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Identification of Measles Virus-Specific Hemolysis-Inhibiting Antibodies Separate from Hemagglutination-Inhibiting Antibodies

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The occurrence of antibodies giving hemolysis inhibition (HLI) but not hemagglutination inhibition (HI) was examined in human convalescent and rabbit hyperimmune sera. HI antibodies, which through their interaction with hemagglutinin components display HLI activity, were removed by absorption with Tween 80-ether (TE)-treated measles virus material. This absorption did not change the titer of non-HI HLI antibodies. After removal of HI antibodies from 16 late measles convalescent sera and three batches of gamma globulin, HLI antibody titers showed a two- to eightfold reduction. The titers of neutralizing antibodies were reduced from 1/4 to 1/20 of the original titers. There was a good correlation between the titers of neutralizing and HLI antibodies both in sera from which HI antibodies had been removed by absorption and in sera spontaneously showing markedly higher HLI than HI antibody titers. HLI antibodies with these characteristics could be identified in HI tests when whole virus instead of TE-treated material was used an antigen and anti-antiserum was added to the tests. In contrast to the situation in human sera, antibodies remaining after removal of HI antibodies from rabbit hyperimmune sera against purified virus particles were detectable in neutralization and HLI tests only in the presence of anti-antiserum. However, virus particles from which the major fraction of all envelope projections had been removed by treatment with 0.004% trypsin induced the production of non-HI HLI antibodies active also in the absence of anti-antiserum. TE and formalin treatment destroyed the hemolytic activity of virus preparations and also their capacity to induce a production of non-HI HLI antibodies.

In a study of the occurrence of antibodies against different structural components of measles virus in late measles convalescent sera from healthy individuals and from patients with subacute sclerosing panencephalitis and multiple sclerosis, a few samples were encountered in which hemolysis-inhibiting (HLI) antibodies were present in much higher titers than hemagglutination-inhibiting (HI) antibodies (11, 19). Analysis of a larger number of samples from multiple sclerosis patients and controls revealed the occurrence of sera displaying this kind of preferential HLI antibody response in about 10% of the population (18). These findings have been interpreted to signify the presence of one or more antigenic components separate from the hemagglutinin in the virus envelope. Since antibodies against this additional envelope component cause an inhibition of virus-specific hemolysis, it will be referred to in this paper as hemolysin, a designation used as an operational term.

Assessment of HLI-specific antibodies can readily be made when these occur in excess over HI antibodies. However, since HI antibodies by their reaction with hemagglutinin indirectly cause an inhibition of hemolysis, the titer of antibodies reacting directly with the hemolysin can not be measured in the presence of these antibodies. A technique is described here for a separate identification of HLI antibodies. It has not been possible to isolate the hemolysin component of the virus and to use this as a specific antigen in immunological test systems. Instead, the technique employed is based on a selective removal of HI antibodies by absorption with hemagglutinin preparations not containing any hemolysin. The occurrence of HLI antibodies in human sera and rabbit hyperimmune sera against different virus products will be described. A special emphasis is put on the analysis of the occurrence of antibodies against different virus envelope components in rabbit hyperimmune sera against products of the kinds

previously used as inactivated measles vaccines.

MATERIAL AND METHODS

Virus and cell cultures. Two different strains of measles virus were used for preparation of antigen material. These were the diluted-passage variant of the Edmonston strain (17) and the LEC strain. The latter strain was originally isolated from a case of subacute sclerosing panencephalitis (1) and was kindly provided by Hilary Koprowski, the Wistar Institute, Philadelphia. In some experiments the Montana strain of Newcastle disease virus was used. All virus materials were prepared in mycoplasma-free Vero cells maintained on Eagle minimal essential medium containing 2% fetal calf serum.

Sera. Late measles convalescent sera (collected 4 to 20 years after recovery from regular measles infections) were obtained from the Virus Department of the National Bacteriological Laboratorium, Stockholm. In addition, serum samples selected from the material included in previous studies on the occurrence of different measles virus antibodies in multiple sclerosis patients and controls (18) were used.

