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Pathogenesis of Rinderpest Virus Infection in Rabbits

II. Effect of Rinderpest Virus on the Immune Functions of Rabbits

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Rinderpest virus infection was shown to induce marked suppression of both humoral antibody response and cell-mediated immunity in rabbits. The virus exhibited a suppressive effect on primary antibody response as indicated by a decrease in numbers of plaque-forming cells (immunoglobulin [Ig]M) and hemagglutinating antibody titers of both IgM and IgG types to sheep red blood cells, whereas there was no detectable effect of the virus on the production of memory cells. Virus-induced suppression of cell-mediated immunity was demonstrated by a decreased rate of proliferative response of peripheral lymphocytes to phytohemagglutinin stimulus and by a depression of delayed-type skin reactions to purified protein derivative. Such suppressive effects were indicated to persist for 14 days or longer. Alteration in phagocytic activity of the reticuloendothelial system was not observed. The relevance of the virus-induced histological lesions in the lymphoid tissues to the virus-induced immunosuppression was discussed.

In the preceding paper (17), we indicated that rinderpest virus infection in rabbits is similar in several aspects to measles virus infection. It is well known that measles virus induces a suppression of cell-mediated immunity such as delayed hypersensitivity to tuberculin and several other antigens (8, 16). However, the mechanism of the immunosuppression is still unclear. Since rinderpest virus was shown to grow primarily in the lymphoreticular cells, similar to the growth of measles virus, rinderpest virus was suspected to affect the immune capacities of the host. In the present study, effects of rinderpest virus infection on humoral antibody response, cell-mediated immunity, and phagocytic activity of the reticuloendothelial system (RES) were examined.

MATERIALS AND METHODS

Virus. The L strain of rinderpest virus was used. Details of the stock virus preparation were described in the preceding paper (17).

Formalin inactivation of virus. The stock virus was inactivated at 37 C for 48 h by addition of 0.025% Formalin. After dialysis against Eagle medium, the inactivated virus was stored at −80 C until used. Inactivation was confirmed by rabbit inoculation.

Rabbits. Albino rabbits, JW-NIBS strain, were used as described previously (17).

Titration of hemagglutinating (HA) antibody. Rabbits were intravenously inoculated with 10^8 sheep red blood cells (SRBC). Serum was collected at intervals and inactivated by heating at 56 C for 30 min, and samples were treated with 0.1 M 2-mercaptoethanol (2-ME) at 37 C for 30 min. Both untreated and 2-ME-treated sera were titrated for HA antibody against a 0.5% SRBC suspension on the microplates by using phosphate-buffered saline containing 0.01% gelatin and 0.1% bovine serum albumin as a diluent. HA antibody titer was expressed as the reciprocal of the highest serum dilution which showed a distinct hemagglutination pattern.

Enumeration of plaque-forming cells (PFC). Number of immunoglobulin (Ig) M PFC in the spleens of rabbits immunized intravenously with 10^8 SRBC was determined by the direct slide technique of Cunningham and Szenberg (5). Since the preliminary experiment revealed production of the maximal number of PFC on the 7th day after immunization, the spleens were harvested on day 7.

Delayed hypersensitivity to purified protein derivative (PPD). Rabbits were sensitized by intramuscular inoculation of 10 mg of heat-killed *Mycobacterium tuberculosis* emulsified in Drakeol (Pennsylvania Refining Corp., Butler, Pa.) into footpads and backs 7 to 8 weeks before virus inoculation. Skin tests were conducted with 1 μg of PPD and were read after 24 and 48 h.

In vitro response of lymphocytes to phytohemagglutinin (PHA). Samples of heparinized blood (50 to 70 ml) were collected from the heart, one-tenth volume of 10% dextran was added, and the mixtures were placed at 37 C for 50 min. The leukocyte-rich upper layer was separated, washed two times by centrifugation at 800 rpm for 5 min, and suspended in

