Antigenically-related Viruses Associated with Infectious Bursal Disease

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SUMMARY

Bursae from chickens infected with infectious bursal disease have been examined by immune electron microscopy. Two types of virus particle have been detected, the larger being 55 to 60 nm in diam. and markedly hexagonal in outline, while the smaller has an appearance similar to that of the parvoviruses with a diam. of 18 to 22 nm. The presence of mixed aggregates and demonstrable antibody-bridging show that the two types of particle have at least one surface antigen in common.

INTRODUCTION

The lymphoproliferative condition of young chickens known as infectious bursal disease (Gumboro Disease) has been recognized as a clinical entity since 1962 (Cosgrove, 1962; Winterfield & Hitchner, 1962). The disease specifically affects the bursa of Fabricius, initially causing severe oedema followed by atrophy. The disease is of interest, not only from a commercial viewpoint, but also immunologically, as young birds affected by the disease are unable to cope satisfactorily with other infections, presumably because of the depression of bursa-derived lymphocytes. Specifically, birds which have been exposed to infectious bursal disease at 1 day of age and are infected by it will not respond efficiently to subsequent routine vaccination against Newcastle disease (Faragher, Allan & Cullen, 1972).

It has been known for some time that the disease is caused by a virus (Helmboldt & Garner, 1964; Cheville, 1967), but attempts to characterize it have led to ambiguous results. Although several workers have studied the virus in the electron microscope, they have frequently been at variance with each other (Cheville, 1967; Petek & Mandelli, 1968; Cho & Edgar, 1969; Rinaldi et al. 1970; Kosters, Becht & Rudolph, 1972). We have, therefore, employed the technique of immune electron microscopy (Almeida & Waterson, 1969) in the hope that by concentrating the particles within immune aggregates, study of virus morphology would be easier.

Results to be described show that infectious bursal disease (IBD) has associated with it two distinct virus particles. The larger particle has a strongly hexagonal outline and a diam. of 55 to 60 nm. The second particle is 18 to 22 nm in diam. and has the appearance associated with the parvoviruses, such as the adeno-associated satellite viruses (Hoggan, 1970). Although these particles are of differing morphologies, they are, nevertheless, antigenically related.
METHODS

Source of infected material. (i) Isolate 12/69 was obtained as a bursal extract from the second chicken passage of infected bursae taken from an outbreak in Cambridgeshire in 1969 and kindly donated by Dr J. T. Faragher, Central Veterinary Laboratory, Weybridge. This material received two further passages in this laboratory; (ii) Isolate BSE/I/72 – a bursal extract from a field outbreak in Suffolk in 1972; (iii) Isolate K/1/73 – a bursal extract from a field outbreak in Kent in 1973.

All laboratory investigations were carried out on extracts prepared by homogenizing bursae each in 1 ml of phosphate buffered saline (PBS) at a pH of 7.2 using a Waring blender. This homogenate was clarified by centrifuging on a bench centrifuge for 30 min at 2000 rev/min. The initial field diagnosis was confirmed by double-diffusion in agar, the technique being basically that described by Woernle (1966).

Antisera. (i) Four-week-old IBD-susceptible birds were infected orally with 1 ml of a 1:20 dilution of fourth passage 12/69 bursal extract. Birds were screened for antibody activity by immunodiffusion in agar gel and finally bled out at 32 days post-inoculation. All test bleeds from 7 days onwards gave a positive reaction in immunodiffusion. (ii) Convalescent serum was obtained from a flock in Suffolk having a history of the disease. It was shown to contain antibody by immunodiffusion.

Immunodiffusion test. The immunodiffusion test was carried out in an agar gel of 1 % (w/v) Difco Noble agar in distilled water with 8 % (w/v) sodium chloride and 0.4 % (w/v) phenol. Microscope slides (3 x 1 in.) were coated with 2.5 ml of agar, and 2 mm diam. wells were cut with an inter-well distance of 3 mm. After the wells were filled, the slides were left overnight at room temperature in a humidified chamber.

Electron microscopy. 0.2 ml of bursal suspension was mixed with 0.1 ml of a 1:50 dilution of convalescent serum and left overnight at 4 °C. The next morning the mixture was centrifuged for 30 min at 17000 g and the supernatant fluid discarded. The pellet was resuspended to the original vol. with PBS and again centrifuged for 30 min at 17000 g. The supernatant fluid was discarded and the pellet used for negative staining.

