PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 449–452 (1978)

Stimulation of Immune Response in Hybrid Mice Following Rauscher Virus Infection[1] (40074)

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Suppression of plaque former cell (PFC) response to sheep red blood cells (SRBC) following Rauscher leukemia virus (RLV) or Friend leukemia virus (FLV) infection has been well documented in several mouse strains sensitive to these viruses (1–3). Likewise, a transient suppression of PFC response has been reported for the virus-resistant C57B1/6 mouse during the first week after FLV infection (4). Ceglowski and Friedman (5) compared PFC immunosuppression by FLV in several strains 8 days after infection and reported that the degree of immunosuppression roughly paralleled that of virus susceptibility, with the only hybrid tested (the B6D2F1 of the C57B1/6 and the DBA) showing even more immunosuppression than its sensitive DBA parent. However, in that study as in most of the previous studies the prime concern was with the immune response of strains known to be sensitive to the virus. Further, many of these studies have been limited in their observation periods to the rather short duration after infection in which confirmation of immunosuppression is usually obtained. On the other hand, recent data of at least one study (4) suggest that stimulation of PFC response by leukemia virus might also be demonstrable in resistant strains if the observation period is sufficiently long. In the present study we have compared the level of PFC response as affected by RLV in the sensitive SJL/J mouse, the resistant C57B1/10 mouse and their resistant hybrid-(SJL/JxC57B1/10)F_1. Evidence for stimulation of PFC response in the resistant mice between the second and fourth week after infection was obtained, with the immune response of the hybrid showing even greater enhancement than that of its resistant parent.

Materials and methods. Mice. The SJL/J and C57B1/10 mice were females obtained from Jackson Laboratories (Bar Harbor, ME) at the age of 6–8 weeks and quarantined before use. The F_1 hybrids were females bred in our own facilities from parents obtained from Jackson Laboratories. All mice were maintained five per cage in sterilized filter-top cages and allowed autoclaved food and water *ad libitum*.

Virus. The NB-tropic strain of Rauscher leukemia virus used was obtained from NIH in 1968 and has been maintained in our laboratories in the SJL/J mouse. Assay for the virus activity was done by a variation of the spleen enlargement dose (SED) technique of Chirigos *et al.* (6), as previously employed by us (7–9). Virus was prepared from a cell free plasma filtrate, buffered with sodium citrate and kept at −72° until use. 0.1 ml of saline diluted virus suspension containing 50 $SED_{50/14}$ units of RLV was injected ip into the mice at approximately 12 weeks of age. Control mice were sham injected with a similarly prepared material not containing virus.

PFC Response. A modification of the Kennedy–Axelrad technique (10) was used to evaluate PFC response. This technique has been shown to yield a greater number of plaques per unit of cells than the Jerne and Nordin technique (11). At the times indicated in the results, either before or after RLV administration, washed SRBC were injected into the virus infected mice and into paired age-matched control mice of the same strain. Five days later the mice were sacrificed, their spleens removed, weighed, gently mashed, and single cell suspensions prepared in PBS. Total nucleated cell number per spleen was determined and then the concentration of the

[1] These studies were supported in part by NIH-NCI Grant No. 1-PO2-CA10438, U.S. Energy Research and Development Administration Contract No. E(11-1)-3097 and HRSF Grant No. T-25.

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0037-9727/78/1573-0449$01.00/0

suspension was adjusted to 2.5×10^6 cells per ml. Two milliliter of this, mixed with guinea-pig complement, was then carefully layered over a monolayer of SRBC afixed to a grided plastic Petri dish with poly-l-lysine. After a 45-min incubation at 37°, the supernate was carefully decanted off and the plates gently rinsed in PBS. The plaques per plate were counted on the damp plates and the number of PFC per spleen calculated. The plaque forming ability of the virus injected mice was compared directly with that for the normal control mice of the same strain run in parallel on each of the experimental days, and the results are reported in terms of percent of the control animal plaque forming ability.

