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Neutralizing Activity in Isolated Serum Antibody Fractions from Visna-Infected Sheep

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The neutralization technique was used to detect specific antibodies against visna virus in isolated immunoglobulin (Ig) G_1, IgG_2, and IgM fractions of visna-infected sheep serum. The neutralizing antibodies were present chiefly in the IgG_1 class. Low amount of activity was demonstrated in the IgM class, but no significant activity was seen in the IgG_2 class.

Visna virus is the cause of a slow infection affecting the central nervous system of sheep (10). The animals usually form detectable neutralizing antibodies against the virus within a few months after inoculation (9). Pétursson (6), using visna-infected sheep serum, reported that the neutralizing antibodies were absent in both the immunoglobulin (Ig) G and the IgM classes and speculated that they might be associated with the IgA class. However, preliminary results of Karl and Thormar (4) indicated that the neutralizing antibody activity was found in both the IgG and IgM classes. These findings are contradictory, and therefore further work on the association of neutralizing antibodies with specific immunoglobulin fractions is necessary.

The serum immunoglobulins of cow and sheep were characterized recently by a number of investigators (1-3, 11). Our paper describes the isolation of immunoglobulin fractions IgG_1, IgG_2, and IgM from visna-infected sheep serum. The antibody activities to visna virus in these isolated fractions were titrated in the neutralization test.

The antiserum used in the present investigation was from a sheep inoculated with visna virus (strain K796) in 1968. This sheep had not shown any clinical signs of the disease but there was an unusually high neutralizing activity in the serum.

The virus neutralization test was carried out as described previously (12). Serial twofold dilutions of the serum and isolated antibody fractions were mixed with an equal volume of visna virus dilution containing approximately 200 mean tissue culture infective doses per 0.1 ml. The mixtures were incubated at 22 C overnight and were then inoculated in 0.1-ml amounts into tissue culture tubes. The tubes were incubated for 14 days before the final reading. The neutralizing activities of the isolated immunoglobulin fractions were expressed in titers per 0.1 ml of protein adjusted to the concentration (in milligrams per milliliter) found in the serum.

Approximately 10 ml of serum was first centrifuged at 33,000 rpm in the Spinco model L-50 ultracentrifuge for 17 h at 4 C. The pellet was mixed with a few drops of the fluid above it, and the mixture was subjected to zone electrophoresis on starch for 16 to 18 h at a potential gradient of 20 V/inch, using barbital buffer (pH 8.6 and ionic strength 0.05) as described by Kunkel (5). Each 0.5-inch (about 1.25-cm) portion of the block was eluted with 0.15 M NaCl, concentrated, and tested in immunoelectrophoresis (8) with potent rabbit anti-sheep serum. Thus, IgG_2 was separated from IgG_1 by starch-block electrophoresis alone. The separation of IgM from IgG_1 was further achieved by gel filtration of selected starch-block eluates on a Sephadex G-200 column, equilibrated with 0.1 M tris(hydroxymethyl)aminomethane-0.15 M NaCl, pH 8.0. Protein concentrations of immunoglobulins were determined (3) by using an extinction coefficient ($E^{1\%}_{280\ (1\ cm)}$) of 13.7.

The immunoelectrophoretic patterns of the whole serum and isolated immunoglobulin fractions, tested with rabbit anti-sheep serum, are shown in Fig. 1. The serum contained several components in the gamma globulin region. The long major arc, extending the length of the gamma globulin region, had two distinct antigenic components, namely IgG_1 and IgG_2. The IgG_1 migrated faster towards the anode than IgG_2. The mixture of IgG_1 and IgG_2 preparation showed a spur of IgG_1 over IgG_2 in immunoelectrophoresis against rabbit anti-sheep serum. The IgM preparation migrated slightly towards the cathode from the point of application. No contamination by other serum proteins could be demonstrated in any of these purified fractions,

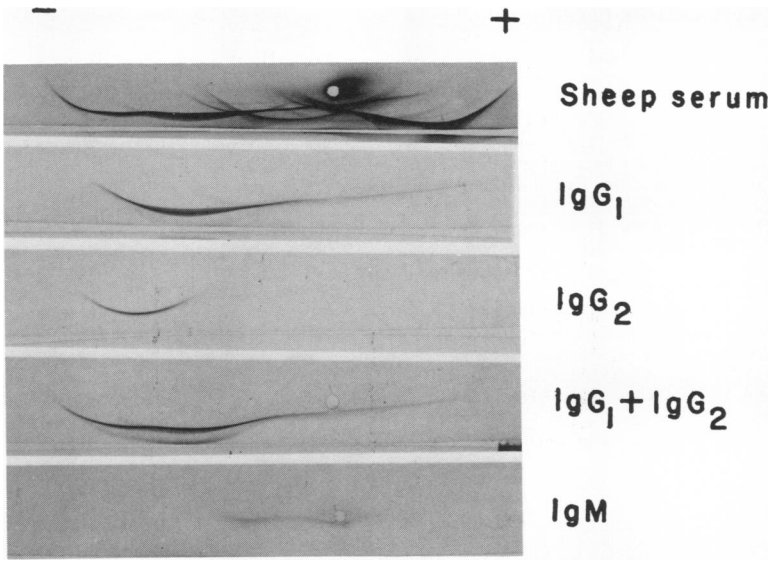

FIG. 1. *Immunoelectrophoretic analysis of sheep serum and isolated immunoglobulins. The troughs contain rabbit anti-whole sheep serum.*

except in the IgG_1 class, which has a minor contamination, probably of x-component (11). To determine whether these purified fractions contained any IgA contaminant, they were examined by the radial immunodiffusion method using rabbit anti-bovine secretory IgA serum (Miles Laboratories, Kankakee, Ill.). None of the fractions showed the presence of IgA. By this method, the whole sheep serum had an IgA level of 0.38 mg/ml.

The visna-neutralizing antibody response in serum and different isolated fractions is shown in Table 1. The visna-neutralizing antibody titer was the highest in the IgG_1 class. A significant amount of antibody activity was also detected in the IgM class, although much less compared with the IgG_1 class. No activity was observed in the IgG_2 class at the concentrations tested.

The serum and isolated immunoglobulin fractions failed to show precipitating, complement-fixing, and passive hemagglutinating antibodies against visna virus.

We made no attempt to isolate IgA, since the concentration of this component has been found to be extremely low in sheep serum and it has not been well defined and characterized (11). Our results confirmed the earlier findings of Karl and Thormar (4), which indicated that the neutralizing antibodies are associated with the IgG and the IgM classes. However, the antibody fractions used by these investigators (4) were partially purified, and no attempt was made to isolate the subclasses of IgG. Since in our study the neutralization titer of the IgG_1 class was higher than that of the serum, and the preparation was free of IgA as found by radial immunodiffusion technique, it was evident that the neutralizing activity was primarily associated with the IgG_1 class.

TABLE 1. *Visna-neutralizing antibody in serum and isolated immunoglobulins*

Sample	Protein content[a] (mg/ml)	Antibody titer to visna
Sheep serum	66.0	8,000
IgG_1	12.0	≥16,000
IgG_2	8.0	<500
$IgG_1 + IgG_2$	20.0	10,000
IgM	4.5	150

[a] Mean protein concentration level of isolated immunoglobulins were taken from the quantitation data of Duncan et al. (3).

Sheep have been found to respond to acute viral infection by an initial formation of the IgM class antibodies followed in a couple of weeks by the formation of IgG type (7). The presence of IgM neutralizing antibodies in visna sheep 6 years after inoculation with the virus probably reflects the persistent nature of the visna virus infection.

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