Adenosine deaminase activity in blood cells of mice*

Age (weeks)	Adenosine deaminase (units/mg protein)** Lymphocytes		Erythrocytes	
	SJL/J	C57BL/6J	SJL/J	C57BL/6J
12	0.43±0.074	0.20±0.027	0.093±0.009	0.089±0.012
22	0.59±0.062	0.23±0.035	0.091±0.008	0.080±0.004
32	0.52±0.077	0.24±0.047	0.096±0.008	0.093±0.005
42	0.48±0.038	0.17±0.022	0.081±0.009	0.078±0.005
52	0.52±0.054	0.16±0.013	0.074±0.010	0.083±0.008

* Values determined from samples of 12–16 mice and expressed as means ± SE. ** 1 unit of activity is defined as the amount of enzyme which deaminates 1.0 µmole of adenosine per min at 25 °C.

cytes were separated by a method which was developed to allow for their isolation from small volumes of peripheral blood[7]. Then, they and the resultant erythrocyte fractions were washed in cold Hanks' balanced salt solution, resuspended in phosphate-buffered saline (pH 7.4), and lysed by freezing and thawing at −60 °C.

Enzyme assay. Lymphocyte and erythrocyte ADA activities were determined spectrophotometrically in a final volume of 1.0 ml by a method described previously[8]. The reaction mixture contained 0.2 mM adenosine in 0.05 M phosphate buffer (pH 7.4), 0.1 unit of nucleoside phosphorylase (or present in erythrocyte lysates), and 0.2 units of xanthine oxidase (Sigma Chemical Corporation. St. Louis, Missouri). After addition of the sample and incubation at 25 °C for 6 min, the absorbance of the solution was recorded at 293 nm for 4 min. ADA activity is expressed as µmoles of adenosine deaminated per min per mg of protein at 25 °C. Protein was determined by the Bio-Rad Assay (Bio-Rad Laboratories, Richmond, California).

Results and discussion. The results, which are summarized in the table, indicate that lymphocyte ADA activities were higher in SJL/J mice than C57BL/6J animals. Further, both strains exhibited little variation in enzyme activity throughout the test period. These findings suggest that a deficiency of lymphocyte ADA is not associated with immune disorders of SJL/J mice or with the development of reticulum cell neoplasms in this strain.

As also shown in the table, ADA activities in erythrocyte lysates were similar in strains SJL/J and C57BL/6J at all ages tested. Thus, it would appear that neither modifications in the immune status of SJL/J mice nor spontaneous tumor development are reflected by alterations in the levels of erythrocyte ADA.

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Local leukocyte mobilization in irradiated or cyclophosphamide-treated rats[1]

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Summary. Sprague-Dawley rats made neutropenic by ^{60}Co irradiation or cyclophosphamide treatment retained a limited capacity for mounting a local leukocyte mobilization (LLM) response. Rats irradiated with 700 rad ^{60}Co lacked an LLM. Rats treated with 100 mg/kg cyclophosphamide showed no LLM following an initial low response when assayed originally.

The plastic skin chamber technique has been developed to study local leukocyte mobilization (LLM) to a skin abrasion site[2,3]. A flexible plastic cup is glued over a skin abrasion and filled with serum or other osmotically buffered fluid. The cup contents are removed at selected time intervals, and the cells are counted or microscopically assayed. Since neutrophils constitute over 95% of the cells found in chambers during the first 24 h, the cells counted are a measure of neutrophil migration to the skin abraison[4]. Also, assay of skin chamber fluid at 24 h provides a superior comparative assessment of an animal's LLM capacity due to kinetic variations[5]. The plastic skin chamber technique has been used to study in vivo LLM as a function of fluid contents in the chamber or various disease conditions in the animal[3–8].

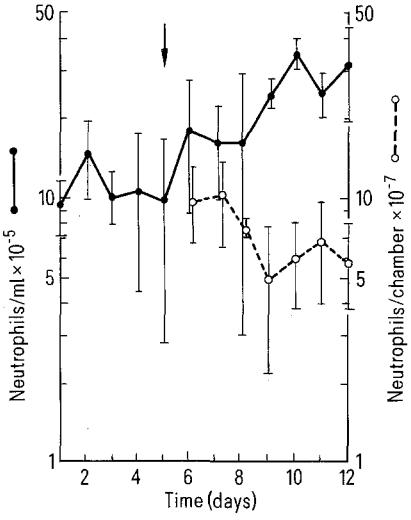
This report is an examination of LLM in female Sprague-Dawley rats, weighing 250–325 g, following exposure to ^{60}Co radiation or cyclophosphamide (Sigma). Rats were irradiated with ^{60}Co at 40 rad/min or administered cyclophosphamide by tail vein injection prior to installing skin chambers. When circulating neutrophil counts in blood drawn from rat tail veins dropped to a nadir 3 or 4 days after treatment, skin chambers were installed on the animals. The chambers were glued over skin abrasions on the right or left lateral thoracic surfaces of the rats. Blood neutrophils were determined by counting white blood cells on a Coulter Counter Model F (Coulter Electronics, Inc.) and making differential counts of blood smears. The plastic skin chambers were filled with sterile physiological saline having 250 µg/ml *Salmonella typhosa* lipopolysaccharide W (Difco). Approximately 24 h later, fluid was removed from the skin chambers, and cells in the fluid were counted and sized with the aid of a Coulter Counter equipped with a Coulter Channelyzer Model H4. Skin chamber fluids were also examined microscopically. Following removal of a chamber from a rat, another plastic skin chamber was installed over a skin abrasion on the opposite thoracic surface of the animal and assayed as described above approximately 24 h later. Control and experimental animals were assayed for the LLM response concurrently, and no attempt was made to determine the LLM of treated rats before irradiation or cyclophosphamide injection.