Statistics. For each data point given a minimum of four virus infected animals was run against the controls. Most points were repeated two or more times and the data averaged in the production of the figures. Mean values and standard errors were calculated using the Student's t test as described by Dixon and Massey (12). The error limits as given in the text and figures are ± 1 SE.

Results. The mean level of PFC per spleen in the normal SJL/J, C57BL/10 and F_1-hybrid mice varied according to the strain, being 204,600 ± 9000, 293,000 ± 15,700 and 431,000 ± 24,500 respectively, which differ significantly from each other at an α level of better than 0.05. At the same time there were no significant differences in the spleen size of these normal mice, indicating that the observed variation in PFC numbers reflected a true difference in the relative proportion of plaque formers per spleen between these strains.

Figure 1 gives the data for PFC response of the virus injected animals, normalized to their respective control values for each strain and day, and presented for comparison purposes in percent of the respective controls. The RLV sensitive SJL/J mouse showed a brief elevation in response when the virus was injected 1 day before or immediately before SRBC administration. This was followed by a depression in response, reaching minimum levels at the 21st day. The C57B1/10 showed contrasting behavior, with only a slight depression in PFC response at day 2, which was much less than that previously reported for FLV infected C57B1/6 (4).

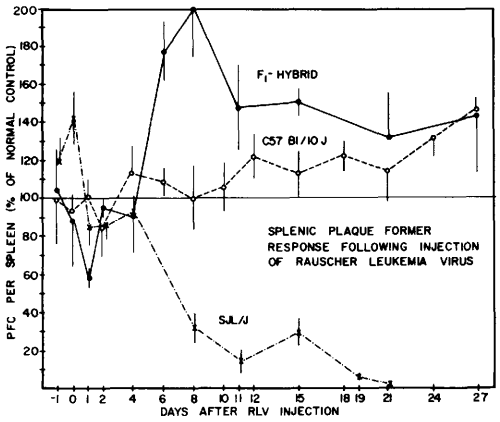

FIG. 1. Change in splenic PFC response as a function of time after RLV infection. Data is normalized to the control values for each strain taken as 100%. Error bars indicate ± 1 SE of the mean. Half error bars are shown where they would otherwise overlap.

Following this there was a recovery and overshoot which first became significant at the $\alpha = 0.05$ level at day 12. The most striking difference seen was that in the hybrid, which showed a PFC response as affected by RLV that was unlike either of its parental strains. First there was a significant immunosuppression, evident at day 1 after virus infection. This was followed by a rapid recovery in PFC, peaking at a level twice the normal by day 8, with a subsequent drop-off but a sustained immunostimulation throughout the remainder of the observation period.

Figure 2 illustrates that the observed levels of PFC response were not a direct function of spleen size following RLV infection. The C57B1/10 showed no significant change in spleen size throughout the duration of the study. The hybrid did show a transient threefold increase, but returned to normal by the termination of the study. Further, the peaks of splenomegaly for the hybrid did not coincide with the peaks of PFC activity. However, it would be correct to say that some of the increases in PFC response were due to the overall elevated number of spleen cells in the infected hybrid. By contrast, although the SJL/J developed the greatest splenomegaly, this strain showed virtually complete suppression of total splenic PFC response.

Discussion. While several authors have previously shown that murine leukemia viruses are immunosuppressive in virus-sensitive

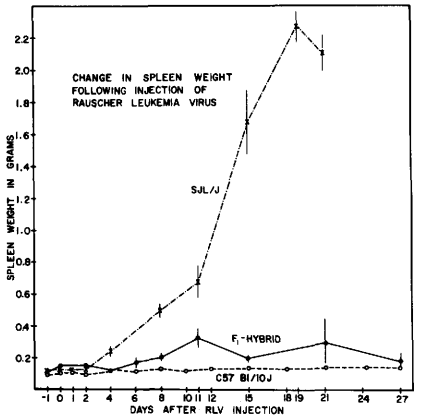

FIG. 2. Variation in spleen weight as a function of time after RLV infection. Error bars indicate ± 1 SE of the mean. Where no error bars are given the SE was less than the size of the data symbols.

