Effect of Friend Leukemia Virus and Rowson-Parr
Virus on Immunological Maturation of Mice

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The effect of neonatal infection with Friend virus (FV) and Rowson-Parr virus (RPV) on the maturation of the capacity to respond to sheep red cells, as measured by the numbers of hemolytic plaque-forming cells in the spleen, was investigated in BALB/c mice. Both viruses affected immunological maturation but there were significant differences between their effects. The development with age of the ability to produce plaque-forming cells in response to antigen was virtually abolished by FV and only slightly impaired by RPV. Furthermore, FV also suppressed the development of background plaque-forming cells, whereas RPV did not.

In the last few years it has been recognized that viruses may have notable consequences on the immunological functions of the host, and this area of investigation is receiving increasing attention (24, 31, 32). There are, however, very few data about the action of viral infections on maturation of immunological competence. The problem may be important because several viruses are vertically transmitted and many virus infections are experienced early in life. Moreover, the immune system might well be more susceptible to the action of viruses during maturation than during maturity.

Infants with congenital rubella have no detectable defect of antibody formation, but their lymphocytes are depressed in the ability to undergo blast transformation in vitro (23, 26). Adult mice, infected at birth with gross passage of a leukemia virus, are unable to reject allografts across a weak histocompatibility barrier and have reduced antibody-forming capacity (14, 27). Adult rats neonatally infected with Moloney virus show reduced antibody response and decreased rate of immunoglobulin synthesis (11, 12). Chickens infected at birth with lymphoid leukemia virus show decreased antibody response at 1 month of age but at 3 and 4 months have no significant impairment of humoral and cellular immunity (13). Lymphocytic choriomeningitis virus (LCM), which in adult mice markedly depresses antibody response, during the neonatal period has only a temporary effect and animals from established LCM carrier colonies, which are infected at very early stages of embryonic development, produce normal levels of antibody (21). Neonatal infection with LCM in C57 B1 mice prevents the development of immunological tolerance to bovine gamma globulin given at 8 weeks of age (28) and in New Zealand mice aggravates the autoimmune disease which develops during adulthood (34). This last property is shared by polyoma virus (34).

A systematic study on the immunological maturation of hamsters neonatally infected with SV40 was recently carried out (16). During the early period of immunological maturation there was a suggestion of slight to moderate depression, but in young adults there was little, if any, impairment of antibody response.

This paper describes the effect that neonatal infection with Friend leukemia virus (FV) (17) or Rowson-Parr virus (RPV) (30), which are known to depress the immune responsiveness of adults, exerts on the maturation of antibody response in mice.

MATERIALS AND METHODS

Mice. Litters were from our colony of BALB/c mice, which were originally obtained from the Cancer Research Department of the London Hospital Medical College, London, and maintained by brother-sister mating.

Viruses. FV was originally obtained from Charlotte Friend. It has been passed in Swiss and DBA mice at the Chester Beatty Research Institute of London from 1959 to 1962 and since then has been through several serial passages in BALB/c mice. It has been freed of the lactic dehydrogenase-elevating virus of Riley (20). In adult BALB/c mice, it produces a progressive anemia and thrombocytopenia, together with gross enlargement of the spleen. The preparation used in these experiments consisted of a single batch of citrated plasma collected from adult BALB/c mice 21 days after infection, stored at -70 C, and titrating 10^{3.5} median infectious doses (ID_{50})/0.1 ml, inoculated intravenously (iv) in adult mice.
RPV was obtained as spleen extract of BALB/c mice from M. H. Salaman. It is further purified by three passages at end-point dilution. Three weeks after infection, the spleens of RPV-infected mice range between 180 and 430 mg. The preparation used consisted of a single batch of citrated plasma collected from adult BALB/c mice 21 days after infection, stored at -70°C, and titrating 10^5 ID_{50}/0.1 ml inoculated iv in adult mice.

