Hemagglutination-Inhibition Test in Rhinovirus Infections of Volunteers

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The hemagglutination-inhibition (HI) test for antirhinovirus antibody was carried out on paired sera from volunteers inoculated with rhinovirus type 3 or type 4 (RV4). The HI test gave results which paralleled the neutralization test and was at least as sensitive as a microneutralization method for detection of serotype-specific antibody. Although high levels of HI antibody in the serum were associated with protection from infection, in the case of RV4 low serum HI antibody levels did not necessarily imply susceptibility to challenge with small doses of virus. HI activity could be measured in concentrated nasal-washing fluids, and this antibody also seemed relevant to protection against infection.

In a recent report Stott and Killington (4) described direct hemagglutination of sheep erythrocytes by certain serotypes of rhinovirus and noted that the agglutination could be inhibited by specific antisera. The relationship of hemagglutination-inhibiting antibody to rhinovirus infection has now been studied in paired pre- and post-infection sera from volunteers inoculated with rhinovirus, and in nasal washings collected before administration of virus.

MATERIALS AND METHODS

Procedures in volunteers. The subjects were healthy adults aged 18 to 50 years who were housed in isolation at the Common Cold Unit and observed by standardized methods (7). Blood samples were collected from the volunteers 3 days before and about 18 days after they had been given a virus inoculum. Nasal washings were collected by instilling 10 ml of Hanks saline into the nasal cavity and collecting the discharged fluid in a petri dish. In most volunteers, this was done before virus inoculation and 2, 3, and 4 days afterwards. Virus inoculation was given as nose drops containing a strain of rhinovirus which had not been passaged in tissue culture. The doses were six or ten 50% infectious doses for HeLa cells (TCD_{50}) of rhinovirus type 3 (RV3), or 2, 5, 9, or 36 TCD_{50} of rhinovirus type 4 (RV4).

Virus isolation. Nasal washings collected after administration of virus were inoculated into rhinovirus-sensitive HeLa cells (5), which were observed for typical cytopathic effect.

Preparation of hemagglutinating antigens. Hemagglutinating antigens were prepared from RV3 (strain FEB) and RV4 (strain 16/60). The viruses were grown in 80-ounce rolled bottles (about 2.365 liters) containing monolayers of rhinovirus-sensitive HeLa cells (5) maintained in Eagle medium with 2% bovine

serum, 5% tryptose phosphate broth, and 30 mM $MgCl_2$. Bottles were inoculated with sufficient virus to produce almost complete cytopathic effect after incubation for 18 to 24 h at 33 C. Cells still adhering to the glass were then shaken off into the medium, which was centrifuged at 2,000 rpm for 10 min. The supernatant fluid was discarded, and the deposit of infected cells, to be used as the source of virus, was suspended in a volume of phosphate-buffered saline equal to about ½₀ the volume of the original medium. The suspension was frozen and thawed twice, then clarified by low-speed centrifugation. The supernatant fluid, which had an infectious titer of $10^{7.5}$ to $10^{8.0}$ TCD_{50} per ml, constituted the hemagglutinating antigen. When used as previously described (4), the titer of these antigens was about 1:32 to 1:128.

Hemagglutination-inhibition (HI) test. Nasal-washing fluids collected before the volunteers received a virus inoculum were tested for the presence of blood, and those which were negative were concentrated fivefold by dialysis against polyethylene glycol 6000. Serum samples and concentrated nasal-washing fluids were absorbed for 3 to 18 h at 4 C with about one-third of their volume consisting of packed sheep erythrocytes. After centrifugation at 4 C the supernatant sera or fluids were inactivated at 56 C for 30 min. The HI test was carried out in microtiter plates using 4 hemagglutination units of antigen. Sera or nasal-washing fluids were allowed to interact with antigen for 1 h at room temperature before addition of erythrocytes, and the test was read after settling overnight at 4 C. Sometimes another reading was made after an additional period of 2 h at room temperature.

Neutralization (N) test. Sera were also tested for neutralizing antibody against approximately 100 TCD_{50} of virus by a micromethod (5), except that the readings were made by cytopathic effect rather than by the color change produced.

