Veterinary Research Communications, 11 (1987) 109-118
Geo Abstracts Ltd, Norwich - printed in England

STRAIN SPECIFIC ANTIBODIES REVEALED BY IMMUNOABSORPTION STUDIES WITH AVIAN INFECTIOUS BRONCHITIS VIRUS

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(Accepted: 28 April 1986)

ABSTRACT

Collins, M.S. and Alexander, D.J. 1987. Strain specific antibodies revealed by immunoabsorption studies with avian infectious bronchitis virus. Vet. Res. Commun., 11: 109-118

Rabbit antisera prepared against the Massachusetts 41 (M41) strain of avian infectious bronchitis virus (IBV) and absorbed with chick embryo immunoabsorbent produced multiple precipitin lines in immunodouble-diffusion (IDD) tests with homologous or heterologous strains of virus. These precipitin lines were all removed by absorption with concentrated M41 virus preparations, but repeated absorption with concentrated, purified preparations of IBV strains: T, Holte, Connecticut, Beaudette or H120 failed to remove all precipitin lines produced to M41 virus, although all those to the heterologous viruses were removed. The remaining line(s) produced with M41 virus by sera absorbed with different heterologous viruses showed identity in IDD tests and was associated with the surface projections of the virus.

INTRODUCTION

Vaccination of chickens against avian infectious bronchitis virus (IBV), aimed at controlling the economically important disease affecting the respiratory and reproductive systems, is used universally where domestic fowl are farmed intensively. However, many problems have been experienced in selecting strains of IBV for use as live or inactivated vaccines due to the confused and complex situation in assessing serotypes of IBV. Generally, it is considered that numerous serotypes of IBV exist, in that isolates may, on serological findings, be placed into

0165-7380/87/$3.50

groups, but members of these serogroups may show cross relationships with strains ostensibly of other serotypes (Bracewell, 1973; Cowen and Hitchner 1975; Darbyshire et al, 1979; Hofstad, 1981; Hopkins, 1974, Wadey and Faragher, 1981) while, more recently, considerable problems have been reported in Holland due to the emergence of variant strains of IBV (Davelaar et al, 1983).

The extremely complex nature of the immunologically detectable relationships between the various strains and isolates of IBV appears to reflect a situation in which several antigens or antigenic determinants are involved in the immune response to IBV and the degree to which these are shared by different viruses governs the level of serologically detectable homology between two viruses. The object of the present paper is to determine if shared and distinct antigens can be detected between strains by immunodoublediffusion reactions with absorbed precipitating antisera.

MATERIALS AND METHODS

Viruses

The method of growth, in embryonated fowls' eggs, source and passage history of the IBV strains used have been described (Collins and Alexander, 1980a). The passage levels of the six strains: Massachusetts 41 (M41), Connecticut, T, H120, Holte and Beaudette used were 6, 9, 4, >120, 20 and >250 respectively.

Virus purification

Viruses were purified by differential centrifugation and sucrose density gradient centrifugation as described (Collins and Alexander, 1980a).

Dissociation of virus antigens

Purified virus preparations containing about 1mg protein/ml were made 4% (v/v) with respect to Triton X-100 (BDH Scintillation grade) and 1.0M with respect to potassium chloride and shaken gently for one hour at 4°C to dissociate antigens for sucrose gradient separation or immuno-doublediffusion analysis.

Antisera

Antisera to IBV strains were raised in New Zealand white rabbits by the injection of 1ml purified virus, 0.5-1.0mg protein, mixed with an equal volume of incomplete Freund's adjuvant subcutaneously above ths capula and in the hind quarters of each rabbit. Five further immunizations were done at two week intervals.

Sera were treated at 56°C for 30 minutes before absorption procedures.

Absorption of sera

An immunoabsorbent prepared from tissue homogenates of 9-10-day-old embryonated fowls' eggs by the method of Ternynck and Avrameas (1976) was used to absorb any rabbit-anti-chicken antibodies present in the sera.

