Autoimmunity Induced in Rabbits by Rinderpest Virus

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During rinderpest virus infection in rabbits, 18S cold hemagglutinating antibody against rabbit erythrocytes and 7S anti-nuclear antibody, which reacts with the nuclei and/or the nuclear membranes in immunofluorescent staining, were demonstrated. Virus infection that affected the thymus-dependent immune functions was speculated to act as a trigger for the production of these two autoantibodies.

The concept that virus may act as trigger for development of autoimmunity has been proposed by demonstration of various types of autoantibodies after virus infection as well as by demonstration of virus-like structures in the tissues of patients with autoimmune diseases (1, 3, 5, 6). Among several candidate viruses as triggers for autoimmunity, measles virus is of particular interest. The lymphocytotoxic antibody which reacts with both the autologous and allogeneic lymphocytes at cold temperature was detected in high frequency in the sera of measles patients (7). The presence of demyelinating antibody, which destroys the myelin sheath in vitro, was also demonstrated during measles infection and was speculated to be involved in pathogenesis of demyelination in measles encephalitis and subacute sclerosing panencephalitis (12). Recently, presence of deoxyribonucleic acid with base sequences complementary to ribonucleic acid of measles virus was revealed in the tissues of patients with systemic lupus erythematosus, suggesting possible involvement of measles virus in pathogenesis of this autoimmune disease (1).

Rinderpest virus, which is classified in the same subgroup as measles virus in the paramyxovirus group, was previously shown to exhibit pathogenicity in rabbits similar to that of measles infection in humans (10, 11). In the present paper, we wish to report the development of two types of autoantibodies, i.e., cold hemagglutinating antibody (HA) and anti-nuclear antibody (ANA), in rabbits during rinderpest virus infection.

MATERIALS AND METHODS

Virus. The L strain of rinderpest virus was used. Details of the stock virus preparation and of the inactivation procedure with formalin were described previously (10). The virus was inoculated intravenously into rabbits.

Rabbits. Albino rabbits, JW-NIBS strain (both sexes; 3 months old), were obtained from a closed colony at the Nippon Institute for Biological Science, Tokyo. They were confirmed to be free from coccidial infection by examinations of the feces for oocysts. SFP-Japanese white rabbits free from infections with coccidium (Eimeria spp.), Mycoplasma, Bordetella bronchiseptica, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella spp., Sendai virus, and rabbit poxvirus were obtained from the colony in our institute.

Titration of cold HA. In a preliminary test, rinderpest virus was confirmed to lack agglutinating activity on rabbit erythrocytes at 4, 25, and 37°C to exclude the possibility of virus-induced hemagglutination. Rabbit sera were separated after the blood clotted by incubation at 37°C for 60 min or at 45°C for 20 min. To the tubes containing 0.25 ml of serial two-fold dilutions of heat-inactivated serum, 0.05 ml of 0.25% rabbit erythrocyte suspension was added, and the mixture was incubated at 4°C overnight. Since the same antibody titers were obtained with autologous and allogeneic erythrocytes, the latter was routinely employed. Agglutination was determined macroscopically by shaking the tubes at 4°C. Subsequently, disappearance of the agglutination pattern upon incubation at 37°C for 30 min was confirmed. The antibody titer was expressed as a reciprocal of the serum dilution that showed positive agglutination. Treatment of the sera with 2-mercaptoethanol was conducted by incubating the sera with 0.1 M 2-mercaptoethanol at 37°C for 60 min.

Detection of ANA. The indirect immunofluorescent technique was used to detect ANA by using diploid human embryonic lung cells as targets. The cells grown on cover slips in Leighton tubes were washed three times with phosphate-buffered saline, followed by a wash with cold acetone, and fixed with acetone at −20°C for 30 min. The cells were incubated at 37°C for 2 h with serial twofold dilutions of serum, washed with phosphate-buffered saline, and then stained at 4°C overnight with goat anti-rabbit gamma globulin serum conjugated with fluorescein isothiocyanate, which was confirmed not to react with the target human embryonic lung cells. The
ANA titer was expressed as a reciprocal of the highest serum dilution that showed nuclear fluorescence.

For determination of the immunoglobulin class of ANA, 0.4 ml of the ANA-positive serum diluted to 1:4 was layered on a linear sucrose gradient (12.5 to 37%, wt/vol) and centrifuged at 35,000 rpm for 16 h in a swinging-type rotor, no. RPS40. As immunoglobulin markers, 0.1 ml of rabbit hemolysin to sheep erythrocytes was also fractionated at the same time. After centrifugation, 5 drops were collected from the pierced bottom of each centrifuge tube and titrated for ANA or hemolysin.

