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Humoral and Cellular Immune Responses in Susceptible and Resistant Strains of Mice Infected with Friend Leukemia Virus (38159)

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Infection of susceptible strains of mice with murine leukemia viruses (MuLV) results in rapid development of lymphoreticular malignancy characterized by lymphoid cell dyscrasia, splenomegaly, and hepatomegaly culminating in the death of the animal (1–3). Recent studies in our laboratory, as well as in other laboratories, have been concerned with the effects of MuLV infection on the immunocompetence of susceptible strains of mice (4–8). Such studies have been concerned primarily toward an understanding of the effects of virus infection on the immune response at the humoral level, using a variety of antigens known to stimulate antibody formation.

More recent studies in our laboratory have been directed toward assessment of host immunocompetence at the level of cell mediated immunity (CMI) in MuLV infected mice (9–12). These studies were performed in light of the recent emphasis on the relative importance of cellular mechanisms in tumor immunity. It is now widely accepted that immune functions based upon activity of thymus dependent (T) lymphocytes have a primary role in the "immunologic surveillance" defense mechanism against malignancies, whether induced by viruses, chemical carcinogens, or spontaneous (13, 14). Thus it appeared to be important to ascertain whether leukemogenic viruses capable of suppressing humoral immune responses also exert an equivalent effect on cell-mediated immunity, not only in virus susceptible mice but also in virus resistant animals. For this purpose studies were performed to determine the effects of inoculation of one of the murine leukemia viruses, i.e., Friend leukemia virus (FLV), into both susceptible and resistant strains of mice to assess effects on both humoral and cell-mediated immunity.

Methods and Materials. Experimental animals. Young adult Balb/c and C_{57} Bl/6 mice, approximately 6–8 wk of age at the time of testing, were obtained either from Flow Laboratories, Inc., Durham, Va., or Jackson Memorial Laboratories, Bar Harbor, Maine. The animals were kept in groups of six to eight in plastic mouse cages and fed water and Purina mouse food ad lib. The Balb/c mice are highly susceptible to infection by FLV, whereas the C_{57} Bl mice are resistant (15, 16).

Leukema virus. A highly infective strain of FLV propagated in this laboratory through adult Balb/c mice for a period of 7–8 yr was used for this study. The stock virus preparation consists of clarified spleen cell homogenates prepared from pooled spleens of mice infected for 2–3 wk (17–19). The stock virus is highly infective for adult Balb/c mice so that 0.1 ml of a 10^{-4} dilution invariably results in splenomegaly and death of 100% of the inoculated animals. The virus preparation is free of Lactis Dehydrogenase virus and other known MuLV. For the present experiments aliquots of the stock virus preparation, titrated for infectivity in Balb/c mice, were kept frozen at −60° until used. Sufficient virus pool for each day's use was thawed quickly and diluted in sterile Hanks' solution immediately before inoculation.

Infection. Individual mice were injected intraperitoneally (ip) with 0.1 ml of graded doses of the stock virus preparation.

Antigens. To determine the humoral immune response mice were immunized ip with

0.5 ml of washed 10% suspension of sheep red blood cells (SRBC). For assessment of cell-mediated immunity mice were inoculated subcutaneously (sc), in several sites, with a total of 0.5 ml complete Freunds adjuvant (CFA) containing mycobacteria (Difco Laboratories, Detroit, Mich.). In some experiments mice were injected in the footpad with 0.1 ml of a standard suspension of BCG (approx 2×10^8 organisms).

Assessment of humoral immunity. At varying times after immunization groups of five to six mice were bled from the retroorbital venous plexus to obtain serum for antibody titration. The animals were killed and each spleen was removed, teased in sterile Hanks' solution with needles and forceps, and the dispersed cell suspension washed by centrifugation in cold Hanks' solution. The number of viable nucleated lymphocytes per cell suspension was determined by hemocytometer count and Trypan blue stain technique. The number of antibody plaque forming cells (PFCs) to sheep erythrocytes was enumerated by the standard localized hemolysis in gel technique exactly as described previously (17–19). The number of PFCs per whole spleen and per million spleen cells plated was calculated from at least two to three plates per spleen. Serum titers were determined by the direct hemolytic assay in microtiter plates using 0.025 volumes of serum dilutions in saline and equal amounts of a 1% suspension of washed sheep erythrocytes and guinea pig complement diluttd 1:10.