**Infection.** Since the rate of immunological maturation varies with litter size (1), only litters of six to eight mice were used for these experiments. Each litter was divided into four groups. Within 20 hr of birth, two groups were intraperitoneally (ip) inoculated with 0.05 ml of either FV or RPV, and the other two groups were inoculated with normal mouse plasma. To prevent leakage, the 30-gauge needle was introduced subcutaneously just above the knee and pushed along the internal face of the thigh until the peritoneal cavity was reached.

**Immunization and measurement of antibody response.** Sheep blood, obtained from the same animal throughout the experiments, was suspended in Alsever's solution. Before use the sheep red blood cells (SRBC) were washed four times in 10 volumes of sterile saline, and the buffy coat was removed. SRBC (2.5 × 10^6) in 0.05 ml of saline were ip inoculated at various ages into one infected and one noninfected group of each litter. With younger mice the same precautions as for infection were used. Four days after SRBC administration, all mice were weighed and killed by exsanguination, and the spleens were weighed and tested for direct plaque-forming cells (PFC) by the method of Jerne et al. (19). The two groups of mice which had not received SRBC were used to study background PFC. Plaques were counted by using a previously described device (G. Petracchi et al., J. Clin. Pathol., in press).

**Statistical analysis.** For each spleen, two plates were prepared and the mean counts were recorded. A minimum of six mice were used per group. The geometric means of PFC per spleen are shown in the figures, together with 95% confidence limits of the geometric mean.

**RESULTS**

BALB/c mice ip inoculated in the first 3 days of life with 2.5 × 10^6 SRBC undergo no detectable antibody response, as judged from the number of direct PFC present in the spleen 4 days after antigen administration. A slight but significant increase in PFC number is seen when the mice receive the antigen at 4 days of age; the increase is specific since it does not occur after inoculation with rabbit red cells. In this study, therefore, mice were immunized at 4 or more days of age.

**Spleen and body weight changes.** Spleen and body weight increases with age of mice neonatally infected with FV or RPV and of controls injected with normal mouse plasma are shown in Fig. 1. Body weight was not significantly affected by RPV infection, whereas FV-infected mice were constantly lighter than the controls. The spleens of FV-infected mice were already significantly enlarged at 8 days of age and by day 19 reached a mean weight of 469 mg (controls 92 mg). After this time the mice began to die. In RPV-infected mice, the mean weight of the spleen was significantly higher than in the controls at 11 days of age, by day 19 was 150 mg, and at 34 days reached 273 mg (controls 141 mg). No deaths occurred in these mice over the entire period of observation. A significant difference between mean spleen weight of immunized and nonimmunized animals was found only in noninfected 34-day-old mice.

**Effect on background PFC.** The mean number of background PFC per spleen in the controls increased steadily from 6.7 at day 8 to 177.9 on day 34. RPV-infected animals gave results indistinguishable from the controls, whereas in FV-infected mice background PFC were constantly markedly depressed and on day 14 and 19 were practically absent (Fig. 2).
FV and RPV

Effect on immune PFC. Figure 3 shows the
effect of neonatal infection with FV and RPV
on the number of PFC present in the spleen of
mice of various ages 4 days after SRBC ad-
ministration. In control mice the number of PFC
increased at a very high rate during the first 3
weeks of life, then more slowly until levels similar
to those found in adult mice were reached at the
age of 1 month. In FV-infected animals the
production of PFC was virtually abolished: the
mean number of PFC per spleen was 8.9 in
mice killed at 10 days (controls 371.6) and 8.7
at 19 days (controls 19,960). The effect of RPV
was much less pronounced. Infected mice on
days 8–11 had about half as many PFC per
spleen as controls, on day 14 there was an ap-
proximately 13-fold reduction, and on days
19, 25, and 34 PFC were again about 50% of
those found in the controls. The differences
between mean numbers of PFC in control and in
RPV-infected mice were significant on days
9–11, 14, 19, and 34.