medium 199 supplemented with 20% normal rabbit serum. The number of viable nucleated cells was counted by staining with trypan blue and was adjusted to 10^6 cells/ml. Eight cultures were prepared by dispensing 1.5-ml samples into small tubes; 0.1 ml of PHA-M (Difco Laboratories, Detroit, Mich.) was added to each of the four tubes, and 0.1 ml of phosphate-buffered saline was added to the remaining four tubes. After incubation at 37 C for 24 h, 1 μCi of [^{3}H]thymidine (Radiochemical Centre, Amersham; specific activity, 5.0 Ci/mmol) was added, and incubation continued for another 24 h at 37 C. The cells were sedimented by centrifugation at 800 rpm for 5 min and then hemolyzed by adding 5 ml of 1% sodium citrate dropwise. After centrifugation, the pellets were resuspended in 5 ml of 5% trichloroacetic acid. The trichloroacetic acid-insoluble fractions were collected on filter papers, washed with ethanol three times, and dried. Radioactivity of the precipitates was counted in a Packard Tri-Carb liquid scintillation spectrometer. The degree of PHA response was expressed as the ratio of the average uptakes of the isotope in the cultures with PHA to those in the cultures without PHA.

Carbon clearance. Rabbits were given intravenous injections of colloidal carbon (India Ink C11/143a, Günther Wagner, Pelikan Werke, Germany) at a dose of 8 mg per 100 g of body weight. At 4, 8, 12, 16, 30, 45, and 60 min, blood was collected from the ear vein, a 0.1-ml sample was mixed immediately with 3 ml of distilled water to lyse the red blood cells, and the carbon content in the blood was measured by a spectrophotometer at 620 nm. Clearance rate was calculated according to the method of Biozzi et al. (1).

TABLE 1. *Effect of rinderpest virus infection on the response of lymphocytes to PHA*

Days postinoculation	Rabbit no.	^{3}H-uptake (counts/min)[a]		Degree of PHA response (PHA+/PHA−)
		PHA−	PHA+	
3	121	139 (65)	52 (47)	0.4[b] (1.1)
	123	60	81	1.4[b]
	124	50	33	0.7[b]
	125	12	23	1.9[b]
7	111	81 (68)	57 (79)	0.7[b] (1.1)
	112	58	65	1.1[b]
	113	82	150	1.8[b]
	114	91	99	1.1[b]
	115	30	23	0.8[b]
14	116	89 (46)	288 (105)	3.2[b] (1.8)
	117	16	25	1.6[b]
	118	53	39	0.7[b]
	119	21	22	1.0[b]
	120	60	153	2.5[c]
28	137	39 (37)	70 (344)	1.8[b] (10.1)
	138	33	1,218	36.9
	139	36	342	9.4[c]
	140	34	43	1.3[b]
	141	45	46	1.0[b]
Control	126	15 (30)	383 (1470)	25.5 (54.5)
	127	37	1,134	30.7
	129	38	420	11.0
	130	17	2,489	146.4
	148	20	924	46.2
	149	39	1,056	27.0
	150	41	3,882	94.7

[a] Numbers in parentheses indicate mean values in each group.
[b] $P < 0.01$.
[c] $P < 0.05$.

RESULTS

In vitro response of lymphocytes to PHA. Five groups of four to seven rabbits each were examined for the proliferative capacity of peripheral lymphocytes to in vitro PHA stimulus. Four groups were inoculated intravenously with the virus and killed at 3, 7, 14, and 28 days, respectively, and one group was used as an uninoculated control (Table 1). On days 3 to 14, a marked suppression of PHA response was observed in all of the rabbits. On day 28, significant suppression remained in four of the five rabbits, whereas one rabbit recovered normal capacity. Thus, it is apparent that rinderpest virus showed a suppressive effect on the proliferative capacity of lymphocytes to PHA for more than 4 weeks after infection.

Skin reaction to PPD. Two groups of rabbits sensitized with *Mycobacterium tuberculosis* were used to examine the effect of virus on delayed hypersensitivity. The first group was infected with the virus, and the other one was inoculated with the Formalin-inactivated virus. At 3, 7, 14, and 28 days, rabbits were examined by skin tests with PPD. All four infected rabbits failed to react with PPD on days 3 to 7 (Table 2). On day 14, one rabbit (R157) restored delayed hypersensitivity, whereas complete or partial suppression remained in the other three rabbits. On day 28, restoration of delayed hypersensitivity was observed in all of the rabbits except the one that still showed a partial suppression. The two rabbits inoculated with the Formalin-inactivated virus consistently reacted positively with PPD, indicating that the suppression was induced by infectious virus and that the repeated skin tests did not show any remarkable suppressive effect.

