Brain Localizing Activity of Hetero- and Isoimmune Sera Directed against Lymphocytic Cells* (33689)

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In studies of the *in vivo* localization properties of mouse isoantibodies prepared in C57BL/6 mice against leukemic DBA/2 tissue, i.e., L1210 cells, we have shown that there is a fixation of the isoantibodies in the brain. There was an 18-fold greater localization of the globulin from the isoantiserum when compared to the localization of the globulin fraction of normal C57BL/6 serum (1).

Previous experiments by others indicated that localization of antibodies in the brain is usually difficult to demonstrate with antibodies prepared in a heterologous species even with antibodies directed against brain tissue. Thus, Williams and Rothfield (2) were not able to achieve preferential localization of rabbit antirat brain antibodies over normal rabbit globulin in the brain even when the antibodies were injected intrathecally. Day *et al.* (3), using the same system, obtained essentially the same results as did Williams and Rothfield following intravenous injection of antibodies. After a passive transfer of short duration of the antirat brain preparation through rats to remove cross-reacting antibodies, Day *et al.* revealed the presence of a minute amount of brain localizing activity in the antiserum. Only three times as large a fraction of the injected dose of the antiserum preparation as of the normal globulin preparation was fixed.

In the present studies, we compared the *in vivo* brain localization properties of heteroantibodies directed against mouse lymphocytic tissues with the isoimmume antisera.

Materials and Methods. The heteroantisera used were those reported previously (4) and were made by injecting rabbits with the following: (a) a mixture of spleen and lymph nodes of DBA/2 mice, (b) lymphocytic leukemia L1210 cells grown in ascites form in DBA/2 mice, and (c) lymphocytic leukemia L1210 cells grown in cell culture. The isoantisera were those directed against ascites L1210 cells of DBA/2 mice and were prepared in C57BL/6 mice as described previously (1).

The IgG was separated from rabbit serum by two successive precipitations with ammonium sulfate at 50 and 33% saturation followed by column chromatography on DEAE-cellulose (5). The globulin fraction of mouse serum was prepared by Na_2SO_4 precipitation (1).

The globulin fraction from antiserum was iodinated with ^{125}I (or with ^{131}I). Control globulin from corresponding unimmunized animals was iodinated with ^{131}I (or with ^{125}I). The radiolabeled antiserum globulin and control globulin were mixed and the paired-labeled mixture was purified by absorption on and elution from tissue sediments. The purified globulins were injected intravenously into DBA/2 mice, which were sacrificed and perfused 18–22 hr later. The perfused brain was assayed for radioactivity in a double channel γ-ray spectrometer. The percentage of the injected dose which localized in the brain was calculated for the antiserum globulin and for the normal serum globulin. The difference between these two is the net localization. The ratio of the net localization in brain to the total of the net localizations in the primary organs (liver, kidney, spleen, lung, and brain) was also calculated.

Results. Table I summarizes the results of *in vivo* localization experiments in which

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TABLE I. *In Vivo* Localization in Brain of DBA/2 Mice of Radiolabeled Purified Globulin from Various Antisera Directed against Lymphocytic Tissues of DBA/2 Mice Paired with Radiolabeled Purified Globulin from Normal Sera.

Immunizing tissue	Sediment used for purification	No. of mice assayed	% of injected dose localized in brain			Total net localization (%) in primary organs	Ratio (net in brain/total net in primary organs)
			Antibody	Normal globulin	Net		
Isoantiserum[a] (C57BL/6 anti-DBA/2)							
Ascites L1210	Cultured L1210	18	0.142	0.012	0.130	3.80	.0343
Heteroantiserum (rabbit anti-DBA/2)							
Ascites L1210	Ascites L1210	4	0.112	0.024	0.088	6.00	.0147
Cultured L1210	Cultured L1210	11	0.114	0.024	0.090	6.43	.0140
Spleen and lymph nodes	Spleen	4	0.070	0.022	0.048	19.20	.0025

[a] Six different pools of isoantisera gave similar results.

purified paired-labeled mixtures were injected intravenously into mice.

