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Localization in Rat Skin Transplants of Purified ^{125}I-Labeled Xenogeneic Histocompatibility Antibody (39633)

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It has been known for some time that properly prepared xenogeneic antisera can distinguish between different major histocompatibility antigens (1). We have now found it possible to prepare, from two different types of xenogeneic antisera, ^{125}I-labeled histocompatibility antibody able to localize *in vivo* with a high degree of specificity in rat skin grafts where only the transplanted skin carries the corresponding antigen. One source of such antisera was from rabbits immunized with a fraction prepared from a tumor originating and carried in Fischer-344 (F-344) rats. A second source was a commercially available horse anti-rat lymphocyte sera (Microbiological Associates, Bethesda, Maryland, Lot. No. 131182).

It was also found that ip injections of ml amounts of this unfractionated horse serum resulted in a high degree of blocking of the localization in such skin grafts of either the labeled antibody prepared from the immune horse serum or a labeled histocompatibility antibody prepared from the sera of allogeneically immunized rats. The rapidity and completeness of this blocking suggests that its cause was the preempting by antibody from the unlabeled sera of antigen sites where the labeled antibody would otherwise have localized.

Materials and methods. Preparation of allogeneic antibody. The general methods used in preparing ^{125}I-labeled antibody to the strong histocompatibility antigen expressed on F-344 rat erythrocytes have already been described (2, 3). Briefly, Buffalo strain rats were immunized by repeated transplants of a tumor induced by methylcholanthrene in an F-344 rat. From such immune sera, γ-globulin was isolated and labeled with ^{125}I. Labeled antibody was isolated by a twice repeated procedure that consisted of the absorption of the antibody onto F-344 erythrocytes, followed by the elution of absorbed antibody from ghosts prepared from these cells. Recently, we have obtained much higher yields of antibody by partially purifying the antibody before labeling.

Preparation of ^{125}I-labeled rabbit antibody. Rabbits were immunized with a "lipoprotein" fraction rich in histocompatibility antigens from the same F-344 carried tumor using a modification (4) of the procedure developed by Manson *et al.* (6). Immunization consisted of four weekly injections of "lipoprotein," each of 6 mg protein content, in complete Freund adjuvant, divided between two im sites. Antisera was obtained 10 days after the last immunization. The γ-globulin isolated from the immune sera was further purified by absorption and elution from additional tumor lipoprotein, labeled with ^{125}I, and *in vivo* absorbed by iv injection into Buffalo strain rats which were bled 2 days later. Antibody was isolated from the sera by absorption on F-344 red cells and elution at pH 3.2 from ghosts prepared from these cells. *In vitro* incubation of a sample of the final product showed 47% of the ^{125}I-label binding to F-344 red cells, 1.1% to Buffalo red cells.

Preparation of ^{125}I-labeled horse antibody. Four-milligram portions of γ-globulin prepared from horse anti-rat lymphocyte sera were labeled with ^{125}I, then *in vivo* absorbed by iv injection into Buffalo rats. From sera obtained 2 days later by heart puncture labeled antibody was separated by absorbing onto F-344 erythrocytes, and elutions from ghosts were prepared from these erythrocytes. In one experiment, from 6.6 mCi of ^{125}I attached to a total of 1.78 mg of horse γ-globulin injected into two rats, 4.9% was recovered in 6 ml of rat sera, and 0.13 μCi as purified antibody. *In vitro* this antibody bound 43% to F-344 red blood cells and 1.6% to Buffalo erythrocytes.

Preparation of ^{131}I-labeled normal Buffalo

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γ-globulin. After labeling 4 mg of normal Buffalo γ-globulin prepared by ammonium sulfate precipitation, the labeled material (300 μCi/rat) was injected iv into normal Buffalo rats. Three days later the rats were bled and the serum was stored frozen until used in double-label experiments.

In vivo studies. Skin grafts were performed as described by Billingham and Silvers (7) with slight modifications. Small pinch grafts weighing less than 0.4 g were cleaned of fat and loose connective tissue. Each Buffalo rat, approximately 150 g in weight, received a Buffalo skin isograft on the left side as a control for an F-344 skin graft on the right side.

