COMPARISON OF AUTOIMMUNITY INDUCTION WITH VIRULENT AND ATTENUATED RINDERPEST VIRUS IN RABBITS

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SUMMARY: Two strains of rinderpest virus which differ each other in virulence to rabbits were compared in their capacity to produce autoantibodies and their effects on the function of the lymphoid system. The virulent L strain induced two autoantibodies, i.e., antinuclear antibody (ANA) and cold hemagglutinating antibody (HA), and suppressed lymphocyte response to phytohemagglutinin and to concanavalin A for at least 4 weeks after infection. The attenuated LA strain, on the other hand, failed to induce the autoantibodies except transient production of cold HA in few animals, although persistent production of virus-neutralizing antibody like that in L strain infection was observed. The suppression of lymphocyte responses to mitogens was limited to a period of 3-7 days after infection. Possible mechanism of the virus-induced autoimmunity was discussed in relation to the immunosuppressive effect of virus infection.

INTRODUCTION

Rinderpest virus belongs to the morbillivirus subgroup of paramyxoviridae which includes measles and canine distemper viruses. The lapinized rinderpest virus L strain is attenuated for cattle, the natural host, but exhibits high virulence in rabbits affecting the lymphoid tissues as a major target like measles virus infection in human (Fukusho and Nakamura, 1940; Yamanouchi et al., 1974a; Yamanouchi, 1980).

L strain further attenuated for rabbits by serial passages in chicken embryos was designated as LA strain (lapinized-avianized) (Nakamura and Miyamoto, 1953). In rabbits inoculated with LA strain, clinical symptoms such as fever and diarrhea are not observed, whereas a marked antibody response to the virus is evoked, indicating the occurrence of efficient virus growth.

In a previous paper (Fukuda and Yamanouchi, 1976), we reported consistent production of two types of autoantibodies, i.e., 7S antinuclear antibody (ANA) and 19S cold hemagglutinating antibody (HA) against rabbit erythrocytes during L strain infection in rabbits, and possible association of virus-induced immunosuppression with the induction of the autoimmunity.
In the present study, we compared these two strains of rinderpest virus, L and LA, in the capacity to induce autoimmunity and immunosuppression. The virulent L strain was found to cause lymphoid necrosis, prolonged suppression of lymphocyte responsiveness to mitogens, and production of autoantibodies. In contrast, the attenuated LA strain failed to induce lymphoid necrosis and autoantibodies except only transient production of cold HA. Suppression of lymphocyte responsiveness was also only transiently noticed.

**Materials and Methods**

*Virus:* Details of the stock virus preparation were described previously (Yamanouchi et al., 1974a). In brief, L strain was prepared as 10% homogenate of the mesenteric lymph nodes and the spleens of the rabbits infected with the virus 5–7 days before, and LA strain was propagated in Vero cell cultures. Infectivity titer of the stock L strain could be titrated only in rabbits and was $10^{3.0}$ ID$_{50}$/ml. Infectivity titer of LA strain was $10^{6.0}$ TCID$_{50}$/ml when titrated in Vero cells.

*Rabbits:* Male albino rabbits, JW-NIBS strain, at 3 months of age in a closed colony were purchased from the Nippon Institute for Biological Science, Tokyo. They were confirmed to be free from coccidial infection by the examination of feces for oocysts.

*Titration of cold HA:* Details were previously described (Fukuda and Yamanouchi, 1976). In brief, rabbit sera were separated after clotting at 37°C for 60 min and inactivated at 56°C for 30 min. Two-tenth milliliters of serial twofold dilutions of the serum was mixed with 0.05 ml of a 0.25% allogeneic rabbit erythrocyte suspension. After incubation overnight at 4°C, the agglutination pattern was examined macroscopically and then disappearance of the agglutination pattern after incubation at 37°C for 30 min was confirmed. The reciprocal of the highest serum dilution showing positive agglutination was expressed as cold HA titer.

*Titration of ANA:* Frozen sections of rabbit spleen, chicken erythrocytes and diploid human embryonic lung (HEL) cells were compared for ANA detection by the indirect immunofluorescent technique in a preliminary study. Although similar results were obtained with these three target cells, HEL cells were selected as target because of the clearest staining pattern. Serial twofold dilutions of rabbit sera were titrated for ANA by the procedure described previously (Fukuda and Yamanouchi, 1976), and the reciprocal of the highest serum dilution showing nuclear fluorescence was taken as ANA titer.