Rabbit hyperimmune sera were prepared by injection of 4 ml of antigen mixed with Freund complete adjuvant intramuscularly followed by an intravenous booster 5 weeks later. The animals were exsanguinated 1 week after the booster.

Serological tests. The techniques for neutralization tests in the absence and presence of anti-antiserum, HLI, HI, and complement fixation tests with purified nucleocapsids were previously described in detail (11). The diluted-passage variant of the Edmonston strain was used in neutralization tests and LEC virus material was used as antigen in other serological tests. HLI tests were performed both in the absence and presence of sheep anti-human or anti-rabbit sera (obtained from the Department of Immunology, the National Bacteriological Laboratorium, Stockholm). Serial twofold dilutions of serum in 0.2 ml were prepared, whereafter 0.1 ml of antigen was added per dilution. After incubation for 1 h at room temperature, 0.1 ml of anti-human or anti-rabbit serum diluted 1:10 was added and the mixtures were incubated for another hour before addition of the erythrocyte suspension. HI tests were carried out with two different antigen preparations, purified whole-virus particles (12) and Tween-ether (TE)-treated virus material (7). Tests with the different antigen preparations were carried out both in the absence and presence of sheep anti-human or anti-rabbit sera. After preparation of serial twofold dilutions of serum in 0.05 ml, 0.025 ml of properly diluted antigen was added. After incubation for 1 h at room temperature, 0.025 ml of anti-human or anti-rabbit serum diluted 1:10 was added. The erythrocyte suspension was added after still another hour of incubation at room temperature.

HI antibody consumption (HIC) tests were used for identification of incomplete hemagglutinin. Serial twofold dilutions of material in 0.05 ml were mixed with 0.025 ml containing 2 HI units of a rabbit hyperimmune serum against whole measles virus. After incubation for 1 h at room temperature, 0.025 ml containing 2 hemagglutinating units of TE-treated measles virus material was added to the mixture, whereafter the samples were incubated for another hour at room temperature. Green monkey (*Cercopitecus aethiops*) erythrocytes (0.05 ml) were then added and the cells were allowed to settle at 37 C. The highest dilution of test samples absorbing HI antibodies, i.e., the last well showing maximal or clear-cut partial agglutination, was considered to contain 1 HIC unit.

Preparation of hemagglutinin devoid of hemolytic activity. Three different procedures were used to prepare antigen material with these properties from concentrated extracellular material. These were (i) TE treatment (7), (ii) Triton X-100 treatment of purified whole-virus material followed by zonal centrifugation in sucrose gradients for preparation of small particle hemagglutinin (12; the detergent in this publication was used under a different trade name, Cutscum), and (iii) Triton X-100 treatment followed by anion exchange chromatography on diethylaminoethyl Sephadex A-25. The major population of small particle hemagglutinin which eluted at 0.25 M NaCl (Norrby, unpublished data) was collected.

Absorption of sera. The procedure used has been described previously (16). Sera were absorbed with antigen in an amount corresponding to at least four times that calculated from HI tests to be required for binding of all HI antibodies. The mixtures were incubated for 1 h at room temperature and 4 C overnight. After centrifugation at 81,000 $\times$ *g* (35,000 rpm, rotor 40, Spinco Division, Beckman Instruments Inc.) for 2 h the supernatant was collected; if needed, absorptions were repeated. Hemagglutination tests were carried out to determine the amount of hemagglutinin possibly remaining in the mixtures. Excess hemagglutinin was removed by absorption with green monkey erythrocytes.

Electron microscopy. Virus material was added to carbon-coated grids. After incubation for 1 min at room temperature, the grids were washed by dropwise addition of 4% sodium tungstosilicate solution in distilled water. Excess fluid was then removed by a filter paper and the specimens were dried in the air. Thereafter they were examined in a Philips EM 200 electron microscope at a primary magnification of 45,000 to 50,000.