Rats were given drinking water 625 μ*M* in KI from 3 days after skin transplantation, and injected with a paired dose of ^{125}I-labeled antibody and ^{131}I-labeled normal γ-globulin 4 days after the transplant. One day later the rats were heparinized and killed by severing the vena cava. Fluothane-O_2 was used for anesthesia during all procedures. Radioactivity measurements were made with an automatic well-type scintillation counter. Variability of results is indicated as the standard deviation of individual values from the mean.

Results. A substantial number of experiments have been carried out showing that a purified, ^{125}I-labeled alloantibody to a strong F-344 histocompatibility antigen will localize preferentially in F-344 skin grafts on Buffalo rats. Some of these results have been published (4, 5). More recent experiments have also shown that prior administration of horse anti-rat lymphocyte sera will largely prevent such localization. Table I shows the results of one such experiment. Horse anti-rat lymphocyte sera was administered ip, in 0.5-ml doses, 2 days before skin transplant, on the day of skin transplant, and 4 days later, on the day of labeled antibody injection. Rat weights averaged 190 g and F-344 skin grafts, 0.31 g, or 0.163% of the average rat weight. The ^{125}I skin values of Table I are computed from values that averaged 18.16% of the injected ^{125}I dose found in the F-344 grafts on Buffalo strain rats that did not receive horse antiserum and 3.51% found in rats that received 3 injections of this horse antisera.

In this experiment the dose of ^{125}I-labeled antibody was 13,696 cpm and of ^{131}I-labeled normal γ-globulin, 14,402 cpm. The ^{125}I content of F-344 skin grafts in rats receiving no horse antiserum averaged 24,292 counts per 10 min (cp10m) above an average background of 97 cp10m. The net ^{131}I count was 562 cp10m. In the rats receiving horse antiserum the F-344 skin grafts averaged a net 4817 cp10m for ^{125}I and 346 cp10m for ^{131}I.

Figure 1 shows the results of a similar experiment in which the ^{125}I label was attached to a histocompatibility antibody prepared from the serum of one rabbit immunized with a fraction prepared from F-344 rat tissue. In this experiment the rat weight averaged 167 g, and the F-344 skin trans-

TABLE I. CONCENTRATIONS OF ^{125}I-LABELED RAT HISTOCOMPATIBILITY ANTIBODY AND ^{131}I-LABELED NORMAL RAT γ-GLOBULIN IN BUFFALO RATS, SOME BEARING F-344 AND BUFFALO SKIN GRAFTS.[a]

	Percentage injected dose in tissue equal to 1% rat weight (mean ± SD)							
	No horse anti-RL sera				3 doses horse anti-RL sera			
	Four grafted rats		Average of two normal rats		Four grafted rats		Average of two normal rats	
Tissue	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I	^{125}I	^{131}I
Blood	2.29 ± 0.71	4.61 ± 0.38	4.76	5.24	2.39 ± 0.48	3.65 ± 0.47	3.44	3.78
Plasma	3.73 ± 1.20	7.75 ± 0.43	7.95	8.72	3.68 ± 0.51	5.80 ± 0.59	5.99	6.45
Buffalo skin graft	1.23 ± 0.51	1.69 ± 0.30			1.09 ± 0.58	1.25 ± 0.26		
F-344 skin graft	111.3 ± 47.8	2.27 ± 0.50			21.54 ± 5.38	1.44 ± 0.33		
Normal Buffalo skin	0.47 ± 0.15	0.60 ± 0.11	0.42	0.36	0.47 ± 0.05	0.46 ± 0.05	0.50	0.55
Lung	0.97 ± 0.31	1.68 ± 0.33	1.88	1.92	0.90 ± 0.15	1.31 ± 0.13	1.31	1.30
Liver	0.46 ± 0.12	0.72 ± 0.07	0.82	0.74	0.46 ± 0.08	0.66 ± 0.14	0.72	0.72
Spleen	0.70 ± 0.24	0.95 ± 0.11	1.24	1.15	0.67 ± 0.10	0.77 ± 0.12	0.88	0.90
Kidney	0.61 ± 0.18	1.10 ± 0.09	1.12	1.40	0.65 ± 0.11	0.94 ± 0.11	0.83	1.00

