Effective Immunotherapy of Friend Virus Infection.* (32006)

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Friend(1) with her agent, and Fink(2) employing the Rauscher virus, have demonstrated that formalinized vaccines prepared from filtrates of leukemic spleens induce significant degrees of immunity in mice against challenge with the respective virulent viruses. Protection against Rauscher virus infection has also been demonstrated with passive immunotherapy(3), and with combined drug and immunotherapy(4).

The development of a method for production of large volumes of Friend virus neutralizing antibody(5) has allowed studies on immunotherapy with the Friend virus (FV). Employing a spleen weight assay(6) an accurate estimation of Friend virus titer in control and treated animals was achieved. This report presents the results of immunotherapy initiated in animals at different stages of infection with Friend virus and its effect on viremia, splenomegaly and survival time.

Materials and methods. The preparation of FV has been previously described(6). Production of FV neutralizing antibody in the form of ascitic fluid has been reported(5). BALB/s male and female mice, 6-8 weeks of age, weighing between 20-24 g obtained from Microbiological Associates, Inc., were used.

Blood collection. At different time intervals, indicated with each Figure, 0.2 ml of blood, collected from animals in each group from the inner canthus of the eye(7), was pooled and diluted with an equal volume of 0.306 M potassium citrate. The blood was centrifuged at 1250 $\times$ *g* for 10 minutes. The supernatant plasma was recovered and again centrifuged (1250 $\times$ *g*) for 5 minutes to insure removal of cells.

Bioassay. Donor plasma was bioassayed by inoculating 0.2 ml of each dilution into each of 5 recipient mice. Recipients were sacrificed for spleen weight determinations 14 days after inoculation(6). Spleen weights were read to the nearest 10 mg using a model P120 Mettler balance.

Results. Initiating treatment with antibody-containing ascitic fluid as late as 4 days after FV infection resulted in a significant reduction of virus (Table I), retardation of splenomegaly (Fig. 1), and increased survival time (Table I).

Results in Fig. 1 (Group 1) show that viremia increased progressively and that virus was detectable as early as 3 days after infection. The progressive increase in spleen weight attained in these recipient mice inoculated with plasma from non-treated controls revealed that virus titers increased with time. Bioassay of plasma from each donor group 21 days after infection showed a decreased splenomegalic response in recipients inoculated with plasma from groups 2-6, indicative of a lowered infectious titer of virus.

A quantitative estimation of viremia in mice of each group was also made 21 days after virus infection (Fig. 2). As expected, a dose-response relationship was achieved between the degree of splenomegaly in recipient mice inoculated with serial dilutions of plasma from non-treated controls. Employing the response of control mice as the standard curve, the viral content of animals in Groups 2-7 was estimated and these values are presented in Table I. Decreases in virus titer were achieved in animals which received treatment as late as 6 days after virus infection.

Reduction in virus titer and retardation of splenomegaly were reflected by an increase in survival time over controls with a significant number of survivors at 120 days. All animals which received treatment beginning 4 hours prior to infection (Group 2) were alive at 120 days. Their spleens were not enlarged, they gained weight and their coat and general health was good. The observations for remaining animals in Group 3 were similar. Of

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TABLE I. Effect of Immunotherapy on Friend Virus Infection.

Group No.*	Treatment schedule† (days)	% Survival — Observation days 30	40	50	60	70	95	120	MST (days)‡	Recoverable virus§ (logs)
1	None	90	70	50	20	0	—	—	51	
2	—4 hr, D_2, D_4, D_6	100	100	100	100	100	100	100	>120	.4
3	D_1, D_3, D_5, D_7	"	"	"	"	"	30	30	84	.8
4	D_3, D_5, D_7, D_9	"	"	"	"	90	80	80	>120	1.0
5	D_4, D_6, D_8, D_{10}	"	"	90	90	70	50	50	>120	1.3
6	D_5, D_7, D_9, D_{11}	"	90	50	30	10	0	—	50	1.7
7	D_6, D_8, D_{10}, D_{12}	"	90	50	40	30	0	—	54	2.8
8	D_6, D_8, D_{10}, D_{12}	"	100	100	100	100	100	100	>120	0

* Groups 1-7 were inoculated intraperitoneally (I.P.) with 0.2 ml of a $10^{-2.3}$ dilution of FV on day 0. Group 8 was non-infected, ascitic fluid treated controls, 10 mice per group.

† Antibody-containing ascites fluid was inoculated at 2% body wt I.P. every other day, for a total of 4 treatments (—4 hr represents treatment 4 hr prior to and D_1 represents 1 day after virus infection).

‡ MST = median survival time.

