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Serological Relationships of the Subcomponents of Human Coronavirus Strain 229E and Mouse Hepatitis Virus Strain 3

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SUMMARY

Antibodies were raised in rabbits against the structural components of human coronavirus strain 229E and mouse hepatitis virus strain 3, prepared from disrupted virus particles. Hyperimmune sera to the subcomponents showed cross-reactions by enzyme-linked immunosorbent assay between ribonucleoprotein antigens of these viruses, indicating the presence of a common antigen(s). None of the other virus structural components showed any cross-reactivity.

The Coronaviridae are a group of large, enveloped viruses ranging in diameter from 80 to 200 nm and possessing distinctive club-shaped surface projections of up to 20 nm in length (McIntosh, 1974; Tyrrell *et al.*, 1978). They all have a ribonucleoprotein (RNP) complex containing a single high mol. wt. single-stranded RNA genome and protein subunits of mol. wt. about 50000. The antigenic relationships of these viruses is complex, although antigenic groupings have been attempted (Pedersen *et al.*, 1978). Two coronaviruses, human coronavirus (HCV) strain 229E and mouse hepatitis virus (MHV) strain 3, have been shown to be serologically distinct (McIntosh *et al.*, 1969; Pedersen *et al.*, 1978; Kraaijeveld *et al.*, 1980). Treatment of purified virus particles with Nonidet P40 (NP40) followed by sucrose density gradient centrifugation resulted in the separation of virus structural components, comprising surface projections, membrane and RNP components (Hasony & Macnaughton, 1981; Macnaughton *et al.*, 1981). This has permitted the preparation of antisera specific for each of these subviral components. In this paper we have used an enzyme-linked immunosorbent assay (ELISA) to study the antigenic relatedness of the subcomponents of HCV 229E and MHV 3.

HCV 229E and MHV 3 were grown and purified as previously described (Macnaughton & Madge, 1978; Macnaughton *et al.*, 1978). Virus structural components comprising surface projections, envelope and RNP were prepared from purified, disrupted virus particles and separated on sucrose density gradients as described previously (Hasony & Macnaughton, 1981; Macnaughton *et al.*, 1981). Subcomponents were shown to be uncontaminated with the other components by labelling their polypeptides with ^{125}I and analysing them on polyacrylamide gels (Hasony & Macnaughton, 1981; Macnaughton *et al.*, 1981). Antisera to the isolated structural components were raised in rabbits as previously described (Kraaijeveld *et al.*, 1980) with simple modifications. Briefly, antiserum was raised using 0·5 ml of purified dialysed virus subcomponents emulsified in an equal volume of complete Freund's adjuvant (CFA) and injected intracutaneously at up to 20 different sites in to the shaven back of a rabbit. Two days before immunization animals received 0·5 ml *Bordetella pertussis* vaccine (Lister Institute of Preventive Medicine, Elstree, U.K.) intracutaneously as an additional adjuvant. Each animal was boosted twice with similar doses by weight of virus components in CFA 4 and 5 weeks after the initial immunization. Rabbits were bled both before and 7 weeks after immunization. HCV 229E antiserum was absorbed with an equal volume of newborn calf serum and MHV 3 antiserum was absorbed with an equal volume of foetal calf serum before use. Sera were stored at −20 °C. The ELISA procedure used has been described previously (Kraaijeveld *et al.*, 1980). An immune serum was considered to be positive for specific

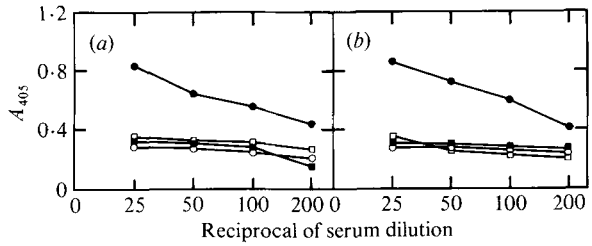

Fig. 1. ELISA of purified RNP against dilutions of rabbit sera to homologous subcomponents. (*a*) HCV 229E RNP and (*b*) MHV 3 RNP. ●, Antiserum to RNP; ○, preimmune antiserum; □, antisera to envelope; ■, antiserum to surface projections. All readings were taken at 405 nm after 20 min.

