Antiinflammatory Reaction Associated with Murine L1210 Leukemia

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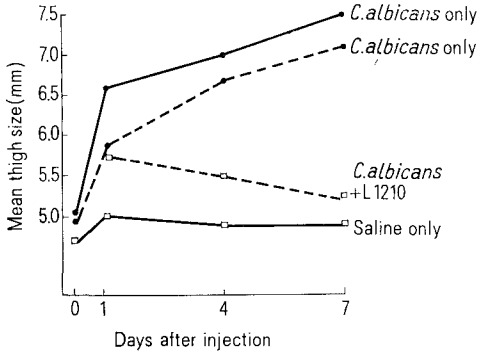
Summary. Mice bearing L1210 leukemia did not show impaired humoral or cellular immune response to antigenic stimulation during the early stage of the tumor, and a depressed response was noted only in the terminal stage. L1210 cells were shown to suppress inflammatory reaction in vivo.

Immunosuppression has been demonstrated in mice infected with murine leukemia viruses[2-5]. The immunosuppressive property of these viruses has been suggested as playing an important role in the leukemogenic process[3,4]. We report in this paper our failure to demonstrate immunosuppression in the early stage of transplantable L1210 leukemia. Furthermore, L1210 cells were shown to elicit an antiinflammatory reaction similar to that reported by FAUVE et al.[6] with other murine malignant cells. It is proposed that the antiinflammatory reaction elicited by tumor cells may permit the tumor to develop in the susceptible host.

Methods and results. The L1210 tumor was originally obtained by LAW et al.[7] in DBA mice following skin paintings with methylcholanthrene. It grows progressively in DBA mice and the F_1 hybrids. An injection of as few as 10 cells is lethal in 100% of the mice, and the length of survival is 10 to 12 days. To measure humoral immune response, groups of DBA/2 mice obtained from the Simonsen Laboratory, Gilroy, California were immunized intraperitoneally with sheep erythrocytes 3 days prior to, on the same day, and 3 and 6 days after tumor transplant. 4 days after the immunization, the spleen was removed for assay of antibody-forming cells by the plaque technique[8]. Sera collected at the same intervals were also tested for hemagglutinin titers using microtiter technique[9]. Table I shows that mice immunized with sheep erythrocytes 3 days prior to tumor transplant, on the same day, or 3 days after tumor transplant had higher numbers of mean antibody-forming cells per spleen than



Effect of L1210 cells on inflammatory reaction elicited by *C. albicans*. Solid lines represent mean readings of 8 animals which received injections of *C. albicans* in left thighs (●—●) and saline in right thighs (□—□). Broken lines represent mean readings of 8 animals which received *C. albicans* in left thighs (●—●) and mixture of *C. albicans* and L1210 in right thighs (□---□).

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Table I. Humoral response of mice bearing L1210 tumor to sheep erythrocytes[a]

Time of immunization relative to tumor transplant[b]	Number of antibody-forming cells per spleen $\times 10^{-3}$	p-value[c]	Hemagglutinin titer	p-value[c]
— 3 days	38.8 ± 2.2	> 0.20	102 ± 10	> 0.20
0 days	47.1 ± 2.1	< 0.05	307 ± 58	< 0.02
+ 3 ays	42.6 ± 3.9	> 0.20	166 ± 26	> 0.20
+ 6 days	25.9 ± 3.1	< 0.02	65 ± 14	< 0.02
Control (without tumor)	38.1 ± 3.2		134 ± 22	

[a] Groups of 10 mice each, immunized i.p. with 10^8 sheep erythrocytes and tests were performed 4 days after the immunization. All figures represent Mean ± SE.

[b] Mice were immunized at 4 intervals: 3 days before tumor transplant (— 3 days), on the same day (0 days), and 3 and 6 days after tumor transplant. Tumor was transplanted by i.p. injection with 5,000 viable L1210 cells from a freshly killed tumor-bearing mouse.

[c] Probability for t-test of means, compared with normal controls without tumor, two-tailed test.

