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Protection of A/He Mice by a Friend Virus Pseudotype Against Challenge with Friend Virus[1] (38743)

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Friend virus (FV) pseudotypes may be prepared either by retrieval by the appropriate helper virus (1, 2) of the FV genome from nonproducer cells grown *in vivo* or *in vitro*, or by taking advantage of the FV-1 gene and passaging, in a restrictive host, a mixture of FV with another helper virus that will suppress the helper virus indigenous to the FV complex (3). The former method has the advantage that the resulting pseudotype is admixed with a single helper virus only and therefore is presumably stable. This is in contrast to the latter method where the pseudotype reverts to the original FV when passaged in a nonrestrictive host. FV pseudotypes prepared by our method, i.e. retrieval from nonproducer cells, may differ from the original isolate of FV in age susceptibility and host range. These differences offer a method to actively immunize, with live virus, some mouse strains normally susceptible to our original FV. Previous studies have shown that A/He mice are resistant at 5 wk of age to inoculation with the Graffi pseudotype of FV [FV(Gi)], but not to FV or the Moloney leukemia virus pseudotype (4). However, when inoculated as newborns they are susceptible to all three viruses. We report the successful active immunization of young adult A/He mice with FV(Gi), but not with Gratti leukemia virus (GiLV).

Materials and Methods. Mice. Female A/HeJax mice aged 8 wk were obtained from Jackson Memorial Laboratories, Bar Harbor, Maine. Female BALB/c mice of the same age came from Simonsen Laboratories, Gilroy, CA, or our own colony; BALB/c mice from both colonies readily accepted skin grafts from each other.

Viruses. The source and passage history of our GiLV have been described (2). The virus used was in the fourth and fifth passage in BALB/c mice in our laboratories. FV(Gi) was prepared by the *in vitro* method (2) and was in the sixth passage in BALB/c mice. FV was in the eleventh passage in BALB/c mice (5). Pools were prepared from virus-infected or normal BALB/c mouse spleen (NMS) (5) and used as 10% cell-free suspensions. The diagnosis of Friend disease was based on microscopic examination of the spleen after formalin fixation and hematoxylin and eosin staining.

Antibody titrations. These were performed using a constant virus-variable serum technique. Twofold serial dilutions of sera inactivated at 56° for 0.5 hr were made from 1:2 through 1:256 in phosphate-buffered saline. These were mixed with an equal volume of 10^{-2} dilution of FV, incubated at 37° for 1 hr, and chilled immediately in ice-cold water. Groups of eight mice were inoculated with 0.2 ml of the mixture and killed 35 days later. The incidence of Friend disease was determined on a basis of spleen size and microscopic examination. A simultaneous virus titration was carried out in similar groups of mice.

Results and Discussion. Protection of A/He mice with FV(Gi). Groups of 5-wk-old mice were inoculated ip with either 0.2 ml of FV(Gi) or a similar volume of GiLV or NMS. Twenty-eight to 35 days later mice were challenged ip with 0.2 ml of either FV, FV(Gi) or NMS, or received no challenge. They were killed on day 56, their spleens weighed and examined histologically for Friend disease. Table I combines the results of three experiments. These show complete protection of A/He mice against challenge with FV by prior inoculation with FV(Gi), but not by GiLV or NMS. In one experiment the mice were bled from the retro-orbi-

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TABLE I. EFFECT OF PRETREATMENT WITH GRAFFI VIRUS AND GRAFFI VIRUS PSEUDOTYPE ON SUBSEQUENT CHALLENGE WITH FRIEND VIRUS IN A/He MICE.[a]

Pretreatment	Challenge	No. mice with Friend disease/ Total inoculated
FV(Gi)	FV	0/26
GiLV	FV	25/31
FV(Gi)	Nil	0/20
FV(Gi)	NMS	0/15
FV(Gi)	FV(Gi)	0/10
Nil	FV	25/31
NMS	FV	20/23
GiLV[b]	Nil	0/17

[a] A/He mice were inoculated ip with the indicated virus and challenged ip 35 days later. They were killed on day 70 and the incidence of Friend disease determined.

[b] The same dose of GiLV inoculated into BALB/c mice of the same age induced lymphatic leukemia in 10/15 animals that were observed for 180 days.

tal plexus on day 32 before challenge on day 35. The blood from mice of each group was pooled. Heat inactivated sera were then titrated for the presence of neutralizing antibody against FV. Neither preinoculation with FV(Gi), GiLV nor NMS induced detectable FV antibody in A/He mice. In each instance the antibody titer of the serum was $<1:8$ against 15 ID_{50} of FV.

Failure of replication of GiLV in adult A/He mice. It appeared curious that FV(Gi) induced protection, but GiLV did not, since it might be expected that both viruses would share the same surface antigens. Further, it appeared to conflict with our earlier observation that GiLV would protect BALB/c mice against subsequent challenge with FV.