[a] Rats were injected iv, 4 days after making skin transplants, with a paired mixture of ^{125}I-labeled antibody to a major F-344 histocompatibility antigen and ^{131}I-labeled normal Buffalo γ-globulin, then killed 24 hr later. Horse anti-rat lymphocyte (horse anti-RL) sera was given, in 0.5-ml doses, ip, 2 days before skin transplant, on the day of skin transplant, and just before labeled antibody injection.

plants, 0.187 g. The ^{125}I skin values of Fig. 1 are calculated from an average of 9.0% of the injected dose found in the F-344 grafts. These rats received no horse serum injections. The ^{125}I dose was 7982 cpm, the ^{131}I dose 6928 cpm. The F-344 skin grafts averaged a net 7157 cp10m for ^{125}I and 149 cp10m for ^{131}I.

Table II shows the results of an experiment similar to the experiment reported in Table I except that the ^{125}I label was attached to a histocompatibility antibody isolated from horse anti-rat lymphocyte sera. This experiment clearly shows localization

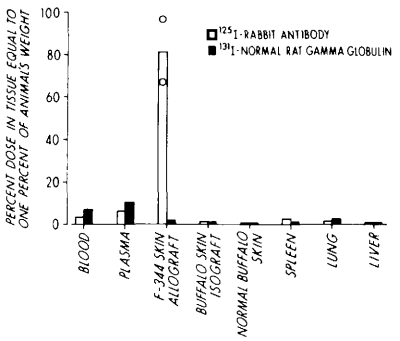

FIG. 1. Distribution of ^{125}I-labeled rabbit antibody recognizing F-344 rat histocompatibility antigen and of ^{131}I-labeled normal rat γ-globulin in Buffalo strain rats carrying transplants of F-344 and Buffalo skin. Rats were injected 4 days after the skin transplants were made, and killed 24 hr later. Values are the average for 2 rats. ○, individual rat values.

of the ^{125}I-antibody in the F-344 skin graft on Buffalo rats and no obvious binding to Buffalo strain tissue. Administration of immune horse serum, given, as ip doses of 0.5 ml, 6 and 4 days before labeled antibody injection, and on the day of injection, has in this case also blocked antibody localization. The ^{125}I concentration in the F-344 skin grafts is lower by a factor of 10 in rats receiving the horse serum.

In this experiment the net ^{125}I dose was 6493 cpm, the ^{131}I dose, 6442 cpm. In rats not receiving horse antiserum the F-344 skin grafts averaged a net 4782 cp10m for ^{125}I and 301 cp10m for ^{131}I. In rats receiving horse antiserum the F-344 skin grafts average a net 442 cp10m for ^{125}I and 182 cp10m for ^{131}I.

Since the labeled antibody of Table II was prepared from horse γ-globulin, one possible explanation or partial explanation for this blocking action on antibody localization was that the horse antiserum injections, over the 6-day period, had induced an immune response to horse γ-globulin that in some way blocked the labeled antibody localization. Therefore, an additional experiment was carried out similar to that reported in Table II except that the administration of immune horse serum was confined to two injections of 0.5 ml each given the day preceding and the day of labeled antibody injection. The labeled antibody again localized with high specificity in the F-344

TABLE II. DISTRIBUTION OF ^{125}I-LABELED HORSE XENOANTIBODY AND ^{131}I-LABELED NORMAL RAT γ-GLOBULIN IN BUFFALO RATS CARRYING F-344 AND BUFFALO SKIN GRAFTS.[a]

