine, followed by one dose of inactivated vaccine as the birds come into lay. The live vaccines serve to give protection to the young bird and to prime the immune response to the later inactivated vaccine (Gough et al., 1977; Box et al., 1980; Finney et al., 1990). Gough et al. (1977) demonstrated that the chickens that had received one dose of live vaccine followed by inactivated vaccine 10 weeks later, were protected against challenge 10 weeks later, as assessed by re-isolation of challenge virus from trachea, oviduct and kidney. Box & Ellis (1985) reported that the response to the inactivated vaccine was poor if given within 8 weeks of a live vaccination.

Passive immunization

Macdonald et al. (1981) inoculated 12-week-old chickens with 2 ml convalescent IBV serum. Two days later they were challenged by intravenous application of the H52 vaccinal strain, a procedure known to result in nephritis. The birds were protected against kidney disease, as assessed by lack of watery droppings, macroscopic and microscopic pathology in the kidney, in contrast to control birds to which non-immune serum had been given. The passive application of immune serum did not protect against respiratory infection, although onset was delayed and of shorter duration.

Variation among genetic lines of chicken in their susceptibility to IBV

Smith et al. (1985) and Cook et al. (1986) demonstrated varying mortality among different breeds of chicken, both when inoculated with a mixture of IBV isolates alone and when in conjunction with a pool of Escherichia coli strains. The IBV predisposed the birds to infection with the bacterium, resulting in increased mortality. Bumstead et al. (1989) continued with this IBV/E. coli infection, showing marked variation in mortality among several inbred lines of White Leghorn chickens. More detailed analysis of two of these lines, line 15I (highly susceptible) and line C (relatively resistant), showed that the rate of virus production and titres in the respiratory tract were similar during the first 4 days after infection (Otsuki et al., 1990). Thereafter, however, titres declined much more slowly in the susceptible line 15I, taking two-fold longer to decline to undetectable levels. In tracheal explants of the two lines there was no difference in the replication profile of the virus, showing that the difference in the birds themselves was not because of an intrinsic difference in the capacity of the trachea to support the replication of IBV. The underlying cause of the difference in vivo might be immunological.

The extent to which infection by a nephropathogenic strain caused mortality was also dependent on the type of chicken (Pensaert & Lambrechts, 1994). Thus, 0% and 2% of a specified pathogen free flock died within the periods 5 to 7 days and 7 to 9 days, respectively, after tracheal inoculation of the B1648 strain. In contrast, 15% and 21% of commercial meat-type birds died in these periods.

Presence and duration of antibody in the serum, trachea and nasal secretions

The humoral immune response to IBV vaccination has mostly been investigated by measuring antibody levels in serum, using enzyme-linked immunosorbent assay (ELISA), VN or haemagglutination-inhibition (HAI) tests. IBV haemagglutinates very poorly until treated with enzyme preparations containing neuraminidases; Alexander et al., 1983; Schultze et al., 1992, and references therein.). However, there have also been a few studies of IBV antibodies in the nose and trachea. It should be stated at the outset that many studies have shown that the presence or absence or titre of serum antibody to IBV does not correlate with protection; that is, vaccinated chickens may be protected against respiratory disease IBV irrespective of the titre of serum antibody (for example, Raggi & Lee, 1965; Ignjatovic & Galli, 1994).

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), a majority of the birds having detectable antibody by 6 days after infection, with titres maximal within 21 days and tending to decline shortly afterwards (Mockett & Darbyshire, 1981). VN antibody was delayed in comparison with ELISA-detected antibody, being first detected in the period 9 to 21 days after infection, peaking within 35 days and remaining level for the remainder of the sampling period (63 days after inoculation), although in 25% (2/8) of the birds the VN antibody was still increasing at 63 days, when sampling ended. The HAI antibodies were first detected at 9 days, peaking at 14 to 17 days and then declining, although in one bird the HAI titre did not peak until much later (as with the VN titre in the same bird).