Effect of rinderpest virus on primary antibody response. Eight groups of five rabbits each were used. Among them, six groups were challenged with SRBC at −4, 0, 3, 7, 14, and 28 days after virus inoculation. The other two groups were left as controls; the first one was immunized with SRBC without virus inoculation, and the second one was uninoculated and unimmunized. On the 7th day of SRBC challenge, spleens and sera were examined for PFC and HA antibodies.

The results of the experiment are summarized in Table 3. Development of PFC was

TABLE 2. *Effect of rinderpest virus infection on skin reaction to PPD*

Inoculum	Rabbit no.	Reading (h)	Skin reaction[a]				
			Preinoculation	3 dpi[b]	7 dpi	14 dpi	28 dpi
Live virus	157	24	15.0	0	0	26.5	15.0
		48	16.0	0	0	20.5	22.5
	158	24	14.5	0	0	0	16.0
		48	17.0	0	0	0	22.5
	159	24	16.4	0	0	0	14.0
		48	18.4	0	0	7.5	14.5
	160	24	14.5	0	0	0	0
		48	18.4	0	0	10.5	14.5
Formalin-inactivated virus	181	24	21.4	21.0	19.4	18.5	18.0
		48	22.4	21.0	21.8	25.5	21.4
	182	24	27.7	20.0	22.8	25.4	22.0
		48	27.4	25.1	27.4	31.4	26.8

[a] Geometric mean of diameters of erythema (in millimeters).
[b] dpi, Days postinoculation.

TABLE 3. *Effect of rinderpest virus infection on antibody response to SRBC*

Group[a]	SRBC challenge (day of virus infection)	No. of spleen cell[b]	No. of PFC/10^6 cells[c]	PFC/spleen[c]	HA antibody titer[d]	
					Untreated	2-ME-treated
1	−4	1.1×10^8	5.5[e]	12.2[e]	6.4	4.6
2	0	8.1×10^7	3.6[e]	9.8[e]	4.8	<2.0[e]
3	3	8.2×10^7	3.2[e]	9.4[e]	<2.0[e]	<2.0[e]
4	7	6.5×10^7	6.0[e]	11.9[e]	2.6[e]	<2.0[e]
5	14	1.9×10^8	6.9[e]	13.6[e]	3.0[e]	<2.0[e]
6	28	1.2×10^8	9.0	15.9	6.0	<2.0[e]
7	Uninfected control	2.7×10^8	8.9	16.8	6.2	3.8
8	Uninfected, unimmunized control	2.4×10^8	1.8	10.3	2.0	<2.0

[a] Five rabbits per group.
[b] Mean total nucleated cell number per rabbit.
[c] Mean PFC ($\log_2$).
[d] Mean antibody titer ($\log_2$).
[e] $P < 0.01$.

significantly suppressed in the rabbits immunized at −4 to 14 days after virus inoculation. The most marked suppression of PFC was observed in the rabbits immunized at 0 to 3 days after virus inoculation, the number of PFC being reduced to a number close to the background level. HA antibody response was also significantly suppressed by the virus infection. The most marked suppression of 2-ME-sensitive antibody was observed in the group immunized at 3 days after infection. Significant suppression was also present in the groups immunized at 7 to 14 days after infection. In contrast to 2-ME-sensitive antibody response, 2-ME-resistant antibody response was significantly suppressed in the groups immunized at 0 to 28 days after infection. Thus, rinderpest virus was proved to suppress both 2-ME-sensitive and -resistant antibody responses to SRBC. Restoration of normal capacity of antibody response was observed at 28 days in the case of 2-ME-sensitive antibody, whereas restoration of 2-ME-resistant antibody response could not be detected at 28 days.

Effect of rinderpest virus on secondary antibody response. Three groups of three rabbits each were immunized two times with SRBC at intervals of 21 days. Group A was used as an uninfected control, group B was inoculated with the virus 3 days before primary immunization, and group C was inoculated 3 days before secondary immunization.

Typical patterns of primary and secondary antibody responses were observed in control group A (Fig. 1). Virus infection before primary immunization significantly suppressed the primary antibody response, as demonstrated in group B, in agreement with the findings shown in Table 3. However, a typical pattern of secondary response was obtained in group B in spite of suppression of primary response, suggesting the presence of memory cells. In group C, which showed a normal pattern of primary response, virus infection before secondary immunization completely suppressed the secondary response.

