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Failure of Peritoneal Exudate Macrophages to Reverse Immunologic Impairment by Friend Leukemia Virus[1] (38637)

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Infection of susceptible strains of mice with Friend leukemia virus (FLV) invariably results in a marked impairment of immune responsiveness (1–4). Such suppression parallels and usually proceeds development of overt symptoms of leukemia, including splenomegaly and blood cell dyscrasia. Systematic studies in this laboratory concerning the nature and mechanism of FLV induced immunosuppression have focused attention on the B-lymphocyte as an important target cell for immunologic impairment (5, 7). Antibody forming cells per se do not seem to be involved directly in the immunologic injury induced by FLV infection. Adoptive cell transfer experiments indicated that depletion of the antibody precursor cell population, presumably B-cells, accounted for the major deficiency in FLV-infected animals. For those experiments different cell types from infected mice were adoptively transferred to irradiated recipient mice challenged with sheep red cells as the test antigen. In reverse experiments it was found that spleen cell suspensions from normal mice could readily transfer antibody forming capability to FLV infected recipients (8, 9). Lymphocytes in particular were most effective in restoring, at least temporarily, immunocompetence to the leukemia virus infected animals. In the present study attempts were made to determine whether macrophages from normal mice could similarly alter the immunodeficiency of FLV-infected animals. For this purpose peritoneal exudate cell suspensions, rich in macrophages were transfused into mice infected with FLV. The mice were then challenged with sheep erythrocytes and the number of antibody producing cells appearing in their spleen enumerated by the hemolytic plaque assay.

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Methods and Materials. Mice. Young adult Female Balb/c mice, 8–12 wk of age, were used for these experiments. They were obtained from Flow Laboratories, Dublin, VA, and weighed 18–20 g each when used. The mice were housed in groups of 5–6 in plastic mouse cages and fed Purina mouse food and water *ad libitum*.

Leukemia virus. A stock preparation of FLV, originally obtained from the American Type Culture Collection, Rockville, MD, and passaged for over 8 yr in this laboratory through young adult Balb/c mice, was used for these experiments (3, 6). The stock preparation consisted of a clarified 10% homogenate prepared from spleens of 20–30 Balb/c mice infected for 18–20 days.

Leukemia induction. Test mice were injected intraperitoneally (ip) with 0.5 ml of a 10^{-1} dilution of the stock virus preparation containing approximately 100–200 ID_{50} of infectious virus. The virus preparation was free of detectable LDH or LCM viruses as contaminants.

Test antigen. Sheep erythrocytes (SRBC) were obtained in Alsever's solution from Baltimore Biologic Laboratories and were washed three times in phosphate buffered saline. Standardized concentrations (v/v) of either 0.5 or 5.0% RBCs were prepared in saline. For immunization 0.5 ml of a given RBC concentration was injected ip into each mouse.

Assessment of antibody formation. The localized hemolysis in gel assay for antibody plaque forming cells (PFC) was performed essentially as described initially by Jerne and associates (10). In brief, dispersed spleen cell suspensions were prepared and 0.1 ml aliquots were incubated in 2.0 ml 1% agar containing an 0.1 ml inoculum of a freshly washed 10% suspension of sheep erythrocytes. The agar-cell mixture was carefully poured onto a previously prepared base

layer of agar in a petri plate and when the upper layer had solidified the plates were incubated at 37° for 1 hr. The plates were then treated with approximately 5 ml of a 1:15 dilution of guinea pig serum as a source of complement and incubated for an additional one hour at 37° until zones of hemolytic plaques appeared on the plates. These plaques were considered due to high efficiency 19S IgM PFCs. The number of PFCs on three or more plates containing the same dilution of a spleen cell suspension was enumerated and the number of PFCs per spleen calculated.

Peritoneal exudate (PE) cell preparation. Balb/c mice were inoculated ip with 0.5 ml incomplete Freund's adjuvant. Twenty-four hr later the mice were killed and the peritoneal cavity was washed with sterile Hanks' balanced salt solution. The cells obtained were then washed three times in the cold with sterile buffer by low speed centrifugation and standardized to a concentration of 1×10^8 nucleated cells per 1.0 ml. In most cases approximately $1–5 \times 10^7$ cells were obtained from each donor mouse. Over 90% of the cells were morphologically identical to macrophages and nearly all of the cells showed phagocytic activity since they rapidly ingested carbon particles. For cell transfer 1×10^7 PE cells were injected ip or iv into recipient mice 18–24 hr before challenge immunization with SRBC. In some experiments 3×10^7 PE pooled cells were injected into each recipient animal.