RESULTS

Demonstration of HLI antibodies in human sera after selective removal of HI antibodies. Absorption experiments were carried out with three different preparations of hemagglutinin devoid of hemolytic activity (see above). Since it was possible that these preparations might contain hemolysin separate from hemagglutinin, a situation precluding any expression of the biological activity of the hemolysin, preliminary experiments were carried out with sera selected to contain HLI antibodies in marked excess over

HI antibodies. It was found that repeated absorptions with large quantities of TE-treated antigen did not change the HLI antibody titer of this type of sera, whereas, with some preparations of the two other types of purified hemagglutinin, a two- to fourfold reduction in the HLI antibody titer occasionally was encountered. TE-treated material was therefore chosen for further absorption experiments.

Sixteen late measles convalescent sera and three batches of gamma globulin were absorbed with TE-treated antigen until all HI antibodies had been removed. The neutralizing and HLI antibody activity of unabsorbed and absorbed samples were tested in parallel, and some representative results are summarized in Table 1. The titer of HLI antibodies remained unchanged or was only slightly reduced by the absorption with TE antigen. All samples except one retained capacity to neutralize measles virus after removal of all detectable HI antibodies. However, the neutralization titers after absorption was 4 to 20 times lower than that of unabsorbed samples. The effect of addition of anti-human serum on the neutralizing activity of sera absorbed with TE-treated antigen was analyzed (data not shown) since it was experienced previously (11) that HLI antibodies appeared to be more prone to show an enhanced neutralization under these conditions than HI antibodies. However, only a twofold and in a few cases a fourfold increase in titers was recorded.

A comparison was made between the neutralization and HLI antibody titers of human sera spontaneously displaying high titers of HLI antibodies in the presence of only low titers of HI antibodies encountered in a previous study (18), and of the above mentioned human sera from which HI antibodies had been removed by absorption with TE antigen (Table 1). A correlation was found between the titers of HLI and neutralizing antibodies in both groups of sera (Fig. 1). The effect of anti-human serum on the HLI and HI activity of antibodies remaining after absorption with TE antigen was also studied (Table 2). The HLI titers remained unchanged or showed a twofold increase. The effect on HI antibody titers was dependent on the type of antigen employed in the test. When TE-treated antigen was used, a slight increase in titers was observed in a few cases. In contrast, titers recorded with whole-virus antigen were markedly increased by addition of anti-human serum. The enhanced HI antibody titers reached levels corresponding to those found in HLI tests.

Characterization of the presence of HLI antibodies before and after absorption with TE antigen in rabbit hyperimmune sera against different measles virus products. Antisera against three principally different types of virus products were studied. These products were (i) virus particles purified by centrifugation in discontinuous sucrose gradients (12), (ii) purified virus particles from which projections had been removed by treatment with low concentrations of trypsin, and (iii) hemagglutinin preparations devoid of hemolytic activity.

Sera against virus particles devoid of hemag-

TABLE 1. *Measles virus-specific antibodies in five late convalescent sera and two batches of human gamma globulin before and after absorption with TE-treated measles virus antigen*

Sample	Time after measles (years)	Absorption with TE antigen	Test for antibody activity		
			Neutralization	HLI	HI with TE-treated antigen
Convalescent serum 1	4	0	1,800	640	320
		+	320	320	<20
Convalescent serum 2	10	0	2,560	320	320
		+	220	160	<20
Convalescent serum 3	21	0	640	80	80
		+	<14	<20	<20
Convalescent serum 4	3	0	320	640	160
		+	320	320	<20
Convalescent serum 5	16	0	450	320	80
		+	160	80	<20
Gamma globulin 1		0	4,500	3,200	1,600
		+	220	1,600	<40
Gamma globulin 2		0	9,000	3,200	1,600
		+	160	1,600	<40

