Maternal Vaccination with Formalin-Inactivated Gross Lymphoma Virus in Rats and Transfer of Immunity to Offspring[1] (37595)

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The offspring of rodents normally acquire immunoglobulins from their mothers before and shortly after their birth (1). Gamma G immunoglobulins are transferred through the placenta while colostrum and milk are the vehicles for IgA and IgG fractions (2). This pattern of transmission of maternal antibodies is of great importance in the protection of newborn rats from viral induction of lymphoma.

It has been demonstrated in earlier work (3) that lymphoma induced by Gross virus in rats is strictly age-dependent since animals older than 2 wk are able both to neutralize the virus and to reject cells which express antigens associated with it (4). However, during the first 2 wk of life rats are highly susceptible to the leukemogenic activity of the Gross virus which can consistently induce the disease in all the recipients (5). Therefore, the only way of protecting infant rats against viral induction of lymphoma is by active immunization of their mothers prior or during their pregnancy (6). Transmission of immunity to Gross lymphoma virus (GLV) from mother to offspring occurred both pre- and postnatally by transplacental and milk passage (7). It was also shown that serum of adult immunized rats completely neutralized GLV *in vitro* in contrast to serum of nonimmunized animals (7).

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In order to develop this system further and to avoid immunization with live virus, the capacity of formalin-inactivated GLV to vaccinate mothers and induce transferable immunity was explored in the present work.

Materials and Methods. Animals. Female 3 mo old rats of three different strains (Wistar/Furth, Sprague–Dawley and Long–Evans) were used for immunization. Their progeny of 1 to 4 days of age were used for leukemia induction.

Virus. GLV used in all experiments was from a pooled stock, prepared by cell-free-filtration of rat lymphoma induced by rat-adapted GLV and stored in liquid nitrogen, as previously described (3).

Leukemia induction. Rats 1 to 4 days old were injected intraperitoneally with 0.1 ml of GLV stock suspension diluted 1:10 in Hanks' solution.

Virus inactivation. Formaldehyde in the form of 10% water solution (formalin) was used for the production of vaccines. Three different schedules were followed:

1. GLV stock suspension inactivated by formalin, brought to a final concentration of 1:500, stored for 7 days at 4°.

2. GLV stock suspension inactivated by formalin, brought to a final concentration of 1:500, stored for 7 days at 25°.

3. GLV stock suspension inactivated by formalin, brought to a final concentration of 1:4000 in the presence of 1 *M* $MgCl_2$, stored for 7 days at 37° according to the procedure of Salk (8).

GLV inactivated according to all three schedules was injected intraperitoneally in the same final concentration as the live virus

in 1–4 day old rats to assess its lack of oncogenicity.

Vaccination. Female rats before or during mating were injected intraperitoneally 3 times, at weekly intervals, with 0.5 ml of GLV inactivated with formalin according to schedules 1, 2 or 3. Control female rats were injected intraperitoneally with similar amounts of live GLV of the same stock suspension stored for 7 days at 4°.

Neutralization in vitro. GLV stock suspension was diluted 1:5 in sterile phosphate buffer solution then mixed 1:1 with the serum to be tested, bringing it to a final dilution of 1:10 and kept for 90 min at room temperature with periodical stirring (7). Rats 1–4 days old were injected intraperitoneally with 0.1 ml of the GLV–serum mixture. Serum of female rats vaccinated with formalin-inactivated GLV was used in one group of newborn rats, serum of female rats immunized with live GLV in a second group, serum of normal nonimmunized female rats in a third group and live GLV in the fourth group of newborn rats.

Results. Vaccination of mothers. Female rats vaccinated with formalin-inactivated GLV gave birth to litters which were injected with live GLV within the first 4 days of life together with litters of nonvaccinated mothers to serve as controls.

According to the results summarized in Table I, of 21 rats, offspring of 3 nonvaccinated mothers, 19 had lymphoma (90.4%) within 95 days after GLV injection. In sharp contrast, only 3 of 57 rats (5.2%), offspring of 6 mothers vaccinated with GLV inactivated with formalin according to one of three schedules, had lymphoma after a similar latency period. Analyzed according to the schedules used for GLV-inactivation, the results show that 2 of 24 babies had lymphoma (8%) when the mothers were vaccinated with GLV inactivated with formalin 1:500 for 7 days at 4°; 1 of 18 babies had lymphoma (6%) when the mothers were vaccinated with GLV inactivated with formalin 1:500 for 7 days at 25°; and none of 15 babies had lymphoma (0%) when the mothers were vaccinated with GLV inactivated with formalin 1:4000 + 1 *M* $MgCl_2$ for 7 days at 37°.