RESULTS

The results of HI and N tests carried out on the same sera appeared well correlated, although titers obtained in the HI test were often slightly higher (Fig. 1). In the case of RV3, titers were almost always somewhat higher by the HI method, although the correspondence between the two tests was generally good. The HI test was slightly more sensitive than the N test for detecting fourfold rises of antibody titer in paired sera; in the case of RV3 one of three fourfold rises in titer shown in the HI test was not detected by the N method, and in the case of RV4 the N test did not detect one of 15 such rises.

Rises in serum HI antibody titer which accompanied infection appeared to be specific to the infecting virus. Seven pairs of sera from volunteers infected with RV4 and three pairs from volunteers infected with RV3, each of which showed rising titers against the homologous virus, showed no change against the heterologous serotype. Similarly, four pairs of sera showing rising titers of N antibody against the nonhemagglutinating serotypes RV2 or RV9 showed no change in HI titer against RV3 or RV4.

Volunteers who had initially low serum HI antibody titers to RV3 were apparently more easily infected, as judged by subsequent virus-shedding, specific symptoms, and fourfold rises in serum HI antibody titer against the homolo-gous virus, than those with initially higher titers (Fig. 2). This parallels previous observation of the relationship between N antibody and resist-
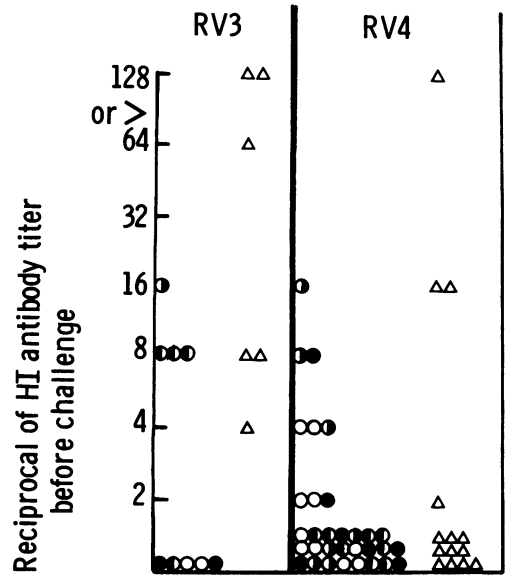

FIG. 2. *Relationship of initial serum hemagglutination-inhibition antibody titers to the results of inoculation of volunteers with RV3 and RV4. Each point represents one volunteer. Symbols:* Δ, *no virus shedding, seroconversion, or symptoms;* O, *virus shedding only;* ◑, *virus shedding and symptoms;* ◐, *virus shedding and seroconversion;* ●, *virus shedding, symptoms, and seroconversion.*
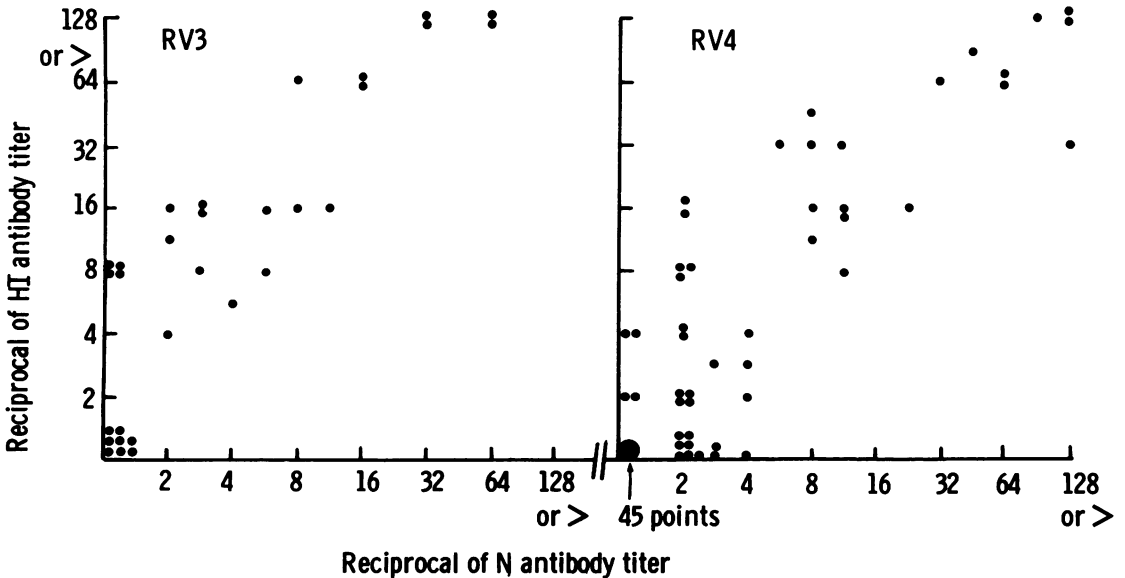

