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Two Antigenic Groups of Human Coronaviruses Detected By Using Enzyme-Linked Immunosorbent Assay

MALCOLM R. MACNAUGHTON,[1]* M. HILARY MADGE,[1] AND SYLVIA E. REED[2]†

Division of Communicable Diseases, Clinical Research Centre, Harrow, Middlesex HA1 3UJ,[1] and Common Cold Unit, Harvard Hospital, Salisbury, Wiltshire SP2 8BW,[2] England

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Paired sera from volunteers inoculated with one of five recently isolated strains of human coronavirus (HCV), AD, GI, HO, PA, and RO, none of which has been grown in tissue culture, or with strain OC38 were tested against coronavirus antigens by enzyme-linked immunosorbent assay. When HCV strains OC43, 229E, or the 229E-related tissue culture-adapted strains PR and TO were used as antigens, it was shown that all strains fell into one of two antigenic groups. The HCV OC43 group was comprised of strains OC43, GI, HO, and RO, and the HCV 229E group contained strains AD and PA as well as the tissue culture-adapted strains PR, TO, and KI. Enzyme-linked immunosorbent assay of the paired sera with the coronavirus mouse hepatitis virus strain 3 as antigen confirmed the relationship of this virus to the HCV OC43 group but not to the HCV 229E group.

Although infections with human respiratory coronaviruses are probably very common (5, 8), the serogroups of these human coronaviruses (HCVs) are poorly defined. Strains that grow in tissue culture have been found to be serologically related to HCV 229E, whereas the organ culture-grown strains may comprise more than one group, sometimes related to HCV OC43, to the murine coronavirus mouse hepatitis virus (MHV) strain 3, and perhaps also to HCV 229E (2, 4, 11).

We have previously reported an enzyme-linked immunosorbent assay (ELISA) for the measurement of antibody rises in sera from volunteers inoculated with four HCV strains (4) which could be cultivated in tissue culture (5). Sera of volunteers inoculated with heterologous or homologous strains showed antibody rises against HCV 229E and related viruses used as antigens. This study has now been extended to include the use of HCV OC43 and MHV 3 and other coronaviruses as antigens and paired sera from volunteers infected with five coronaviruses recently isolated in organ culture but not so far grown in tissue culture. The antigenic relationships of these HCVs to one another and to MHV have been determined.

MATERIALS AND METHODS

Viruses. Two prototype HCV strains, 229E (3) and OC43 (10), and seven HCVs isolated from the nasal washings of subjects with natural colds (5) were used. The seven isolates were identified as HCVs by electron microscopy after passage in tracheal organ cultures or after inoculation of volunteers followed by passage in tissue or organ cultures (5). Two of these isolates, HCV PR and HCV TO, were readily adapted to growth in monolayer cultures of MRC continuous cells (5), whereas the other five isolates, HCV AD, HCV GI, HCV HO, HCV PA, and HCV RO, could only be passaged in human fetal tracheal and nasal organ cultures. Strain HCV OC38, obtained from K. McIntosh and reported to be serologically identical to HCV OC43 (9), was used instead of HCV OC43 for inoculation of volunteers.

Virus antigens. HCV 229E, PR, and TO antigens were prepared in MRC continuous cells by a method previously described (4), with minor modifications; the input multiplicity was one infectious particle per cell, and the cultures were maintained in 2% rabbit serum or fetal bovine serum and harvested 32 h after inoculation. Crude virus suspensions resulting from one freeze-thaw cycle and centrifugation at 4,000 rpm for 30 min and having infectivity titers of 10^6 to 10^7 plaque-forming units per ml (7) were used as antigens.

HCV OC43 antigen was grown in suckling mouse brains (9) and used as a 10% mouse brain suspension in Dulbecco phosphate-buffered saline "A." It contained 10^8 to 10^9 particles per ml by electron microscopy particle counts (7). The same virus was also grown to lower titers in BSC-1 cells as described by Monto and Rhodes (12).

Other HCV strains were grown in human fetal tracheal and nasal organ cultures as described previously (5). Media harvested 3 to 7 days after inoculation of the cultures were used as antigens in ELISA.

MHV 3 was grown in secondary mouse embryonic fibroblasts. The input multiplicity was one infectious particle per cell, and the cultures were incubated at 37°C for 72 h in Eagle minimal essential medium in

† Present address: Central Public Health Laboratory, London NW9 5HT, England.

2% fetal bovine serum. Cell suspensions were frozen, thawed, and clarified as described above. Supernatant fluids containing from 10^8 to 10^9 particles per ml as determined by electron microscopy (7) were used as antigens.

Sera. The methods used with adult volunteers taking part in experiments at the Common Cold Unit, Salisbury, England have been described previously (1, 5, 6, 13). The paired sera used in the present study were collected from volunteers who developed colds after an inoculation of HCV or from controls given a saline inoculation. Postinfection sera were collected about 3 weeks after virus inoculation. The experiments were approved by the Ethical Committee of Northwick Park Hospital, Harrow, England.