Mockett (1985) also compared ELISAs using purified S and M proteins with the conventional virus-coated ELISA; the primary and secondary antibody response profiles were very similar, VN antibody again later than ELISA antibody.

Following infection with a live IBV vaccinal strain there was a good primary immunoglobulin (Ig)M response (Mockett & Cook, 1986; Martins et
As expected, the primary IgM response peaked, and declined, before that of the IgG response (Figure 1) (Martins et al., 1991). 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 (Figure 1). Mockett & Cook (1986) suggested that measurement specifically of serum IgM would be useful in defining recent infection. In contrast to the response after IBV infection, vaccination with inactivated virus produced almost no IgM response, as well as a poor IgG response (Figure 1) (Martins et al., 1991).

A single and double vaccination with inactivated IBV resulted in HAI titres of 1/28 and 1/219, respectively, by 3 to 4 weeks after vaccination (Gough et al., 1977). A single inoculation of 3 µg purified IBV induced maximum titres of HAI and ELISA antibody of 6 log₂ and 12 log₂, respectively, by 3 to 4 weeks after vaccination (Cavanagh et al., 1986). VN antibodies peaked 1 week later, at 5 log₂. Titres decreased eight-fold within 4 weeks of peaking.

Holmes (1973) measured VN antibody in nasal washings after challenge of chickens that had received either a single live virus infection or two intra-muscular inoculations (4 weeks apart) with inactivated IBV. Serum antibody levels rose rapidly in both groups. Nasal antibody rose more slowly in the group that had received inactivated virus than in the group that had received a primary inoculum of live virus.

**Figure 1.** Antibody induction by inactivated and live IBV vaccination, from Martins et al. (1991). Profile of IBV-specific IgM and IgG responses of unprimed 14-week-old chickens to live attenuated vaccine (H120) given by eye-drop or intramuscularly (i.m.), and to inactivated vaccine (oil emulsion) given i.m. The IgM and IgG were measured using an ELISA. The ELISA results (as absorbance values) were plotted graphically, and the areas corresponding to each immunoglobulin class were cut out and weighed. The chickens were challenged with virulent IBV 24 days after vaccination. IgG: live vaccine, eye-drop (■); live vaccine, i.m. (▲); inactivated vaccine, i.m. (●). IgM: live vaccine, eye-drop (□); live vaccine, i.m. (△); inactivated vaccine, i.m. (○).

**Induction of protection by subviral and vectored vaccines**

There is no doubt that the IBV S protein, when inoculated on its own, can induce protective immunity. The proportion of chickens being protected may be dependent on the manner by which the S protein is presented to the host. There is also evidence that the N protein can prime protective immune responses, and one report that the N protein on its own induced protective immunity.

**Immunization with the S protein**

Whereas a single inoculation of 10 µg IBV induced tracheal protection in 40% of 10 chickens, all birds were susceptible after immunization with IBV from which the S1 subunit had been removed by urea, indicating that the S1 subunit was required to induce protective immunity (Cavanagh et al., 1986). The S1 that had been released by treatment with urea was dialysed against decreasing concentrations of urea, and eventually in the absence of urea; this might have enabled some renaturation of the protein. Sedimentation analysis indicated, as expected, that the S1 that had been released by urea was in monomeric form; that is, it was no longer a multimeric (dimer or trimer) molecule with quaternary structure. Notwithstanding, 22% and 44% of 10 chickens that had been inoculated on four occasions with 3 µg S1 developed VN and HAI antibody, respectively. There were no positives following three inoculations. Protective immunity was not assessed in that experiment.

Ignjatovic & Galli (1994) dispersed the proteins of a nephropathogenic IBV using non-ionic detergent and purified S1, N and M by affinity chromatography using monoclonal antibodies. Chickens were inoculated on four occasions, 50 µg for each dose. Chickens that had been immunized with N or M proteins did not develop protective immunity (assessed by non-recovery of challenge virus) to either the trachea or kidney following challenge. However, 71% and 86% of 10 chickens inoculated with S1 developed VN and HAI antibody, respectively. There were 3 positives following three inoculations. Protective immunity was not assessed in that experiment.