glutinin projections were included since it was fortuitously found that they contained excess amounts of HLI antibodies over HI antibodies. The trypsin treatment of virus particles was carried out in the following way. Virus particles were banded at the interphase between 54 and 20% sucrose in discontinuous sucrose gradients as described previously (12). Preparations with an HA titer of about 320 were then mixed with green monkey erythrocytes in a final concentration of 20% and incubated for 3 h at 4 C. The virus-erythrocyte aggregate was washed twice in excess volumes of physiological NaCl at 4 C and finally resuspended in a buffer of pH 7.8 (at 37 C) containing 0.05 M tris(hydroxymethyl) aminomethane-hydrochloride, 0.1 M NaCl, and 0.01 M $CaCl_2$ containing trypsin (2× crystallized, Fluka AG, Buchs, SG, Switzerland) in a final concentration of 0.004%. The carbobenzoxy-D-phenylalanyl-L-phenylalanyl-*N*-nitro-L-arginine tripeptide SV-4814 was also added to the mixture in a final concentration of 50 μg/ml to prevent virus-induced hemolysis (10). After incubation for 1 h at 37 C in a water bath, the erythrocytes were removed by centrifugation. The eluate, which was completely devoid of hemagglutinating activity but contained HIC test-positive material, was centrifuged in a discontinuous sucrose gradient of the type mentioned above. The distribution of different activities is illustrated in Fig. 2. Particles associated with nucleocapsid-complement fixation activity sedimented to the interface between 54 and 20% sucrose. They did not display any hemolytic activity. In the electron microscope (Fig. 3), these particles appeared to represent virions from which the major part of all projections had been removed. The trypsin-treated particles frequently displayed a certain degree of breakdown causing a release of their nucleocapsid component. The monovalent hemagglutinin demonstrated by its HIC test activity remained on the top of the gradient.

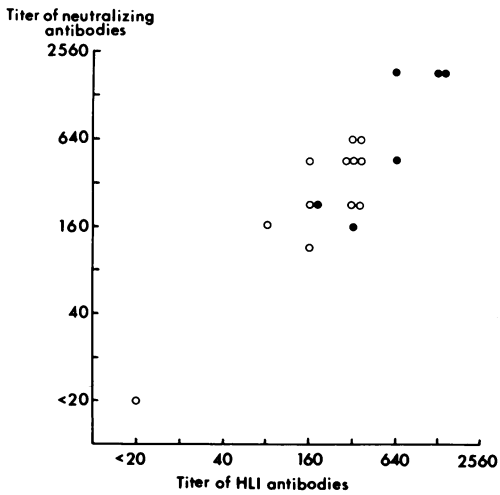

FIG. 1. *Correlation between titers of neutralizing and HLI antibodies in sera (●) with high titers of HLI antibodies in the presence of low titer HI antibodies included in a previous study (20) and in sera (○) with similar characteristics after removal of HI antibodies by absorption with TE-treated antigen.*

Preparations of nonhemolytic hemagglutinin included TE-treated material (7), small-particle hemagglutinin (12), and material treated with 3.7% formaldehyde for 1 h. The latter treatment was previously shown to destroy the hemolytic activity without impairment of hemagglutinin activity (15). Rabbit hyperimmune sera against TE-treated and formalin-inactivated vaccine products used in previously described field trials (13, 14) were included in the study.

Table 3 gives a summary of antibody activi-

TABLE 2. *Demonstration of HLI antibodies in sera lacking HI antibody activity demonstrable with TE-treated measles virus antigen, by HI tests with purified whole virus as antigen and addition of anti-antiserum*

Sample[a]	HI antibody titer with:				HLI antibody titer[b]	
	Whole-virus antigen[b]		TE-treated whole virus antigen[b]			
	−	+	−	+	−	+
Convalescent serum 1	<20	160	<20	<20	320	640
Convalescent serum 6	<40	5,120	<40	<40	2,560	2,560
Convalescent serum 7	40	1,280	40	160	2,560	5,120
Convalescent serum 8	<10	640	<10	20	1,280	2,560
Gamma globulin	<40	1,280	<40	<40	1,600	3,200

[a] Convalescent sera 1 and 6 and gamma globulin were absorbed with TE-treated measles virus antigen for removal of HI antibodies demonstrable with TE-treated antigen, whereas the other two sera spontaneously contained HLI antibodies in pronounced excess over HI antibodies.