There was preferential localization in mouse brain from heteroantisera against L1210 cells as well as from the isoantiserum. Rabbit antiserum against spleen and lymph node showed only some localization in the brain. The normal globulin preparations passed through the same purification process were assayed simultaneously in the same animal with the antiserum and showed appreciably less localization, less than 10% of the value for isoantiserum and about 20–30% of the value for the heterologous antisera.

Since the contents of localizing antibody differed from one antiserum to the other, the ratio of the localization in brain to the total localization in the primary organs is also given. On this basis, the isoantiserum clearly showed a larger ratio than the ratios observed with the rabbit sera against L1210 cells (0.034 vs 0.014). The ratio for the rabbit antiserum prepared against mouse spleen and lymph node was even lower than the ratios for the rabbit antisera against the L1210 cells. The lower ratios apparently resulted not only from a lower (but still significant) localization in the brain, but also from a greater localization of antibodies in other organs such as kidney and liver. For the sera used, it appears that the antibodies in the isoantiserum showed a somewhat greater localization in brain than did those from the heteroantisera.

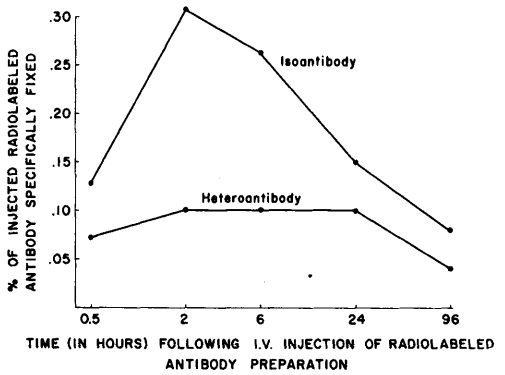

FIG. 1. Time course of rabbit and mouse anti-L1210 antibody fixation in the brain.

The time course of isoantibody fixation to brain was compared to the time course of heteroantibody fixation (Fig. 1). As shown, the heteroantibody preparation reached a peak level in 2 hr following intravenous injection and remained at that level for at least 24 hr. The isoantibody preparation reached a peak level also in 2 hr, a level which is much higher than that of the heteroantibody, but the isoantibody preparation was removed from the brain quite rapidly thereafter, decreasing to 50% of the peak level at 24 hr and to 25% at 96 hr.

Discussion. The present results seem to indicate that to achieve antibody localization in brain following intravenous injection one does not have to produce antibodies to brain

tissue. Antibodies directed against L1210 cells derived from animals with lymphocytic leukemia exhibited a quite marked preferential localization in brain. Isoantibodies against L1210 cells showed a higher localization than did heteroantibodies. It was further shown that localization of antibodies in the brain is a rapid process reaching peak levels at 2 hr following the i.v. injection followed by relatively rapid removal. We do not yet know whether the brain localizing antibodies are directed against antigens of nerve cells, although it seems likely that the responsible antigen is found in the vascular beds of the brain because of the rapid uptake of the antibodies.

The nature of the brain antigens responsible for antibody fixation has not been determined. They might be antigens similar to those described by Reif and Allen (6) which are present on thymocytes, leukemic cells, and adult brain. It should, therefore, be noted that our antisera were all produced against lymphocytic components. We also do not know whether the antigens that fix the isoantibodies are the ones that fix the heteroantibodies.

Summary. Isoantibodies prepared in C57BL/6 mice against lymphocytic leukemia cells of DBA/2 mice (L1210) exhibited *in vivo* localization in the brain of DBA/2 mice. Rabbit antisera prepared against the same cells exhibited a lower localization in DBA/2 brain, while rabbit antibodies prepared against normal lymphoid organs of DBA/2 mice exhibited a much lower localization. The localization of both the isoantibodies and the heteroantibodies was very rapid. Maximal amounts of antibodies were fixed to the brain 2 hr after i.v. injection.

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