The S1 protein has also been expressed in *Spodoptera frugiperda* cells from a recombinant *Autographa californica* baculovirus (Song et al., 1998). When chickens were inoculated with these cells containing expressed S1, 43% and 0% of seven birds were protected (assessed by non-re-isolation of challenge nephropathogenic virus) in the kidney and trachea, respectively. Protection rose to 50% and 25%, respectively, of eight chickens after three inoculations.

The S1 protein has also been expressed in *SARS and vaccination against IBV* 575

**Figure 1.** Antibody induction by inactivated and live IBV vaccination, from Martins et al. (1991). Profile of IBV-specific IgM and IgG responses of unprimed 14-week-old chickens to live attenuated vaccine (H120) given by eye-drop or intramuscularly (i.m.), and to inactivated vaccine (oil emulsion) given i.m. The IgM and IgG were measured using an ELISA. The ELISA results (as absorbance values) were plotted graphically, and the areas corresponding to each immunoglobulin class were cut out and weighed. The chickens were challenged with virulent IBV 24 days after vaccination. IgG: live vaccine, eye-drop (■); live vaccine, i.m. (▲); inactivated vaccine, i.m. (●). IgM: live vaccine, eye-drop (□); live vaccine, i.m. (△); inactivated vaccine, i.m. (○).
fowlpoxvirus expressing S1 induced IBV-specific antibodies (Wang et al., 2002). After challenge with IBV, there was somewhat less recovery of challenge virus and less tracheal damage than in controls, and only mild clinical signs. Greater protection was achieved following a single oral application of a fowl adenovirus expressing S1 (Johnson et al., 2003). Protection (assessed by non-re-isolation of challenge virus) was obtained in 90% and 100% of 10 to 13 chickens in two experiments.

Immunization with the N protein

The first study of the role of the IBV N protein in immunity was by Boots et al. (1992). The N protein was in the form of a bacterially expressed fusion protein with β-galactosidase. Initial experiments were with mice, inoculated in the footpad. A single inoculation resulted in popliteal lymph node cell proliferative responses to IBV. Delayed-type hypersensitivity responses were obtained when mice had been inoculated with: (a) inactivated IBV on two occasions; (b) inactivated IBV followed by N protein; and (c) N protein followed by inactivated IBV.

Experiments were subsequently undertaken with chickens. Following a single intramuscular inoculation of inactivated IBV, 1/8 birds were protected against respiratory challenge, as assessed by ciliary activity in the trachea, and none were protected in a group inoculated with N protein. Other chickens were inoculated with either inactivated virus or N protein, and then inoculated a second time, 6 weeks later, with inactivated IBV. After challenge, 8/8 and 8/10 chickens in the two groups, respectively, developed protective immunity (Table 3). The extent of protection was assessed as being slightly less when immunofluorescence was used to detect challenge virus in tracheal cells (Table 3). In a third group in which birds had been inoculated with β-galactosidase without any N protein, and then inoculated with inactivated IBV, some chickens had protective immunity, induced by the secondary inoculation with inactivated IBV (Table 3). Linear regression analysis showed that this was statistically less than in the group that had been primed with the N protein. The authors concluded that immunization with the N protein had induced protective immunity by activation of cytotoxic or helper T-cell responses.

Two intramuscular immunizations of chickens with a plasmid expressing the N protein, or a fragment of the N protein, induced immune responses that protected the birds from infection by IBV (applied by eye-drop and intranasally), as evidenced by mark reduction in replication of the challenge virus (Seo et al., 1997b). A fragment of the N protein comprising the carboxyterminal 120 amino acid residues was sufficient to induce protection. Yu et al. (2001b) expressed this fragment in chickens using a single inoculation of a fowlpoxvirus recombinant. Upon challenge with a homologous strain and one heterologous strain of IBV, the birds did not develop clinical signs, although some replication of challenge virus was detected by an ELISA. The birds were not protected against challenge with a different heterologous strain.