hosts (1–3, 5, 13, 14), evidence for a possible stimulatory effect had been limited to the C57B1/6 strain (4, 5), and only suggestive at best. The data of this paper gives positive evidence that enhancement of the humoral antibody plaque forming cell response can be demonstrated following leukemia virus infection. The significance of this lies in the fact that humoral antibody formation is known to play a role in the induction of immunity to leukemia virus in otherwise sensitive mice following the injection of killed virus and leukemia cells (15–17). For other strains, including the group to which the C57B1/10 belongs, it has also been established by *in vitro* studies that one control of resistance to infective oncorna virus rests directly at the level of the target cell, unmediated by immune response (18–20). In that regard the level of PFC response of the hybrid is of particular interest, in that the F_1 possesses alleles for both sensitivity and resistance to the virus. The much greater stimulation of plaque formers suggests that the influence of the virus is more pronounced in the hybrid than in its resistant parent, perhaps strongly activating an immune response to the leukemia antigens. The elevated spleen weights also indicate an effect of the virus in the hybrid resulting in some cellular proliferation, but not to the extent observed during leukemogenesis in the SJL/J mouse, and clearly reversible. This suggests that some initial oncogenic activity may have occurred which the animal was able to successfully overcome. Full leukemogenesis did not result, however, as peripheral white counts done at the terminal point of the study showed only a slight elevation, and none of the animals died of Rauscher disease. In contrast to the $B6D2F_1$ hybrid of the DBA and C57B1/6, which shows immunosuppression and succumbs to leukemia virus (5), we have found this hybrid survives RLV infection (21).

Because Rauscher virus is NB-tropic (22), the Fv-2 locus would be expected to play the primary role in the genetic determination of resistance to the virus. This gene governs spleen focus formation and splenomegaly, with the sensitivity allele, Fv-2^s, being dominant over Fv-2^r. The C57B1/10 is Fv-2^r (18), while the SJL/J is felt to be Fv-2^s, similar to other Swiss-Webster derived mice (18, 23). Therefore, like the $B65D2F_1$, one would expect this F_1 hybrid to be almost as sensitive to Rauscher virus as its SJL/J parent. Since it is not, it would seem likely that other genes in this cross may be masking the effect of the Fv-2 locus. In the related Friend virus system, Chesebro and Wehrly (24) have reported that hybrids of C57B1/10 with either BALB.B. or A.BY, which are also Fv-$2^{r/s}$, likewise show a high incidence of recovery from splenomegaly and leukemia. From their results they postulated that this may be due to other non-H-2 genes associated with the H-2^b configuration, but not found in H-2^d mice. Whether any of these other susceptibility genes are also present in the SJL/J mouse, which is H-2^s, is as yet unknown. However, if they were it might account for the observed resistance of the hybrid used in this present study to Rauscher leukemia and its recovery from splenomegaly. Additional work remains to be done to establish this point, as well as the specific response of this hybrid to the spleen focus forming virus component of the Rauscher complex (25). In so doing, this hybrid may prove to be a useful model, analogous to that of Chesebro and Wehrly (24), for the study of genetic factors governing RLV sensitivity and the development of immune response to viral leukemia antigens.

Summary. The effect of Rauscher leukemia virus injection on the splenic PFC response to SRBC was measured in a mouse strain sensitive to the virus (SJL/J), one resistant to

the virus (C57B1/10), and their F_1 hybrid (SJL/JxC57B1/10)F_1. In contrast to previous findings of a suppressive effect of Friend virus on PFC response in another hybrid of different resistant and sensitive strains, a significant immunostimulation was seen. Immunostimulation was also seen in the resistant parent when the PFC response level was measured 12 days or later after RLV infection, while the PFC response of the sensitive parent was nearly completely suppressed. Evaluation of the effect of RLV on the splenic masses of the three strains indicated a mild transient splenomegaly in the hybrid; a pronounced splenomegaly in the sensitive parent; and no effect on the resistant strain. Comparison of the data suggests that the change in splenic PFC number in the hybrid following RLV infection occurs as a function partially independent of changes in the total splenic mass.

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Received July 29, 1977. P.S.E.B.M. 1978, Vol. 157.