ELISA procedure. The ELISA used to detect antibody rises in human paired sera has been described previously (4, 6). When HCV 229E group antigens prepared in medium containing fetal bovine serum were used, the human sera were mixed with equal volumes of fetal bovine serum and held for 16 h at 4°C before dilution and testing. Absorbance values were read after 30 min at 405 nm in a Flow Tikertek Multiscan photometer. Dilutions of antigens and sera producing a satisfactory range of absorbance values for postinoculation sera were determined as previously described (4, 6). All serum samples were tested at 1:50, 1:100, and 1:200, and all antigens were used at 1:50 except for HCV OC43, which was used at 1:200.

RESULTS

Detection of antibody rises in human paired sera by ELISA. Table 1 shows the ELISA absorbance values obtained by using HCV strains 229E, PR, TO, and OC43 and MHV 3 as antigens against dilutions of the paired sera from a volunteer inoculated with HCV AD. The highest ratio of postinoculation to preinoculation absorbance values obtained at any of the serum dilutions tested was called the ELISA ratio, and ratios of 2 or more were considered to represent significant antibody rises (4, 6). Hence, sera from this volunteer had significant antibody rises to HCV strains 229E, PR, and TO, as indicated by ELISA ratios of 4.8, 3.8 and 4.0, respectively. However, no antibody rises to HCV OC43 and MHV 3 were observed, since the ELISA ratios were 1.2 and 1.1, respectively.

TABLE 1. *ELISA of coronaviruses against dilutions of a pair of human sera*[a]

Coronavirus antigen	ELISA absorbance values against the following serum dilution[b]					
	1:50		1:100		1:200	
	Pre	Post	Pre	Post	Pre	Post
HCV 229E	0.33	1.60	0.19	0.86	0.11	0.37
HCV PR	0.48	1.84	0.29	0.91	0.20	0.47
HCV TO	0.49	1.97	0.30	1.14	0.25	0.66
HCV OC43	0.17	0.20	0.12	0.13	0.10	0.10
MHV 3	0.14	0.15	0.10	0.11	0.06	0.06

[a] Paired sera from a volunteer inoculated with HCV AD.

[b] Pre, Preinoculation; post, postinoculation.

Table 2 summarizes the results of similar ELISAs done on serum pairs from 50 volunteers given HCV OC38/43, the five recent HCV isolates not cultivable in tissue culture, or saline. Seven of eight pairs of sera from volunteers developing colds after inoculation of HCV OC38/43 showed significant ELISA ratios (antibody rises) when tested against OC43, and six of the seven also showed rises against MHV 3. No significant rises were found when 229E, PR, or TO antigen was used, although ratios up to 1.5 were occasionally found, and mean ratios up to 1.3 were recorded. Serum pairs from volunteers given two other HCV isolates, RO and HO, showed similar patterns of response; significant ELISA ratios were detected against HCV OC43 and MHV 3, but not against HCV 229E and related strains. The number of rises against MHV 3 was smaller and the ratios recorded were somewhat lower than those against HCV OC43. A third HCV isolate, GI, gave somewhat poorer responses against HCV OC43 and none against MHV 3.

Sera from volunteers given two other HCV strains, PA and AD, showed a different pattern of responses. Significant rises were found in 9 of the 12 PA paired sera and in 7 of the 8 AD paired sera against HCV strains 229E, PR, and TO. No significant rises were obtained in these sera when HCV OC43 and MHV 3 were used as antigens. Paired sera from three control volunteers given saline inocula and who did not develop colds showed no significant rises against any antigen. In addition, serum pairs from 25 volunteers given the various organ culture coronaviruses described above but who did not develop colds were tested against the same antigens and showed no significant rises.

Attempts to detect antibody rises by using other HCV antigens. Numerous attempts were made to grow the HCV strains RO, GI, HO, PA, and AD in tissue culture, but in only one case, that of PA, were limited effects observed in first and second passages in MRC continuous cells. HCV strains RO, GI, HO, PA, and AD grown in organ culture and HCV OC43 grown in BSC-1 cells were used as antigens in the ELISA test. None of the serum pairs tested showed significant antibody rises against these antigens when a variety of different antigen and serum dilutions was used.