§ The amount of recoverable virus was estimated by comparing the log mean spleen weight of recipient mice sub-inoculated with antibody-treated donor plasma, to the non-treated control standard curve (Fig. 2).

the remaining 8 animals in Group 4, all had spleen enlargement ranging from 1 + (3 animals), to 2 + (3 animals), and 4 + (2 animals). In Group 5 there were 3 animals with 1 + and 2 animals with normal spleens at 120 days. Spleen enlargement, as determined by palpation of 1 + or greater was considered in these studies as indicative of the Friend disease. Necropsy of animals expiring during the 120 day observation period revealed enlarged spleens ranging from 1.0 to 3.5 g. Of particular interest were the greatly en-

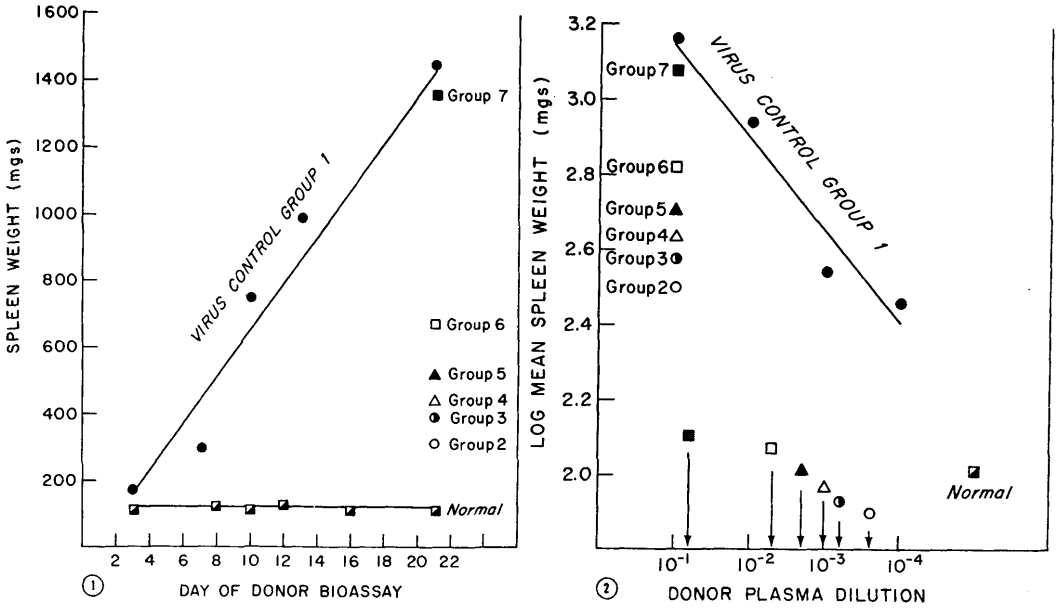

FIG. 1. Splenomegaly in recipient animals sub-inoculated with donor plasma. Donor blood was collected on the indicated day of bioassay; the plasma was diluted to 10^{-1} and 0.2 ml was inoculated into each of 5 recipient mice. Recipients were sacrificed 14 days after inoculation for spleen weights.

FIG. 2. Determination of virus titer in control and treated animals. Blood was collected from donor animals 21 days after virus infection, and 0.2 ml plasma at 10^{-1} to 10^{-4} dilution of control and 10^{-1} of treated donor plasma was sub-inoculated into each of 5 recipient mice. Recipients were sacrificed 14 days after inoculation for spleen weight.

larged livers containing discreet white nodules (2-4 mm) observed in animals expiring after 60 days.

Discussion. The more effective therapy achieved in animals receiving treatment 4 hours prior to virus infection may be attributable to a prophylactic effect. It appears that sufficient circulating antibody was present, even after 4 hours, to neutralize the injected virus. Fink(2) has demonstrated that animals were protected when treated with one injection of homologous neutralizing antibody 30 hours prior to Rauscher virus challenge.

The lower number of survivors and increase in survival time achieved in Group 3 when compared to Group 2 and 4 was unexpected. A possible explanation for this is that antibody administered 1 day after infection was ineffective in that FV undergoes eclipse and cannot be detected till 2 to 3 days later (6,8). A 3-5 day eclipse period has also been reported for the Rauscher virus(9). It would appear that since FV is detectable by the 3rd day after infection, treatment on this day and on the 5th and 7th day after infection resulted in neutralization of extracellular virus.

The therapeutic effect achieved in Groups 4-5 must be attributable to the presence of circulating antibody sufficient to neutralize extracellular virus.

The white liver nodules observed in animals expiring after 60 days have been described by Metcalf(10) as microtumor nodules of reticulum cells, and indicate that uncontrolled proliferation of reticulum cells had occurred.

Despite the excellent therapeutic response achieved by initiating treatment as late as 3 or 4 days after infection, symptoms of the Friend disease were still evident. The inability of the treatment regimens initiated 24 hours after infection to completely suppress virus replication may be attributable to several factors: (1) insufficient number and volume of treatments to completely neutralize extracellular virus; (2) inability of neutralizing antibody to penetrate virus-infected cells which were replicating virus; (3) inability of neutralizing antibody to reach distal organs such as brain which is known to harbor murine leukemia viruses and which therefore could serve as a reservoir for reinfection.

Studies are presently in progress to clarify the possible contribution of each of these factors in explaining the relative refractiveness of viremic animals to completely successful immunotherapy.

Summary. Treatment of FV infected mice with homologous antibody at different stages of viremia resulted in a significant reduction of infectivity titers and of splenomegaly with a concomitant increase in life span. When treatment was begun 4 hours prior to virus infection the animals survived longer than 120 days with no apparent symptoms of the disease. The therapeutic effects achieved in animals treated after virus infection are considered to be due to the ability of the injected antibody to neutralize extracellular virus as it is liberated which thus delays the induction of the Friend disease.

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