Antibodies to antigens shared by various virus strains were absorbed by treatment of sera with purified virus preparations, concentrated about 300-fold from the original allantoic fluids. A 0.8ml volume of purified virus, containing about 2-3mg protein per ml was pelleted and resuspended in 0.4ml of anti-serum. The mixture was stored at room temperature for one hour and then centrifuted at 16000g for 45 minutes to remove virus. This was repeated at least three times for each absorbing virus and for the final step the virus - antiserum mixture was left overnight at 4°C.

Immunodoublediffusion (IDD) tests

IDD tests were done in one % (w/v) agarose gels (HSA type : Litex, Denmark) containing one % (v/v) Triton X-100 in phosphate buffered saline pH7.2 (PBS). Gels were cast to a depth of 1.5mm on 2.5 x 7.5cm microscope slides and a plastic template was used which enabled 25µl amounts of antigen and sera to be presented to 0.8mm diameter wells which were 5mm apart. After adding the samples, gels were left for 24 hours at room temperature then washed thoroughly in PBS with a final wash in distilled water before drying and staining with Coomassie brilliant blue.

Sucrose gradient separation

Dissociated virus antigens were separated by application to a 20ml 15-55% (w/w) sucrose gradient in 0.01M TRIS-HCl buffer pH7.2 containing 1% Triton-X-100 and 1.0M KCl. Centrifugation was for 60 hours at 100,000g and 20 fractions were taken from each gradient.

RESULTS

Immunodoublediffusion (IDD) in agarose gels with M41 antisera prepared in rabbits, run against purified virus treated with Triton X-100 and KCl produced multiple precipitin lines to both homologous and heterologous viruses (Fig 1). All precipitin lines were removed by treatment of M41 antiserum with concentrated M41 virus, but absorption of M41 antiserum with T strain virus concentrate resulted in diminution of the number and intensity of the precipitin lines to both viruses with each absorption step, until only two precipitin lines remained against M41 virus and none were seen with T strain. The presence and intensity of these lines were unaffected by further absorption steps. Similar results obtained by absorption with Connecticut and H120 viruses are shown in Fig 1. Exhaustive absorption with these viruses and strains Holte and