*Virus neutralization test:* Neutralizing antibody was titrated in Vero cells grown in semi-microplates (Coastar 3524) with LA strain as a challenge virus (Yamanouchi et al., 1969). The reciprocal of the highest serum dilution protecting 50% of the cell cultures from the viral cytopathic effect was taken as neutralizing antibody titer.

*Response of lymphocytes to mitogens:* Peripheral blood was tested directly
for the response to concanavaline A (Con A; Difco Lab., Detroit, MI). Heparinized blood was obtained from the ear vein, diluted to 1:10 and 50 μl was mixed with 1 μg per 10 μl of Con A in 96 well-microplates (Linbro S-MRC 96). After incubation at 37°C for 48 hr in a CO₂ incubator, 0.2 μCi per 10 μl of ¹²⁵IUDR was added, and the mixtures were incubated overnight. The radioactivity of the trichloroacetic acid-insoluble fraction was measured with a Beckman Biogammacounter.

The spleen and mesenteric lymph nodes were minced and filtered through wire-mesh screen. The lymphocytes were suspended in RPMI 1640 medium supplemented with 20% normal rabbit serum to make a cell concentration of 5×10⁶/ml. A 100-μl portion of the cell suspension was put into each well in microtest plates (Falcon 3040) and added 2.5 μg of Con A or 1.5 μg of phytohemagglutinin (PHA; Difco Lab.). After incubation at 37°C in the CO₂ incubator for 48 hr, 1 μCi of ³H-thymidine (Amersham Corp., specific activity 24 Ci/m mol) was added and the mixture was incubated overnight. The cells were collected by an automatic cell harvester (Labo Science, Tokyo), and the radioactivity in the filter paper disc was determined in an Aloka liquid scintillation counter.

Responsiveness of lymphocytes from all the three sources was expressed as stimulation index (S.I.) calculated by dividing the isotope count in the presence of mitogen by that in its absence.

**Results**

**Development of Virus-Neutralizing Antibody and Autoantibodies**

Two groups each consisting of four rabbits were inoculated with L or LA strain, and their sera were examined every week for virus-neutralizing antibody and for cold HA. As shown in Fig. 1, virus-neutralizing antibody was first detected 5 days post inoculation (pi) with L strain and 7 days pi with LA strain, reached the maximum titer around 3 weeks pi, and showed a persistent pattern of antibody production in the animals inoculated with either virus strain. It is noteworthy that the maximum antibody titer in the animals inoculated with LA strain was 10 times higher than that in those inoculated with L strain.

The pattern of development of cold HA is shown in Fig. 2. In the animals inoculated with L strain, cold HA was first detected 4 days pi. The titers increased to the maximum about 4 weeks and became undetectable 9 weeks later. In contrast, the infection with LA strain induced cold HA production in three of the four rabbits only 7 days pi.

Development of ANA after inoculation with L strain was examined with the serum samples collected in several experiments. As shown in Fig. 3, ANA started to develop a week pi, increased to the maximum titer about 2 weeks pi, and then decreased to negative by 6 to 7 weeks pi.

The four rabbits inoculated with LA strain were bled weekly from 1 to 12 weeks pi. All the sera were negative for ANA (data not shown).
Fig. 1. Time course of virus-neutralizing antibody.
The sera of rabbits inoculated either with L or LA strain were examined for virus-neutralizing antibody in Vero cells with LA strain as a challenge virus. Antibody titers in $\log_{10}$ are shown in the ordinates.

Fig. 2. Time course of cold hemagglutinating antibody (HA).
The sera of rabbits inoculated either with L or LA strain were examined for cold HA by incubating with allogeneic erythrocytes at 4°C overnight. Antibody titers in $\log_{10}$ are shown in the ordinates.
Fig. 3. Time course of antinuclear antibody (ANA).
Rabbits inoculated with L strain were examined for ANA by the indirect immunofluorescence technique with human embryonic lung cells as targets. Antibody titers in log₂ are shown in the ordinates.

Fig. 4. Response of peripheral blood lymphocytes to Con A.
Heparinized blood obtained from each of four rabbits was examined for lymphocyte response to Con A in the presence of ²²³I UdR. Abcissae indicate days after virus inoculation. Ordinates indicate stimulation indices.
Responsiveness of Lymphocytes to Mitogens

Three groups each consisting of four rabbits were inoculated with L or LA strain or uninoculated. The blood samples obtained from the ear vein at intervals were examined for the responsiveness of circulating lymphocytes to Con A. As shown in Fig. 4, a marked suppression of lymphocyte response to Con A compared with that of the control group was observed 3–28 days pi in the rabbits inoculated with L strain, and only 3–7 days pi in those inoculated with LA strain.