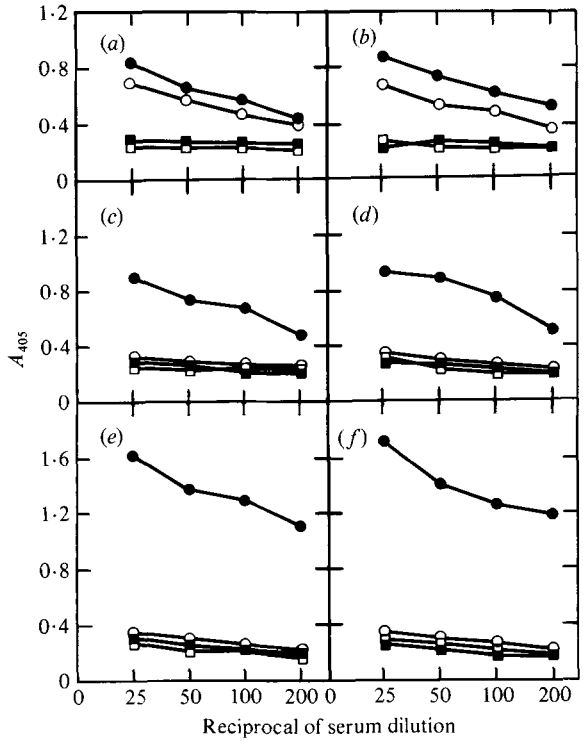

Fig. 2. ELISA of purified subcomponents against dilutions of homologous and heterologous subcomponent rabbit sera. (*a*) HCV 229E RNP; (*b*) MHV 3 RNP; (*c*) HCV 229E envelope; (*d*) MHV 3 envelope; (*e*) HCV 229E surface projections; (*f*) MHV 3 surface projections. ●, Homologous subcomponent antisera; ○, heterologous subcomponent antisera; ■, preimmune homologous subcomponent antisera; □, preimmune heterologous subcomponent antisera. All readings were taken at 405 nm after 20 min.

antibodies when the ratio of the immune to preimmune serum absorbance values at the same dilution was 2 or more.

The reactions of HCV 229E structural components with antisera to HCV 229E components and of MHV 3 structural components with antisera to MHV 3 components were determined by ELISA. Fig. 1 (*a*) shows the reaction of HCV 229E RNP against antisera to HCV 229E structural components and Fig. 1 (*b*) the reaction of MHV 3 RNP against antisera to MHV 3 structural components. In both cases a positive reaction was observed between RNP and antisera to RNP, but not between RNP and antisera directed against the

other structural components. Similar results were obtained using purified membrane and surface projection components with antisera directed against the structural components: positive reactions were only observed between a subcomponent and its homologous antisera. These results show that there was no contamination detectable by our ELISA of the components or antisera.

Chequerboard titrations were done to obtain dilutions of antigen and antisera which produced comparable absorbance values by ELISA (Kraaijeveld *et al.*, 1980). These dilutions were then used for the detection of the cross-reactivity of the different virus structural components with homologous and heterologous sera. Typical experiments are shown in Fig. 2. The results of ELISAs of antisera to HCV 229E and MHV 3 RNPs against HCV 229E RNP (Fig. 2*a*) and MHV 3 RNP (Fig. 2*b*) show that in both cases there was a positive reaction with the heterologous RNP as well as the homologous RNP. The reactions were significantly above the level with preimmune sera, although the heterologous reactions were lower than the homologous reactions. Antisera to HCV 229E and MHV 3 RNPs did not react by ELISA against purified HCV 229E RNA isolated as described previously (Macnaughton & Madge, 1978), indicating that the cross-reactions were not due to RNA components of RNP. ELISAs of antisera to envelope against HCV 229E (Fig. 2*c*) and MHV 3 (Fig. 2*d*) envelope, and antisera to surface projections against HCV 229E (Fig. 3*e*) and MHV 3 (Fig. 2*f*) surface projections, produced no significant heterologous reactions. In these cases, the heterologous reactions were similar to the preimmune sera, although there were strong homologous reactions.

We have previously reported no cross-reactions between HCV 229E and MHV 3 by ELISA (Kraaijeveld *et al.*, 1980). In that study, only external components were examined as the ELISA plates were coated with whole virus particles and the sera produced in rabbits was, presumably, mainly directed against the surface projections, as the envelope and RNP components of HCV 229E and MHV 3 are not good immunogens in rabbits (Hasony & Macnaughton, 1981; Macnaughton *et al.*, 1981). We have confirmed these results in that no cross-reactions were observed in heterologous membrane and surface projection ELISA reactions. However, a clear antigenic relationship has now been revealed between the RNPs of HCV 229E and MHV 3, indicating that they contain some common antigenic determinant(s). This confirms and extends the work of Yaseen & Johnson-Lussenburg (1978) who reported that HCV 229E RNP could be detected by immunodiffusion using antisera to MHV and the porcine coronaviruses, transmissible gastroenteritis virus and haemagglutinating encephalomyelitis virus. Similar cross-reactions have been observed between the RNPs of other virus groups, such as orthomyxoviruses and rhabdoviruses. Further studies are in progress to analyse the serological relationships between the subcomponents of other coronaviruses and to relate these results to those obtained for other virus groups.

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