Nature of protective immune responses to IBV

Collisson and colleagues (Seo & Collisson, 1997b; Pei et al., 2003) have shown that cytotoxic T-cell responses in chickens to IBV infection correlated with initial decreases in infection and clinical signs. Cytotoxic T-cell activity was major histocompatibility complex restricted, and lysis was mediated by CD8+/CD4- cells. Adoptive transfer of IBV-infection-induced αβ T cells bearing CD8 antigen protected chicks from challenge infection (Seo et al., 2000; reviewed in Collisson et al., 2000). Earlier work has been reviewed by Dhinakar Raj & Jones (1997). Tissues from chickens infected with two field strains of IBV were assayed for interferon, which was readily detected in the trachea and lung but at only low levels in the plasma, kidney, liver and spleen (Otsuki et al., 1987).

Final comments

If there were to be a resurgence of SARS in humans, one would anticipate that the authorities

Table 3. Priming of tracheal protection by primary immunization of chickens with IBV N protein (modified from Boots et al., 1992)

| Primary immunization | Ciliary activity assay | Immunofluorescence assay |
|----------------------|------------------------|--------------------------|
|                      | Challenge after primary immunization | Challenge after secondary immunization | Challenge after primary immunization | Challenge after secondary immunization |
| Inactivated IBV      | 1/8                     | 8/8                      | ND                      | 7/8                     |
| β-galactosidase−IBV N protein fusion protein | 0/8 | 8/10 | ND | 7/10 |
| β-galactosidase      | 0/8                     | 8/12                     | ND                      | 4/12                     |

*Chickens were challenged by eye-drop 4 weeks after primary immunization.

*The secondary immunization was inactivated IBV. Chickens were challenged by eye-drop 4 weeks after secondary immunization.

*ND, not done.
would rapidly put into operation the various surveillance and control measures that proved successful in early 2003. If vaccines had been developed, vaccination might be included, especially for health care workers and contacts in the wider community. Coronaviruses can establish persistent infections, in at least a proportion of their hosts, resulting in chronic asymptomatic shedding, with subsequent problems for containment of the disease. For this and other reasons, a decision to apply a SARS vaccine would not be taken lightly. Notwithstanding, what type of vaccine might be used?

The poultry industry prefers to use live vaccines rather than inactivated ones. The former are cheaper to make and buy, and easier/cheaper to apply. These financial considerations would presumably not be paramount in the case of a resurgence of SARS in humans. Efficacy would be an important criterion for use of a particular vaccine, although that also applies in the poultry industry, of course. Safety of a SARS vaccine might be deemed more important than efficacy, assuming that a vaccine had an acceptable, if not complete, efficacy. In general, inactivated vaccines are considered to be safer than live attenuated vaccines, although the safety of inactivated vaccines cannot be taken for granted.

Unless a future SARS outbreak were to get rapidly out of control, it would seem unlikely that a conventional live attenuated vaccine would be used; the potential risk of vaccine-related problems (reactions) might be deemed too high. Genetic manipulation, in addition to traditional approaches, will be used as a means of attenuating *SARS coronavirus* (Kuo *et al.*, 2000; Thiel *et al.*, 2001; de Haan *et al.*, 2002a,b; Casais *et al.*, 2003; Ortego *et al.*, 2003). However, a desirable outcome cannot be guaranteed and fears of vaccine-related problems will still be harboured. However, given the almost total susceptibility of the global human population, the lethal outcome of 10% of cases, and the huge strain put on the health care services, and economies, a killed vaccine, subunit vaccine or vectored vaccine might be used in the first instance, among selected groups of people.

