

PASSIVE TRANSFER OF TRANSPLANTATION IMMUNITY

III. INBRED GUINEA PIGS*, ‡

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In guinea pigs, when tuberculin sensitivity was passively transferred by lymphoid cells sensitized to tubercle bacilli and labeled with tritiated thymidine, the transferred cells were readily identified at the PPD skin test site (1). Further, the transfer of tuberculin sensitivity was not achieved by transferring BCG-sensitized cells enclosed in Millipore chambers (2). In mice, however, when transplantation immunity was passively transferred by lymphoid cells sensitized to homologous tissues and labeled with tritiated thymidine, few or no tagged cells were observed in the skin rejection sites. In addition, successful accelerated rejection of homologous skin grafts was accomplished by transferring cells, sensitized to the homologous skin, enclosed in Millipore chambers (3). The differences observed in the transfer of tuberculin sensitivity and transplantation immunity in guinea pigs and mice respectively might therefore have been due to the use of two different species.

This investigation was designed to study transplantation immunity in inbred guinea pigs by (a) the passive transfer of cells sensitized to homologous tissues and labeled with tritiated thymidine, and (b) the passive transfer of sensitized cells enclosed in Millipore chambers. The results were similar to those in mice and corroborated that different immunologic mechanisms were operative in transplantation immunity and tuberculin sensitivity.

Materials and Methods

Two inbred strains of guinea pigs, strain 2 and strain 13, were used throughout the study. Intrastrain histocompatibility has been demonstrated by persistence of isografts for 20 weeks (4) and 2 years (5).

Skin Grafting Procedure.—Full thickness grafts from the body integument were found to have such a thick dermis that the number of successful takes of autografts and isografts was low. Hence, split thickness body skin or full thickness ear skin was used. For split thickness grafts the entire body skin was stripped from a guinea pig. The skin was stretched over a

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sterile wooden board and strips of split thickness grafts were removed with a Blair-Brown skin graft knife. The split thickness skin segments were cut to desired size and placed on sterile, saline-soaked gauze pads until needed.

To sensitize donors, four $1\frac{1}{2} \times 1\frac{1}{2}$ cm grafts were applied to full thickness skin defects created on the dorsum of the animal, inferior to each axilla and over each posterior iliac crest. These four skin grafts provided stimulation of the regional axillary and inguinal lymph nodes.

The test skin grafts were 2 x 4 cm rectangles and were applied on full thickness skin defects created over the posterior thoracic wall. All the grafts were fitted and sutured to the surrounding skin with 4-0 silk sutures. After placement, the grafts were perforated to permit escape of serum from the graft bed. The transplants were not covered with dressings and were inspected daily. With this technique successful takes could be distinguished from failures; and further, the cause of unsuccessful takes could be ascertained; *i.e.*, whether due to immunologic rejection or surgical manipulation.

TABLE I
Skin Homograft Survival Times between Strain 2 and Strain 13 Guinea Pigs

Type of skin graft	No. of grafts	Strain		Mean of homograft survival times \pm SE*
		Donor	Recipient	
First set	26	13	2	9.7 \pm 0.98
	12	2	13	9.1 \pm 0.64
Second set	12	13	2	5.6 \pm 0.28
	12	2	13	5.9 \pm 0.46

* Standard error of the mean.

To determine the normal first and second set homologous skin graft survival times between strain 2 and strain 13 guinea pigs, twelve exchange first set grafts and, 4 weeks later, twelve exchange second set grafts were studied. The skin graft survival times were also determined in an additional fourteen strain 13, skin grafts on strain 2 animals since this combination was used solely in the Millipore chamber experiments. The data are recorded in Table I. These norms were needed because Bauer (4, 5) had used a different technique of skin grafting and had reported first set survival times of 7.7 to 7.8 days between the two strains.