TABLE I. Vaccination with Formalin-Inactivated GLV.

Mothers		Offspring[a]		
Total	Treatment	Injected	Lymphoma	Protection (%)
3	None	21	19	9
2	Inactiv. GLV[b]	24	2	92
2	Inactiv. GLV[c]	18	1	94
2	Inactiv. GLV[d]	15	0	100

[a] All injected ip at 1–4 days of age with 0.1 ml GLV diluted 1:10.

[b] GLV inactivated with formalin 1:500 for 7 days at 4°.

[c] GLV inactivated with formalin 1:500 for 7 days at 25°.

[d] GLV inactivated with formalin 1:4000 + 1 *M* $MgCl_2$ for 7 days at 37°.

TABLE II. Neutralization of GLV with Sera of Vaccinated Rats.

GLV mixed with	Newborns injected[a]	Lymphoma	Percent
None	9	8	88
Serum of nonimmunized	13	9	69
Serum of immunized			
with live GLV	18	1	6
with inactivated GLV	23	0	0

[a] All injected ip at 1–4 days of age with 0.1 ml of GLV–serum mixture.

Neutralization in vitro. In comparative experiments GLV was mixed *in vitro* with sera of vaccinated, immunized and nonimmunized rats and injected in 1 to 4 day old rats. The results according to Table II, show that of 23 newborn rats injected with GLV mixed *in vitro* with serum of rats vaccinated with formalin-inactivated GLV, none had lymphoma (0%), and only one of 18 newborn rats injected with GLV mixed *in vitro* with serum of rats immunized with live GLV had lymphoma (6%).

In contrast, 9 of 13 newborn rats (69%) injected with GLV mixed *in vitro* with serum of nonimmunized rats and 8 of 9 newborn rats injected with plain GLV (88%) had lymphoma.

Discussion. The present experiments have

shown that formalin treatment of GLV achieves an effective inactivation of this virus without alteration of its antigenic properties.

Maternal immunization with formalin-treated GLV conferred protection to the progeny which had lymphoma in only 5% when injected with a lethal dose of GLV. In contrast 90% of the progeny of nonimmunized mothers had lymphoma when challenged with the same dose of virus.

Of the three schedules used to inactivate the virus, the best results were obtained with formalin 1:4000 at 37° in the presence of 1 *M* $MgCl_2$. This procedure was used by Salk (8) who demonstrated that all 3 types of poliomyelitis virus could be inactivated with retention of adequate antigenicity. In the presence of 1 *M* $MgCl_2$ the curve of inactivation by formalin shows much less tailing-off, which otherwise occurs due to aggregation of virus particles (9).

Neutralization of GLV *in vitro* with serum of rats immunized with inactivated virus was as efficient as that with serum of rats immunized with live virus. In both instances, the neutralization of GLV was almost complete, as shown by bioassays in newborn rats.

Formaldehyde was previously used in the preparation of numerous virus vaccines since the first inactivation of rabies virus was reported by Cumming in 1914 (10). In addition to influenza, measles, adeno and polioviruses (9) oncogenic viruses such as Rous (11), Friend (12) and Rauscher (13) were also successfully inactivated with formalin.

Both Friend and Rauscher viruses are strongly antigenic in the mouse and therefore vaccination with formalin-inactivated viruses resulted in good titers of neutralizing antibodies and protection against challenge with live virus (13, 14). Yet this may not be applicable to a situation in which an organism by virtue of congenital infection becomes immunologically tolerant to a virus (15). In contradistinction to both Friend and Rauscher leukemias which can be induced in adults, the Gross lymphoma occurs only in rats which were injected with the virus in very early life. To avoid induction of tolerance during intrauterine life and/or infection in the early postnatal period, immunization of mothers with subsequent passive transfer of immunity to offspring has been attempted (6, 16, 17). Using live Friend virus to immunize Swiss mice mothers, protection was obtained in 60–70% of the offspring subsequently challenged, although occasional transmission of virulent virus to babies was also noted (16). In our earlier work, when live GLV was administered to female rats before or during pregnancy, protection against a lethal dose of virus given to newborns was achieved in all cases (6). In the present work similar effects were obtained with inactivated virus, removing thus the danger of occasional induction of lymphoma in the mother and passage of virulent virus to the progeny.