The experience of single applications of inactivated IBV is not greatly promising in the context of SARS among selected groups of people. A single application of inactivated IBV is not greatly promising in the context of SARS; a single application of inactivated IBV might be used, as expected, not more so than inactivated virus. In two studies, protection of the trachea was achieved after three vaccinations in 70% and 59% of chickens, respectively. Protection of the kidney was less efficient (10% and 25%, respectively). Vectored SARS vaccines might be considered likely to be less potentially problematic, with regard to safety, than attenuated *SARS coronavirus*. Perhaps such a vaccine might be used if an outbreak developed into an epidemic or pandemic. In this regard the results of the investigation by Johnson *et al.* (2003) are very promising; a single oral application of a non-pathogenic fowl adenovirus expressing the IBV S1 protein gave 90+% protection, as good as is obtained by conventional attenuated IBV.

In the event that any type of live vaccine was used against SARS, an inactivated SARS vaccine might subsequently be applied, to build upon the initial vaccination and perhaps provide at least medium-term protection. The experience with IBV is very good in this regard.

If *SARS coronavirus* were to re-emerge in humans, its S1 protein might not be the same as that of the 2002/2003 outbreak. Research with IBV has indicated that differences of only 5% of S1 protein amino acid can reduce cross-protection. Consequently, S1 differences among *SARS coronavirus* isolates must not be viewed complacently. The finding that the IBV N protein plays a beneficial role in immunity suggests that the SARS virus N protein should not be overlooked in a SARS vaccine development programme.

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SARS and vaccination against IBV

Die Beschreibung der genetischen Stabilität und evolutionären Flexibilität von IBV wird in einer Vielzahl von Arbeiten dargestellt, darunter in der Arbeit von Medical Materials and Methods. Die IBV-Pate (Meleagris gallopavo und das Ringlasen (Phasianus colchicus) ein Coronavirus der Gruppe 3, während das SARS Coronavirus vorläufig in die Gruppe 4 eingeordnet wurde und die anderen bekannten Mammalier-Coronaviren zu den Gruppen 1 und 2 gehören.

Das IBV vermehrt sich nicht nur in Respirationsstrukturen (z. B. Nase, Trachea, Lungen und Luftsäcken verbunden mit Verursachung einer respiratorischen Erkrankung) sondern auch in Nieren (assoziiert mit gering- bis hochgradiggradiger Nephritis), Ödödum und in vielen Teilen des Verdauungstrakts (Ösophagus, Proventriculus, Duodenum, Jejunum, Bursa Fabrici, Zäkaltionsilen, Rektum und Kloake, dort jedoch gewöhnlich ohne klinische Auswirkungen. Das Virus kann persistieren und wird mit Beginn der Legetätigkeit (im Alter von 4-5 Monaten) wieder ausgeschieden, wobei die Virusaktivierung als Konsequenz aus dem damit verbundenen Stress angesehen wird.

Verschiedene genetische Hühnerlinien unterscheiden sich im Ausmaß der durch IBV verursachten Mortalität sowie hinsichtlich der Virus Clearance nach der akuten Infektionsphase.

In den 1950iger Jahren wurden (durch Passagen in embryonierten Hühnerembryonen) attenuierte IBV-Stämme als Lebendvirusvakzinen einge führt. Einige Jahrzehnte später folgten Inaktivatvakzinen zur Boosterung der Schutzwirkung bei Legehennen. Lebendvirusimpfstoffe werden gewöhnlich bei Masthühnern am ersten Lebenstag appliziert. Unter experimentellen Bedingungen kann dies zu einer sterilen Immunität führen, wenn die Tiere mit einem virulenten homologen Virus belastungsinzisiert werden. Obwohl 100 % der Tiere geschützt sein können (gegen klinische Symptome und Zilenverlust in der Trachea), zeigen manchmal 10 % der Küken keine schützende Immunantwort. Die Schutzwirkung ist kurzfristig. Neun Wochen nach der Vakzination mit hoch attenuierten Impfstämmen wird der Beginn des Abfalls sichtbar. Das IBV besitzt eine Vielzahl von Serotypen (definiert durch Neutralisationstests), wobei die Kreuzschutzwirkung oft gering ist. Daraus folgt, dass Hühner zwei bis drei Wochen nach der Erstvakzination mit dem gleichen oder einem anderen Serotyp revakziniert werden sollten.