Transfer of Labeled Cells.—Donor guinea pigs were sensitized to the homologous strain by skin grafts and intraperitoneal injection of 5×10^7 spleen cells 1 day after grafting (Fig. 1). The donor guinea pigs were injected intraperitoneally with 0.25 μ c of tritiated thymidine per gm body weight every 8 hours for 3 days, from the 5th to 7th days after grafting. On the 8th day the regional lymph nodes and spleens were removed and raked into balanced salt solution (Hanks') containing 20 per cent polyvinylpyrrolidone by volume and 5 units of heparin per ml. The resulting cell suspension was passed through a 100 mesh nylon gauze filter and centrifuged at 1500 RPM for 10 minutes. The supernatant was discarded and the sediment of packed cells was resuspended in the balanced salt-PVP solution. Aliquots of the resulting suspension were taken for total cell counts and smears for autoradiography. The labeled, sensitized lymphoid cell suspensions were injected intravenously at a dose of one to two donors for each isologous guinea pig. The recipients were divided into two groups. One group received a homologous skin graft 2 days after passive transfer of labeled sensitized lymphoid cells and the other group received grafts simultaneously with the intravenous

transfer of labeled lymphoid cells. The animals were sacrificed at daily intervals up to the 5th day after grafting. The skin homograft, spleen, lymph nodes, liver, and kidneys were processed for autoradiography.

Transfer of Sensitized Lymphoid Cells in Millipore Chambers (Fig. 2).—In all the Millipore experiments, strain 2 guinea pigs were used as donors and recipients of lymphoid cells; strain 13 guinea pigs were used as the homologous sensitizing and testing strain.

Donor strain 2 guinea pigs were sensitized in one of three ways. One group received a single second set strain 13 skin graft 3 weeks after the primary sensitizing skin grafts had sloughed. A second group was sensitized by first set skin grafting and by the intraperitoneal

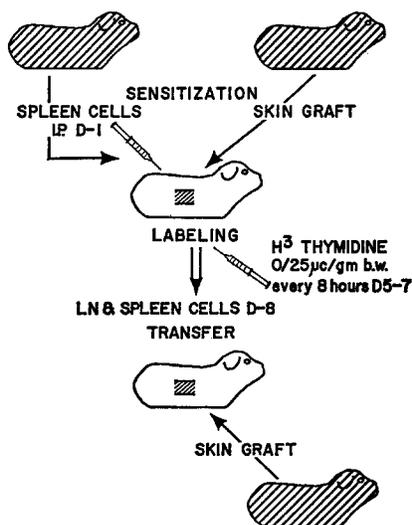


FIG. 1. Experimental model for passive transfer of transplantation immunity by tritiated thymidine-labeled lymphoid cells in inbred guinea pigs. (D = day; LN = lymph node; b.w. = body weight).

injection of 5×10^7 spleen cells 1 day after grafting. A third group was sensitized as the second group, and in addition received a second intraperitoneal injection of 5×10^7 homologous spleen cells 2 weeks after slough of the primary graft.

The sensitized lymphoid cells were harvested from the regional lymph nodes and spleens of the donors at the following times: Group 1, the 6th day after application of the second set graft, Group 2, the 8th day after skin grafting, and Group 3, the 6th day after the last intraperitoneal spleen cell injections. The lymphoid cell suspensions were prepared in the same manner as described for the experiments involving passive transfer of labeled lymphoid cells. The suspensions were placed in Millipore chambers 25 mm in diameter with VC membranes¹ having a pore size of 0.1μ and a thickness of 150μ . The technique of preparing the chambers has been previously reported (3). Each chamber was filled with approximately 3×10^8 lymphoid cells in an 0.3 ml volume.

The filled chambers were placed subcutaneously around the periphery of the graft bed prepared on the dorsa of the isologous recipient guinea pigs. The homologous skin graft was

¹ Obtained from Millipore Filter Corporation, Bedford, Massachusetts.

then sutured into place. The skin grafts were scored in the gross and when graft rejection was apparent the animal was sacrificed and sections of the graft and graft bed were taken for histology. The Millipore chambers were examined for possible leakage and the contents processed for microscopic examination.

For controls, Millipore chambers containing non-sensitized isologous lymphoid cells were placed subcutaneously around homografts of skin in 8 guinea pigs. The grafts were scored in the gross and examined microscopically in the same manner as those of the experimental groups.