Vaccination of females with an inactivated oncogenic virus represents a model which may also be applicable to the prevention of oncogenesis in other animal systems.

Acute leukemia in children displays a striking temporal resemblance to the Gross lymphoma in rats. The peak incidence at 5–6 yr of age in humans is comparable to that of 60–70 days in rats, when allowance is made for the difference in the respective life spans. This may suggest that in humans too, induction of acute leukemia and perhaps that of other neoplasias, takes place during that postnatal period of maximum susceptibility, when passive maternal immunity is steadily decreasing while the baby's own immune system has not yet fully matured. This would appear to be the optimal period during which infection and/or tolerance could be induced in man by an oncogenic virus.

Immunization of females with an inactivated or attenuated virus, as it is now practiced against rubella infection, may well be the answer for the prophylaxis of acute leukemia in children and perhaps of other forms of cancer.

Summary. Newborn rats are highly susceptible to viral induction of lymphoma, and become naturally resistant after 2 wk of age. Protection during this period can be conferred by transfer of maternal immunity which occurs pre- and postnatally, by transplacental and milk passage.

Vaccination of mothers was effectively achieved with formalin-inactivated Gross

lymphoma virus (GLV), resulting in passive immunization of the progeny. Only 5% of the offspring of vaccinated mothers had lymphoma when injected with a lethal dose of virus, as compared to 90% of the offspring of nonvaccinated mothers. Complete neutralization of GLV *in vitro* with serum of vaccinated mothers was demonstrated by subsequent bioassays.

Vaccination of females with an inactivated oncogenic virus represents a model which may also be applicable to the prevention of oncogenesis in other animal systems.

1. Halliday, R., Proc. Roy. Soc. Ser. B. **144**, 427 (1955).

2. Humphrey, J. H., and White, R. G., "Immunology for Students of Medicine." Davis, Philadelphia (1970).

3. Ioachim, H. L., and Berwick, L., Int. J. Cancer **3**, 61 (1968).

4. Ioachim, H. L., Cali, A., and Sinha, D., Cancer Res. **25**, 132 (1965).

5. Ioachim, H. L., Sabbath, M., Andersson, B., and Keller, S., J. Nat. Cancer Inst. **47**, 161 (1971).

6. Ioachim, H. L., Cancer Res. **30**, 2661 (1970).

7. Ioachim, H. L., Keller, S. E., Gimovsky, M. L., and Shepherd, S., Cancer Res. **33**, 107 (1973).

8. Salk, J. E., Ann. New York Acad. Sci. **61**, 1023 (1955).

9. Davis, B. D., Dulbecco, R., Eisen, H. N., Ginsberg, H. S., and Wood, W. B., "Microbiology." Harper & Row (Hoeber), New York (1970).

10. Cumming, J. G., J. Infec. Dis. **14**, 33 (1914).

11. Fink, M. A., and Rauscher, F. J., J. Nat. Cancer Inst. **26**, 519 (1961).

12. Friend, C., J. Exp. Med. **109**, 217 (1959).

13. Fink, M. A., Rauscher, F. J., and Chirigos, M., *in* "Viruses Inducing Cancer" (W. J. Burdette, ed.), p. 50. Univ. of Utah Press, Salt Lake City (1966).

14. Friend, G., *in* "Viruses inducing Cancer" (W. J. Burdette, ed.), p. 51. Univ. of Utah Press, Salt Lake City (1966).

15. Old, L. J., and Boyse, E. A., Fed. Proc., Fed. Amer. Soc. Exp. Biol. **24**, 1009 (1965).

16. Mirand, E. A., Grace, J. R., Jr., and Buffett, R. F., Nature (London) **209**, 696 (1966).

17. Buffett, R. F., Grace, J. R., Jr., and Mirand, E. A., Proc. Amer. Ass. Cancer Res. **8**, 8 (1967).

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