In view of these apparently contradictory results, groups of 7-wk-old A/He and BALB/c mice were inoculated with 0.2 ml of GiLV or NMS. Thirty-five days later they were challenged with $10^{3.7}$ ID_{50} of FV and killed on day 70. The results (Table II) showed that GiLV did not protect A/He mice against subsequent challenge with FV. Since the results in BALB/c mice showed only partial protection this part of the experiment was repeated. In the second experiment, 7 of 20 BALB/c mice immunized with GiLV and challenged with FV developed Friend disease compared with 15/15 control animals ($P < 0.001$).

TABLE II. RESULTS OF IMMUNIZATION OF A/He AND BALB/c MICE WITH GiLV.[a]

Strain of mouse	Immunization	Challenge	No. with Friend disease/ Total inoculated
A/He	GiLV	FV	27/27
A/He	GiLV	Nil	0/12
A/He	NMS	FV	6/6
BALB/c	GiLV	FV	14/20
BALB/c	GiLV	Nil	0/16
BALB/c	NMS	FV	5/5

[a] A/He and BALB/c mice were inoculated with 0.2 ml of either GiLV or a suspension of normal mouse spleen and challenged ip 35 days later with $10^{3.7}$ ID_{50} of FV. They were killed on day 70 and the incidence of Friend disease determined.

Since neither FV (Gi) nor GiLV appeared to induce neutralizing antibodies against FV, an alternative explanation was sought. One possibility was that the GiLV was not replicating in young adult A/He mice and therefore produced little or no immunologic response. To test this hypothesis a simple and relatively rapid assay for the presence of GiLV was used. It was based on the observation that GiLV can act as a helper to retrieve defective FV from a noninfectious FV-induced reticulum cell sarcoma grown *in vitro* (FVTCT) (2). This tumor, syngeneic for BALB/c mice, has been shown to contain the FV genome that can be readily retrieved by lymphatic leukemia viruses of the FMRGi group. Therefore, groups of 7-wk-old A/He and BALB/c mice were inoculated with 0.2 ml of the same pool of GiLV used in the previous experiments. Twenty-one days later they were killed, the spleens of each strain pooled, and a 20% suspension made in sucrose stabilizer. Litters of newborn BALB/c mice were inoculated with 0.05 ml ip of these suspensions. When the mice were 24 or 25 days old they were inoculated sc with 4×10^5 FVTCT cells. Twenty-one days later, when tumors 1–2 cm in diameter had developed, the mice were killed, and their tumors were removed aseptically. Tumors from

mice inoculated with spleen extracts from A/He mice previously inoculated with GiLV were divided into three pools consisting of five tumors each (group A). These were inoculated into 20 newborn mice. Tumors from mice inoculated with spleen extracts from BALB/c mice previously inoculated with GiLV were divided into two pools consisting of four and five tumors respectively (group B). These were inoculated into 13 mice. Each pool had been made into cell-free extracts that were inoculated ip in 0.1 ml amounts into newborn BALB/c mice. Animals were observed for the development of Friend disease.

FV was retrieved from both pools prepared from the tumors arising in group B mice, all 13 animals dying of Friend disease 28–36 days later. This indicated that GiLV was present in the spleens of the BALB/c mice, and therefore was able to act as helper in the retrieval of defective FV from mice with FVTCT tumors. None of the 20 group A mice developed Friend disease after an observation period of 150 days, indicating the probable failure of GiLV to replicate in 7-wk-old A/He mice, with the subsequent lack of immunity against challenge with FV.

It is not yet known if leukemia viruses other than Friend are defective. The studies presented here demonstrate a practical method of taking advantage of the defectiveness of FV to prepare an avirulant pseudotype that can be used for successful active immunization.

Summary. Prior inoculation of 7-wk-old A/He mice with the Graffi pseudotype of Friend virus protected the animals against subsequent challenge with Friend virus. Graffi leukemia virus itself did not induce protection, probably because it failed to replicate in these mice.

1. Fieldsteel, A. H., Kurahara, C., and Dawson, P. J., Nature (London) **223,** 1274 (1969).
2. Fieldsteel, A. H., Dawson, P. J., and Kurahara, C., Int. J. Cancer **8,** 304 (1971).
3. Steeves, R. A., and Eckner, R. J., J. Nat. Cancer Inst. **44,** 587 (1970).
4. Dawson, P. J., and Fieldsteel, A. H., Int. J. Cancer **11,** 484 (1973).
5. Fieldsteel, A. H., Dawson, P. J., and Bostick, W. L., Proc. Soc. Exp. Biol. Med. **108,** 826 (1961).
6. Fieldsteel, A. H., Dawson, P. J., and Scholler, J., J. Nat. Cancer Inst. **36,** 71 (1966).
7. Fieldsteel, A. H., Kurahara, C., and Dawson, P. J., Int. J. Cancer (1975).

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