[b] − and + indicates absence or presence of anti-human serum in the tests.

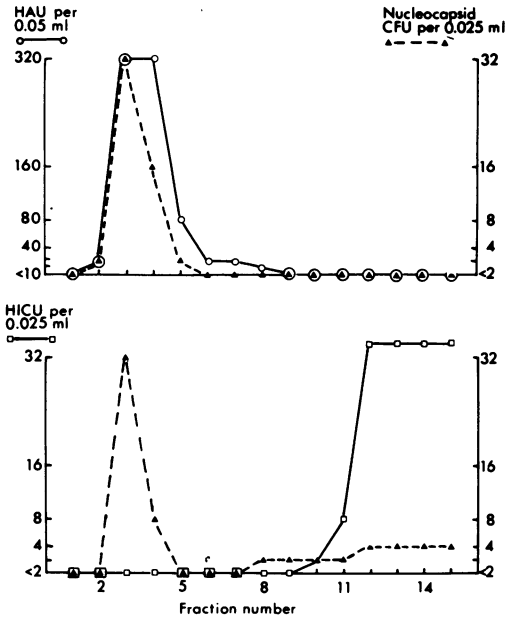

FIG. 2. *Distribution of biological activities of untreated, purified measles virus particles (upper part) and the same products after absorption to red cells and elution with 0.004% trypsin (lower part) after centrifugation in a discontinuous sucrose gradient (6 ml of 54% and 16 ml of 20% sucrose) at 18,000 rpm for 2 h in Spinco SW25 rotor. The following biological activities were recorded: hemagglutination (○), HIC test (□), nucleocapsid complement fixation (▲).*

ties in various serological tests of rabbit hyperimmune sera against the different virus preparations before and after absorption with TE-treated antigen. Somewhat unexpected, absorption with TE-treated antigen in most cases removed not only all detectable neutralizing and HI antibody activities but also HLI antibody activity in sera against whole virus. However, in the presence of sheep anti-rabbit serum, significant antibody titers were recorded in all three tests. Antisera against projection-less virus particles contained only low titers of HI antibodies and no or only small quantities of neutralizing antibodies. However, the sera contained HLI antibodies in titers markedly exceeding HI antibody titers. The titer of HLI antibodies was not significantly reduced by absorption with TE-treated antigen. Similarly, the HI antibody titer demonstrable with whole-virus antigen and addition of anti-rabbit serum remained after absorption with TE-treated antigen. Absorption with TE-treated antigen of sera against small-particle hemagglutinin and TE- and formalin-treated virus materials removed all detectable antibody activity in neutralization, HLI, and HI tests carried out both in the absence and presence of anti-rabbit serum.

Importance of antibodies against different normal cell components for antibody activities of rabbit hyperimmune sera against measles virus products after absorption with

