Anti-Immunoglobulin G Hemagglutination-Inhibition Test for Influenza

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The sensitivity of the hemagglutination-inhibition test for influenza was significantly increased by the addition of species-specific anti-immunoglobulin G serum.

The principle of employing anti-globulin serum to enhance the titer of agglutinins against red blood cells and bacteria, first introduced by Moreschi (9, 10), also proved effective in detecting incomplete Rh antibodies (3). Subsequently, the anti-globulin technique was extended to include agglutination reactions with several bacterial species (7, 11, 14), rickettsiae (4), and mycoplasma (1). Recently, the technique has been utilized to increase the sensitivity of both the precipitin reaction (H. Daugherty, Bacteriol. Proc., p. 169, 1971) and serum neutralization of viruses (2, 5, 6, 15, 16). This preliminary report describes the use of anti-immunoglobulin (Ig) G serum in the hemagglutination-inhibition (HI) test for influenza.

Reagents and immunobiological preparations employed in HI tests consisted of: influenza A (PR8) infectious allantoic fluid; acute and convalescent human sera (courtesy of Walter Dowdle, Center for Disease Control, Atlanta, Ga.); human sera from retired coal miners (courtesy of N. LeRoy Lapp, Cardio-Pulmonary Branch, Appalachian Laboratory for Occupational Respiratory Diseases, Morgantown, W.Va.); normal goat serum (Pentex, Inc., Kankakee, Ill.); goat antiserum to human, chicken, guinea pig, bovine, mouse, and monkey IgG (Microbiological Associates, Bethesda, Md.); goat antiserum to human IgA, IgM, IgD, and IgE (Hyland Laboratories, Los Angeles, Calif.); and erythrocytes (RBC) from adult chickens. All sera were heat inactivated at 56°C for 30 min; acute and convalescent human sera, as well as goat antiserum to the various globulin fractions, were treated either with kaolin (13), trypsin-periodate (12), or receptor-destroying enzyme (8).

HI tests were performed by the standard microtiter system (8) with rigid plastic microtitration plates containing 96 "V"-bottom wells. In a typical test, each well received 0.025 ml of diluent (phosphate-buffered saline, pH 7.2); test sera in 0.025-ml amounts were serially diluted in twofold steps. All serum dilutions then received 0.025 ml of four virus hemagglutinin units. After incubation at room temperature (22 to 24°C) for 1 hr, 0.025 ml of a 1:20 dilution of either goat antihuman IgG or normal goat serum was added to each well. After incubation at room temperature for 30 min, each well received 0.05 ml of 0.5% chick RBC suspension to give a total volume of 0.125 ml. Appropriate controls consisted of combinations of the different reagents with cells. Test patterns were read after incubation at room temperature for 1 to 2 hr. The HI titer was the reciprocal of the highest test serum dilution that completely inhibited hemagglutination.

Preliminary experiments revealed that the incorporation of anti-human IgG serum into the conventional HI test for influenza [reacting influenza A (PR8) or influenza B (Great Lakes) with homologous antiserum] increased the HI titer of human test sera 8- to 16-fold. Cross-reactions between virus and heterologous antiserum did not occur in the presence of anti-IgG serum. Results of a determination on the optimal concentration of anti-IgG serum required to achieve maximal enhancement of test serum titers indicated that dilutions from 1:10 to 1:40 were most effective (Table 1). A 1:20 dilution of anti-human IgG serum was routinely used in subsequent HI tests. In the presence of high concentrations of anti-IgG serum, a prozone of hemagglutination was noted. This occurred in all wells that received the 1:2 dilution of anti-IgG serum. The phenomenon could not be attributed to the presence of incomplete antibodies, because of the negative findings obtained by the antiglobulin test of Coombs (3). As controls consisting of anti-IgG serum alone or in mixture with serum reagents failed to induce hemagglutination, the prozone of hemagglutination probably was related to the virus that may have been freed.
from its union with specific antibody by the action of excess anti-IgG serum.

The data indicate that the sensitivity of the anti-IgG HI test was greater than that of the conventional HI test for the serological diagnosis of influenza (Table 2). Based on the number of sera showing a fourfold or greater rise in titer, four out of six sera gave a positive response by the anti-IgG test, whereas one of six was positive by the conventional HI test.

The capacity of goat antiserum to IgG of different animal species to enhance the HI titer of human serum was determined (Table 3). Although maximal increases in titer were obtained with both anti-human and anti-monkey IgG sera, in general, the reactivity of anti-IgG serum in the test was species specific. The cross-reactivity between anti-monkey IgG and human test sera probably is the consequence of a common primate antigenic determinant. This has been encountered in anti-IgG serum neutralization tests with viruses (6). When different classes of immunoglobulins—anti-IgA, anti-IgM, anti-IgD, or anti-IgE—were introduced into the HI test, there was no enhancement of HI titer of test serum. This indicates that the reaction is influenced by the class specificity of the anti-immunoglobulin.

That the sensitivity of the HI test for influenza is increased by the incorporation of species-specific anti-IgG serum agrees with reports on efficacy of this serum in other serological reactions (1, 4, 6; H. Daugherty, Bacteriol. Proc., p. 169, 1971). This test may prove useful in detecting and estimating low concentrations of viral antibodies and in revealing subtle, common antigenic relationships among virus strains. The principle of the test may be applicable to other viral HI systems.

LITERATURE CITED