of chromosome breaking compounds such as alkylating agents (11,12).

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Immunosuppressive Activity of Rabbit Antisera Directed against Mouse Lymphocytic Leukemia L1210* (32741)

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In the course of studies on the *in vivo* localizing properties of antibodies directed against the lymphocytic leukemia L1210, it was found that the antibodies exhibited marked localization in spleens of the injected mice as did antibodies directed against homogenates of mouse spleen and lymph nodes. Such localization in spleen was seen with anti-L1210 sera prepared against cells grown in continuous culture as well as those in the ascites form.

In view of the fact that antibodies against lymphocytic cells are known to suppress antibody formation(1), it was of interest to determine whether the antibodies to cultured leukemic cells and to ascites cells would cause immunosuppression in mice.

The effect on the immune response was evaluated by determining the concentration of plaque forming cells in the spleen and by determining the titer of humoral hemagglutinins 4 days following a single injection of sheep erythrocytes into mice. The results are reported here.

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Materials and Methods. The mice used throughout this study were 2 months old, male inbred DBA/2.

The L1210 cells grown in a suspension cell culture were obtained from Dr. G. E. Moore. Some of the characteristics of these cells were described(2). The ascites form of the L1210 cells was maintained in male DBA/2 mice and was transplanted weekly by intraperitoneal injections.

Antisera were prepared in rabbits against the following materials: (i) L1210 leukemia cells grown in cell culture, (ii) L1210 leukemia cells grown *in vivo* as ascites tumors in DBA/2 mice, and (iii) homogenates of DBA/2 spleens and lymph nodes.

The leukemia cells from either source were washed 3 times with 50 volumes of Ringer's solution. Three rabbits were injected with cultured L1210 cells and 4 rabbits with ascites L1210 cells. Each rabbit received 2–3 injections at 2-week intervals of a cell suspension in Freund's adjuvant equivalent to 0.25–0.4 ml of packed cells. The injections were made intradermally (in the footpads), subcutaneously, and intramuscularly. The

rabbits were bled 2–3 weeks following the last adjuvant injection, and then periodically following intraperitoneal booster injections which were given without adjuvant. Antisera from different bleedings and rabbits were pooled to give an anti-cultured L1210 antiserum and anti-ascites L1210 antiserum.

Spleens and lymph nodes (inguinal, mesenteric, axillary, brachial, and superficial cervicals) were homogenized in a blender for 1 min with an equal volume of saline. An amount equivalent to 0.5 ml of this homogenate in complete Freund's adjuvant was injected 3 times into each of 4 rabbits at 2-week intervals. The injections were made intradermally (in the footpads), subcutaneously, and intramuscularly. Four weeks after the last injection, a booster series of 2 injections (without adjuvant) with a week interval between them was given. The rabbits were bled a week later, and then another time following an additional booster injection. The sera from the different bleedings and different rabbits were pooled to give an antispleen and antilymph node antiserum.

The antibody plaque technique employed was carried out essentially according to the method of Jerne *et al.*(3,4). The plaque tests were carried out in disposable petri dishes of 6 cm diameter. The volumes of the 2 agar layers had been adjusted to the size of plates used. In our tests we incubated the plates in an ordinary incubator rather than in a CO_2 incubator used by Jerne.

Hemagglutination was carried out as follows: serial, twofold dilutions of inactivated (56°C, 30 min.) mouse sera were made in Difco hemagglutination buffer at pH 7.2. To 0.3 ml of each serum dilution, 0.1 ml of an 8% suspension of sheep RBC was added. The tubes were shaken and kept at room temperature for 18–20 hours. The end point of agglutination was taken as the last dilution showing macroscopic agglutination.

Immunoelectrophoresis was carried out on microscope slides with the LKB apparatus.

Results. In the experiment outlined in the tables, mice of Group no. 1 were injected intraperitoneally with 0.5 ml of a 20% suspension of sheep erythrocytes in Eagle's medium (untreated control). Those of Group no. 2 were injected intraperitoneally daily with 0.25 ml of pooled normal rabbit serum (from 5 rabbits) for 6 successive days. Mice of Group no. 3 were injected daily intraperitoneally with 0.25 ml of the antispleen and antilymph node antiserum. Mice of Groups 4 and 5 were injected daily intraperitoneally with 0.25 ml of the anti-cultured L1210 antiserum and anti-ascites L1210 antiserum, respectively for 6 successive days. On the eighth day (the second day after the last serum injection), groups 2–5 were injected with 0.5 ml of a 20% suspension of sheep erythrocytes. All the mice were bled and their spleens taken for plaque assay at the fourth day after injection of erythrocytes.

Plaque forming cells in nontreated and in serum treated mouse spleens. Three portions of each spleen cell suspension were tested. Spleen cells were counted with the aid of a Coulter counter.

Table I gives the distribution and the mean of plaque forming cells per 10^6 spleen cells of nontreated and serum treated mice. It can be seen that treatment with normal rabbit serum prior to immunization with sheep erythrocytes caused a significant decrease in the number of plaque forming cells as compared to nontreated mice ($0.05 > p > 0.02$). The values of the plaque forming cells in spleens of antiserum treated mice were therefore compared to the values of the plaque forming cells in spleens of normal serum treated mice. It is evident that treatment with both antispleen-lymph-node antiserum and with anti-ascites L1210 antiserum caused a significant decrease in the number of plaque forming cells in the spleens of the treated mice, as compared to the normal serum treated mice ($p < 0.01$).