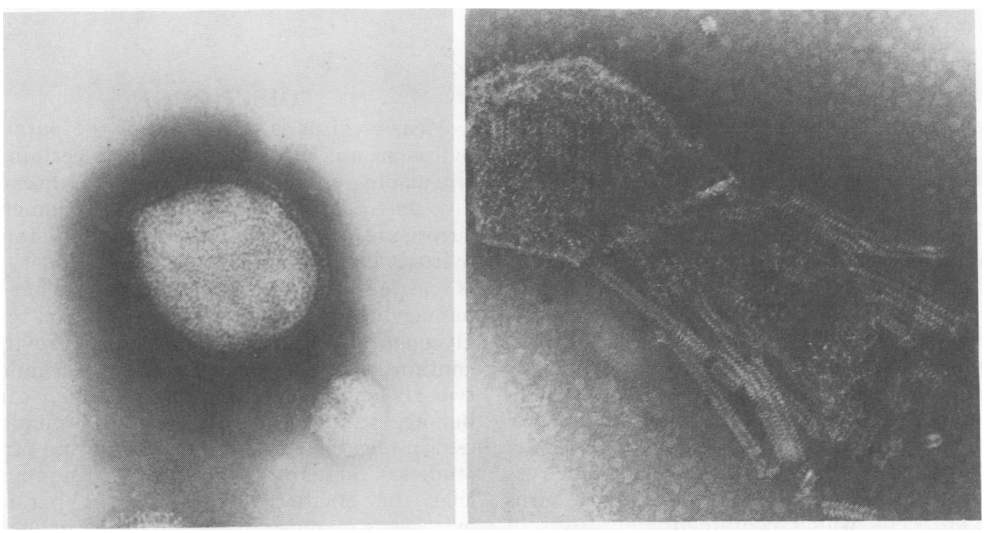

FIG. 3. *Ultrastructure of intact (left) and trypsin-treated (0.004%) measles virus particles (right). Note the presence of a few remaining projections in the fragile trypsin-treated particle releasing nucleocapsids. Negative contrasting with sodium tungstosilicate. Magnification: ×140,000 (left picture), ×92,000 (right picture).*

TABLE 3. *Measles virus-specific antibodies in rabbit hyperimmune sera against different virus products before and after absorption with TE-treated measles virus antigen*

Antiserum against:[a]	Absorption with TE antigen	Test for antibody activities[b]					
		Neutralization		HLI		Whole-virus HI[c]	
		−	+	−	+	−	+
A							
1	0	4,500	10,240	6,400	25,600	6,400	12,800
	+	<160	640	<160	1,280	80	640
2	0	5,120	10,240	12,800	25,600	6,400	25,600
	+	<160	640	160	1,280	<160	1,280
B							
1	0	<20	40	1,280	1,280	160	1,280
	+	<20	56	640	1,280	160	1,280
2	0	<20	<20	320	1,280	80	1,280
	+	<20	<20	160	640	40	640
C							
1	0	640	1,800	1,280	2,560	640	1,280
	+	<40	<40	<40	<40	<40	<40
2	0	900	3,600	2,560	10,240	1,280	2,560
	+	<40	<40	<40	<40	<40	<40
D							
1	0	1,800	2,560	1,600	3,200	3,200	3,200
	+	<40	<40	<40	<40	<40	<40
2	0	640	640	320	640	320	640
	+	<20	<20	<20	<20	<20	<20
E							
1	0	160	220	320	640	160	640
	+	<20	<20	<20	<20	<20	<20
2	0	220	450	320	1,280	160	320
	+	<20	<20	<20	<20	<20	<20

[a] A, Purified whole-virus antigen; B, projectionless virus particles; C, small particle hemagglutinin; D, TE-treated purified whole virus products; E, formalin-treated whole virus-products. Designations 1 and 2 refer to different sera.

[b] − and + indicate absence or presence of anti-rabbit serum in the tests.

[c] After absorption with TE antigen, sera did not contain HI antibodies demonstrable in tests with this antigen.

TE-treated material. Results of different tests to elucidate the possible importance of antibodies against normal cell constituents in different measles virus serological test are summarized in Table 4. Absorption of antisera against purified virus particles with TE-treated virus antigen and, in addition, uninfected Vero cells did not remove antibodies detectable in HLI tests and whole-virus HI tests in the presence of anti-rabbit serum. However, antisera against uninfected Vero cells carried a certain antibody activity in the latter two types of tests. This antibody activity was characterized by pronounced prozone phenomena and was readily removed by absorption with uninfected Vero cells. Antisera against Newcastle disease virus in Vero cells which contained high titers of homologous antibodies did not carry any detectable antibody activity in measles virus HI and HLI tests even in the presence of anti-rabbit serum.