To analyze the suppressive effect of virus infection on lymphocytes in the lymphoid tissues which serve the major target for virus, the spleen and mesenteric lymph nodes were collected and examined for the lymphocyte response to Con A or PHA (Fig. 5a). The suppression of response of spleen lymphocytes to both Con A and PHA was noticed 7 days pi with L strain but restored the responsiveness to almost the normal level 28 days pi. In the rabbits inoculated with LA strain, no marked suppression of response of spleen lymphocytes was observed 3, 7, or 28 days pi except in one rabbit 3 days pi.

As shown in Fig. 5b, the lymphocytes of the mesenteric lymph nodes re-
lymph nodes were examined for lymphocyte response either to Con A or to PHA.

a: response of spleen lymphocytes, b: response of mesenteric lymph nodes

sponded to both mitogens almost 10 times more strongly than did those of the spleen. In the rabbits inoculated with L strain, a marked suppression of the lymph node lymphocyte response to both mitogens was observed 7 and 28 days pi, whereas a transient suppression was observed only 3 days pi in the rabbits inoculated with LA strain.

These results indicate that the infection with L strain induced immunosuppression on lymphocytes of the mesenteric lymph nodes persisting for at least 28 days and that the infection with LA strain showed a similar suppressive effect only in such an early stage as 3 days pi.

DISCUSSION

Virus infection has been suspected of being involved in human autoimmune diseases, although only indirect evidences, which indicate spontaneous development of autoantibodies associated with certain virus infections, are available (Asherson, 1968; Laitinen, Uesikari and Vaheri, 1972; Phillips and Christian, 1973; Shirodaria et al., 1979). Experimental evidences for virus-induced auto-
TABLE I

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<th>Strain</th>
<th>Clinical sign</th>
<th>Virus-neutralizing antibody</th>
<th>ANA (weeks pi)</th>
<th>Cold HA (weeks pi)</th>
<th>Suppression of lymphocyte response to mitogens</th>
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<td>Peripheral blood (days pi) Spleen (days pi)</td>
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<td>L</td>
<td>+</td>
<td>Persist</td>
<td>1-7</td>
<td>1-10</td>
<td>3-28</td>
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<tr>
<td>LA</td>
<td>-</td>
<td>Persist Negative</td>
<td>1</td>
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<td>3-7</td>
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immunity in laboratory animals are also very limited (Levy, 1977). Rinderpest virus is considered to have unique characteristics as it seems to be the only virus which consistently induces ANA production upon experimental infection. In the previous report, (Fukuda and Yamanouchi, 1976), we suggested that the destructive effect of virus on the lymphoid tissues may be followed by the production of autoantibodies.

In the present study, two types of rinderpest virus, virulent and attenuated for rabbits, were compared in the capacity to produce autoantibodies and their effects on the lymphoid tissues. Histopathological lesions in the lymphoid tissues of the rabbits infected with virulent L strain were studied previously in detail. Briefly, the lesions consisting of lymphoid necrosis and giant cells appear 3 days pi reaching the maximum 5–7 days pi and are rapidly repaired by 14 days pi. Almost all the lymphoid tissues are affected; especially the mesenteric lymph nodes and such other gut-associated lymphoid tissues as appendix, sacculus rotundus, and Peyer's patches are most severely affected and are considered to be the major growth site of the virus. In addition, marked depletion of thymocytes is observed in the thymus (Yamanouchi et al., 1974a; Chino and Yamanouchi, 1974). In contrast, attenuated LA strain was recently found to produce mild lesions consisting only of giant cell formation in the lymphoid tissues, whereas lymphoid necrosis is not observed (Chino, Kobune, Fukuda, and Yamanouchi, unpublished data). The presence of giant cells can be taken as an indication of virus growth in situ, since the accumulation of nucleocapsids of rinderpest virus in giant cells were demonstrated ultrastructurally in the lymphoid tissues of cattle (Tajima and Ushijima, 1971) and in Vero cells (Kobune et al., 1981). Therefore, LA strain may grow in the lymphoid tissues without exerting any marked destructive effect on the lymphoid tissues.