The number of neutrophils migrating to plastic skin chambers on control rats was reproducible when the fluid used to

Local leukocyte mobilization* in rats treated with cyclophosphamide or irradiated with cobalt-60

	Controls	Cyclophosphamide (40 mg/kg)	Cyclophosphamide (100 mg/kg)	Irradiated (450 rad)	Irradiated (700 rad)
Skin chamber 1	$9.99\times10^7\pm2.84\times10^7$	$3.01\times10^7\pm1.86\times10^7$	$6.48\times10^6\pm5.86\times10^6$	$5.02\times10^7\pm2.52\times10^7$	None detected
Circulating neutrophils per ml of blood	$2.32\times10^6\pm0.48\times10^6$	$6.80\times10^5\pm5.4\times10^5$	$1.56\times10^3\pm4.33\times10^3$	$9.50\times10^5\pm2.66\times10^5$	$2.56\times10^5\pm0.87\times10^5$
Skin chamber 2	$8.16\times10^7\pm2.25\times10^7$	$4.05\times10^7\pm1.76\times10^7$	None detected	$5.10\times10^7\pm2.42\times10^7$	None detected
Circulation neutrophils per ml of blood	$1.89\times10^6\pm0.42\times10^6$	$7.80\times10^5\pm4.20\times10^5$	$4.20\times10^4\pm4.06\times10^4$	$1.04\times10^6\pm0.31\times10^6$	$2.54\times10^5\pm1.65\times10^5$

*Expressed as the mean ± the 95% confidence limits. 14 control rats and 5 experimental rats were used for each treatment listed.



Neutrophils per skin chamber and circulating neutrophils per ml at different days for control rats. The arrow indicates the day of attachment of the first skin chamber. The error bars indicate 1 SD. 4 rats were used in this study.

fill the chamber was physiological saline containing 250 µg/ml lipopolysaccharide. When the lipopolysaccharide concentration was below 100 µg/ml, neutrophil recovery was variable. Also, cells observed in fluids recovered from the skin chambers were over 95% neutrophils. Analysis made by the Coulter Channelyzer Model H4 of a plot size distribution of cells in fluid removed from skin chambers implanted on control rats indicates that the cells were about 215 μm^3 in volume, and the cell population was fairly uniform. These results verify microscopic examinations of chamber fluids which contained a high percentage of neutrophils. Further, chamber fluids contaminated with bacteria were not included in the tabulation. The figure shows neutrophil counts for blood samples and chamber fluids for a series of control rats that had plastic skin chambers attached to them for 7 consecutive days. Plastic skin chambers were installed on rats following assay of neutrophils in blood for 5 consecutive days. Circulating neutrophil levels may have increased following installation of skin chambers due to the effect of endotoxin introduced into the animals in the skin window fluids. Also, the stress of skin abrasion, removal of neutrophils in chamber fluids, plus minor local infection at the skin abrasion site all may have contributed to granulocytosis. The LLM response was highest for the first 2 skin chambers attached to the rats and decreased for the remaining 5 chambers. The decrease in the number of neutrophils in chambers on days 3–7 may be due to the necessity of placing the plastic chamber over a patch of skin that had been abraded for a previous skin chamber. These results limited determination of the LLM response to the first 2 skin chambers placed on the rat.

Rats made neutropenic by either ^{60}Co irradiation or cyclophosphamide treatment retained a limited capacity for mounting an LLM response. Presented in the table are the numbers of neutrophils collected from skin chambers and the neutrophil counts determined on blood samples from the same rats. The number of circulating neutrophils in cyclophosphamide-treated or irradiated rats followed a pattern similar to the one described by Host[9]. However, rats presented with the additional challenge of skin chamber implantation responded by increasing circulating neutrophils even though an LLM response was not detected in skin chamber 2 in the 100 mg/kg-cyclophosphamide and 700-rad treatment groups. Perhaps neutrophils present in circulation in these treatment groups lacked the chemotactic abilities of those circulating in less severely stressed rats. This reduced chemotactic ability may be due to any of a number of factors, including immaturity, defective migration capacities, and other neutrophil defects.

It is clear that the LLM response depended on the number of circulating neutrophils in rats. Animals injected with 40 mg/kg cyclophosphamide or exposed to 450 rad ^{60}Co had an LLM response. Apparently these levels of cyclophosphamide or ^{60}Co irradiation were unable to eliminate neutrophil precursor cells, neutrophil release into circulation, and an LLM response following this level of stress. Even 100 mg/kg cyclophosphamide was insufficient to block an LLM response on the first day that skin chambers were installed on rats. On the other hand, 700 rad ^{60}Co exposure eliminated the LLM response even though blood neutrophil counts were in the 10^5 per ml range. Rats irradiated with 700 rad ^{60}Co may have damaged cells and produced more potent chemotactic attractants than lipopolysaccharide in skin chambers, thus reducing LLM into chamber fluids. Also, neutrophils in such irradiated animals may have a reduced ability to migrate, marginate, or undergo chemotaxis. These possibilities as well as others may be addressed using the skin chamber technique in rats.

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