Thus, rinderpest virus appeared not to affect the production of memory cells, but to affect the late stages of antibody response such as proliferation or differentiation of antibody-forming cells.

Effect of rinderpest virus on carbon clearance. Three groups of four rabbits each were examined for phagocytic activity of RES; the first group was left as an uninfected control, and the other two groups were inoculated with the virus at 3 or 7 days before the carbon clearance test.

As a result, the clearance rate of the control group was determined to be 0.0120 ± 0.0033. In the virus-infected rabbits, clearance rates were 0.0093 ± 0.0004 at 3 days and 0.0080 ± 0.0009 at 7 days after infection, respectively. Although there was a tendency of slight decrease in the clearance rate after virus infection, the difference was not statistically significant ($P = 5\%$).

DISCUSSION

Since an initial observation of von Pirquet (16) on tuberculin anergy in measles patients, evidence which indicates the measles virus-induced suppression on cell-mediated immunity is accumulating (8). The present study clearly demonstrates that rinderpest virus, which belongs to the same medipest subgroup of paramyxoviruses as measles virus, also affects the immune system of rabbits.

Rinderpest virus infection suppressed humoral antibody response to SRBC as well as cell-mediated immunity such as delayed hypersensitivity to PPD and proliferative response of lymphocytes to PHA. Suppression of antibody response by rinderpest virus was previously reported by Penhale and Pow (15), who demonstrated that the antibody production to chicken RBC was markedly suppressed in the rabbits which developed fever after inoculation of the L strain of rinderpest virus. However, they failed to detect the suppressive effect in the rabbits which did not develop fever after virus inoculation. Since this strain of virus is highly virulent in rabbits and successful infection should result in fever response (17), their negative results in the rabbits without fever may have been caused by a failure of infection. In the present study in which 1,000 mean infective doses of the virus was used, all of the infected rabbits consistently developed fever accompanied by suppression of both humoral and cell-mediated immunity.

Several mechanisms have been proposed for the virus-induced suppression of antibody response (6, 12). Antibody production is generally considered to proceed through the following steps: (a) uptake and processing of antigen by macrophages; (b) antigen recognition by T and B cells; (c) proliferation of antibody-forming cells accompanied by production of memory cells; and (d) differentiation into plasma cells. The suppressive effect of virus on the macrophage level was discussed in the case of Friend leukemia virus. Competition for the common precursor cells of antibody-forming cells between virus and immunogen have also been

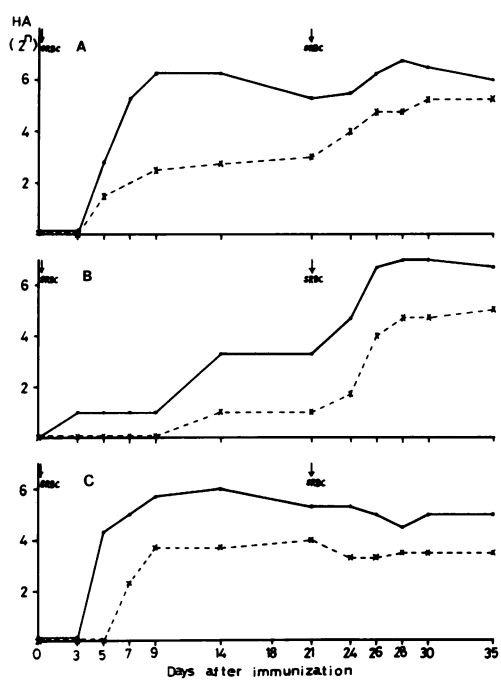

FIG. 1. *Effect of rinderpest virus on primary and secondary antibody responses to SRBC. A, Uninfected control; B, infected with virus 3 days before primary immunization; C, infected with virus 3 days before secondary immunization. Symbols:* ●——●, *Mean HA titer of the untreated group;* ×-----×, *mean HA titer of the 2-ME-treated group.*

