act as regulatory signals capable of modulating the immune response. Therefore, it is tempting to speculate that the contacts between nerve endings and cells of the immune system: 1) might influence or modulate the cell cycle of lymphoid cells or their capacities for reacting in immune responses or, 2) modify the microenvironments of lymphoid organs through the modulations of reticular cells.

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Effect of cyclophosphamide on development of reticulum cell sarcoma in SJL/J mice[1]

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Summary. Studies to determine the effect of cyclophosphamide (CY) on the development of reticulum cell sarcoma (RCS) in SJL/J mice indicated a dependence on the duration of the test period. Age also appeared a factor of importance. Thus, a comparison of tumor incidences at 52 weeks of age showed maximal inhibition when CY was administered at 40 weeks, minimal inhibition when the drug was given at 30 weeks, and intermediate inhibition when treatment was initiated at 10 and 20 weeks. Consistent with these findings, long-term treatment of 40-week-old SJL/J mice with low doses of CY resulted in an increase in the mean survival time and in a reduction in the incidence of RCS.

The SJL/J strain of mice was derived from noninbred Swiss Webster stock by brother × sister matings for 30 generations. Initial reports emphasized the susceptibility to development of type B reticulum cell sarcoma (RCS) in high incidence, and the shared features of this spontaneous disease of mice and Hodgkin's disease of man[2,3]. Thereafter, other characteristics of the strain were described such as low erythrocyte counts (males), a high incidence of spontaneous amyloidosis (old females), resistance to tolerance induction, a high susceptibility to induction of delayed hypersensitivity, paraproteinemia and antinuclear antibodies[4–6].

Consistent with the many immunologic manifestations, most attempts to inhibit the development of SJL/J neoplasms have had their basis in a modification of immune functions. These include treatment with antilymphocytic serum or corticosteroids[7,8], as well as thymectomy or splenectomy and the transplantation of allogeneic bone marrow[9,10].

The purpose of this study was to determine the influence of an immunosuppressive drug, cyclophosphamide (CY), on neoplasia in SJL/J mice. A brief description of preliminary findings has been reported previously[11].

Materials and methods. Animals. Female SJL/J mice were obtained from the closed breeding colony maintained in the Animal Services Center, University of Alabama in Birmingham. The mice were caged in groups of 3 or 4, and provided with food and water ad libitum.

Test procedure. CY was prepared at a concentration of 15 mg/ml in cold 0.85% saline and administered by i.p. injection (1.5 mg/mouse/week). The age of the mice varied among the groups from 10 to 40 weeks and, in most tests, the period of treatment was either 6 or 12 weeks in length. Groups of control animals received weekly an i.p. injection of 0.1 ml of 0.85% saline, or they were not injected.

Tumor incidence. Experimental and control mice were killed at 40, 52 and 60 weeks of age, or they were held until moribund. Thereafter, the animals were necropsied for evidence of neoplasia. The findings of enlargement of Peyer's patches, the mesenteric lymph node complex, other lymph nodes and/or the spleen were accepted as indicative of RCS. In those cases in which the gross observations seemed questionable, sections of appropriate organs were prepared by routine histologic methods and examined microscopically to allow for a definitive diagnosis. Differences in tumor incidence between experimental and control mice were evaluated for statistical significance by the contingency test.

Results and discussion. Experiment 1. Two groups of 10-week-old female SJL/J mice were given a series of 6 or 12 weekly injections of CY. For controls, 15 animals received

Table 1. Effect of duration of CY treatment on the incidence of RCS in SJL/J mice

Treatment	No. of mice	Age at onset of treatment (weeks)	No. at 40 weeks	No. with RCS at 40 weeks	Percent with RCS at 40 weeks
CY (6 weekly injections)	26	10	26	18	69.2
CY (12 weekly injections)	34	10	33	13	39.4[a]
Saline (12 weekly injections)	15	10	15	11	73.3

[a] Significant inhibition at $p < 0.05$.