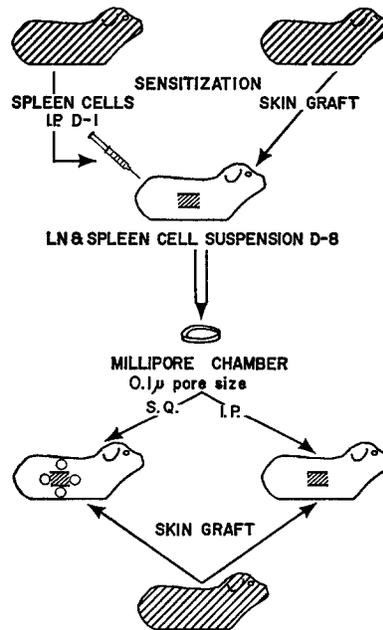


FIG. 2. Experimental model for passive transfer of transplantation immunity by sensitized lymphoid cells in Millipore chambers. (S.Q. = subcutaneous).

To determine specificity of the rejection process, in 7 strain 2 recipients, lymphoid cells enclosed in Millipore chambers and sensitized to strain 13 tissues were placed subcutaneously around a specific strain 13 skin graft and a non-specific skin graft from an albino guinea pig bred at random.

RESULTS

Transfer of Labeled Cells.—Following the passive transfer of 3 to 6×10^8 labeled sensitized lymphoid cells into isologous recipients there was evidence of accelerated homograft rejection in 9 of 14 recipients (Table II). All guinea pigs sacrificed after the homograft had been in place for 3 days or longer showed evidence of rejection. Despite a 20 per cent label of the transferred cells and

effective passive transfer of homograft immunity only 22 labeled cells were observed in 168 autoradiographs of graft rejection sites.

Labeled lymphoid cells were found without difficulty in the spleen and lymph nodes of the recipient isologous guinea pigs as late as 7 days after cell transfer and were rarely seen in the non-lymphoid parenchymal organs 48 hours after cell transfer.

TABLE II
Passive Transfer of Tritiated Thymidine-Labeled Sensitized Lymphoid Cells in Inbred Guinea Pigs

Guinea pig	Day of sacrifice		No. of cells transferred ($\times 10^6$)	Per cent cells labeled	Condition of graft* (+ to +++++)	No. of labeled cells at graft site†
	Age of graft	Duration of cell transfer				
	days	days				
3A	1	1	363	22	0	2
3B	1	3	363	22	0	0
4A	2	2	492	18	0	0
4B	2	4	492	18	0	3
7A	2	2	422	21	0	1
7B	2	4	422	21	+	1
6A	3	3	589	17	+	5
6B	3	5	589	17	+	1
8A	3	3	524	20	++	3
8B	3	5	524	20	+	5
9A	4	4	514	18	++	1
9B	4	6	514	18	+++	0
10A	5	5	461	21	+++	0
10B	5	7	461	21	+++	0

* 0 = no rejection, + = 0 to 25 per cent epithelial necrosis, ++ = 25 to 50 per cent epithelial necrosis, +++ = 50 to 75 per cent epithelial necrosis, ++++ = 75 to 100 per cent epithelial necrosis.

† Total of 22 labeled cells found in 168 autoradiographs of skin graft rejection.

Transfer of Cells in Millipore Chambers.—Passive transfer of transplantation immunity was achieved in two of the three experimental groups (Table III). Cells obtained from donor guinea pigs sensitized by skin grafting and by intraperitoneal spleen cell injection, passively transferred transplantation immunity when enclosed in Millipore chambers. The addition of a second spleen cell injection 2 weeks after sloughing of the first set skin graft did not significantly increase the effectiveness of the sensitized cells. However, lymphoid cells obtained from donors sensitized with second set skin grafts did not effect a statistically significant decrease in homograft survival times when passively transferred within Millipore chambers ($p < 0.2$). There was no evidence of

accelerated skin graft rejection in the 8 control guinea pigs which received nonsensitized isologous cells in Millipore chambers.