Virus neutralization test. Neutralizing antibody was titrated in Vero cells as described previously (8). The neutralizing antibody titer was expressed as a reciprocal of the serum dilution that protected 50% of the cultures from viral cytopathic effect.

RESULTS

Three experiments were conducted and are summarized in Tables 1 and 2. The time course of neutralizing antibody development was examined in rabbits in experiment 1. Neutralizing antibody developed 1 week after inoculation and persisted during the experimental period for 13 weeks (Fig. 1).

The time course of cold HA development is summarized in Table 1. In uninoculated rabbits and those inoculated with formalin-inactivated virus, cold HA titers were 4 or lower. In contrast, a rise of cold HA was noted in all four rabbits infected with live virus 1 to 2 weeks previously. The antibody reached a maximum titer of 64 at 4 to 8 weeks; thereafter, the titers tended to decrease in three rabbits to below the normal level of 4. Cold HA was found to be sensitive to 2-mercaptoethanol treatment, indicating the presence of 19S antibody. Since the rise of cold HA was observed in rabbits infected with live virus but not in those with inactivated virus, it may be concluded that virus infection was responsible for the production of cold HA.

Development of ANA was observed 2 weeks after inoculation of live virus (Table 2). The ANA was detected by one of two types of fluorescence, i.e., fluorescence in the nuclei, except the nucleoli, and fluorescence in the nuclear membranes (Fig. 2). At 2 to 4 weeks after inoculation, ANA reached maximum titers ranging from 16 to 256 and disappeared 1 to 5 weeks later. Except in the one rabbit (no. 288) that showed a transient rise of ANA with a titer of 8 at 2 weeks after inoculation of inactivated virus, ANA was not observed either in uninoculated rabbits or those inoculated with inactivated virus. After fractionation of ANA-positive sera in the sucrose gradient, ANA was demonstrated only in the fraction corresponding to 7S rabbit hemolysin.

DISCUSSION

The present study revealed transient production of 19S cold HA and 7S ANA during rinderpest virus infection in rabbits. It has been reported that coccidial infection leads to production of autoantibodies in rabbits (3). Development of cold HA has also been noticed in mycoplasma infection in humans (3). However, the rabbits employed in the present experiments were free from the coccidial infection. Similar development of autoantibodies was demonstrated in the SPF rabbits and in the conventional ones. Therefore, the possibility of coccidium- or mycoplasma-induced autoantibodies can be excluded. Alternatively, participation of

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* In experiment 2, SPF rabbits were employed.

Not done.
an immune complex of virus antigens and specific antibody, such as cryoglobulin, might occur during hemagglutination. However, viremia was demonstrated to occur between 3 and 7 days after inoculation of rinderpest virus (10), whereas cold HA was present for 6 weeks or longer. Moreover, rinderpest virus lacks agglutinating activity against rabbit erythrocytes. Therefore, this possibility seems to be unlikely.

There was a marked difference between the time course of neutralizing antibody and those of the autoantibodies, the former exhibiting a persisting pattern and the latter exhibiting a transient pattern. The autoantibodies were demonstrated in rabbits inoculated with live virus but not in those inoculated with inactivated virus. These results may exclude the possibility of antigens cross-reacting to the autoantigens during autoantibody production, and suggest that the autoantibody production was triggered by virus infection.

Allison et al. (2) proposed that a subpopulation of T cells is involved in feedback control of the proliferation of autoantibody-producing B cells. In rabbits, development of autoimmunity after removal of the thymus and/or the appendix was observed, indicating key roles for these central lymphoid tissues in controlling the development of autoimmunity (4, 9). In previous studies, rinderpest virus was shown to affect the morphology and function of both the central and peripheral lymphoid tissues. Lymphoid necrosis leading to severe depletion of the lymphocytes was induced transiently 3 to 7 days after infection (10). Marked suppression of the proliferative response of peripheral lymphocytes to phytohemagglutinin, delayed hypersensitivity to tuberculin, and antibody production against sheep erythrocytes were generally observed 3 to 14 days after infection (11). Transient production of the two autoantibodies seemed to follow these destructive effects of virus on the lymphoid tissues. Therefore, the destructive effect of
rinderpest virus on T cells may act as a trigger for the production of autoantibodies.

ACKNOWLEDGMENTS
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LITERATURE CITED

Fig. 2. Fluorescence pattern of ANA. (a) In the nuclei; (b) in the nuclear membranes.