Einmalige Applikation von inaktiviertem Virus hat allgemein zum Schutz von 50 % der Hühner geführt. Zwei Applikationen rufen laut einiger Veröffentlichungen einen 90-100%-igen Schutz hervor, in anderen Untersuchungen blieb er jedoch unter 50 %. Unter Praxisbedingungen im Feld werden Inaktivatimpfstoffe bei Legehennen eingesetzt, die vorher zwe- oder dreimal mit attenuierten Lebendvirusvakzinen geimpft worden sind. Dies erhöht die Schutzwirkung bei den Legehennen gegen Legeleistungseinbrüche und induziert einen anhaltenden Antikörperlevel, der auf die Nachkommen weitergegeben wird.

Das große Spikeprotein (S) umfasst eine S2-Untereinheit im Karboxy-Ende (ungefähr 625 Aminosäuren), die das S in der Virushülle verankert, und eine S1-Untereinheit am Ami no-Ende (ungefähr 520 Aminosäuren), von der angenommen wird, dass sie größtenteils den distalen Bulbusstiel des S formt. Die S1-Untereinheit (gereinigt aus dem IBV, exprimiert unter Verwendung von Baculovirus oder exprimiert in Hühnern mit Hilfe inaktivierter Vektor-Hühner-Baculovirus) induzierte Virusneutralisierende (VN) Antikörper.

Obwohl eine schützende Immuantwort induziert wurde, waren mehrfache Inokulationen erforderlich und der Prozentsatz geschützter Hühner war zu niedrig (< 50 %) für die kommerzielle Anwendung. Bemerkenswerterweise induzierte die Exprimierung von S1 in Hühnern unter Verwendung eines apathogenen
Vektor-Hühner-Adenovirus in zwei Experimenten eine Schutzwirkung bei 90 bzw. 100 % der Tiere. Unterschiede von weniger als 5 % in den S1- Sequenzen können in geringen Kreuzschutzwirkungen resultieren. Un terschiede im S1 von 2-3% (10-15 Aminosäuren) können den Serotyp ändern, was vermuten lässt, dass eine geringe Anzahl von Epitopen immunodominant ist hinsichtlich der neutralisierenden Antikörper.

Anfangsuntersuchungen zur Bedeutung des IBV-Nukleokapsidpro teins (N) für die Immunität legten nahe, dass die Immunisierung mit in Bakterien exprimiertem N, obwohl nicht direkt Schutz auslösend, die Induktion der Schutzwirkung bei einer nachfolgenden Inokulation mit inaktiviertem IBV verbesserte. In einer anderen Studie führten zwei intramuskuläre Immunisierungen mit einem N exprimierenden Plasmid zu einer schützenden Immunität.

Die Grundlagen der Immunität gegen IBV sind nicht gut erforscht. Serumantikörper korrelieren nicht mit der Schutzwirkung, doch lokalen Antikörpern wird eine wichtige Rolle zugesprochen. Die adaptive Übertragung von durch IBV-Infektion induzierten alpha/beta-T-Zellen mit CD8-Oberflächenannten schützten Hühnchen gegen eine Belas tungsinfektion.