Table 2. Effect of age at the onset of CY treatment on the incidence of RCS in SJL/J mice

Treatment	No. of mice	Age at onset of treatment (weeks)	No. at 52 weeks	No. with RCS at 52 weeks	Percent with RCS at 52 weeks
CY (12 weekly injections)	16	10	14	6	42.9[a]
CY (12 weekly injections)	16	20	16	7	43.8[a]
CY (12 weekly injections)	22	30	22	14	63.6
CY (12 weekly injections)	30	40	30	5	16.7[b]
Control	12	–	12	11	91.7

[a] Significant inhibition at $p < 0.05$; [b] significant inhibition at $p < 0.01$.

Table 3. Effect of long-term CY treatment on the incidence of RCS in SJL/J mice

Treatment	Age at onset of treatment (weeks)	Percent of mice with RCS 52 weeks	62 weeks	75 weeks	86 weeks	104 weeks
CY (weekly)	40	4.2	8.3[a]	25.0[a]	54.2[b,c]	–
Saline (weekly)	40	8.3	45.8	75.0	79.2	87.5[d]

[a] Significant inhibition at $p < 0.01$; [b] significant inhibition at $p < 0.05$; [c] the last mouse died at 86 weeks of unknown causes; [d] the last mouse with RCS died at 104 weeks. 2 animals died with fibrosarcoma and of unknown causes at 129 weeks and 133 weeks, respectively.

injections of saline over a period of 12 weeks. All mice were killed and examined for evidence of neoplasia at 40 weeks of age.

The results of this experiment are summarized in table 1. It can be seen that treatment for 6 weeks had no effect on the incidence of RCS. By comparison, a significant reduction in the tumor incidence was observed among mice treated for 12 weeks. These findings suggested that long-term CY therapy might have an inhibitory influence on the development of SJL/J neoplasms. This possibility was supported by results obtained in a related test with 10-week-old animals (data not presented in the table). Thus, the tumor incidence at 52 weeks of age was 24.1% (7/29) among mice which received 18 injections of CY at biweekly intervals, as compared to 90% (9/10) in saline-injected controls (significant inhibition at $p < 0.01$).

Experiment 2. Four groups of SJL/J females received 12 weekly injections of CY beginning at 10, 20, 30, or 40 weeks of age. A 5th group of control animals was not injected.

The results obtained are presented in table 2. It would appear that age is a factor since administration of CY to 40-week-old mice inhibited the development of RCS to a greater extent than treatment at 10 or 20 weeks of age. Further, initiation of therapy at 30 weeks had no significant influence on the tumor incidence ($p > 0.05$). This finding proved of interest because a previous study had shown that proliferation occurs in the mesenteric nodes of SJL/J mice at a mean age of 222 days[12]. As yet, however, there is no explanation for this apparent relationship between the onset of preneoplastic changes and refractoriness to CY treatment.

Experiment 3. In the final experiment, 24 SJL/J mice were treated with an indefinite series of weekly injections of CY beginning at 40 weeks of age. For controls, an identical number of animals received weekly injections of saline. The cages were checked periodically for sick or moribund mice; all animals were examined for RCS as described above.

Table 3 shows that the development of RCS was inhibited by long-term administration of CY at low doses. Further, the mean survival time was increased from 486 ± 159 (SD) days (controls) to 525 ± 73 (SD) days (experimentals). It should be noted, however, that 3 control animals lived longer than any of the experimental mice. This finding seems best interpreted as evidence for a CY-induced toxicity with prolonged therapy.

CY is known to be a potent inhibitor of humoral antibody production[13] and, by way of agreement, evidence has been obtained to indicate a preferential toxicity of the drug for B lymphocytes[14]. Further, CY has been shown to affect cell-mediated responses[15]. The effects are variable, but, in the case of enhanced responses, they appear to have their basis in an inactivation of suppressor T lymphocytes[16]. Such a mechanism of action could account for the observation that treatment of 40-week-old SJL/J mice inhibits the development of RCS since this strain manifests a number of T-cell deficiencies in later life[17]. It may also be a factor in the reduction of tumor incidence when therapy is initiated at 10

or 20 weeks of age, but other possibilities exist such as an effect of CY on the emergence of tumor cells. Present data are insufficient to allow for a conclusion.