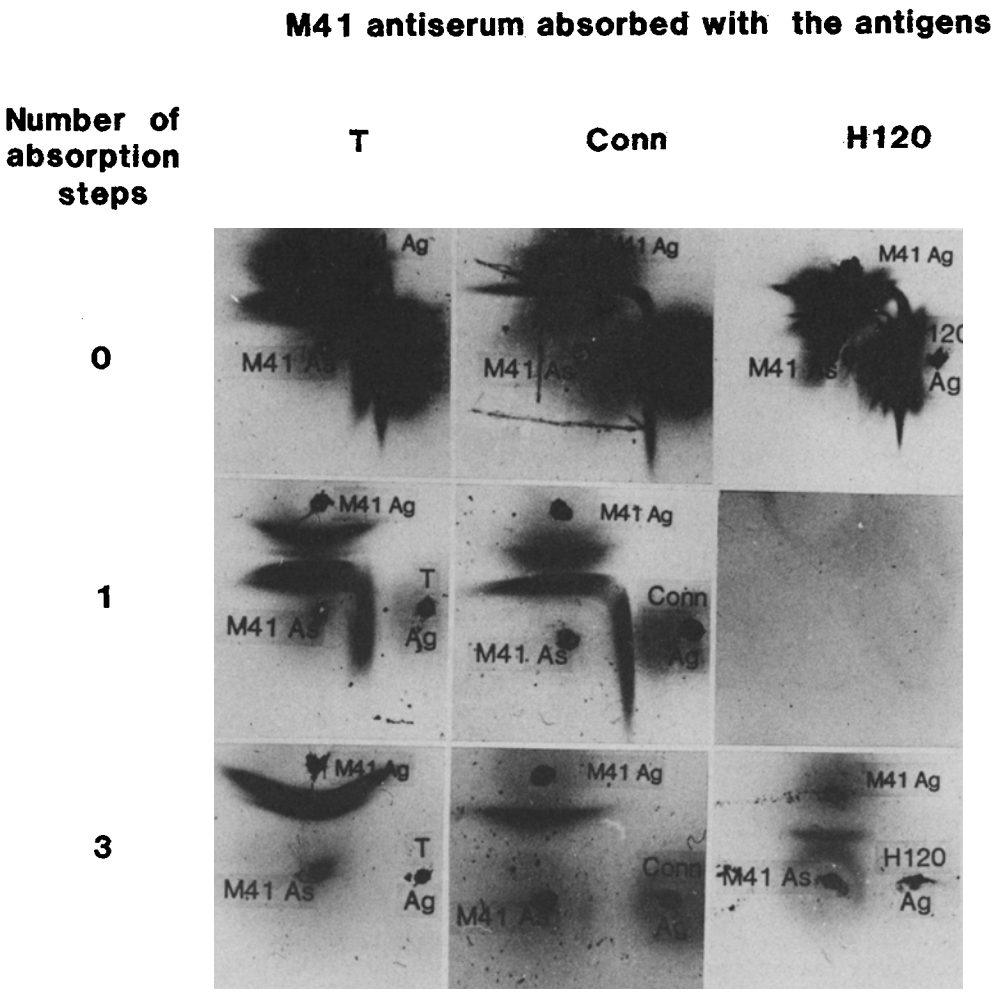

Figure 1. Effect of absorption of rabbit antiserum raised against Massachusetts 41 with heterologous IBV strains on immunodoublediffusion against the homologous strain. M41 = Massachusetts 41, T, Conn = Connecticut, H120

Beaudette (results not shown) resulted in only one precipitin line to M41 being apparent in most gels. However, occasionally the precipitin line seen between M41 virus and antiserum absorbed with these viruses appeared to represent two very closely situated lines similar to those with T strain absorbed serum.

The remaining precipitin lines to M41 in sera absorbed by heterologous viruses showed identity regardless of the viruses used for absorption (Fig 2), indicating that the variations detectable between the different strains used and M41 were associated with the same antigen or antigens.

Collins and Alexander (1980b) were able to isolate M41 surface projections by solubilization of purified virus with Triton X-100 and KCl and separation by sucrose density gradient centrifugation. In the present study a modification of this method was used which resulted in pelleting the densest material through the gradient but gave a wider spread of the other material throughout the gradient. Examination of the fractions from sucrose gradient centrifugation of disrupted M41 by negative contrast electron microscopy showed the small peak between fractions 7-10 as consisting of rosettes of surface projections, while fractions corresponding to the less dense material consisted of amorphous material. IDD results of the fractions taken from a typical gradient of solubilized M41 virus against M41 antiserum absorbed with T strain antigen are shown in Fig 3. Precipitin lines were seen with fractions 7-9 which corresponded to the peak containing the surface projections. Fraction 12, which contained the densest part of the larger protein peak also gave a precipitin line which showed identity with the line produced by fraction 9. A further precipitin line was produce by fractions 12-16 which was distinct from the line produced by fractions 7-9. Similar results were obtained in IDD tests with a similar sucrose gradient of disrupted M41 virus and M41