DISCUSSION
RPV, which has been recently isolated from
FV preparations (30), has been shown to depress
antibody response in adults (8), where it induces
a slight and transient hyperplasia of the spleen
followed, after several months, by development
of reticulum cell lymphomas in a high proportion
of animals (Rowson, personal communication).
The immunodepression exerted by FV in adult
mice has been extensively studied (2–5, 7, 9,
15, 18, 25, 29, 33, 35). Although RPV can be
easily isolated from FV, numerous attempts to
obtain FV free from RPV have failed (unpub-
lished data). This suggests that special relations-
ships might exist between the two viruses and
implicates that the preparations of FV used in
these experiments contained large amounts of
RPV.

The results reported in this paper clearly show
that both FV and RPV injected at birth depress
immunological maturation as judged by the
acquisition of the capacity to produce spleen
PFC after SRBC administration. However, con-
siderable differences between the effects of
the two viruses were observed.

FV induced a rapidly progressive splenomegaly
which was accompanied by reduction of body
growth and followed by early deaths. In infected

FIG. 2. Numbers of background plaque-forming
cells to sheep red blood cells at various ages in the
spleen of unimmunized mice inoculated at birth with
Friend virus (■) or Rowson-Parr virus (▼) or normal
mouse plasma (●). Bars represent 95% confidence
limits of the mean.

FIG. 3. Numbers of immune plaque-forming cells to
sheep red blood cells (SRBC) at various ages in the
spleen of mice inoculated at birth with Friend virus
(■) or Rowson-Parr virus (▼) or normal mouse plasma
(●) and immunized with 2.5 × 10⁸ SRBC 4 days be-
fore sacrifice. Bars represent 95% confidence limits
of the mean.
animals, the gradual increase of background PFC number with age and the response in PFC after SRBC were virtually abolished. Immunosuppressions of this extent by FV have not been described in adult mice.

The effects of RPV were much less severe and compared well with those produced in adults (8). Appearance of background PFC proceeded at a normal rate, and, after immunization, infected mice had about 50% fewer PFC than the controls, except on day 14 when there was a 13-fold reduction in PFC count. Body growth was normal and the spleens were moderately enlarged. Degree of immunodepression and degree of splenomegaly did not correlate. At the time of greatest immunodepression, the ratio of infected spleen weight per control spleen weight was 1.7, whereas at 34 days it was 2.2. As observed after adult infection (8), the slight impairment of antibody response caused by neonatal infection with RPV persists for a long time since it has been found also in 10-week-old mice (unpublished data).

Many hypotheses have been proposed to explain how viruses depress immune functions. In the case of FV, injury of immunocompetent cells in the lymphoid organs by neoplastic proliferation may be an important factor since peritoneal lymphocytes, that are not exposed to this injury, show normal immunological reactivity in vitro (6) until late in the course of the disease (2, 3). In the case of RPV, other factors must be responsible in view of the minimal pathological changes that it induces in the early stages of infection. Immunological competition is an obvious candidate, (8) but the occurrence of immunodepression after neonatal infection makes it unfeasible. A contribution of RPV to the immunodepressive activity of FV preparations cannot be ruled out, since FV has not yet been freed from RPV and RPV replicates more rapidly than FV (unpublished data).

The observation that neonatal infection with FV depresses background PFC contrasts with previous findings in adult mice, in which unchanged (25, 35) or increased (18) numbers of background PFC have been found after FV infection. A different effect of FV on preexisting and on developing background PFC is possible. If PFC development is stimulated by antigens cross-reacting with SRBC (10), the same mechanisms which suppress antibody response in actively immunized animals might prevent their formation. Alternatively, the cells after having met the stimulating antigens might not reach the spleen. In adults FV does not interfere with migration of normal lymphoid cells to the spleen (5), but nothing is known about lymphocyte circulation in very young FV-infected mice.

FV is not effectively transmitted from parents to offspring (22). It is suggestive to speculate that this characteristic is a consequence of the severe damages that FV induces in newborn mice. In this respect, it will be of some interest to see whether RPV, which is well tolerated by newborns and immunologically closely related to FV (unpublished results), is vertically transmitted.

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LITERATURE CITED