Zusammenfassend lässt sich sagen, dass attenuierte Lebendvirusimpfstoffe einen guten, aber kurzfristigen Schutz gegen homologe Belas tungsinfektionen hervorrufen, wobei eine Minderheit der Tiere eine schlechte Immunantwort zeigen können. IBV-Inaktivatvakzinen sind nur ungenügend wirksam, wenn sie nur einmal und ohne Vorvakzination mit einer Lebendvirusvakzine angewendet werden. Zwei Applika tionen von inaktiviertem IBV sind viel effizienter, doch dies ist ein kommerziell nicht rentabler Vorschlag für die Geflügelindustrie. Für den Schutz der menschlichen Bevölkerung jedoch dürften die Kosten und die Logistik für eine mehrfache Applikation einer SARS-Inaktivatvakzine akzeptabler sein, zumal wenn die Anwendung auf bestimmte Zielgruppen wie Beschäftigte im Gesundheitswesen und hoch gefährdete Kontaktpersonen beschränkt wird. Die Applikation eines SARS-Imm pfstoffs sollte möglichst limitiert bleiben auf eine minimale Anzahl von Zielpersonen, die überwacht werden können, da einige geringe Individuen, wenn sie sich mit dem SARS-Coronavirus infizieren, asymptomatische Virusausscheider werden können und somit ein Risiko für die nicht vakinierte Bevölkerung darstellen können. Beim Blick auf die Zukunft stimmt die hohe Wirksamkeit des IBV-S1-Subunit-exprimierenden Vektor-Hühner-Adenovirus optimistisch für eine SARS-Lebendvirus-Vakzine mit der Möglichkeit der Inkorporierung des N-Proteingens, falls das für nötig erachtet werden sollte.

REVISION

Desarrollo de vacunas frente al SARS: experiencia de la vacunación frente al coronavirus de la bronquitis infecciosa aviar

Las vacunas frente a la bronquitis infecciosa (IB) de pollos (Gallus gallus domesticus) han sido las más exitosas y ciertamente las más comúnmente usadas de las vacunas frente a enfermedades causadas por coronavirus, siendo las otras frente a coronavirus bovino, camello, felino y porcino. El virus de IB (IBV), junto con otros coronavirus relacionados genéticamente como el del pato (Meleagridis gallopavo) y faisán común (Phasianus colchicus), forman el grupo 3 coronavirus, mientras que el coronavirus del SARS se encuentra en el grupo 4 y el resto de coronavirus de mamíferos en los grupos 1 y 2.

El IBV se replica no únicamente en tejidos del aparato respiratorio (incluida la cavidad nasal, traquea, pulmones y sacos aéreos, causando enfermedad respiratoria), sino también en el rinón (asociado con nefritis de diferente intensidad), oviducto, y en varias partes del tracto digestivo – esófago, proventriculo, duodeno y yeyuno, bolsa de Fabricio, tonsilas ceceales, recto y cloaca, habitualmente sin efectos clínicos. El virus puede persistir, siendo reexcretado al inicio de la puesta (a los 4 o 5 meses de edad), probablemente como consecuencia del estrés de la entrada en puesta.

Las diversas líneas genéticas de pollos presentan diferencias respecto a la mortalidad causada por la infección con IBV en pollos y respecto a la eliminación del virus tras la fase aguda.

Las cepas vivas atenuadas de IBV (mediante pases en huevos de pollo embrionados) fueron introducidas como vacunas en 1950s, y un par de décadas después fueron seguidas por las vacunas inactivadas para prolongar la protección en aves de puesta. Las vacunas vivas se aplican normalmente en aves de carne al día de edad. En condiciones experimentales esto puede resultar en una inmunidad estétil cuando los animales son desafíos con un virus homólogo. Aunque el 100% de los pollos puede estar protegido (frente a la sintomatología clínica y a la pérdida de actividad ciliar de la traquea), en ocasiones el 10% de las aves vacunadas no responden con una respuesta inmunidad. La protección suele durar poco y empieza a declinar a las nueve semanas tras la vacunación con vacunas basadas en cepas muy atenuadas. Existen varios serotipos de IBV (definidos mediante técnicas de neutralización), pero la protección cruzada suele ser pobre. En consecuencia los pollos son revacunados con el mismo u otro serotipo, dos o tres semanas después.