DISCUSSION

The results confirm the value of the ELISA in detecting significant antibody rises against HCV

TABLE 2. *Antibody rises in paired sera of volunteers inoculated with HCV strains*

Inoculum	No. of serum pairs	Antigen[a]				
		HCV				MHV 3
		229E	PR	TO	OC43	
HCV OC38/43	8	0; 1.1-1.5; 1.3	0; 1.1-1.4; 1.2	0; 1.0-1.4; 1.2	**7; 1.2-17.9; 5.7**	**6; 1.3-8.5; 3.7**
HCV RO	11	0; 0.9-1.6; 1.3	0; 0.9-1.6; 1.3	0; 1.0-1.6; 1.3	**7; 1.0-7.5; 2.9**	**3; 0.9-2.5; 1.5**
HCV GI	5	0; 1.0-1.5; 1.3	0; 1.1-1.4; 1.2	0; 1.1-1.3; 1.2	**2; 1.3-4.4; 2.1**	0; 1.0-1.6; 1.3
HCV HO	3	0; 0.8-1.5; 1.2	0; 0.9-1.2; 1.1	0; 0.8-1.5; 1.2	**3; 4.2-12.0; 7.2**	**2; 1.0-4.0; 2.3**
HCV PA	12	**9; 1.1-5.8; 2.5**	**9; 1.2-6.1; 2.6**	**9; 1.2-7.1; 2.9**	0; 1.0-1.3; 1.2	0; 0.7-1.3; 1.1
HCV AD	8	**7; 0.9-4.8; 3.3**	**7; 0.9-4.0; 2.6**	**7; 1.1-4.2; 2.8**	0; 0.8-1.5; 1.2	0; 0.9-1.4; 1.1
Saline	3	0; 1.0-1.2; 1.1	0; 1.0-1.2; 1.1	0; 1.0-1.1; 1.1	0; 1.1-1.3; 1.2	0; 1.0-1.2; 1.1

[a] For each antigen, the figures show the number of serum pairs giving ELISA ratios of 2 or more, the range of ratios obtained, and the mean ratio. Serum-antigen combinations in which any of the serum pairs gave significant ELISA ratios of 2 or more are boldfaced.

in human sera. Antigens prepared in tissue culture or in suckling mouse brain and which were known to contain at least 10^6 particles per ml were satisfactory, but others prepared in organ culture were not, presumably because they contained fewer particles.

The HCV strains examined fell into two distinct antigenic groups when tested by ELISA. As previously reported (4), the HCV 229E group includes four strains cultivable in MRC continuous cells, 229E, TO, PR, and KI (the latter not being used in this study). To these we now add the strains PA and AD, which are clearly related to 229E, TO, and PR, but which have so far proved impossible to grow in tissue culture. Although HCV isolates cultivable in tissue culture have generally been assumed to show a close relationship to 229E (8), most organ culture strains hitherto examined by various methods not including ELISA have cross-reacted with 229E only partly (2) or not at all (11). The cross-reaction of HCV strains PA and AD with 229E but not with OC43, demonstrable by ELISA, therefore represents a newly described combination of serological with biological properties. It should be noted that the criteria used for deciding that a rise in titer has occurred are very rigorous and that minor antigenic differences would be missed with these criteria. Use of other techniques and, where possible, use of hyperimmune animal sera would probably help to elucidate the cross-relationships between the new and older strains. However, paired sera from volunteers given HCV strains AD, RO, HO, GI, or PA showed no consistent fourfold-rising titers in the OC43 hemagglutination inhibition test (S. E. Reed, manuscript in preparation).

The relationship of HCV OC43 to MHV 3 was confirmed in this study. Although sera against two recent organ culture HCV strains, RO and HO, also showed a partial reactivity with MHV 3, five pairs of sera against a third organ culture strain, GI, did not do so, indicating that this cross-reactivity is a variable property.

It is not clear what antigenic determinants were involved in these ELISAs, as relatively crude HCV preparations containing whole and disrupted particles were used. Thus, both surface and internal HCV components were available as antigens. However, it is likely that the antibodies detected in these human sera were directed primarily against the surface components, in the form of projections, since most of the antibody made during infection has been shown to be against these components, with only small amounts of antibody made against membrane or ribonucleoprotein components (6). Thus, the antigenic differences observed between the HCV 229E group and the HCV OC43 group viruses may reflect a lack of common external antigens, although they may nevertheless share antigenically related internal components not detected by the present method.

This ELISA should be valuable in detecting human respiratory and other infections with viruses related to HCV 229E and HCV OC43; infections due to all of eight recent HCV isolates have been detected serologically when these two antigens are used. It is not yet clear whether HCVs that show no cross-reactivity with 229E or OC43 strains by ELISA exist or whether only two antigens will prove adequate to detect the majority of infections with such viruses. However, our ELISA did not detect significant antibody rises in the sera of 12 of the 47 volunteers who developed colds after inoculation with HCVs (Table 2). In a previous study with ELISA, antibody rises were detected in the sera of all volunteers who developed colds after inoculation with several HCV 229E group viruses (4). However, those colds were generally more severe than the colds observed in this study.

Future studies are required to develop the ELISA for clinical use in detecting all infections caused by HCVs.

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