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Heterogeneity in filterability of erythrocytes from malaria *(Plasmodium berghei)*-infected blood

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Summary. Erythrocytes from *Plasmodium berghei*-infected blood show a decrease in deformability with increasing parasitaemia, as measured by filterability through polycarbonate sieves. A major fraction of cells carrying mature parasites and a smaller fraction carrying ring-stage parasites account for the obstruction of filtration, while the remaining infected cells do not contribute to the decrease in filterability. The relation of filterability to metabolic status in infected cells is discussed.

The deformability of erythrocytes plays an important role in their passage through capillaries and associated processes. Erythrocytes of reduced deformability may not be capable of passage through restricted areas of circulation such as those in the spleen, and are thereby removed from the circulation[2,3]. In some malaria infections[4-6], such as those resulting from *Plasmodium knowlesi, Plasmodium coatneyi* and *Plasmodium yoelii* YM, it is believed that reduced deformability could lead to capillary obstruction in the brain contributing to cerebral complications. Apart from cerebral complications, other lesions in malaria which involve altered rheologic properties of the erythrocytes include renal failure and liver necrosis[4,5]. Filterability of erythrocytes through micropores having similar dimensions to those of capillaries has been widely used[7-9] as an indicator of deformability. Studies with infected simian erythrocytes[4] have indicated that alterations in the microcirculation are correlated with their filterability. It is unclear, however, whether a similar decrease in filterability occurs generally with all malaria infections, or only in those with cerebral complications or prominent microcirculatory disturbances. Although *P. berghei* infection of the mouse is normally not associated with cerebral involvement, many of the pathological lesions could be related to alterations of the rheological properties of the erythrocytes. This paper reports a decrease in the filterability of *P. berghei*-infected mouse erythrocytes, and the heterogeneity of this decrease in erythrocytes carrying parasites at the same stage of maturation.

Materials and methods. *P. berghei*-infected blood was obtained by cardiac puncture of infected Swiss mice, 5 days after i.p. inoculation with 10^7–10^8 parasitized erythrocytes. No reticulocytosis ensued during this period, as observed by Brilliant Cresyl Blue staining. Cells from normal and infected blood were collected in ACD solution and washed 3 times with either a buffer containing 25 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 86 mM glucose and 50 mM Na_2HPO_4, pH 7.4 or (in ATP depletion experiments) with a buffer

Changes in parasitaemia, filtration time and ATP content of erythrocytes from *P. berghei*-infected blood on repeated filtration

Source	Number of filtration	Parasitaemia (%) Total	Ring	Mature	Filtration time (sec)	ATP content (nmole/10^9 cells)
Normal	1–4	–	–	–	5.3± 0.7	64.9±14.0
Infected, day 3	1	12.6	5.7	6.9	326 ±10	61
	2	6.5	3.9	2.6	12.8	66
	3	3.4	2.2	1.2	7.1± 1.6	52
Infected, day 5	1	40.3	6.4	33.9	>1800	58
	2	22.0	4.4	17.6	>1800	76
	3	20.0	4.4	15.6	77.4	72
	4	13.0	3.5	9.5	6.0± 2.3	68
Normal, ATP-depleted	1	–	–	–	139	2
Infected, day 3, ATP-depleted	1	12.0	–	–	463	2

Erythrocyte suspensions (1.0×10^9 cells/ml) were repeatedly applied on 3 μm polycarbonate filter under 10 cm water pressure, using a new filter each time. Before each filtration, the parasitaemia and ATP content was determined and the erythrocyte concentration appropriately adjusted.