FIG. 1. *Correlation between serum antibody titers measured by the hemagglutination-inhibition and microneutralization methods. Points represent observations on single sera.*

ance to infection with rhinovirus (1, 3, 6). In the case of RV4 the relationship between serum antibody and protection against infection was less clear-cut. Moderate or high levels of serum antibody against RV4 were uncommon in the population studied, and the few individuals who initially had titers of 1:16 or above appeared relatively insusceptible. However, a significant proportion (10 of 33) of those volunteers who initially had no serum antibody (HI titer less than 1:2) were not infected by the challenge doses of RV4 used (2 to 36 TCD_{50}), and the failure to infect these individuals was not related to the challenge dose within the range tested.

Fig. 2 also indicates that, for both RV3 and RV4, virus shedding was a more sensitive criterion of infection than either symptoms or seroconversion, that subclinical infections were common, and that symptomatic infections did not occur in individuals with serum HI titers above 1:8.

HI antibody against the challenge virus was measured in concentrated nasal-washing fluids from 19 volunteers inoculated with 9 or 36 TCD_{50} of RV4 and from 6 volunteers inoculated with 10 TCD_{50} of RV3; low titers, up to 1:8, were detected in 8 individuals. Measurement of nasal HI antibody appeared at least as useful as serum HI antibody for assessing resistance to infection, but failure to detect either serum or nasal HI antibody in an individual did not necessarily imply susceptibility (Table 1).

DISCUSSION

It appears that the HI and N tests measure the same serotype-specific antibody but that the HI test, when compared with a microtiter neutralization test, has the advantage of slightly greater sensitivity, and correspondingly

a slightly greater ability to detect fourfold antibody rises in paired sera. Some human sera contained nonspecific agglutinins for sheep erythrocytes to titers up to 1:8, which were active in this test and could obscure low titers of HI antibody. However, these agglutinins were generally removed by absorption of the sera and were often inactive at room temperature. Nonspecific inhibitors caused no apparent difficulty in the test. The HI test apparently did not have the subgroup specificity of the indirect hemagglutination test described by Faulk et al. (2).

The HI test may evidently be used as a speedier alternative to the N test for screening volunteers for susceptibility to rhinovirus challenge, e.g., for studies of antiviral drugs. However, in the case of RV4 neither the HI nor the N test carried out on serum was wholly satisfactory for selecting susceptible individuals, because volunteers with initially low serum antibody titers were not always infected even by the higher of the challenge doses used. The reasons for this failure to infect all the serum antibody-free volunteers are probably complex, because although nasal antibody was clearly relevant to protection, it evidently did not always account for the volunteers' resistance. The ability of rhinoviruses to attach to ciliated epithelial cells, to establish an infection, and to spread widely in the nasal epithelium may also be influenced by factors relating both to virus virulence and to host susceptibility which are as yet undefined.

LITERATURE CITED

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TABLE 1. *Relationship of virus shedding to initial levels of HI antibody in serum and nasal-washing fluid*

| Challenge virus | No. of volunteers | Incidence of virus shedding in individuals with indicated initial HI antibody status[a] | | | | |
| | | Nasal antibody | | Serum antibody | | Neither serum nor nasal antibody |
		Yes	No	Yes	No	
RV3	6	1/3	3/3	1/3	3/3	2/2
RV4	19	2/5	10/14	3/4	9/15	8/11

[a] Numerator, number of volunteers shedding virus; denominator, number of volunteers tested.