Anti-cultured L1210 antiserum was also active as an immunosuppressive agent although not as active as the former antisera as compared to normal serum treated mice ($0.05 > p > 0.02$).

Hemagglutinin response to sheep erythrocytes in nontreated and in serum treated mice. Table II shows the distribution of the hemagglutination titer for the various mouse sera 4 days after immunization with sheep erythrocytes.

TABLE I. Plaque Forming Cells (PFC) in Spleens of Mice Treated with Rabbit Antisera Directed Against Mouse Leukemia L1210 and Against Lymphocytic Tissues.

Experimental group	Serum used	No. of spleens used	No. of spleens giving indicated range of PFC per million spleen cells[a]							PFC per million spleen cells (mean ± SE)
			0–30	30–60	60–90	90–120	120–150	150–200	over 200	
1	None	17	0	3	3	2	4	2	3	131 ± 19
2	Normal	10	0	1	7	0	2	0	0	85 ± 8
3	Anti-spleen and lymph node	10	6	3	1	0	0	0	0	28 ± 7
4	Anti-cultured L1210	10	2	2	4	2	0	0	0	52 ± 12
5	Anti-ascites L1210	10	6	3	1	0	0	0	0	30 ± 5

[a] Each spleen was tested in triplicate. The values are means of the 3 determinations.

It can be seen that the suppression of hemagglutinin titer by treatment with rabbit sera paralleled the suppression of plaque forming cells. The largest suppression was caused by antispleen-lymph-nodes antiserum and by anti-ascites L1210 antiserum. Less suppression was caused by anti-cultured L1210 antiserum and only a slight suppression by normal serum.

Antibody response to the injected rabbit sera. Some mouse sera were tested for antibodies against the injected rabbit sera. The rabbit antisera used for injection were subjected to electrophoresis and the immunoelectrophoresis patterns were developed with the individual mouse sera. All the tested mouse sera showed 1–2 precipitate arcs which corresponded to rabbit serum proteins of the mobility of α or β globulins. There were no differences between the groups in respect to the number of lines formed.

Discussion. The immune response of DBA/2 mice to sheep erythrocytes could be suppressed significantly by treatment of these mice with rabbit antisera directed against lymphocytic leukemia L1210. Antisera against the cultured cells and ascites cells were both effective. The degree of immunosuppression was comparable to the one induced by treatment with an antiserum directed against spleen and lymph nodes. Both anti-L1210 antisera apparently contained antibodies directed against those antigenic components which are important in the immunosuppression phenomenon. Apparently the cultured cells had not lost these components during culture.

The distribution of these antigens in the various tissues has not yet been very thoroughly documented. Although Waksman (5) and Jeejeebhoy (6) presented data indicating that none of these antigens are present in cer-

TABLE II. Hemagglutinin Response in Mice Treated with Rabbit Antisera Directed Against Mouse Leukemia L1210 and Against Lymphocytic Tissues.

Experimental group	Serum used	No. of sera assayed	No. of sera showing indicated hemagglutinin titer					
			<15	15	30	60	120	240
1	None	16	0	0	2	9	3	2
2	Normal	10	0	0	2	7	1	0
3	Antispleen-lymph-nodes	10	4	5	1	0	0	0
4	Anti-cultured L1210	9	2	2	4	1	0	0
5	Anti-ascites L1210	10	2	5	3	0	0	0

tain other tissues or cells (polymorphonuclear cells or liver), there was a report of Levey and Medawar (7) that L cells and mouse epidermal cells did contain some of the antigens since antisera directed against them had immunopressive properties, and were capable of prolonging skin homografts. These authors concluded that the antigens are not unique for lymphocytic cells.

Our results, like the results of other investigators (8,9) show that injection of normal rabbit serum caused a certain degree of immunosuppression. The nature of the suppression remains to be investigated.

Summary. Rabbit antisera directed against cultured mouse lymphocytic leukemia L1210 cells as well as those directed against the ascitis form were shown to be immunosuppressive when injected into mice prior to immunization with sheep erythrocytes. The extent of immunosuppression was comparable to the one induced by injection of antisera directed against mouse spleen and lymph node antigens. The degree of immunosupression was quantitated by assaying the number of antibody forming cells by plaque formation in a sheep erythrocyte medium, and by titration of humoral hemagglutinins.

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Intracellular Distribution of Calcium-45 in Arteries of Normal and Hypertensive Dogs* (32742)

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Evidence has accumulated suggesting that the calcium ion plays an important role in maintenance of the blood pressure level in man both in normotension and in some forms of secondary hypertension. This evidence has been reviewed recently by several authors (1, 2). In addition, well documented changes occur in the electrolyte and water composition of walls of small and large arteries in hypertension (3). These changes include increases in calcium content of small arteries (4) and of arterioles (1) in experimental hypertension, and suggest that alterations in arterial wall calcium metabolism may accompany or underlie hypertension. In view of these relationships and findings, it is of interest to study the intracellular distribution of calcium of arterial wall in normotension and hypertension. The technique of differential centrifugation is appropriate for such an investigation.

Although the intracellular distribution of ^{45}Ca in cardiac muscle has been reported (5,6), there are no published simliar studies of vascular wall tissue. In the present study radial arterial, carotid arterial, and descending

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