Cellular immunity. Mice sensitized by sc inoculation of CFA containing the mycobacteria were asayed for development of cellular immunity at various times thereafter by means of the direct and indirect macrophage migration inhibition assay (20, 21). In brief, spleen cells were washed in cold Hanks' balanced salt solution, resuspended in Medium 199 containing 10% heated fetal calf serum, and packed in capillary tubes sealed at one end with paraffin wax, exactly as described previously (11, 22, 23). The capillaries were cut at the fluid-cell interface and incubated in Sykes Moore chambers with 50 μg PPD/ml Medium, or with Medium alone, for 24 hr at 37°. The area of migration and percent inhibition of migration were calculated by measuring the enlarged image of the migrating cells. A significant inhibition ($P < 0.05$) of migration was considered to be any value greater than 20% inhibition.

Titration of virus. The presence of FLV in spleens of all mice given virus was determined by preparing saline homogenates of portions of each spleen used for antibody or macrophage migration determination and injecting aliquots into susceptible young Balb/c mice, which were then sacrificed 7, 14, or 21 days later to determine development of splenomegaly and other overt symptoms of FLV leukemia.

Results. In agreement with previous studies, there was a marked and prolonged depression of the humoral immune response, both serum titer and PFCs, to sheep erythrocytes in the susceptible Balb/c mouse strain (Table I). Immunosuppression was apparent even when mice were immunized on the same day as infection, or 1 day after infection,

TABLE I. Effect of Friend Leukemia Virus Infection on the Hemolytic Antibody Responses of Balb/c and C_{57}Bl Mice to Sheep Erythrocytes

	Balb/c			C_{57}Bl		
Time between infection and immunization[a]	PFC/spleen	Percent of control	Mean serum titer	PFC/spleen	Percent of control	Mean serum titer
Controls (no FLV)	75,100	—	1:128	56,700		1:96
−1 day infected	12,000	16	1:48	6800	12	1:46
−3 days infected	3750	5	1:32	2288	4	1:24
−7 days infected	585	<1	1:16	62,000	106	1:96

[a] Mice infected ip with FLV on day indicated before challenge immunization; all mice tested for splenic PFC 4 days later.

at a time when there was no overt disease observable. FLV could be readily recovered from the spleens of these mice at the time of antibody plaque assay. Earlier studies had demonstrated that such immunodepression was dependent upon the virus dose and the time of administration (17, 18). Higher doses of virus resulted in greater levels of immunosuppression, whereas a longer time interval between infection and immunization also resulted in a greater degree of depression. Also, earlier studies had shown that C_{57}Bl mice appeared to be resistant to immunosuppression when infected with virus 7–10 days after infection, regardless of FLV dose (24). Thus it was surprising that the C_{57}Bl mice infected 1 or 3 days prior to immunization showed a consistent and significant depression in the number of PFCs (Table I). The suppression was of a magnitude similar to that observed in the susceptible Balb/c mice infected at the same time interval before challenge immunization. Serum antibody titers were also reduced, on the average of three- to four-fold.

In contrast to the continued suppression of the PFC response in the Balb/c mice, the C_{57}Bl animals rapidly recovered their immunocompetence so that mice of this strain, when infected with FLV 7 days before immunization, developed the normal numbers of PFCs. The response at this time was equal to or even greater than those of control immunized C_{57}Bl mice. FLV was readily recovered from spleens of the C_{57}Bl mice injected 1 or 3 days before immunization, but not from the spleens of mice injected 7 days earlier.

The direct macrophage migration inhibition assay for demonstrating cellular immunity *in vitro* showed that FLV infected Balb/c mice lost this capacity shortly after infection with FLV. For these experiments mice were sensitized with CFA 20–30 days prior to infection. Control, noninfected Balb/c mice showed evidence of cellular immunity to the mycobacteria when their spleen cells were tested *in vitro* in Sykes Moore chambers containing PPD. The antigen induced a 45% inhibition of macrophage migration as compared to control cultures without antigen (Table II). Within a day after infection of Balb/c mice there was much less migration inhibition evident with PPD. Similarly, at days 3 and 7 there was a further reduction in migration inhibition, since spleen cell cultures from these animals were much less inhibited by PPD than were the control cultures.