Seven strain 2 guinea pigs were grafted with both a strain 13 and an indifferent skin graft and received sensitized cells in Millipore chambers. The grafts were removed 6 days after transplantation. Six of the 7 specific strain 13 grafts showed partial to complete rejection as compared with only 1 of the 7 non-

TABLE III
Passive Transfer of Homograft Immunity in Guinea Pigs by Sensitized Lymphoid Cells in Millipore Chambers

No. of guinea pigs	Method of sensitization: (strain 13 → strain 2)	No. of cells transferred ($\times 10^6$)	Mean of homograft survival times (\pm SE)	p values
10	Second set skin graft	960	8.0 \pm 0.42	<0.2
19	Skin graft plus i.p. spleen cells	770	6.4 \pm 0.22	<0.01
7	Skin graft plus i.p. spleen cells \times 2	840	5.7 \pm 0.70	<0.01
8	Controls (no sensitization)	760	9.3 \pm 0.88	
26	First set skin grafts		9.7 \pm 0.98	

TABLE IV
Rejection of Specific Strain 13 and Non-Specific Skin Grafts (Random-Bred) by Sensitized Cells in Millipore Chambers

Extent of skin graft rejection (6th post graft day)	Specific graft (strain 13)	Non-specific graft (random-bred)
<i>per cent</i>		
100	3	0
75	3	0
50	0	0
25	0	1
0	1	6

specific skin grafts (Table IV). These results indicated that the effect of sensitized cells enclosed in Millipore chambers was specific.

Gross examination of the Millipore chambers at autopsy of the guinea pigs revealed no evidence of perforation of the membranes or separation of the membranes from the lucite rings. Microscopic examination of the membranes showed no cells traversing the Millipore membranes.

DISCUSSION

The findings reported in this study of the passive transfer of transplantation immunity in inbred guinea pigs are similar to the findings previously reported in mice (3). Passive transfer of transplantation immunity was accomplished

without the physical presence of the sensitized cells at the site of graft rejection. The specific agent responsible for skin graft rejection in both species was a substance liberated by the sensitized cells, capable of penetrating Millipore chambers, and effecting graft rejection. Thus, it would appear that the difference in the immunological mechanisms of homograft rejection and delayed sensitivity of the tuberculin type are truly different and do not represent a species difference.

Of importance was the method of sensitization of donor cells capable of transferring transplantation immunity when enclosed in Millipore chambers. Sensitization by a first set and second set skin graft was inadequate, although this method was efficacious in adoptive immunity. This failure might have resulted from insufficient sensitization because of the short exposure of the host to the second antigenic stimulus of the second set graft. In second set rejection there was early vascular occlusion, perhaps preventing the spread of antigen within the host. In contrast, the effectiveness of adding spleen cells to the sensitization of the donor might be explained by another mechanism. The dissociated spleen cells entered the circulation and inhabited all lymphoid structures resulting in a more generalized antigenic stimulus as compared with solid tissue grafts which primarily stimulated the regional lymph nodes.

The use of inbred guinea pigs for tissue transplantation studies presented problems not encountered in inbred mice. The first problem was the paucity of information available regarding the precise skin graft survival times between strain 2 and strain 13 guinea pigs. Since our skin grafting technique differed from that of Bauer (4, 5), it was necessary to establish our own rejection times.

Second, the process of skin graft rejection in the guinea pig and mouse differed. In the mouse second set rejection involved dermal infiltration of mononuclear cells followed by vascular occlusion and rapid death of the thin epithelium within 1 to 2 days. In the guinea pig, the dermal reaction was more intense and inflammatory cells were predominantly found at the dermal-epidermal junction but the epithelium appeared histologically normal up to, and occasionally, at the time of, graft slough. This normal histologic appearance of the epithelium just prior to slough obscured accurate gross scoring of the grafts.

SUMMARY

Passive transfer of transplantation immunity was accomplished in inbred guinea pigs with tritiated thymidine-labeled lymphoid cells sensitized to homologous tissues. Autoradiographs of the homologous skin graft sites disclosed the presence of relatively few or no labeled cells at the site of rejection.

Passive transfer of transplantation immunity was also accomplished with sensitized lymphoid cells enclosed in cell-impenetrable Millipore chambers. Previous studies with passive transfer of tuberculin sensitivity in guinea pigs revealed that the specifically sensitized cells could be easily found at the site of

challenge in the presence of specific antigen and were ineffective when enclosed in Millipore chambers. It appeared, then, that the homograft reaction and delayed sensitivity of tuberculin type were achieved by different immunologic mechanisms within the same species.

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