Table II. Footpad and lymphocyte transformation test results with PPD[a]

Testing time relative to tumor transplant[b]	Increase of footpad swelling (%)	*P*-value[c]	Lymphocyte transformation stimulation index[d]	*P*-value[c]
0 days	52 ± 3	< 0.05	3.3 ± 0.5	< 0.02
+ 3 days	56 ± 3	< 0.05	2.4 ± 0.4	N.S.
+ 6 days	54 ± 3	< 0.05	2.2 ± 0.2	N.S.
+ 9 days	28 ± 3	< 0.02	1.2 ± 0.1	< 0.05
Control (without tumor)	36 ± 2		2.4 ± 0.3	

[a] Groups of 10 mice each, sensitized s.c. at 5 sites of the dorsum with 0.5 ml complete Freund's adjuvant containing 5 mg of killed *Mycobacterium tuberculosis* 21 days prior to tumor transplant.
[b] Tests were performed on the same day (0 days), and 3, 6 and 9 days after tumor transplants. All figures represent Mean ± SE.
[c] Probability for *t*-test of means, compared with normal control, two-tailed test. N.S. denotes not significant.
[d] Stimulation index = $\frac{\text{cpm of cultures with PPD}}{\text{cpm of cultures without PPD}}$

non-tumor-bearing controls. A reduction in the mean antibody-forming cells was noted with the group immunized 6 days and tested 10 days after the tumor transplant. A similar response was noted with hemagglutinin titers, in that a significantly lowered titer was noted only in the terminal stage of the tumor.

Cellular immune response was measured by sensitizing mice with killed *Mycobacterium tuberculosis* H37 Ra (Difco Laboratories, Detroit, Michigan) 3 weeks prior to tumor transplant. Footpad testing was performed by injecting 0.025 ml sterile saline in the left hind footpad and an equal volume of 50 µg PPD (Connaught Laboratories, Toronto, Canada) in the right footpad. Footpad swelling was measured with sliding calipers 24 h after the injection. The percentage of increase of swelling of the PPD-injected over the saline-injected footpad was calculated. In vitro lymphocyte transformation tests with PPD were performed with spleens removed from the mice. Cultures containing 2×10^5 spleen cells per ml were set up in RPMI 1640 medium with 10% fresh human serum[10]. PPD, 10 µg/0.1 ml, was added to 'stimulated' cultures in triplicate while the control cultures received saline only. After a 24-h incubation in a carbon dioxide incubator, 2 µCi of ^{3}H-thymidine (specific activity 6 Ci/mmole, Schwarz-Mann, Orangeburg, N.Y.) was added to each of the cultures. After an additional 10 h of incubation, the unincorporated ^{3}H-thymidine was removed by washing the cultures with 5% trichloroacetic acid. The incorporation of ^{3}H-thymidine was determined in a liquid scintillation counter. As shown in Table II, with the exception of animals tested 9 days after the tumor transplant, no depression of footpad swelling or in vitro lymphocyte transformation was observed with tumor-bearing mice.

Several murine malignant tumors have been shown by FAUVE et al.[6] to elicit an antiinflammatory response both in vivo and in vitro. We investigated the possibility of a similar response with L1210 tumor. Acute inflammatory reaction was induced in mice by i.p. injection of 3 ml of RPMI medium and checked for total number of peritoneal exudate cells and percentage of neutrophils 6 h later. Table III shows that the injection of normal mouse kidney cells in RPMI 1640 medium did not reduce the mean total of peritoneal exudate cells or the percentage of neutrophils. However, the injection of L1210 cells significantly reduced the total peritoneal exudate cells and the percentage of neutrophils. The effect of L1210 cells on the inflammatory reaction elicited by *Candida albicans* was also studied by injecting 10^8 *C. albicans* cells intramuscularly into the left thighs of mice and a mixture of 10^8 *C. albicans* and 50,000 L1210 cells into the right thighs. The thighs were measured daily with sliding calipers. At the end of one week, the animals were killed and thighs removed for histopathological study. The Figure shows significant reduction in the localized swelling with the

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Table III. Effects of L1210 cells oni nflammatory response[a]

Substance injected	Total peritoneal cells $\times 10^{-6}$ (mean ± SE)	*P*-value[b]	Neutrophils (%) (mean ± SE)	*P*-value[b]
None	3.5 ± 0.2		0	
RPMI 1640	11.2 ± 0.9		60.3 ± 2.5	
50,000 normal mouse kidney cells in RPMI 1640	13.4 ± 1.2	N.S.	61.5 ± 3.9	N.S.
50,000 L1210 cells in RPMI 1640	3.8 ± 0.3	< 0.001	14.3 ± 1.4	< 0.001

[a] Each group consisted of 8 mice. 6 h after injection, total cell counts were performed with peritoneal washings and percentages of neutrophils were estimated by counting 500 cells in Giemsa stained smears.
[b] Compared with injection of RPMI 1640 alone. N.S. denotes not significant.

injection of the mixture of *C. albicans* and L1210 cells. Histopathological study by a pathologist without the knowledge of the experimental design confirmed this finding.