	Percentage injected dose in tissue equal to 1% rat weight (mean ± SD)			
	Group 1 (No horse anti-RL sera)		Group 2 (three doses horse anti-RL sera)	
Tissue	^{125}I	^{131}I	^{125}I	^{131}I
Blood	2.01 ± 0.13	5.26 ± 0.43	1.32 ± 0.24	3.90 ± 0.39
Plasma	3.08 ± 0.24	8.48 ± 0.52	1.85 ± 0.33	6.09 ± 0.57
Buffalo skin graft	0.91 ± 0.19	1.34 ± 0.40	0.43 ± 0.34	1.33 ± 0.24
F-344 skin graft	41.9 ± 6.0	2.68 ± 0.66	4.15 ± 1.11	1.72 ± 0.37
Normal Buffalo skin	0.29 ± 0.07	0.62 ± 0.06	0.38 ± 0.09	0.49 ± 0.20
Lung	0.93 ± 0.18	1.87 ± 0.21	0.60 ± 0.17	1.40 ± 0.09
Liver	0.41 ± 0.07	0.82 ± 0.07	0.33 ± 0.08	0.59 ± 0.07
Spleen	0.82 ± 0.12	0.81 ± 0.13	0.40 ± 0.09	0.55 ± 0.14
Kidney	0.53 ± 0.07	1.17 ± 0.15	0.36 ± 0.06	0.94 ± 0.17

[a] Each group, with and without injection of 0.5-ml doses of horse anti-rat lymphocyte (horse anti-RL) sera over a 6-day period, consists of four rats. All rats were injected iv, 4 days after receiving skin transplants, with a mixture of ^{125}I-labeled horse antibody reacting with an F-344 strong histocompatibility antigen and ^{131}I-labeled normal Buffalo γ-globulin. Rats were killed 24 hr after injection.

skin grafts on otherwise untreated Buffalo rats. The administration of the unlabeled horse antiserum reduced this localization by a factor of 6.7.

Discussion. The experimental results reported here show that it is possible to prepare, from xenogeneic antisera produced in rabbits or horses, labeled histocompatibility antibody able to localize after iv injection with a high degree of selectivity in rat skin grafts where only the skin transplant carries the corresponding antigen. The reason for the presence of the histocompatibility antibody in the horse anti-rat lymphocyte sera remains to some extent a matter of speculation. Presumably the rat cells, or a portion of the rat cells used for the horse immunization carried a strong histocompatibility antigen that is expressed in F-344 rats.

The most likely cause of the rapid blocking by horse antiserum of labeled antibody localization in target tissues is the saturation of antigen sites in the skin transplant by histocompatibility antibody present in the horse antiserum. This effect might be supplemented by an antibody induced reduction in the number of available antigen sites, an effect termed antigenic modulation by Old and Boyse and their co-workers (8, 9). Since the horse anti-rat lymphocyte serum is also a powerful immunosuppressant, and this might also affect antibody localization, the postulated mechanisms for the blocking action would be better established if experiments using unlabeled histocompatibility antibody isolated from horse antiserum showed a similar blocking action.

Our discovery of the blocking action of the horse antisera was due to experiments in which the immunosuppressive effects of this horse serum was used, by the technique of Kellen (10), to permit the growth in Buffalo strain rats of tumors induced by methylcholanthrene in F-344 rats. It turned out that localization of labeled histocompatibility antibody in these tumors was much lower than that found in experiments that were identical except that immunosuppression was induced by whole body X irradiation plus cortisone injections.

If similar antibodies are present in antihuman lymphocyte sera, a possibility would seem to exist that the survival of transplants of human kidney and other organs in patients treated by anti-lymphocyte sera might sometimes be influenced by the presence of this type of antibody.

Summary. It is shown that appropriate xenogeneic rabbit and horse antiserum, as well as allogeneic rat serum, can serve as sources from which ^{125}I-labeled antibody to a strong histocompatibility antigen expressed on Fischer-344 strain rat cells can be prepared. After iv administration all three types of antibody would localize preferentially in F-344 skin grafts made 4 days earlier on Buffalo strain rats. After 24 hr, ^{125}I localization ranged from 10 to 20% of the injected dose on F-344 skin grafts averaging 0.3 g weight on 150–190-g Buffalo rats. The horse serum used as an antibody source was a commercially available anti-rat-lymphocyte serum. Prior ip administration of two or three doses of 0.5 ml of this serum largely blocked uptake by F-344 skin grafts of both the ^{125}I-labeled alloantibody and the ^{125}I-labeled xenogeneic histocompatibility antibody prepared from this horse antiserum.

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