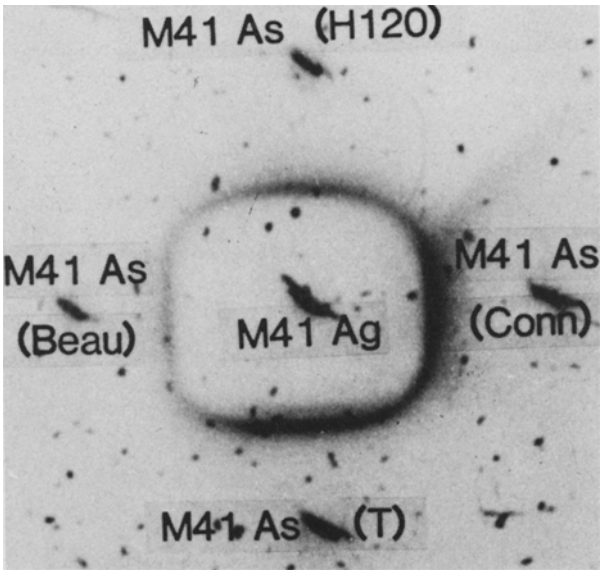

Figure 2. Identity of detectable variation seen in immunodoublediffusion tests with M41 antigen against M41 rabbit antiserum absorbed X3 with the virus strains indicated:- H120, Beaudette, Connecticut and T.

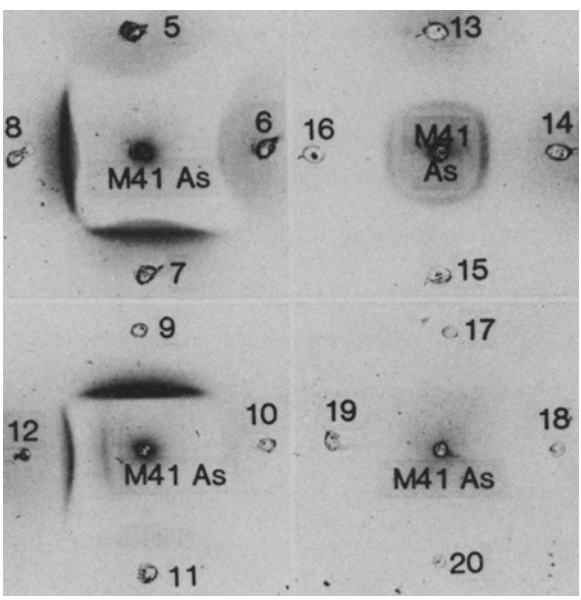

Figure 3. Immunodoublediffusion of fractions from sucrose density gradient centrifugation of disrupted M41 virus against M41 rabbit antiserum absorbed X3 with T virus, numbers indicate fractions. Electron microscopy revealed surface projections in fractions 7-10

antiserum absorbed with Connecticut virus (results not shown).

DISCUSSION

The results obtained in the present study indicate that while a considerable number of antigens of the different strains of IBV may be identical, one, or two, of the virus antigens has at least one antigenic site that is distinct for the different strains. IDD using absorbed antisera and fractions from a sucrose gradient in which the surface projections were separated from other viral material indicated that the antigen responsible for this specificity is associated with the surface projections which would be in keeping with the major role of these in haemagglutination and neutralization (Mockett et al, 1984).

One unexpected result from the present study was the evidence for antigenic variation among viruses that are considered to be of the same serotype i.e. M41, H120 and Beaudette. Clearly, if the surface projections are important antigens in protective immunity, some antigenic sites must be shared by these viruses and account for the serological relationshops seen in neutralization and cross-protection studies, but the variation reported here are in keeping with those detected by more stringent methods of detecting variation such as oligonucleotide fingerprinting (Clewley et al, 1981) and monoclonal antibodies (Mockett et al, 1984). Similarly these results are consistent with the findings of Holmes and Finney (1985) that higher haemagglutination inhibition titres could be detected in vaccinated hens using the homologous H120 strain as antigen than using the M41 strain as antigen.

The results in the present study may offer some explanation for the frequently confusing serological relationships reported between different strains of IBV. Cross relationships between viruses representing osten-

sibly different serotypes ("group titres") may be due to antigens other that those associated with the surface projections, whereas variation within a serotype ("type titres") may be related to the degree of relationship of antigenic determinants on the surface projections.

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