CORONAVIRUSES, A NEW GROUP OF ANIMAL RNA VIRUSES

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SUMMARY

In the last few years, strains have been isolated from various human common cold epidemics which resemble avian infectious bronchitis virus (IBV) so much, both morphologically and in other properties, that they have been called IBV-like viruses. It has also been found that the mouse hepatitis virus is morphologically identical with them.

All these viruses seem to constitute a group of their own and are known to possess at least the following common properties: 1) size 80–160 mμ; 2) characteristic surface structure; 3) sensitivity to lipid solvents; 4) ribonucleic acid content; 5) low density of infectious units (1.18–1.19 in sucrose gradient); 6) development in the cytoplasma by budding into cisternae or vesicles.

The nucleocapsid structure of these viruses is still unknown. Because of the characteristic appearance, recalling the solar corona, the name coronaviruses has been suggested. Besides avian infectious bronchitis virus and mouse hepatitis virus, this virus group at present includes five IBV-like human strains: B814, 229E, OC43, LP, and EVS.

Some comparisons in cross-neutralization tests have been made among the viruses of the group. The results showed that they all seem to belong to distinct serotypes. However, it was necessary to use several neutralization methods since no common method applicable to all the strains was available. Hence, conclusions based on those results must be treated with reserve. First, the adequate serological studies can disclose how closely related the viruses are and whether there is a speculative possibility that the IBV strains which are ubiquitous in almost all poultry populations can serve as reservoirs for human common cold infections, e.g. through mutation of IBV or hybridization of animal and human coronaviruses.

Avian infectious bronchitis virus (IBV) attracted the interest of veterinary virologists only a few years ago. It was the only one of the known agents causing respiratory infections in poultry that was difficult to classify. On the basis of its chemical and physical properties it was generally thought to be closest to the myxoviruses but outside the influenza and paramyxovirus subgroups (16).

The electron-microscope technique of negative staining (6,18) reveals, however, that the virus particles have a structure which differs from all the viruses then known. The main difference is in the projections of the virus, which are considerably fewer than in the influenza virus or other known myxoviruses. The projections are club- or pear-shaped, their outlines are vague and "smudged" in the electron microscope, and they are distributed fairly uniformly around the circumference of the virus particles.

IBV now has a more general interest, for in the last few years strains have been isolated from various human common cold epidemics (32,21,25) which resemble IBV so much both morphologically and in other properties that they have been called IBV-like viruses (25,26). It has also been found that the mouse hepatitis virus (MHV) is morphologically identical with IBV and IBV-like viruses (5,32).

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1) Size, 80–160 mμ
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The nucleocapsid structure is still unknown. Because of the characteristic appearance, recalling the solar corona, the name coronaviruses has been suggested by a group of virologists (J. D. Almeida; D. M. Berry; C. H. Cunningham; D. Hamre; M. S. Hofstad; L. Mallucci; K. McIntosh; D. A. Tyrrell). This suggestion has been accepted by the members of the Myxovirus Study Group under the International Committee for the Nomenclature of Viruses.

True IBV causes a disease known as avian infectious bronchitis. The disease has been known for a long time. The first report

on it was published in 1931 (30), and the virus etiology of the disease was suggested in 1933 (10) and was established conclusively in 1936 (3). In its classical form, the disease is peracute, causing bronchitis, tracheitis, and sinusitis in chicks aged 1–3 weeks, and mortality can be 40–90%. The disease affects adult birds also, though the mortality is negligible and the symptoms disappear in 8–14 days (4,15). Some IBV strains can cause nephritis and nephrosis in addition to respiratory symptoms (12,34). The disease may occur in chickens also in such a clinically mild form that the only observation made is a decrease in egg production. The disease pattern may be changed considerably by secondary bacterial infections.

It has now been found that IBV is ubiquitous in its distribution in many European countries, and precipitating antibodies can be demonstrated in most poultry flocks with gel diffusion precipitation (2,16,19,20,35,36). About 10 different serotypes have been established in cross neutralization tests with the IBV strains isolated up to the present (24). There appears to be some cross-immunity between IBV strains, although neutralization tests do not demonstrate it (23). All the strains have common precipitinogens (35). A hemagglutinin associated with the virus has been described (7), but it has not been possible so far to demonstrate specific inhibition of these hemagglutinations when using chicken anti-IBV serum (13). The suitability of the complement fixation test has been studied (31), but it has been disregarded in the serology of IBV, perhaps for the simple reason that since avian antiserum ordinarily does not fix complement, the direct complement fixation test is not suitable.

IBV has been found so far to cause disease naturally only in chicken. This is in contrast to some other avian respiratory diseases, such as Newcastle or psittacosis, which can produce diseases in humans. No clinical infection of humans with IBV has been reported. A report was recently published on neutralization of IBV by human sera (28). In this study, the sera of one group of individuals who were associated with poultry and a control group with limited poultry associations were tested for their ability to neutralize IBV. Reactors were found only among individuals who worked closely with poultry, and the percentage of reactors in this group was 41.4. These findings indicate that the reactors were infected with IBV or with another agent antigenically related to IBV. The former possibility seems to be more probable since only

the sera of the individuals associated with poultry were positive. More reliable conclusions can be drawn after cross-serological studies of positive human and avian sera.