Spleen cells from C_{57}Bl mice sensitized with mycobacteria continued to show evidence of sensitization to the T.B. antigen when assayed *in vitro* by the migration inhibition procedure despite prior infection of the mice with FLV. As can be seen in Table II, control, noninfected C_{57}Bl mice sensitized with mycobacteria showed normal migration inhibition when their spleen cells were cultured in the presence of PPD. Similarly, spleen cells obtained from mycobacteria sensitized C_{57}Bl mice given FLV either 1, 3, or 7 days prior to assay also showed a significant degree of migration inhibition when cultured with PPD. There was no evidence of impairment of this activity, considered an *in vitro* correlate of delayed hypersensitivity.

Discussion. These experiments show that shortly after injection of FLV into genetically resistant C_{57}Bl mice there is a marked but yet transient inhibition of humoral antibody formation as assesed at the level of individual plaque forming cells and hemolytic serum antibody to sheep erythrocyte antigens. This early but significant depression of humoral antibody response is similar to the marked suppression of antibody formation to SRBC in FLV susceptible Balb/c mice. However, in contrast to the situation with the Balb/c animals, where immunosuppression remains evident throughout the course of the disease, there is a rapid and striking return to normal immunoresponsiveness to sheep erythrocytes in the C_{57}Bl mice given FLV 7 days earlier. These mice not only failed to show any suppression of the hemolysin response, but often developed a slight enhancement as compared to the responses of uninfected control mice.

It is noteworthy that FLV could be recovered in high titer from the spleen, as well as other organs, and serum of C_{57}Bl mice infected with virus 1 or 3 days before challenge immunization. The virus was asayed at the time of PFC determination. Thus virus was present at least 5–7 days after infection. The relatively large numbers of virus particles per unit spleen weight or milliliter serum sug-

TABLE II. Effect of Friend Leukemia Virus Infection on Macrophage Migration Inhibition of Balb/c and $C_{57}Bl$ Mice Sensitized with Mycobacteria.

Time between infection and sensitization[a]	Balb/c		$C_{57}Bl$	
	Percent inhibition[b]	Percent of control	Percent inhibition[b]	Percent of control
Controls (no FLV)	45	—	40	—
−1 day infected	22	49	37	93
−3 days infected	17	38	40	100
−7 days infected	15	33	44	115

[a] Groups of mice sensitized with CFA 3 wk earlier and infected with FLV on day indicated relative to day of assay.

[b] Percent of migration area of spleen cells incubated with PPD *in vitro* compared to migration of same cells without PPD.

gested that the virus was replicating in these mice without inducing any signs of disease or pathology. In contrast, mice infected 7 days before challenge immunization did not show evidence of virus when tested 4 days later (plus 11 days). Thus the presence of virus correlated with the transient depression of immune responsiveness, supporting the belief that virus replication and immunosuppression are concomitant events and probably related (5, 7).

Although immunosuppression occurs throughout the course of the leukemogenic process in susceptible Balb/c mice it seems unlikely that viremia and/or leukemogenesis are unequivocally required for immunosuppression. This seems evident from the findings with the resistant $C_{57}Bl$ mice, since immunosuppression occurred only transiently early in the course of disease. These mice did not go on to develop leukemia. Thus it is apparent that leukemogenesis is not necessary for FLV-induced immunosuppression. Such an interpretation is also supported by the many observations with other systems indicating that nononcogenic viruses, such as LCM, are capable of inducing a transient but significant depression of humoral antibody formation (4, 7). Although progression of a tumor virus infection into an active leukemogenic process may still be related to a concomitant immunosuppression, the studies concerning humoral antibody formation show that impairment of immunity by a leukemia virus is not necessarily followed by leukemogenesis.