Results. Earlier studies had shown that mice infected with FLV show a marked impairment of antibody formation to sheep erythrocytes (3, 7). Transfer of $5–10 \times 10^6$ splenocytes from normal donor mice into syngeneic recipients infected with FLV induced a readily detectable adoptive immune response in that many more hemolytic PFCs developed after challenge immunization with sheep RBCs as compared to the response of untreated FLV-infected recipients. In the present study PE cells obtained from normal donor mice were injected into similar FLV-infected mice. As controls normal noninfected mice were given the same number of donor PE cells. Mice were then challenged with an inoculum of either a 0.5 or 5.0% suspension of SRBC. The number of PFCs appearing in the spleen of these mice was determined 4 days later. Mice infected with FLV 2 days before the challenge immunization showed a slight to moderate suppression of the PFC response, especially when the challenge dose of RBCs was 5%; the lower dose of antigen stimulated a lower PFC response in both control and infected mice (Table I). For example, normal control mice given the 5% antigen dose responded with approximately 60 thousand PFCs per spleen 4 days after challenge immunization; injection of 10 million normal PE cells into these animals 24 hr prior to challenge immunization resulted in somewhat fewer PFCs (36 thousand per spleen). Mice infected with FLV 2 days before immunization responded with 25,000 PFCs per spleen (approximately a 60% depression as compared to the control mice). When 2-day infected mice were given PE cells there was no enhancement but rather a moderate further depression of the PFC response appeared in their spleen (an average of about 14,000 PFCs). When the challenge dose of antigen was reduced to 0.5% RBCs untreated controls responded with an average of 9400 PFC per spleen; control mice given 10 million PE cells developed an average of 9300 PFCs per spleen. Mice infected with FLV two days earlier responded with about 7,700 PFCs per spleen and infected mice given PE cells developed about 7000 PFCs per spleen (Table I).

When mice were infected with FLV eight days before challenge immunization there was a markedly greater immunodepression (Table II). Again, however, transfer of 10 million PE cells from normal donors to these animals had no significant affect on the depressed immune response. As can be seen in Table II, control mice in these experiments immunized with 5.0% RBC responded with many PFCs per spleen; the response was nearly 50-fold greater than that in mice infected with FLV 8 days earlier. Transfer of PE cells to the control mice caused a moderate reduction in the PFC response (approximately 35%), while transfer of the same number of PE cells to the 8-day FLV infected mice had no significant affect on the PFC response (2110 vs. 1910 PFCs per spleen for PE cell treated as compared to untreated FLV-infected mice, respectively). When the dose

TABLE I. EFFECT OF TRANSFER OF PE CELLS FROM NORMAL DONOR MICE TO CONTROL MICE AND MICE INFECTED 2 DAYS EARLIER WITH FLV.

Mouse group[a]	PE cells transferred (10^7)[b]	PFC response per spleen[c] 5.0% RBC	0.5% RBC
Normal control	−	61,000 ± 6900	9420 ± 850
	+	36,100 ± 4100	9310 ± 330
FLV infected	−	25,300 ± 6700	7720 ± 1930
(2 days)	+	14,400 ± 1600	7060 ± 1990

[a] Groups of 8–12 mice, either normal or FLV infected (0.5 ml of 10^{-1} dose of virus).

[b] PE cells from normal syngeneic mice injected ip into recipient mice 24 hr before challenge immunization with 0.5 ml of indicated dose of RBCs.

[c] Average PFC response 4 days after immunization.

TABLE II. EFFECT OF TRANSFER OF PE CELLS FROM NORMAL DONOR MICE TO CONTROL MICE AND MICE INFECTED 8 DAYS EARLIER WITH FLV.

Mouse group[a]	PE cells transferred (10^7)[b]	PFC response per spleen[c] 5.0% RBC	0.5% RBC
Normal control	−	99,000 ± 6140	9310 ± 2680
	+	61,500 ± 7700	5930 ± 2200
FLV infected	−	1910 ± 450	196 ± 58
(8 days)	+	2110 ± 610	313 ± 68

[a] Groups of 15–20 mice, either normal or FLV infected (0.5 ml of 10^{-1} dose of virus).

[b] PE cells from normal syngeneic mice injected ip into recipient mice 24 hr before challenge immunization with 0.5 ml of indicated dose of RBCs.

[c] Average PFC response 4 days after immunization.

of antigen was reduced (0.5% RBCs) the PFC responses of both control and infected mice were also reduced about ten-fold. Transfer of PE cells, even in this situation, did not significantly alter the PFC responses of FLV infected mice.

In additional experiments of the same type the number of PE cells used for transfer was increased three fold (3×10^7 cells per recipient), with exactly the same results as those observed with the lower number of PE cells. Furthermore, when PE cell suspensions were treated by repeated adsorptions onto glass plates to obtain the adherent cell population consisting almost entirely of macrophages, the same results were obtained. The adherent cell preparation consisted of approximately 99% macrophages, nearly all of which rapidly ingested carbon particles. Transfer of 10^7 or 3×10^7 glass adherent cells into FLV infected mice did not alter immunologic impairment to SRBC.