To carry out negative staining, each pellet was suspended in a small amount of distilled water until the solution was just opalescent. Some of this solution was mixed with an equal vol. of 3 % (w/v) phosphotungstic acid adjusted to pH 6.0. A drop of this mixture was placed on a 400-mesh carbon–formvar-coated grid and excess fluid withdrawn with filter paper. Immediately on drying, the specimen was examined in a Philips 300 electron microscope at a plate magnification of x 60000.

Controls run in parallel with this system were: (i) addition of normal chicken serum to the bursal suspension in place of convalescent serum; (ii) addition of convalescent serum to normal bursal suspension; (iii) two unrelated viruses – the avian adenovirus, chick embryo lethal orphan (CELO, Phelps strain) and poliovirus type 1 (Brunhilde strain) – were mixed in a test-tube. A mixture of antisera to these two viruses was added as before and processed in the same way.

RESULTS

Immunodiffusion

The agar-gel system used has been found to yield two specific lines with IBD positive bursae. All of the bursae used in the present study gave two precipitin lines showing identity to those present in a control positive antigen. Of the two sera employed, the recovery serum from the field consistently gave better results. Serum obtained from laboratory-immunized
Fig. 1. An aggregate, typical of those found in each of the homogenates prepared from bursae infected with infectious bursal disease after addition of recovery serum. Two different types of particle, each having virus-like morphology, are present. The larger particle has a diam. of 60 nm and a distinct hexagonal outline but does not fit into any established virus group. The smaller particle has a diam. of approximately 18 nm and is morphologically similar to the parvovirus group.
Infectious bursal disease

Birds was of lower titre and showed some non-specific precipitin activity, presumably against bursal proteins present in the original inoculum.

Electron microscopy

Examination of bursal homogenates to which antiserum had been added revealed numerous aggregates containing particles with a typical virus morphology (Fig. 1). However, the aggregates were unusual in as much as they were made up of particles showing two distinct morphologies and sizes. The larger particle has a diam. of 55 to 60 nm and is markedly hexagonal in outline. Regularly arranged subunits could be seen on the surface of these particles, but it was not possible to resolve the actual number building up the capsid.

The smaller particle has a size-range of 18 to 22 nm and displays an appearance similar to adeno-associated viruses (AAV). Subunits could occasionally be resolved (Fig. 2) and the arrangement appears similar to that present in the bacteriophage φ×174 (Hall, MacLean & Tessman, 1959). This in turn would mean that this small particle would have 12 subunits, one at each apex of an icosahedron.

High-power magnification of the aggregates containing the two types of particle showed that it was possible to visualize individual antibody molecules forming bridges between large and small particles (Fig. 3).

Examination of the control specimens to which normal chicken serum has been added revealed randomly distributed 60 nm particles (Fig. 4), but the smaller particle could not be recognized with certainty without antibody mediated aggregation. Normal bursa treated with recovery serum showed a total absence of any type of virus particle. The mixture of poliovirus and adenovirus, to which a mixture of homologous antisera to both viruses had been added, yielded aggregates composed of only one virus type, and in no case was a mixed aggregate of both types of virus seen (Fig. 5).

Discussion

In the present study we have been able to examine infected bursal material from three clinical outbreaks. In each instance the electron microscope revealed that these isolates contained not one but two types of virus particle. It could be argued that occasionally a virus-infected organ may be super-infected by an unrelated virus which is irrelevant to the

Fig. 2. A higher-power micrograph of the smaller particles. There is very little antibody present and it is possible to visualize subunit arrangement. The two particles indicated by arrows show four subunits arranged in a diamond formation. This arrangement is present on particles having 12 subunit construction and has been shown for the parvoviruses.

Fig. 3. A small group of large and small particles linked by antibody. The arrow indicates an area where single antibody molecules can be seen joining large and small particles. This finding is put forward as evidence that the large and small particles found in association with infectious bursal disease have at least one surface antigen in common.

Fig. 4. When infected bursal homogenate was examined without the addition of antiserum it was extremely difficult, if not impossible, to recognize the smaller particle. However, the larger particle, as shown here, could still be recognized with ease.