suggested in Friend and Rauscher leukemia viruses (6, 12). In the present experiments, virus infection before primary immunization resulted in significant suppression of the primary antibody response, but the secondary antibody response was not affected (Fig. 1, group B), in agreement with the previous findings by Penhale and Pow (15). Virus-induced suppression was observed as late as 14 days after infection, when the virus antigen was eliminated from the circulation. These results may suggest that the virus does not affect the early steps of antibody production, such as processing and recognition of antigen, but affects primarily the late steps of antibody response, possibly those of proliferation or differentiation of antibody-forming cells. Virus infection before secondary immunization did not show a significant effect on the preexisting antibody titers but suppressed the secondary antibody response (Fig. 1, group C). A suppressive effect on HA antibody titers in the sera was not observed in the group immunized at 4 days before virus inoculation, in spite of significant decrease in numbers of PFC (Table 3, group 1). These results may suggest a lack of virus effect on antibody production by the plasma cells which were already differentiated before virus attacked the lymphoreticular cells. However, the reason for the discrepancy in virus effects at various levels of serum antibody and PFC (Table 3, group 1) is not elucidated. A similar phenomenon was observed in the case of cytomegalovirus (14). Since PFC usually consist of large lymphoblasts as well as plasma cells, one might speculate upon the relative susceptibility of the former cell population to virus effects in contrast to resistance of the latter. In rabbits, antibody-producing cells are thought to be derived from the gut-associated lymphoid tissues (GALT) such as the bursa of Fabricius in chickens (4). Severe involvement of the GALT by viral cytopathic effect can be taken as a cause of the virus-induced suppression of antibody response. It is also possible that the virus growth in these lymphoid tissues results in nonlethal alterations of immune cell competence, as postulated in cytomegalovirus infection of mice (14).

There seems to be a slight difference in persistence of suppressive effect between 2-ME-resistant and -sensitive antibodies; the former appeared to be suppressed for longer periods than the latter. However, such differential effect on IgG and IgM antibodies cannot be explained at the present moment. The possibility of participation of different lymphoid tissues as a central organ for development of IgG- and IgM-producing cells was proposed by Hanaoka et al. (9). A detailed histopathological analysis of virus effect on the GALT and other lymphoid tissues might be helpful in clarifying this problem.

Suppression of cell-mediated immunity by rinderpest virus was demonstrated by depressed skin reaction to PPD and in vitro proliferative response of peripheral lymphocytes to PHA. The possibility of direct virus effect on lymphocytes in vitro as a cause of the suppressed PHA response can be ruled out, since the suppression was detected as late as 14 to 28 days after infection, when the viremia disappeared. In measles patients, skin reaction and in vitro response of lymphocytes to various antigens are generally suppressed, whereas in vitro PHA response is not impaired (8). Since the PHA-responding cell is considered mainly as a T cell (3), it is possible that rinderpest virus affects T cells more severely than does measles virus. Virus growth in the thymus-dependent area and induction of necrosis of the lymphoid follicles, which severely involves both lymphoid and reticular cell elements, can be taken as a primary cause of suppression of cell-mediated immunity, as suggested in lymphocytic choriomeningitis virus infection of mice in which the virus induced marked destruction of the thymus-dependent area with concomitant suppression of cell-mediated immunity (10).

Besides the virus effect on T cells, the effect on macrophages may have to be considered. Facilitation by the phagocytic cells of T-cell response to a nonspecific stimulus such as PHA was suggested by Oppenheim et al. (13). In measles patients, a depressed response of lymphocytes to a suboptimal dose of PHA was reported, indicating a possible virus effect on macrophages (7). Skin reaction to PPD is suggested to be generated by a step of production of humoral mediators such as migration inhibition factors by specific immune lymphocytes followed by a step of accumulation of bone marrow-derived cells which may possibly be macrophages (2, 11). Therefore, it will be interesting to examine the effect of rinderpest virus on production of humoral mediators to find the virus effect on macrophages as well as on lymphocytes.

In the present study, rinderpest virus did not show any detectable effect on phagocytic activity. Since the carbon clearance rate was obtained as a total activity of RES, it is difficult to find any implication of the RES function on the rinderpest virus-induced suppression of both humoral and cell-mediated immunities from the present data.

It is difficult to find an analogy between the

mechanism of immunosuppression by measles virus and that by rinderpest virus. However, the present system may serve as a useful model for the study of virus effects on immune systems, since rabbits are relatively convenient laboratory animals in immunological research.

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