This lack of absolute correlation between immunosuppression and leukemogenesis is further supported by results of the studies concerning CMI to mycobacteria in $C_{57}Bl$ and Balb/c mice. Whereas the humoral immune response was transiently suppressed in $C_{57}Bl$ and Balb/c mice given FLV, there was no evidence of suppression of cellular immunity in the $C_{57}Bl$ mice when assayed by migration inhibition experiments with PPD. On the other hand, such suppression was readily achieved in susceptible Balb/c mice. The absence of a discernible effect of FLV on cell-mediated immunity in $C_{57}Bl$ mice suggests that this parameter of immune responsiveness is either less sensitive to impairment by FLV, or that this virus is less capable of altering T-lymphocyte populations involved in the response to mycobacterial antigens in $C_{57}Bl$ mice.

Earlier adoptive cell transfer studies with B- and T-lymphocyte populations had suggested that, at least in susceptible Balb/c mice, FLV affects preferentially B cells involved in antibody formation early in the course of infection (25, 26). Thymus dependent lymphocyte function related to cell cooperation for hemolysin formation seemed unaffected early in the course of infection. The results of the present studies with FLV-resistant $C_{57}Bl$ mice show the absence of an effect on migration inhibition, considered an example of T-lymphocyte function; Balb/c mice which develop leukemia after FLV infection evinced a rapid and pronounced de-

pression of migration inhibition activity. Both uninfected mice, either Balb/c or C_{57} Bl strains, showed strong responses to PPD by the migration inhibition assay after sensitization with CFA.

A number of studies by other investigators appear to be pertinent to the results presented here. Dent *et al.* first observed that cellular immunity, represented by the allograft response to non-H_2 transplantation antigens, is moderately suppressed in mice infected neonatally with Gross pasasge A virus (27). More recent studies concerning allograft reactions to H_2 alloantigens have shown that mice infected with FLV as adults show prolonged graft rejection times, suggesting that cell-mediated immunity may be affected by FLV (28). On the other hand, Schneider and Dore reported that graft rejection is normal in FLV infected mice (29). However, independent analyses of their data by Dent suggests that an impairment of the graft rejection response did occur in their experiments, although to a moderate degree (7).

It is also noteworthy that Chirigos *et al.* observed that preinfection of mice with several murine tumor viruses, including FLV, effectively blocked regression of transplanted tumors which are normally rejected by noninfected control mice. Such regression is considered to be mediated by cellular immunity (30). Similarly, both Borella (31) and Hayry *et al.* (32) have reported that infection of mice with Rauscher virus impairs the *in vitro* stimulation of spleen lymphocytes to phytohemagglutinin, a response considered a correlate of cellular immunity. Thus there appears to be strong evidence, although not unequivocal, that cellular immunity may be impaired in susceptible strains of mice infected with MuLV. The observed resistance of C_{57}Bl mice not only to virus induced leukemia, but also to depression of cell-mediated immunity suggests that there may be a correlation between lack of inhibition of cellular immune responsiveness with resistance to FLV infection. Those genes which control susceptibility to leukemia viruses may be closely linked with the immune response genes associated with cell-mediated immunity (presumably T-lymphocyte mediated), but not with humoral immunity (dependent upon B-lymphocyte activity). If this is the case, then further analyses of both humoral and cell-mediated immune functions in resistant and susceptible strains of mice should clarify some of the questions concerning the interrelations between tumor virus infection and various immunologic functions.

Summary. Infection of susceptible (Balb/c) and resistant (C_{57}Bl) strains of mice with Friend leukemia virus (FLV) revealed a dichotomous effect in terms of immunosuppression to sheep erythrocytes, assessed at the level of individual antibody forming cells and serum hemolysins, and to mycobacterial antigens, assessed at the level of cell-mediated immunity using a macrophage migration inhibition assay. Balb/c mice infected with FLV showed a prompt and prolonged depression of antibody responsiveness to the sheep erythrocytes, as well as marked impairment of cellular immunity to the mycobacterial antigens. In contrast, C_{57}Bl mice, resistant to FLV induced leukemogenesis, showed a transient but significant depression of antibody formation to sheep erythrocytes, but no depression of cell-mediated immunity to mycobacteria. These divergent effects appeared to reflect the relative importance of cell-mediated versus humoral immunity in resistance to leukemia virus induced leukemogenesis.

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