DISCUSSION

Measles virus is a member of the paramyxovirus group. However, it displays certain distinguishing characteristics, e.g., the absence of any detectable neuraminidase (4). Somewhat surprisingly it has been found that in paramyxoviruses exhibiting neuraminidase activity this is carried by the same glycopeptide as the hemagglutinin activity (21). In addition to this glycopeptide, the envelope of paramyxoviruses contains a smaller glycopeptide (6). Similarly, two glycoproteins, the larger of which carries hemagglutinating activity, has also been identified in measles virus particles (3). From further studies of Sendai virus (20) it has been proposed that the smaller glycoprotein of paramyxoviruses is involved in virus-induced hemolysis, cell fusion, and penetration of virus into cells. On the basis of the effect of certain carbobenzoxy tripeptides, it was proposed that these three biological activities might represent dif-

ferent expressions of one of the biologically active components also in measles virus (10). The smaller glycoprotein of paramyxoviruses has not as yet been identified by use of immunological techniques.

In previous studies (11, 18, 19), it was found that in a certain fraction of human sera HLI antibodies occurred in marked excess over HI antibodies. This observation was further confirmed in the present study, and in addition, a technique was developed to detect antibodies giving hemolysis inhibition by a reaction with virion surface components different from the hemagglutinin. These antibodies could be detected after selective removal of HI antibodies by absorption with TE-treated antigen. The fact that the absorption did not reduce the titer of antibodies causing a direct HLI indicates that the TE treatment not only dissociates the hemagglutinin and hemolysin components of the measles virus envelope but also causes a destruction of the latter component(s).

In conjuction with the present studies, attempts have been made to identify the possible occurrence of isolated hemolysin by use of an antibody blocking test employing sera containing HLI antibodies in titers markedly higher than HI antibodies. These attempts hitherto have been unsuccessful, possibly indicating that the amount of hemolysin may not suffice to allow a detection by use of this type of test which has a relatively low sensitivity. It might be possible to get an estimate of the relative frequency of occurrence of hemagglutinin and hemolysin in the measles virus envelope by use of immune electron microscopy employing specific serological reagents. This technique has been used in studies of influenza virus surface components (5).

In contrast to paramyxoviruses, ortomyxoviruses have hemagglutinating and neuraminidase activities carried by two different glycoproteins. Antibodies against the neuraminidase do not by themselves give any efficient HI. However, after addition of anti-antiserum neuraminidase, antibodies could be readily detected in HI tests (2). Similarly, it was found in the present study that after additional anti-antiserum measles virus HLI antibodies also could be detected in HI tests. However, this was possible only when whole virus and not TE-treated material was used as antigen in the test.

Rabbit hyperimmune sera against different measles virus components behaved differently from human sera after absorption with TE-treated measles antigen. Whereas antibodies remaining after absorption of the latter sera gave a direct HLI and neutralizing activity, anti-antibody had to be added to detect the corresponding activities in rabbit sera against whole virus. In contrast, a separate and direct HLI antibody activity was detectable in rabbit hyperimmune sera against measles virus particles treated with trypsin, although these HLI antibodies gave a poor neutralization both in the absence and presence of anti-antiserum. The relatively higher efficiency of human antibodies in different serological tests might relate

TABLE 4. *Effect of antibodies against normal cell components in HI and HLI tests with purified whole measles virus antigen in the absence and presence of anti-rabbit serum*

Antiserum against:	Absorbtion with:	Test for antibody activity			
		HLI[a]		Whole-virus HI[a]	
		−	+	−	+
Purified whole-virus measles antigen	TE-treated measles antigen	80	1,280	80	640
	TE-treated measles antigen and uninfected Vero cells	160	1,280	40	640
Uninfected Vero cells[b]	0	<20	320[c]	<5	80[c]
1	Uninfected Vero cells	<20	<20	<20	<20
	0	<20	80[c]	<5	<5
2	Uninfected Vero cells	<20	<20	<20	<20
Purified whole NDV antigen prepared in Vero cells[d]	0	<10	<10	<10	<10

[a] − and + indicate absence or presence of anti-rabbit serum in the tests.