In contrast to L strain, infection with LA strain induced only transient production of cold HA and failed to produce ANA. Table I shows comparative immunopathological responses to each of the two virus strains. The big differences between them are the absence of lymphoid necrosis and ANA, shorter duration of suppression of lymphocyte responses to mitogens, and the lack of clinical signs in the infection with LA strain. Previous histopathological ex-
aminations disclosed that L-strain infection causes marked destruction of T cells leading to necrosis of the T-dependent area in the lymphoid tissues, hence the presence of lymphoid necrosis was assumed to be relevant to the immuno-suppressive effect of virus on T cells (Yamanouchi et al., 1974a; Chino and Yamanouchi, 1974). Therefore, the absence of lymphoid necrosis and the induction of only transient immunosuppression in LA-strain infection suggest that the inhibitory effect of LA strain on T cells is much milder than that of L strain.

Evidences for the control of auto-reactive B cells by suppressor T cells are accumulated mainly in the NZB mouse model (Chused et al., 1978) and in adult thymectomy experiments (Carnaud, Carreire and Bach, 1977). Thus, the loss or malfunction of suppressor T cells leading to activation of B cells are postulated as an important autoimmune mechanism. In this aspect, the lack or slight response of autoantibody production in LA-strain infection in contrast to the consistent autoantibody production in L-strain infection may indicate that the viral inhibitory effect on T-cell functions is responsible for the autoantibody production, possibly by affecting suppressor T cells.

Polyclonal activation of auto-reactive B cells caused by rinderpest virus-induced failure of T-cell control as observed in NZB mouse (Chused et al., 1978) remains for future study on L-strain infection. However, the consistent development of both ANA and cold HA in L-strain infection appears to require certain mechanisms in addition to the suppression of the T-cell function, possibly a modification of the specific autoantigens by the virus. Rinderpest virus was recently found to contain two envelope proteins, hemagglutinin and F protein (Sato, Hayami and Yamanouchi, unpublished data). Although the effect of these envelope proteins on the rabbit erythrocytes is unknown at the present, they may possibly modify the surface of the erythrocytes as do measles virus envelope proteins which are able to agglutinate or lyse the primate erythrocytes. Such virus-modified erythrocytes are presumed to serve as autoantigen for cold HA. As a possible candidate of autoantigen for ANA, cellular constituents released from virus-destroyed lymphocytes may be considered. Thus, virus may exhibit carrier effect on antigenic determinants of the erythrocytes or the cellular constituents

<table>
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<th>Lesions in lymphoid tissues</th>
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<td>Necrosis</td>
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<td>Giant cells</td>
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of lymphocytes in addition to the suppressive effect on the lymphocyte function, resulting in production of cold HA or ANA.

It is interesting that cold HA is produced as early as 4 days pi. This antibody is of IgM type as previously reported (Fukuda and Yamanouchi, 1976). The results are compatible with the early appearance of IgM antibody to virus envelope protein in the rabbits infected with L strain 3 days before (Kobune, Ito and Yamanouchi, 1976). Rapid occurrence of viremia caused by iv inoculation of virus may be responsible for such early antibody responses.

The virus-neutralizing antibody was equally produced by either virus strain showing a persistent pattern. Apparently virus-specific antibody response proceeds independently from the production of autoantibodies. Generally, antibody response in infection with an attenuated virus is lower than that with virulent one. In this aspect, it is noteworthy that the attenuated LA strain induced antibody response to the virus 10 times more strongly than that induced by virulent L strain. The infection with L strain was previously shown to suppress antibody response to sheep red blood cells as well as the proliferative response of lymphocytes to mitogens (Yamanouchi et al., 1974b). In such infection with microorganisms that leads to nonspecific immunosuppression, possible suppression involving not only the immunizing antigen but also the infecting microorganism itself has been suspected (Mims, 1977). It might be suggested that the suppressive effect of virus on antibody response to virus itself caused a lower antibody response to virus in L-strain-infected rabbits than that in LA-strain-infected ones.

Thus far, no histological lesion of autoimmune disease has been detected in L strain-infected rabbits nor in LA strain-infected ones, in spite of autoantibody production in the formers. The transient nature of the autoantibody response may not fully account for such autoimmune lesions as depositions of immune complexes. Since rinderpest virus is rapidly eliminated from the lymphoid lesions (Yamanouchi et al., 1974a), it seems necessary to prolong virus growth in the lymphoid tissues to produce autoimmune lesions. However, several attempts at immunosuppression or immune potentiation on L-strain-infected rabbits were not successful in making the virus persistent (unpublished data).

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**References**


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