The report by Hartley *et al.* (22) can probably be regarded as the first study to indicate the occurrence of human infections caused by coronaviruses. They performed serological tests on military personnel at three posts in different parts of the USA. The specimens were taken in connection with respiratory epidemics in the winter months. The paired sera examined revealed that 21.8% of the patients had developed a fourfold or greater rise in complement fixation antibody to MHV. The number of serologically positive cases at the three posts varied between 17.0 and 24.1%. The reliability of the results was confirmed with plaque neutralization tests in cell cultures. The controls were negative for the ordinary human respiratory viruses. The authors concluded that the results they obtained may be due to a cross reaction between MHV and a known human respiratory virus. Some possibilities propounded were that the servicemen might have contracted MHV infection from mouse excrement, or that the vaccines used on them were contaminated with MHV. Finally, it was suspected that the reactions might be due to a still unknown human virus which would be closely related serologically or belong to the same group as MHV. This last-mentioned theory appears more probable, especially as both MHV and human IBV-like viruses have since been proved to belong to the same group. Morris *et al.* (29) made a serological study of MHV and the virus isolated from human patients with African epidemic icterus (AEI), and assumed that infections with AEI or a closely related virus might account for the frequent presence of MHV antibodies in human sera. To the best of my knowledge, AEI virus has not yet been properly characterized and classified.

Tyrrell and Bynoe (33) isolated the first human strain of the corona group from a typical case of the common cold by using human embryonic tracheal and nasal organ cultures. This strain, B814, produced colds in volunteers but could not be detected or propagated in cell cultures. A year later, a report was published by Hamre and Procknow (21) on the common cold epidemic raging in the winter of 1962 among students of Chicago University. Those workers isolated from five patients and one healthy person a medium-sized ether-labile virus strain, 229E, by using secondary human embryo kidney cells. All the patients from whom the virus

was isolated also showed a fourfold or greater antibody rise in complement fixation and neutralization tests. Strain 229E was subsequently adapted to the WI-38 strain of human embryo fibroblasts. In comparative studies (1), B814 and 229E proved morphologically identical to each other and to IBV, but inoculations of volunteers provoked no antibody increase against 229E in sera from volunteers who developed colds after inoculation with B814 (9). It can be concluded from this that these two human strains belong to different serotypes.

In addition to the two human virus strains mentioned above, additional viruses with the same characteristics were isolated in organ cultures by McIntosh *et al.* (25). The isolations were made in the winter of 1965–1966 during an acute upper respiratory illness among employees of the National Institute of Health in Bethesda. Six virus strains were recovered; they bore a close morphological resemblance to IBV. Two of them were successfully adapted intracerebrally in suckling mice. Using mouse brain antigen, paired sera from 59 patients were tested by the complement fixation test. Eighteen of these individuals (including the five from whom the virus strains were isolated) developed a fourfold or greater rise to the two mouse brain-adapted IBV-like viruses, which were shown to be serologically identical.

The same team (5) also reported that MHV was morphologically identical with the members of the IBV-like group and that mouse immune sera to MHV fixed complement in the presence of the antigens of the isolated IBV-like virus. The reactions were two- or fourfold lower than those seen with the homologous virus strain. The MHV had been found previously to be ether-labile and RNA-containing, and to develop by budding into cytoplasmic cisternae (11,14,27). Comparison in cross-neutralization tests of strain 229E, the above-mentioned mouse-adapted IBV-like virus OC43, MHV, and IBV, showed that they all belonged to distinct serotypes (26). However, it was necessary in this study to use several methods for the neutralization tests since no common method was available that was applicable to all the strains. Hence, conclusions based on the results must be treated with reserve.

It will be apparent from the foregoing that different methods have been used to propagate human coronaviruses. Most of the isolates have been propagated only in organ cultures, while cell cultures have failed. However, viruses of type 229E multiply in cell cultures of human embryo kidney cells and human embryo lung

fibroblasts. It was recently established that a continuous human embryo lung cell line L132 seems to be suitable for isolation and propagation of all known human coronaviruses (8), including the recently isolated LP and EVS strains, which seem to have a morphology typical of coronaviruses. The strains produce a clearly discernible cytopathic effect in L132 cells about five days after inoculation. This observation has considerably improved the possibilities of serologic comparison of human coronaviruses.

On the other hand, it appears probable that animal coronaviruses are not adaptable, at least not easily, to L132 cells. Consequently it is hardly likely that we can find a cell culture method which is suitable for the culture of all coronaviruses. This will limit somewhat their serological comparison. There is already some evidence that cross-reactions between individual viruses may exist and that not all members of the group are necessarily species-specific (17,22,28). The serology of viruses of the corona group doubtless constitutes a highly interesting investigation object.

Coronaviruses do in fact constitute a group, and the most rewarding way of studying them is undoubtedly through working teams that include both veterinary virologists and specialists in human coronaviruses.

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