[b] Designations 1 and 2 refer to two different sera.

[c] Prozone phenomena occurred; only two dilutions including the one at the end point gave inhibition of hemolysis.

[d] HLI titer with homologous antigen in the absence of anti-rabbit serum was 2,560. NDV, Newcastle disease virus.

to the presence of antibodies against a wider spectrum of envelope antigens than in rabbit hyperimmune sera. It is also possible that varying properties of antibodies of different species origin could be of importance in determining their biological activity. Concerning the difference between rabbit hyperimmune sera against untreated and trypsin-treated virus particles, it is possible that the latter treatment exposes envelope structures inducing antibodies, which combine with virions to give an efficient HLI but only a poor neutralization.

The capacity of varying proteolytic enzymes to remove surface projections of myxoviruses is well established, and in parallel studies it was described that bromelain efficiently removes surface projections of measles virus (3). In the present study, low concentrations of trypsin were employed since it was found in previous studies that the measles virus hemagglutinin and the receptors on erythrocytes are highly sensitive to the action of this enzyme (8). Taking advantage of this dual effect of trypsin, an efficient technique for obtaining a high degree of purification of projection-less virus particles and free projections was developed. By electron microscopy, the trypsin-treated particles still contained a few detectable projections. It is possible that these may represent the structures responsible for the production of HLI antibodies in rabbits immunized with purified projection-less virus particles. However, it is equally possible that other structures may be involved and it should be emphasized that no information is available as concerns the relative amount of the original concentration of hemolysin which remains in the virus envelope after the treatment. It is of interest that a similar difference in relative susceptibility of hemagglutinin and neuraminidase surface projections of influenza virus to treatment with protolytic enzymes was recently observed (N. G. Wrigley, personal communication).

A possible importance of antibodies against normal cellular constituents in hyperimmune sera needs evaluation when sensitizing antibodies are identified in HLI and neutralization tests. It was found that antibodies against noninfected cells display activities in these tests, but by use of absorption experiments it could be excluded that antibodies of this kind were responsible for the sensitizing antibody activity of rabbit hyperimmune sera against different purified virus products.

A possible significance of measles HLI antibodies in vivo for protection against the disease should be further analyzed. This may be of particular interest with regard to the evaluation of the relatively poor protecting capacity of hitherto used inactivated measles vaccines (9, 13, 14). Two types of inactivated measles virus vaccines have been tested. One is a formalin-inactivated vaccine and the other a purified hemagglutinin product prepared from TE-treated material. Both these vaccines induced good amounts of neutralizing and HI antibodies. However, it was experienced that these antibodies do not provide satisfactory protection upon exposure to wild virus. About 50 to 100 times more antibodies induced by killed vaccines as compared to neutralizing and HI antibodies supplied in the form of passive immunization with gamma globulin were needed to give a corresponding degree of protection. Further, exposure to wild virus or to vaccine virus in certain cases caused aberrant reactions interpreted to represent Arthus phenomena. One way of explaining this situation would be to assume that the inactivated vaccines employed have been defective with regard to a certain antigen quality. The hemolysin of the virus might be the crucial component in this context, since both types of treatments used for preparing inactivated vaccines appear to have destroyed this component. As shown here, rabbit hyperimmune sera against TE- and formalin-killed products absorbed with TE-treated antigen did not contain any antibodies with HLI and neutralizing activity detectable in the absence or presence of anti-antiserum. Further studies by use of serological techniques presented in this publication have shown that neither of the two types of killed measles virus vaccines are capable of inducing HLI antibody production in humans (E. Norrby et al., manuscript in preparation).

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