Discussion. This study fails to demonstrate immunodepression in mice bearing L1210 leukemia until the terminal stage of the disease. Our data support the observation of STUTMAN[11] who indicated that immunosuppression had minimal effects on tumor development induced by chemical carcinogens. Using methylcholanthrene or urethan, STUTMAN did not detect significant differences in tumor incidence or latent period of tumor development between the immunologically deficient nude mice and their normal immunologically competent siblings. Recently, GILLETTE and COX[12] showed that T-cell deficiency did not affect the tumor incidence and latency of mice with methylcholanthrene induced primary tumor.

FAUVE et al.[6] reported that the development of murine teratocarcinoma did not impair the cellular immunity of the host against *Listeria monocytogenes*. Their data actually showed an increased resistance against *L. monocytogenes* by the tumor-bearing animals. They further demonstrated that several malignant cells elicited an antiinflammatory response and suggested that this antiinflammatory property might have allowed the tumor to develop in the susceptible host. We show in this study that L1210 cells are also capable of suppressing the inflammatory response. This antiinflammatory reaction may possibly facilitate the rapid growth of the tumor in the absence of immunodepression.

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Staphylococcus epidermidis in the Circulating Blood of Normal and Thrombocytopenic Human Subjects: Immunological Data

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Summary. Immunological studies, performed with human sera and rabbit antisera upon some strains of *Staph. epidermidis* carried in the circulating blood of normal and thrombocytopenic subjects, indicate that the reactivity is very low and almost completely related to antigenic properties common to *Staph. aureus*.

The presence of cell wall deficient (CWD) forms of Gram positive Cocci has been recognized within the platelets of normal human subjects[1]; foetal blood drawn at birth from the umbilical cord showed a comparable situation[2]; patients affected by thrombocytopenia from autoimmune disease carried in addition a relevant number of conventional forms free in the circulation and phagocytized by leucocytes[3]. Following BERGEY[4], such microorganisms may be recognized as belonging to various strains of *Staphylococcus epidermidis*. Here we describe the results of experiments carried out in order to compare some antigenic properties of our isolates with those of a strain of *Staphylococcus aureus*, and also to evaluate the immunological situation of normal and thrombocytopenic human subjects with regard to such microorganisms.

Methods. The sera of 2 autoimmune thrombocytopenic patients and sera from normal subjects were used. Their reactivity was tested against some strains of *Staph. epidermidis* cultured from the blood of normal and thrombocytopenic subjects and against *Staph. aureus* Rose ATCC 14154.

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Table I. Human sera: each specimen tested against *Staph. aureus* Rose and some strains of *Staph. epidermidis*

Immunological tests	No. and origin of serum specimens	*Staph. aureus* Rose		*Staph. epidermidis*				
		n of positivities/ *n* of proofs	End point of positive sera	*n* of strains	*n* of positivies/ *n* of proofs		End point of positive sera	
Slide agglutination	2 thrombocytopenic	2/2	160	6	7/12[a]	1/12[b]	5–20[a]	10[b]
	4 normal	4/4	320–1280	6	15/24[a]	5/24[b]	5–20[a]	5–10[b]
Complement fixation	2 thrombocytopenic	none	–	4	none	–	–	–
	32 normal	8/32	50	4	3/128[a]	none[b]	10[a]	none[b]
Indirect agglutination	2 thrombocytopenic	none	–	4	none	–	–	–
	3 normal	3/12[c]	512[c]	4	3/12[c]		3–32[c]	
Indirect immuno fluorescence	2 thrombocytopenic	2/2	5	4	8/8[a]	1/8[b]	5[a]	5[b]
	3 normal	3/3	10[d]	4	12/12[a]	3/12[b, c]	5[a]	5[b]

[a, b] Before and after absorption of the sera with *Staph. aureus* Rose. [c] The positive data refer to a single serum specimen showing a strong agglutinating activity towards *Staph. aureus* Rose (titre 1280). [d] Strong positivity, further dilutions not tested.