Las aplicaciones únicas de virus inactivado normalmente protegen a < 50% de los pollos. Se ha descrito que dos aplicaciones pueden llegar a proteger al 90 del 100% de las aves, pero en otras ocasiones se ha descrito una protección por debajo del 50%. En la práctica de campo, las vacunas inactivadas se utilizan en aves de puesta que han sido previamente vacunadas con dos o tres vacunas de virus vivo atenuado. Esto aumenta la protección en las aves de puesta frente a las pérdidas de producción de huevos e induce una nivel de anticuerpos séricos sostenido y que pasa a la progenie.

La glicoproteína de la espícula (S) comprende una subunidad carbono/terminal S2 (de aproximadamente 625 aminoaídos), que incluye la S en el envelloitor viral, y una subunidad amino-terminal S1 (de aproximadamente 320 aminoaídos) que forma la parte distal de la parte bulbosa de la S. La subunidad S1 (purificada de un virus de IBV, expresada en baculovirus o en aves mediante un vector de virus de viruela aviar) induce anticuerpos neutralizantes (VN). Aunque se indujeron respuestas inmunes protectoras, se requirieron múltiples inoculariones y el porcentaje de pollos protegidos fue demasiado bajo ( < 50%) para poder ser aplicada comercialmente. Cabe remarcar, que la expresión de S1 en aves mediante un vector de adenovirus avar no patógeno indujo una protección de entre 90 y 100 de los pollos en dos experimentos. Diferencias de tan sólo el 5% en las secuencias de la S1 resultaron en una protección cruzada baja. Diferencias de entre el 2 y el 3% (de 10 a 15 aminoaídos) en la S1 pueden cambiar el serotipo, lo que sugiere que un número bajo de epitopes son immunodominantes respecto a los antígenos neutralizantes.

Los estudios iniciales sobre el papel que juega la proteína de la nucleocapside (N) de IBV en la inmunidad sugieren que la inmunización con proteína N expresada en bacterias, aunque no induce protección directamente, mejoró la protección inducida por una inocularia subsecuente con IBV inactivado. En otro estudio, dos inmunizaciones intramusculares con un plásmido que expresaba N indujeron una inmunidad protectora. La base de la inmunidad frente a IBV no está totalmente clara. Los niveles de anticuerpos en suero no se correlacionan con la protección, aunque se reconoce que los anticuerpos locales juegan un papel importante. La transferencia de linfocitos T CD8 alfa beta inducidos mediante una infección con IBV, protegieron a los pollos frente a una infección experimental.

En conclusión, las vacunas vivas atenuadas inducen una buena protección, aunque de corta duración, frente a la infección experimental homóloga, pero una minoría de individuos puede responder pobremente. Las vacunas de IBV inactivadas no son suficientemente eficaces cuando se administran únicamente una vez y no se realiza una primovacunación con una vacuna viva. Dos aplicaciones de una vacuna inactivada de IBV son mucho más eficaces, aunque no parece una propuesta viable desde el punto de vista comercial para la industria avícola. Así, el costo y la logística de una aplicación múltiple de una vacuna inactivada frente al SARS serían más aceptables para la protección de una población humana, especialmente si está limitada a grupos de alto riesgo, como trabajadores relacionados con temas sanitarios o personas con alto riesgo de contacto. La aplicación de la vacuna frente al SARS es quizás mejor limitarla a un número de individuos de riesgo que puedan ser monitorizados, ya que algunas personas vacunadas podrían, al estar infectadas con el coronavirus del SARS, volverse excretores asintomáticos del virus, siendo un riesgo para la población no vacunada. De cara a un futuro próximo, la alta eficacia de un vector de adenovirus aviar que expresa la subunidad S1 del IBV proporciona optimismo con respecto a una vacuna viva de SARS, y si se cree necesario podría haber la posibilidad de incluir el gen de la proteína N.