Discussion. The results of the present experiments do not support the view that impairment of phagocytic function of PE cell monocytes constitutes a major defect in FLV-infected mice evincing a state of immunosuppression. In contrast, the present results show that even when PE cell suspensions rich in macrophages are transfered in relatively large numbers to FLV-infected mice, either early or later in the course of leukemogenesis, there is no significant affect on depressed immune responseiveness to sheep erythrocytes.

Although there have been many studies concerning the interrelationship between tumor viruses and the immune response mechanism, it is still unclear how a leukemia virus such as FLV impairs antibody formation (1–4). Extensive studies concerning the cellular mechanisms involved in the immune response to an antigen such as sheep erythrocytes in FLV-infected animals have pointed towards the B-lymphocyte population as the major target for virus induced immunosuppression. However, it should be noted that Odaka initially suggested that FLV

may impair immunity by adversely affecting macrophages (11, 12). FLV replicates readily in macrophages *in vitro* and it is presumed that such infected cells could have impaired functional activity. However, the results of the present study show that immunologic impairment in FLV-infected mice cannot be modified by transfer of PE cell suspensions rich in macrophages from normal donor mice. Furthermore, correlary studies in this laboratory have indicated that the "processing" of sheep erythrocytes is also unimpaired in FLV-infected animals (7, 13). For example, injection of ^{51}Cr labeled RBCs into FLV-infected mice results in as a rapid an uptake of the radiolabeled antigen into the spleen and liver as occurs in normal noninfected animals. Thus there seems to be no unequivocal evidence that impaired phagocytic function parallels immunodepression in infected animals.

The slight to moderate depression of PFC responsiveness in control mice given the 10 million normal PE cells suggests that transferred cells may result in a more rapid degradation of the injected SRBC so that a smaller effective dose of antigen is left to stimulate immune responsiveness. However, if this were the case, then it seems apparent that the same effect is not occurring in FLV-infected mice. The transfer of additional PE cells, either ip or iv to infected mice did not depress further, or alter in any way, the already impaired antibody response to the RBCs. The degree of immunosuppression of infected mice depended on the day of infection; this suppression was not significantly altered by infusion of PE dells from the normal donors. Mice infected with FLV 2 days before antigen responed with many more PFCs than did the mice injected with FLV 8 days earlier. Nevertheless, there was no effect apparent in either group of infected mice. It should be noted that in other experiments (unpublished) it was found that injection of the same number of PE cells from normal donor mice into 8-day FLV infected mice on either the day of challenge immunization or 1 day later also failed to influence the level of immunosuppression. Thus the absence of an effect on PFC responsiveness in infected mice given PE cells 1 day before RBC immunization does not appear due merely to the time of cell transfer.

Despite the absence of any direct evidence for altered macrophage function in FLV infected mice, it seems important to note that earlier histologic studies on the ultrastructure of spleens of infected mice suggested a marked increase in phagocytic activity of macrophages, including an almost overwhelming ingestion of debris and necrotic material by phagocytic cells (14). Those studies suggested that macrophages may be involved in attempts to remove dead and/or destroyed cells and tissue material, as well as virus particles; such cells could be so committed to "scavanging" of endogeneous material that they might be unable to "process" normally an exogenous particulate antigen such as sheep RBCs. Nevertheless, as shown in the present study, transfer of additional macrophages to infected mice did not alter immune impairment. On the other hand, as shown in previous studies, transfer of unfractionated splenocytes, in relatively large numbers, to FLV infected animals readily restored immunocompetence, at least temporarily. However, this appeared due to the transfer of immunocompetent cells *per se*, which could respond to challenge injection of sheep erythrocytes in the recipients and directly form antibody. Such results suggest there is no immunosuppressive "factor" in FLV-infected animals which could adversely affect the immunocompetence of normal antibody forming cells transferred from uninfected donor mouse spleens. The role of the macrophages present in the whole spleen cell population in this transfer response is unknown. Thus attempts should be made to purify macrophages from normal spleen cell suspensions to determine if such cells are more effective than macrophages present in PE cell suspensions. However, even in the absence of such additional studies it seems likely from the present study that the defect in immunocompetence of FLV infected mice lies mainly with impairment of antibody forming cells and their precursors and not to antigen processing cells *per se*.

Summary. Transfer experiments with peritoneal exudate macrophages from normal donor mice were performed to determine if

a defect of normal macrophage function or activity was a major or contributing factor to the immunosuppression characterizing leukemia virus infection of mice. Challenge immunization of Friend leukemia virus-infected mice with sheep erythrocytes resulted in markedly depressed hemolytic antibody responses, as compared to responses of normal noninfected mice. When PE cell suspensions rich in macrophages were transferred from normal donor mice to leukemia virus infected recipients there was no affect on the FLV-induced impairment of the immune response. Similar transfer of PE cells to normal uninfected mice generally resulted in a moderate depression of the expected immune response. In no case did the PE cells enhance the immune responses in normal or virus-infected mice.

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