Fig. 5. It could be postulated that the type of mixed aggregate shown in Fig. 1 could result from the presence of two particle types in the homogenate which would non-specifically become mixed in the final aggregate. This was controlled by mixing CELO and polio virus in one tube and then adding mixed antibodies to both viruses. No mixed aggregate could be found. (a) Shows a group consisting solely of poliovirus particles while (b) is composed entirely of the adenovirus. These two micrographs are present on the same photographic plate but have been presented as two separate micrographs.
clinical condition. However, it seems extremely unlikely that such a contamination would occur repeatedly at such widely distributed locations and over a period of three years. In addition, the mixed aggregates, found on examination of each isolate, point to the fact that the two viruses are antigenically related.

The control experiment, using a mixed preparation of an avian adenovirus and poliovirus, shows that when no antigenic relationship exists, mixed aggregates are not obtained. That an antigenic relationship exists between the 60 nm and 22 nm particles of IBD is further supported by the finding of bridging molecules between them, since it is a basic fact of immunology that the IgG antibody molecule has two identical binding sites, and that linkage of two structures by antibody is proof that there are identical binding sites on each of the structures (Almeida & Waterson, 1969).

The large particle associated with infectious bursal disease has a diam. of approximately 60 nm and, is, therefore, considerably smaller than adenovirus, but the appearance of the two particles described above is similar to that of adenovirus and its associated satellite viruses (Mayor et al. 1965). For the adeno system it is known that the satellite virus is genetically incomplete and is incapable of replicating unless adenovirus is present. However, there does not seem to be any serological relationship between the capsids of adenovirus and the satellite (Hoggan, 1970). This leads to the interesting speculation that the particles found in association with IBD may be a virus and dependent satellite, with the satellite sharing at least some structural proteins with the larger particle. It is clearly a matter of interest to discover whether infectious bursal disease can be produced by the larger particle alone or whether the small particle is obligatory.

Examination of the particle morphology reveals that the 60 nm particle structure is built up of distinct subunits and that the overall form is clearly hexagonal. It has been shown that cubic viruses possess icosahedral symmetry, but only display icosahedral form when there is a considerable number of subunits (Almeida, Waterson & Fletcher, 1966). For example, adenovirus contains 252 subunits and Tipula iridescent virus an even greater number and both of these viruses have well-marked icosahedral form. On the other hand, viruses, such as feline picornavirus, which has 32 subunits, appear spherical (Almeida et al. 1968). The larger particle of IBD displays a marked hexagonal outline which implies a relatively large number of subunits. However, in conjunction with this fact, it has an overall diam. of less than 65 nm, so that it will be difficult to determine the actual number of subunits. Previous reports on the morphology of the virus associated with IBD have described it as a reovirus (Cheville, 1967; Petek & Mandelli, 1968; Rinaldi et al. 1970; Kosters et al. 1972). However, a reovirus has a distinct double-layered capsid while the present virus has only one shell; indeed, the 60 nm particle does not seem to belong to any established morphological group. However, trout pancreatic necrosis virus shows a morphology similar to the large particle described here although the trout virus has a diam. of 74 nm, significantly larger than the one described in this study (Kelly & Loh, 1972).

The 18 to 22 nm particle displayed a morphology closely similar to the paroviruses and in several instances it was possible to resolve the pattern associated with a 12 subunit particle. It is of interest in this respect that in the Results section we described the smaller particle as having an appearance similar to the bacteriophage φ×174, while Mayor et al. (1965) described the adeno-associated viruses as also being similar to φ×174. Although only circumstantial evidence, it nevertheless strengthens the view that in IBD we are looking at a virus-satellite system similar to that found with adenoviruses.

Finally, it is of interest that there have been conflicting reports on both the appearance and properties of the infectious bursal agent. Most frequently it has been described as a
Infectious bursal disease

reovirus, but it has also been suggested that it could be a picornavirus (Cho & Edgar, 1969). These conflicting views could be resolved by the present finding that infectious bursal disease has associated with it not one, but two virus particles. From both virological and immunological viewpoints, these two viruses could form a unique system because, although morphologically dissimilar, they, nevertheless, appear to have surface antigens in common.

REFERENCES


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